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A practical approach to diagnosing and treating infertility by the generalist in obstetrics and gynecology

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Summary

Purpose: To present a diagnostic and treatment paradigm for infertility designed for the obstetrician gynecologist generalist. Materials and Methods: Simple methods of tubal evaluation, e.g., the hysterosalpingogram (HSG) and post-coital test to evaluate both male and cervical factor are discussed. Treating paradigms will be discussed for ovulatory disorders and luteal phase defects. The role of the OB/GYN generalist on performing surgery in the modern era will be mentioned. Results: If an HSG shows a unilateral hydrosalpinx the generalist should consider performing the unilateral salpingectomy since the advent of in vitro fertilization-embryo transfer (IVF-ET) with a de-emphasis on surgery has made the reproductive endocrinologist/infertility specialist (REI) less skillful in laparoscopic surgery. The REI rarely performs tuboplasty today. Not only does the exclusive treatment in the luteal phase with progesterone save the women money and side effects (including multiple births), but may actually improve pregnancy rates compared to the usual technique of follicle stimulating drugs plus intrauterine insemination. Conclusions: Because the generalist will not be tempted to suggest therapies, e.g., IVF-ET because this effective therapy is the best option for the financial success of the REI, but at the expense of financial depletion of the patient, there is plenty of room for generalists taking over as the first line physicians for infertility rather than just a referral service. Reproductive endocrinologists/infertility will almost invariably perform IUI each month even if not doing IVF which is also profitable to the REI, but costly in time and money to the patient. In contrast, the generalist, aimed with the knowledge that IUI does not improve pregnancy rates if the post-coital test is normal, will save the patient and/or the insurance money if the woman conceives. Obviously certain circumstances, e.g., bilateral blocked fallopian tubes or very severe oligoasthenozoospermia (but not teratozoospermia) will prompt an immediate referral to an REI.

Key words: Post-coital test; Progesterone therapy; Laparoscopic surgery; Salpingectomy; Follicle maturing drugs.

Introduction

The obstetrician/gynecologist generalist is frequently the main primary care physician for women. Many generalists list their practice as OB/GYN and infertility. It is reasonable for the OB/GYN specialist to offer infertility services initially rather than an automatic referral to a specialist in reproductive endocrinology and infertility (REI) similar to the primary care physician or general internist having the right to treat a person for probable peptic ulcer disease without an immediate referral to the gastroenterologist or treat a person for a urinary tract infection without immediately referring the patient to a nephrologist or infectious disease specialist. Of course if the problem is not corrected in a reasonable amount of time then a referral to the specialist in that field would be a reasonable approach.

The objective of this editorial is to try to provide a practical diagnosis and treatment philosophy for the OB/GYN generalist to attain the goal of a successful pregnancy that is within the scope of reasonable diagnostic and treatment “tools” available to the generalist. Furthermore this manuscript will try to provide a philosophy that will help the generalist to determine the appropriate infertility specialist to whom to refer the couple if the generalist is unsuccessful in helping the couple to achieve a pregnancy. Finally the guidelines will be provided so that the generalist will know when is the right time for the referral to an REI, i.e., what is a reasonable time for a given treatment before more involved procedures are needed.

Evaluating and treating fallopian tube and pelvic pathology

The most common cause of infertility around the world is damaged fallopian tubes. This is most commonly caused by
previous sexually transmitted diseases with adhesions from endometriosis as the next most common factor.

The usual first type of investigation for tubal disease is the hysterosalpingogram (HSG). The sonohysterogram does not provide an adequate assessment of the fallopian tubes though is very good for evaluating uterine cavity abnormalities.

Though patent fallopian tubes do not necessarily negate a pelvic mechanical factor, e.g., phimosis of the fimbria, or tubal or ovarian adhesions interfering with tube oocyte pick-up, most REIs will not proceed to a laparoscopy at this point if the fallopian tubes appear normal but seek another remediable infertility factor.

If a laparoscopy is the next logical procedure to be performed, years ago, the REIs had more extensive training in laparoscopic surgery than the generalist. Today when I review the charts of patients seeking another opinion concerning their unsolved infertility problem I usually find that the previous REIs have not suggested a laparoscopy but rather always seems to push their patients toward in vitro fertilization-embryo transfer (IVF-ET). This means that REIs, and especially the fellow in REI, are becoming less skilled in performing laparoscopic surgery. Unfortunately this push away from laparoscopy and toward IVF-ET may be financially motivated. Thus fellows are no longer trained in intricate tubal microsurgery and thus there has been a tendency for less surgically skilled but more cerebral type OB/GYN residents seeking fellowships in REI. Thus it is probable time that there is a shift toward the generalist rather then the REI performing laparoscopy for infertility. The obstetrics/gynecologist generalist interested in the field of infertility should take extra training in laparoscopy surgery to become the surgical successors. Of course at the same time they should get extra training in hysteroscopic surgery.

The gynecologist should be aware that when faced with bilateral hydrosalpinges during laparoscopy, the physician should be prepared to preferably perform a bilateral salpingectomy because the infectious material in these diseased fallopian tubes can infiltrate the uterine cavity and prevent implantation with IVF-ET [1-3].

Some clinicians were under the impression that as long as one tube was open that salpingectomy is not needed, but it has been established that even a unilateral hydrosalpinx can impair fertility and it should be removed [4]. One case showed that removing a unilateral hydrosalpinx can allow pregnancy through natural conception as long as the other fallopian tube is normal even if endometriosis is suspected by symptoms or signs. Of course it is important for the generalist to be familiar with proper technique to not only maximize infertiltiy potential, but to prevent subsequent ectopic pregnancies even with IVF-ET. However on occasion, especially related to the presence of dense adhesions, salpingectomy is not feasible, and therefore a tubal ligation is performed to at least impede infiltration of infectious material to the uterine cavity.

The majority of women with endometriosis can conceive by correcting ovulatory dysfunction [7]. Since attempts at removing endometriosis can lead to a diminished ovarian reserve, it is probably best not to perform a laparoscopy initially even if endometriosis is suspected by symptoms or signs. Nevertheless there are data suggesting that at least in some women, and as in those who fail to conceive after correcting luteal phase defects or the luteinized unruptured follicle (LUF) syndrome, removing endometriosis through laparoscopy can improve fecundity [8-11].

At one time the REI was more skilled than the generalist in performing laparoscopic surgery. Today probably because IVF-ET results in a quicker and more definite pregnancy (but unfortunately also because performing IVF-ET is far more profitable to the REI than laparoscopic surgery), many REIs are not as skilled as in previous time in this surgical technique. Thus a skilled generalist should not be afraid to advise the patients that if simple measures performed by the generalist that will be further described in this editorial are not effective in achieving a pregnancy and the opinion of an REI is obtained, if the REI suggests IVF-ET, and this is not an affordable option, to make an appointment with the generalist to perform a laparoscopy with ablation of endometriotic implants and possible lysis of adhesions. Of course it is important for the generalist to be familiar with proper technique to not only maximally improve fertility outcome but also to relieve pain [12].

Ovulation disorders – women with regular menses

There are three phases of ovulation – attaining a mature dominant follicle (18-24 mm average diameter with a serum estradiol (E2) >200 pg/mL), releasing the oocyte from the follicle (as defined by shrinkage of the follicle by at least five mm two days after the LH surge), and the production of adequate progesterone (and E2 also) by the corpus luteum that is formed from the dominant follicle minus the oocyte [13].

There is evidence that a small majority of women who have infertility, regular menses, and have luteal phase de-
fects make mature follicles [14]. One study found that 77% of these women achieved a pregnancy in six months with just luteal phase progesterone support vs. only 17% with follicle maturing drugs [14]. Yet 64% of the failures who had taken follicle maturing drugs conceived when placed on progesterone support exclusively during the next six months [14].

Thus for infertile women with regular cycles, I usually will perform pelvic sonography to evaluate follicular size beginning 16 days before their earliest expected menses. If the follicle is ≥ 18 mm, a serum E2 will be obtained. If the follicle is less than 18 mm, they will be asked to return when the follicle is expected to reach 18 mm, considering that follicles grow at about 2 mm per day. If follicular maturation is reached two days later, a repeat ultrasound is performed to see if the oocyte released from the follicle [15]. If the follicle reached maturity and the oocyte released, the woman is treated exclusively with vaginal progesterone (progesterone vaginal suppositories 200 mg morning and bedtime, or Crinone vaginal gel 8% am or pm, or Endometrin vaginal tablets 100 mg twice or three times per day. One way to determine if the dosage of progesterone is sufficient is to perform a pelvic sonogram at mid-luteal phase. If the endometrial echo pattern did not convert to a homogeneous hyperechogenic pattern, the dosage of progesterone should be increased at that moment and the dosage started higher the next cycle [16]. What if the oocyte did not release? Studies show that failing to release the oocyte could be an isolated phenomenon. However, the majority of women who fail to release the oocyte the first time will fail in succeeding cycles [15]. Thus therapy with either a single injection of 10,000 units of human chorionic gonadotropin is given or if this fails leuprolide acetate (now using its agonistic effect to raise endogenous LH and FSH) one mg every 12 hours with two or three dosages [17].

At first glance the OB/GYN generalist may think that the proposed diagnostic paradigm presented above is beyond the scope of the generalist, but should be relegated to the infertility specialist. Unfortunately the aforementioned more scientific approach is not taken by most reproductive endocrinologists. Instead the majority seems to practice a type of scripted or universal treatment protocol that is not specifically geared to specific problems. Most might try even in women with regular menses three cycles of clomiphene citrate with intrauterine insemination (IUI), three cycles of FSH injection and IUI, and then push them into IVF-ET. Usually they do not prescribe progesterone in the luteal phase for IUI cycles, just IVF cycles.

Intrauterine insemination (IUI)

The best cervical mucus is about 40 hours before ovulation and coincides with the peak serum E2. Right at the time of ovulation the cervical mucus may have already regressed. So the theory holds that even if one has a normal post-coital test maybe the sperm lacks longevity of fertilization potential, so placing the sperm past the cervix directly into the uterine cavity so that there is a closer proximity to ovulation may help some women to achieve pregnancies. However, we presented data at the 2011 American Society of Andrology meeting showing a 25% pregnancy rate per cycle in couples with corrected infertility factors having normal post-coital tests without IUI vs. 26% with IUI added [18]. Thus there is the opportunity for a generalist interested in infertility to take over the cases that do not require IVF-ET. The generalist could do their own ultrasounds, either personally or with an ultrasound tech or could refer them to a local ultrasound facility. Similarly in the “old days” REIs had to develop their own endocrinology laboratory to get same-day results for serum estradiol, progesterone, LH, and FSH. Today all commercial laboratories provide this service. Suppose, however, the generalist is too busy to be able to deal with the day by day decisions that this paradigm requires. Yet, the generalist is reluctant to immediately refer to the REI because he is aware of the patient’s limited finances and the generalist’s experience is that the expensive aforementioned “shot-gun” approach with a push toward IVF-ET is the norm for the local REI specialist. It would be appropriate for the generalist to try limited treatment, described below aimed at improving the women’s fertility potential, but with an educated guess as to what the problem may be. Thus this approach has potential to help with little potential to harm or diminish the couple’s infertility problems.

Evaluating male factor and cervical factor

For couples where the female partners have regular menstrual cycles, the wife is asked to return 15 days before her earliest expected menstrual cycle having intercourse the night before. Finding any sperm moving in a forward manner through the cervical mucus will establish that there is probably not a sperm issue or cervical mucus problem. If the post-coital test is subpar one may have the couple try this again in two days. If still not good then a semen analysis can be ordered or the generalist could ask the couple to bring in a fresh specimen and after placing a drop of sperm on the slide with a coverslip, the generalist can get a reasonably good idea if there seems to be an adequate amount of sperm or not. If the sperm concentration of motile sperm seems reduced, the usual tendency is to refer to a general urologist or a fertility trained urologist. Some urologists will evaluate whether a varicocele is present and if so recommend varicocelectomy. There is little evidence that this procedure helps improve sperm count and motility [19]. Referral to the REI will generally lead to IUI then to IVF with intracytoplasmic sperm injection (ICSI) or directly to IVF with ICSI. However, if the generalist simply obtains a serum FSH and testosterone on the male partner finding an
FSH and testosterone level in the low to low normal range could lead to improvement of the sperm and pregnancy through normal intercourse, by simply treating the male partner with clomiphene citrate 25 mg daily and giving the couple up to six to eight months to achieve a pregnancy before referral to a urologist or REI [19]. Thus, to reiterate, if the post-coital test shows any sperm progressing in the cervical mucus at least eight hours after intercourse, it is probably sufficient not to obtain a formal semen analysis. Even if the sperm concentration falls below the low normal level of ≤ 10 x 10⁶/mL, it does not necessarily mean that any treatment other than intercourse is necessary. We presented data that with natural intercourse infertile women achieved following correction of female infertility factors a 22% six-month pregnancy rate with less than 2.5 x 10⁶/mL sperm concentration a respectable 69% pregnancy rate with 2.5 to < 5 x 10⁶/mL, 81% with 5 to ≤ 10 x 10⁶/mL, and even 81% with those with 5 to < 10 x 10⁶/mL, which was equal to the pregnancy rate for those with superior sperm motile density of > 15 x 10⁶/mL [20]. Thus I think for the OB/GYN generalist it is appropriate to forego a formal semen analysis if the post-coital test is adequate, which means demonstrating at least one sperm moving across a few high powered fields. If a gynecologist wants, he/she can send out the first few semen specimens but place an aliquot of carefully mixed sperm on a slide and try to develop an educated guess as to the concentration and motility if the post-coital test is below par to gain insight as to whether the problem is a male factor or cervical factor. Of course the reader may question the soundness of this suggestion of sperm evaluation because it eliminates morphology. Indeed sperm morphology at one time was considered the best way to detect a subnormal male [21]. However, though we were a minority at that time, we challenged this test as being able to detect a subfertile male [22]. Indeed in the modern era most fertility centers do not place much value on this test [23-25]. Kruger’s test for strict morphology uses 4% normal as the cut-off for detecting subfertile males. We have presented a scientific presentation at the 2012 American Society of Andrology meeting showing that even only 1% normal sperm does not adequately detect the subnormal male.

When we evaluate the male in our practice we always measure the hypo-osmotic swelling test and antisperm antibodies [25]. Most REIs do not assess these very important tests when they perform semen analyses. We will measure for antisperm antibodies on the sperm if the post-coital test is normal because a male may have antibodies that block the attachment of the sperm to the zona pellucida even if immobilizing antibodies are absent [26]. Nevertheless most times significant antisperm antibodies will be immobilizing antibodies and cause a poor post-coital test [26]. Subnormal HOS tests which allow fertilization but failure of the embryo to implant for some reason would not be evaluated by over 95% of REIs [27]. So if the couple fails after many months of treatment, a simple referral to an REI will still miss low HOS tests or antisperm antibodies so the OB/GYN generalist would be better served to find a laboratory that performs these tests and send the male partner there. Fortunately the frequency of this abnormality is only about 5% in males < 40, 16% in males 41-49, and 25% in males > 50 [27]. If the post-coital test is subpar and the semen analysis appears normal even by observation of a drop, and if the mucus appears to have subpar quality, checking the serum E2 and P levels can help the OB/GYN generalist to know if the timing was right. If so one could repeat the post-coital test the following month and one could treat the women with guaifenesin 600 mg extended release tablets twice daily from day 1 until ovulation to attempt to improve cervical mucus quality [28].

Anovulation

What about women who appear to be anovulatory based on oligomenorrhea or amenorrhea? A simple measurement of E2 and FSH can help the physician to determine if the woman appears to have adequate oocyte reserve. If so, for the OB/GYN generalist my recommendation would be to treat the women with letrozole rather than clomiphene citrate [29]. The main reason for this suggestion is that clomiphene citrate is more apt to create hostile cervical mucus [30]. Furthermore letrozole can sometimes enable women with polycystic ovarian syndrome, especially obese women, to ovulate when clomiphene citrate fails. Letrozole is less likely to adversely affect endometrial thickness [29]. Finally it is more likely to induce mono-follicular ovulation. If 2.5mg for five days does not induce ovulation, as evidenced by follicular maturation studies with ultrasound and measurement of serum E2 and P, the dosage can be increased to five mg per day. Sometimes one does not need to induce another menstrual cycle with medroxyprogesterone acetate but merely start the increased dosage if there is no evidence of ovulation within ten days of stopping the letrozole. It is still important to supplement the luteal phase with progesterone since there is usually persistent luteal phase defects and thus increased miscarriage risk in women taking follicular maturation drugs [31].

Women with diminished oocyte reserve

I think that for the OB/GYN generalist it is wise not to measure the day 3 serum FSH at all for fear this will panic the physician and prompt an immediate referral to an REI thinking that time is running out. This seems to be a very provocative statement so I will explain why I make this statement. The large majority of REIs are under the wrong impression that a high day 3 serum FSH or a low inhibin B or anti-Müllerian hormone level is predictive that a woman even if chronologically young, has oocyte quality more akin to perimenopausal women [31].
In vitro fertilization is considered the ultimate method of achieving a pregnancy in an infertile woman. As far back as 1988 a high day 3 serum FSH was found by one of the leading IVF centers in the world to be associated with poor responders to exogenous gonadotropin, and even more important, was the observation of very poor pregnancy rates even if normal appearing embryos were transferred [33].

Even with all of the recent improvements in IVF-ET in recent times, one of the world’s leading IVF centers concluded that if the day 3 serum FSH ever exceeds 15 mIU/mL (even once), the live delivery rate is zero even after the transfer of normal appearing embryos [34]. However, we subsequently published data showing that despite the development of only one embryo in women whose serum FSH levels were all over 15 mIU/mL the clinical pregnancy rate was about 40% per transfer and the live delivered pregnancy rate 33% on the 65% who had a six- to seven-cell embryo [35]. The explanation for the dichotomy between these opposite conclusions is that the very poor pregnancy rate found in some IVF centers will was not as much related to extremely poor quality oocytes. but related to iatrogenic meiosis issues and downregulation of implantation factors by raising the serum FSH too high, by using high dosage FSH stimulation in an effort to create more follicles [35].

Mild stimulation, on the other hand results in pregnancy rates following IVF-ET in women with diminished oocyte reserve comparable to those with women with normal reserve [36]. Despite our aforementioned publications which occurred in the same journal as the Roberts et al. article [34], most REIs when faced with a woman with increased FSH will try high-dose FSH with or without IVF or will try to convince the couple that they should go directly to donor oocytes. The OB/GYN generalist has a better chance of achieving a pregnancy with just luteal phase progesterone on the third day of temperature rise on a BBT especially in women ≥ 30 years of age. Doing just a little more would include performing an HSG and fitting the couple in for a one time post-coital test.

For those generalists who would rather not treat infertility at all it is hoped that this editorial will better enable the OB/GYN to refer to the REI that would be best suited for his/her patient. Certainly the OB/GYN generalist should be reluctant to refer a woman of less financial means and an insurance that does not cover IVF-ET to an REI whose tendency is to perform IVF-ET or practicing herd medicine (three cycles of clomiphene citrate and IU, three cycles FSH injections, and IUI then IVF-ET on everyone).

As the primary care physician of women, no matter what stage a referral was made to an REI, the generalist may tell the woman to make an appointment for a month or two after the initial consultation with the REI so that the generalist can help decide if the treatment paradigm suggested by the REI is more favorable for the patient or the REI. If not satisfied, the generalist may recommend a different REI for a second opinion.

It should be noted that methods of truly diagnosing inadequate progesterone therapy during the luteal phase have not been developed adequately. The future eventually will allow rapid measurement of an immunomodulatory protein known as the progesterone induced blocking factor (PIBF) which suppresses natural killer cell activity in the vicinity of the maternal fetal interface [38]. After normals are determined, women falling below a certain PIBF level will either be given progesterone or their dosage will be increased. Alternatively, evaluation of human endometrial genome of a biopsied specimen in the luteal phase may determine when there is a deficiency or alteration of gene targets that are present during the window of implantation that may influence successful implantation [39]. Until the time that these tests are commercially available, it is reasonable to empirically treat women with progesterone in the luteal phase, especially if the woman is ≥ age 30.

Suggestions for a very busy OB/GYN generalist

For those OB/GYN generalists whose busy schedule precludes evaluating a given individual at specific given times of the schedule then the generalist should not offer clomiphene citrate but consider prescribing vaginal progesterone on the third day of temperature rise on a BBT especially in women ≥ 30 years of age. Doing just a little more would include performing an HSG and fitting the couple in for a one time post-coital test.

For those generalists who would rather not treat infertility at all it is hoped that this editorial will better enable the OB/GYN to refer to the REI that would be best suited for his/her patient. Certainly the OB/GYN generalist should be reluctant to refer a woman of less financial means and an insurance that does not cover IVF-ET to an REI whose tendency is to perform IVF-ET or practicing herd medicine (three cycles of clomiphene citrate and IU, three cycles FSH injections, and IUI then IVF-ET on everyone).

As the primary care physician of women, no matter what stage a referral was made to an REI, the generalist may tell the woman to make an appointment for a month or two after the initial consultation with the REI so that the generalist can help decide if the treatment paradigm suggested by the REI is more favorable for the patient or the REI. If not satisfied, the generalist may recommend a different REI for a second opinion.

It should be noted that methods of truly diagnosing inadequate progesterone therapy during the luteal phase have not been developed adequately. The future eventually will allow rapid measurement of an immunomodulatory protein known as the progesterone induced blocking factor (PIBF) which suppresses natural killer cell activity in the vicinity of the maternal fetal interface [38]. After normals are determined, women falling below a certain PIBF level will either be given progesterone or their dosage will be increased. Alternatively, evaluation of human endometrial genome of a biopsied specimen in the luteal phase may determine when there is a deficiency or alteration of gene targets that are present during the window of implantation that may influence successful implantation [39]. Until the time that these tests are commercially available, it is reasonable to empirically treat women with progesterone in the luteal phase, especially if the woman is ≥ age 30.

References

Female pattern hair loss (FPHL) is very common with an estimate of over 21 million women with this condition in the United States alone [1]. It is more common as women age so that it is present in 55% of women ≥ age 70, but it is present in about 10% of women between the ages of 20-30 and increases with advancing age [1].

Though increased androgens may cause FPHL, the same pattern may be observed in the presence of normal androgens. Thus the term androgenic alopecia has been changed in females to FPHL [2]. When a four-mm punch biopsy is taken from the central portion of the hair loss (preferably not temporal location), there is usually found increased numbers of miniaturized hair follicles that are the type for fine short hairs known as vellus hairs. In a given area of skin there are always some terminal and vellus hairs together but normally the ratio of terminal to vellus hair is over 3:1, and in FPHL the ratio is smaller [3]. Furthermore there is a reduction in follicle depth and a greater percentage of hairs are in the telogen phase (resting) than the anagen (growing) phase [4]. The same histology is found whether the serum androgens are elevated or not [5].

From looking at the phenotype of genetic males with a 5α hydroxylase deficiency (an enzyme that converts testosterone to dihydrotestosterone, DHT) it seems clear that DHT was the more important androgen at the hair shaft level. Thus these individuals despite normal testosterone levels did not grow a beard and did not develop androgenic alopecia. Also they did not develop prostate enlargement. Therefore it was clear that DHT and not testosterone was a more potent binding hormone to the androgen receptor in the hair shaft and also the prostate. Subsequently it was discovered that there are two isoenzymes of 5α reductase and it is type II that has a greater affinity for the androgen receptor [6]. Thus if there is an increase in circulating testosterone, e.g., from polycystic ovarian syndrome or adult onset congenital adrenal hyperplasia, the increased exposure of the hair shaft to the testosterone will allow more DHT produced at the hair shaft by normal amounts of 5α reductase enzyme. This would cause hirsutism but also possibly hair loss on the head in a male pattern distribution, especially if there is also a genetic predisposition [7, 8]. However, alopecia may also develop if there are normal levels of circulating testosterone but an increase of the 5α reductase type II enzyme in the hair shaft, thus creating more DHT locally. Estrogen may compete with testosterone to inhibit 5αreductase and conversion to DHT. Thus sometimes women will develop FPHL because of estrogen deficiency as seen in post-menopausal women [9].
There may be other factors other than androgen effect at the hair shaft level for FPHL. There was an extremely interesting case of FPHL in a genetic male but with complete testicular feminization syndrome [10]. There may be microvascular insufficiency, inflammatory conditions, or even insulin resistance itself without androgen increase an etiologic factor [11]. With insulin resistance there is an increase of insulin-like growth factor and this may stimulate more 5α reductase enzyme at the hair shaft.

In overt severe hypothyroidism progressing to myxedema, there is evidence that 57% of the women lose hair [12]. In milder cases of hypothyroidism the frequency and degree of hair loss is considerably less. The hair loss is mostly related to breakage of terminal hairs so the appearance of thinning is related mostly to areas of diminished length. The hair may get curly also. In contrast, thyrotoxicosis leads to hair that appears to be thin but it mostly related to thinner diameters of terminal hairs [12].

Alopecia areata (and totalis/universalis)

Alopecia areata is not uncommon but it occurs much less frequently (about 1.7%) in women than FPHL [13]. It tends to affect younger women than FPHL [14].

Patchy hair loss is referred to as areata, total hair loss on the head as totalis, and all body hair as universalis. The characteristic hair looks like an exclamation mark with the hair tapered proximally and wider distally.

In the acute stage hair follicles have a “swarm of bees” appearance with the hair follicles in a peribulbar lymphocytic infiltrate. Autoimmunity probably plays a strong role though it may be influenced by emotional stress [15]. Generally, the younger the age of onset the worse the prognosis.

Diagnostic work-up

The typical evaluation for alopecia involves measuring, first of all, serum androgens. For women who appear to be ovulating, we measure the total and free testosterone and 17-hydroxyprogesterone in the follicular phase. A serum dehydroepiandrosterone sulfate may be obtained but it has less importance in diagnosis and treatment.

Though the free testosterone is the more clinically active androgen, for some reason in androgen increased states, e.g., polycystic ovarian syndrome with classic ultrasound appearance of ovaries and typical high LH to FSH ratio (> 1.8:1) we will frequently find the total testosterone elevated but not the free testosterone. Thus we measure both and an increase in total testosterone will lead to methods of suppressing the source of T excess production, whether it be ovary, adrenals, or both. Therapy will be subsequently discussed.

From the thyroid standpoint obvious overt hypothyroidism will be treated with thyroid hormone replacement. Subclinical hypothyroidism with a serum free thyroxin (T4) level in the normal range with a serum TSH above 2.5 microIU/mL will usually be treated with a low dose of thyroid hormone. The exception is if the free T4 is above midnormal where we may repeat the blood test in six to eight weeks without treatment.

We look for iron deficiency but usually only treat with iron if there is an obvious iron deficiency anemia. Possibly a lack of ferritin has been hypothesized to cause hair loss by causing a shift from the anagen to the telogen phase [16, 17].

Cushing’s disease is so rare that we only measure cortisol levels if there are some symptoms or signs consistent with this diagnosis.

As reproductive endocrinologists the present authors do not do scalp biopsies. However honestly, it has not been their policy to refer these patients either to dermatologists for the purpose of a biopsy.

Authors’ present treatment

Increased Androgens

If the results suggest polycystic ovarian syndrome, the most common increased androgen state, an oral contraceptive is one of the first line therapies. Suppression of the increased serum LH is considered as a first line therapy because it will not only lower the elevated androgens, but also diminish the active free T by increasing sex hormone binding globulin. Furthermore the oral contraceptive will provide the necessary progesterone to prevent endometrial hyperplasia related to anovulation.

Though any oral contraceptives may be effective by lowering elevated androgens, improved efficacy may be gained by using oral contraceptive with a progestin that has less androgenic properties (all progestins in oral contraceptives are derived from androgens and have 19 instead of 21 carbon backbones). The present authors’ normal preference is norgestimate, and to reduce the progestin dosage even more they prefer the ones with three graduated dosages. For polycystic ovaries, they generally recommend the addition of spironolactone 100 mg twice daily to the oral contraceptive. One of the main side effects of spironolactone is to cause ovulation disturbance and irregular cycles. The oral contraceptives will generally overcome this problem. Spironolactone can reduce androgen levels without oral contraceptives so the two may be added. However, the main beneficial extra effect may be by blocking the androgen receptor thus further reducing the adversity of the remaining testosterone still circulating.

If we were to use triple therapy the next drug we would use would be a 5 α reductase inhibitor – finasteride or dutasteride. As mentioned they inhibit conversion of T to DHT. The one-mg dosage approved for male alopecia does not seem to be effective for women with FPHL [18]. However at five mg (the dosage used for benign prostate hypertrophy) benefits has been demonstrated [19, 20].
An update on the treatment of female alopecia and the introduction of a potential novel therapy

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For alopecia areata (and totalis and universalis) it is difficult to determine the efficacy of various topical and intranasal therapies because the condition is known for spontaneous remissions. For a discussion of these types of therapy the reader is referred to the review by Alkhalifah [30].

A novel therapy – case report

A 62-year-old woman was seeking help for alopecia of six years duration. Though it had an initial insidious start, it considerably exacerbated in the last 1.5 years. She makes note that her twin brother has a full head of hair as did her mother and her father was only mildly hair deficient. The loss of head hair was not accompanied by facial or body hirsutism but actually the opposite in that she was losing axillary hair and losing patches of pubic hair which sometimes returned.

She has been on raloxifene 60 mg/day starting at age 47. With the notice of increasing hair loss, her gynecologist placed her on estrogen but it did not help the hair loss. The patient also lost her eyebrows totally and her eye lashes were thin. She was taking thyroid hormone for several years and her serum thyroid stimulating hormone level was normal. The clinical picture seemed to be more consistent with a type of progressive alopecia universalis, the totalis portion showing no remission but steady progressive loss, and the pubic hair behaving more in an areata fashion with remissions and exacerbations.

Close questioning found that this woman had atopic dermatitis behind the ears, and she suffered from migraine headaches and pelvic pain. The present authors explained that there is a condition that is related to hypofunction of the sympathetic nervous system that leads to a variety of pain disorders (including migraine headaches and pelvic pain) [31].

One of the main functions of the sympathetic nervous system is to control cellular permeability and hypofunction leads to the inability to filter chemicals and toxins from absorbing into tissues, thus evoking an inflammatory reaction [32]. Indeed, evidence to support this theory has been provided by the quick and effective relief of pain, e.g., migraine headaches and pelvic pain (which plagued this woman) with the sympathomimetic amine dextroamphetamine sulfate [33-37].

There is a variety of skin disorders that respond to sympathomimetic amines including eczema or atopic dermatitis [38]. Since the aforementioned patient’s condition had some suspicions of alopecia, which is an inflammatory cause of hair loss, the present authors recommended the experimental use of dextroamphetamine sulfate rather than antiandrogens which they hoped would not only help the alopecia but relieve the pain from migraines, pelvic pain, and even her atopic dermatitis.

After three months of therapy with 30 mg per day of dextroamphetamine sulfate, her pelvic pain and headaches have completely disappeared along with the eczema. In addition for the first time in six years, she has shown moderate regrowth of head hair, complete restitution of body hair, and she is noticing the development of eyebrows.

Though it is possible that the hair improvement is merely related to spontaneous remission of the inflammatory state as seen in alopecia areata, the quick response in head hair despite no improvement in six years suggests that the sympathomimetic amine therapy may have been responsible for the improvement.

New considerations for diagnosis and treatment based on recent literature review

Based on relatively good safety record from minoxidil 2% twice daily for FPHL and effectiveness of at least some type of hair re-growth in the Cochrane systematic review, the present authors will consider adding topical minoxidil to their treatment paradigm [29]. However, the statement that “there is a lack of evidence for the effectiveness of some of the widely used treatments such as spironolactone, cyproterone acetate, finasteride, and laser comb therapy” will not dissuade the authors from using these therapies which they have personally observed to be reasonably effective in many but not all women. Furthermore, though there may not be enough rigor of design to satisfy the criteria set by Van Zuuren et al., for inclusion in the systematic review (thus leading to the aforementioned lack of support for these therapies), there are indeed studies supporting these other aforementioned therapies and to the present authors’ knowledge, no studies proving a lack of efficacy. Thus they will continue the use of spironolactone, finasteride, and laser hair comb therapy for alopecia (cyproterone acetate is not accessible in the United States).

The impressive response to the present case of alopecia, possibly of the inflammatory type, to sympathomimetic amines has to make the authors consider that perhaps other treatment refractory alopecia states may also respond to this treatment in some women. The present authors are considering using it on women where they think the problem is FPHL, but the woman does not respond to therapy to a dermatologist for purpose of histologic diagnosis. They may consider performing the punch biopsies ourselves. However, they would consider therapy no matter what the histologic diagnosis (FPHL or inflammatory hair loss) and then determine if dextroamphetamine sulfate is only effective in the inflammatory type or both.
References


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Molecular aspects and clinical methods for preserving ovarian reserves in women receiving cancer treatment

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Summary

Cancer prevalence is high, and of importance to cancer sufferers is the long term survival and normal activities resumption. Moreover, pregnancy is drawing interest for preserving ovarian reserves in post-chemotherapy affected women, especially of younger ages. The gonadotoxic effect of cancer treatment, involves mechanisms that are not fully understood, mainly due to the variety of molecular pathways triggered once therapeutic agents applied. Reported rates of premature ovarian failure after the treatment effect and the application of various treatment protocols, differ extensively due to the protocol itself but also due to the age of treated patients. Several options for preserving ovarian reserves are currently employed in the clinic, such as ovarian transposition, embryos cryopreservation and the use of gonadotropin-releasing hormone (GnRH) and its agonists/antagonists, but most of them are still under investigation. This paper reviews these methods and the molecular mechanisms that are possibly involved in the action of agents such as GnRH.

Key words: Ovarian reserves; Molecular; Clinical; Cancer; Treatment.

Introduction

It is estimated that approximately 6 million new cases of cancer, either hematological malignancies or solid tumors, are recorded worldwide every year. About 4 million of them, concern women of all ages and about one million women are survivors of childhood cancer [1].

In developed countries, both screening and use of chemotherapy and radiotherapy, have led to a dramatic reduction in mortality rates of cancer. Additionally, a growing number of women postpone childbearing to older age, due to social or financial causes. It is for such reasons that women younger than the age of 45 years pose a great demand for preserving fertility.

However, cytotoxic therapy is associated with damages to body tissues and cells other than the targeted tumor cells, significant morbidity and long-term physical and psychological effects. Among them, ovarian toxicity is an important and common long-term adverse event of curative chemotherapy and radiotherapy [2]. Since, many of these patients are young, they suffer premature ovarian failure (POF) which annihilates the reproductive function of the ovaries.

This article, reviews the literature, discussing the effects of chemo- and radio-therapy on ovarian toxicity and provides more detail on the possible molecular mechanism for the effect of cancer treatment on female fertility.

Chemotherapy

All chemotherapeutic drugs, have an adverse effect on ovarian tissue, by interrupting vital cell processes and arresting the normal cellular proliferation cycle. Most of the available data for ovarian failure after chemotherapy, is based on leukaemias, lymphomas, Hodgkin’s disease, and on some solid tumors such as breast cancer. However, there is an increasing number of patients with no malignancy who are being treated successfully with chemotherapy due to autoimmune diseases, such as systemic erythematous lupus, rheumatoid arthritis as well as some hematological diseases [1, 3-5].

Chemotherapeutic agents, can be grouped into five classes based on their mode of action: 1) alkylating agents, 2) aneuploidy inducers, 3) topoisomerase II inhibitors, 4) antimetabolites, and 5) radiomimetics [2]. Chemotherapeutic drugs are used as a monotherapy or in combination with other agents in order to increase their anti-tumor effects, but this also leads to an increase in their adverse events. Ovarian damage and failure, is a common long-term side effect of thermotherapy.

The final impact of chemotherapy to ovarian function depends on factors such as the patient’s age, the therapeutic protocol, and the dose of the drug administered [1, 6-8].

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Several studies have attempted to clarify the impact of age in determining the effects of chemotherapy on ovarian function. Older women have in general a higher incidence of complete ovarian failure and permanent infertility comparing with younger women [1, 9-11]. These results, can be explained by the larger deposits of follicles that young women bear [12]. Primordial follicles are diminished with age. At puberty, about 300,000 follicles are present and functioning in the ovaries, progressively declining with age to 100 at the time of menopause [12, 13].

In recent years, there is a great interest in new protocols of treatment of all types of cancer. The vast majority of these protocols are based on combinations of chemotherapeutics agents, and results in ovarian failure depend on the agents that are used. Alkylating agents, pose the highest risk in causing ovarian failure [2]. Cyclophosphamide, the most common agent in this category, can cause ovarian fibrosis and also follicular and oocyte depletion. Meier reports that the substances of this class, are closely associated with the greatest risk among all chemotherapeutics protocols for ovarian dysfunction [14]. Bines et al. [15], reviewed reports on ovarian destruction after post-adjuvant chemotherapy applied on premenopausal breast cancer survivors and concluded that the protocols that included cyclophosphamide, have the highest rate of chemotherapy-related amenorrhea.

Cisplatin and its analogs can also cause ovarian failure. Studies with cisplatin treatment in mice, have shown that different types of chromosomal damage are associated with genetic effects in the oocytes which result in early embryonic mortality and aneuploidy [16].

Recently, Li et al. and Blumenfeld, have shown that the combination of a gonadotropin-releasing hormone (GnRH) agonist with a GnRH antagonist completely prevented the flare-up effect and enhanced the protective effect of the ovary from cisplatin-induced gonadotoxicity in rats [17, 18]. In addition, agents such as vinca alkaloids can cause aneuploidy. Many experiments in mice have shown that the use of these agents results in malformed fetuses [19]. Anthraccline antibiotics have been implicated in dominant lethal mutations in maturing / preovulatory oocyte in female mice. Etoposide, can also cause aneuploidy in oocytes and pericentric lesions, leading to malformed embryos. The present authors’ knowledge up to now, does not allow to draw a safe conclusion regarding the effects of antimetabolites on female germ cell.

Finally, studies have shown that young women treated with chemotherapy agents prior to menarche, exhibited a delay at the start of menstruation, but all had their menarche reappear shortly after cessation of the treatment. Most of young women treated after menarche, developed amenorrhea, while some others treated with very mild drug regimen, had irregular cycles. Data from endocrinological studies, have shown that primary ovarian failure was rare and occurred in adolescent girls only when chemotherapy and radiotherapy were used in combination. In girls with regular menstrual cycles, when treated with high dose of chemotherapeutics agents, an ovulation or inadequate luteal phase could be observed due to hypothalamic effects of stress, anxiety, and emotions associated with the malignant disease [13, 20].

On many occasions, treatment may be shifted from one protocol to another, also taking in effect combined therapies, but for each disease only a few characteristic protocols are commonly used. Therefore, it is very practical to analyze the risk for ovarian failure according to disease type. For example, use of combined chemotherapy in early-stage of Hodgkin’s lymphoma can significantly reduce long-term ovarian dysfunction [2].

Radiotherapy

Radiotherapy has adverse events on gonadal function. The degree of the injury depends on factors such as the dose, the irradiation field, and the patient’s age with older women, being at a greater risk of damage [1, 2].

Radiotherapy, is used to treat pelvic and abdominal diseases, such as cervical and rectal cancer. It is also used for thyroid cancer, while cranio-spinal radiotherapy is used for central nervous system malignancies. Another application of radiotherapy, is in Hodgkin’s lymphoma and other hematological malignancies, when pelvic lymph nodes are influenced by the disease before bone marrow transplantation occurs, when total body is being irradiated.

Previous studies have shown that doses of about 30 Gy used in the case of brain tumors, can cause long-term hypogonadotropic hypogonadism in children [21]. Wallace et al. [22, 23], demonstrated that the estimated dose at which half of the follicles are lost in humans (LD50) is four Gy. Every patient exhibits different sensitivity to radiation damage which may be pre-determined genetically, but it seems that the age factor is the most important, as younger women are more likely to preserve their ovarian function, due to the greater primordial follicle reserve [13]. Additionally, where possible, shielding of the ovaries is used, or the radiation field is restricted in order to avoid direct irradiation to the ovaries [5, 24, 25].

Lashbaugh and Casarett [26], indicated in their study that women younger than 40 years of age are less sensitive to ovarian failure after radiotherapy, with an estimated dose of 20 Gy being required to produce permanent ovarian failure, while about six Gy are required for older women. A possible explanation for this is that younger women have better quality follicles, and the cell membrane is more resistant to damages. As the oocyte membrane seems to be among the less sensitive membranes to radiation, older women with poor quality follicles seem to have lost this protective factor.

Chiarelli et al. [27], presented in their study the relationship between the risk of premature ovarian failure and the total dose of abdominal-pelvic irradiation, in order to study the long-term effects of radiotherapy in young women. With doses < 20 Gy, the relative risk was 1.02, at dose of
20-35 Gy the risk was 1.37, and at doses > 35 Gy the relative risk of premature ovarian failure was 3.27. The percentage of females who suffered from infertility after radiotherapy, co-related with the patients’ age at the time of treatment and was restricted to women who were irradiated after puberty. Also, the percentage of women who suffered infertility, correlated with increasing dosage of radiotherapy: a dose of 20–35 Gy causes 22% rate of infertility and doses > 35 Gy led to a 32% rate of infertility [27].

In addition, Thibaud et al., showed that total body irradiation of < ten Gy given in a single dose before puberty causes a high ovarian failure rate of about 50-80% [28]. On the other hand, Vini et al., concluded that radiotherapy in distant areas like thyroid pose a low risk of permanent damages to the ovaries and these patients can have normal pregnancies after this treatment [29].

Many studies have attempted to show the radiation effect on the uterus and on subsequent pregnancy outcomes [30-32]. Uterine radiation is closely associated with infertility, spontaneous miscarriage mainly in the first trimester, and intrauterine growth retardation [33]. These effects are probably caused by the changes in the uterine musculature and blood flow, as well as from hormone-resistant endometrial insufficiency caused by radiotherapy. A review by Critchley and Wallace, suggests that steroid hormone replacement therapy can be used to improve uterine damage after irradiation, but only in young women. Moreover, it is also known that there is a close relationship between radiotherapy and obstetric complications. Patients who have received radiation treatment in childhood or puberty, show a higher rate of complications such as spontaneous abortions, preterm labor, and low-birthweight infants when compared to the general population, but there is no study to prove the relationship between radiation and teratogenicity [34-39].

In conclusion Langan et al., report that younger women at treatment with chemotherapy were associated with a higher frequency of normal ovarian function post-treatment, whereas adding total body radiation to the regime was associated with a high risk of ovarian failure [40].

Molecular aspects of chemotherapy and radiotherapy effect on the ovaries

Chemotherapy and radiotherapy direct effect

Chemotherapy and radiotherapy constitute the main two therapeutic regimes either as standalone approaches or in combination with other non chemo- or radio- therapeutic agents (e.g. monoclonal antibodies) in order to treat cancer [41, 42]. There is still a necessity for extensive work to be performed on dissecting the molecular events that are triggered in the ovary after different therapeutic regimes have been applied to the patient.

In general though, the main feature of both these therapy regimes is that they induce tissue and subsequent cell and DNA damage [43, 44]. Both of these regimes have also been shown to exert effects on reproduction aspects of the ovary such as the ovarian reserve and fertility.

One example of a classic chemotherapeutic agents is cisplatin. Cisplatin, once introduced into the cells via the assistance of a copper transporter (CTR1), is activated via a series of reactions in which one of the chloride ligands is displaced by water in a slow manner. The complex of cisplatin and water is formed eventually binds DNA exhibiting a predilection for nucleophilic N7 sites on purines on the nucleic acid chain [45].

At first, the formation of monoadducts is observed. These monoadducts take part in reactions that lead to the formation of inter-strand and also intra-strand crosslinks. The cytotoxic effects of such platinum agents depend on the formation of these crosslinks. Cisplatin’s cytotoxic effects may also depend on the formation of adducts that cause conformational changes in the DNA chain, that leads to impairment of separation of the two DNA strands thus impairing the DNA replication and synthesis [46]. These lesions upon formation are able to be recognized by DNA proteins that trigger DNA damage repair and/or apoptosis signaling and the platinum caused cell death is mediated by cell cycle arrest that occurs in the G2 phase of the mitotic cycle [47]. DNA repair includes the nucleotide excision repair pathway (NER) [48, 49], the DNA mismatch repair mechanism (MMR) [50, 51], and the homologous recombination repair pathway [52]. Other chemotherapeutic agents include cyclophosphamide, doxorubicin, melphalan, busulphan etc, that act in various ways in achieving their effect on the target cells [53-55]. Cyclophosphamides and alkylating agents cause dose-dependent destruction of oocytes and also follicular depletion [56-58].

In the case of the ovaries, the gonadotoxic effect of various chemotherapeutic agents such as cisplatin, usually affects oocytes, granulose, and theca cells in a way that may be detrimental to the ovarian reserve. Agents such as cyclophosphamide and melphalan may pose a risk to gamete formation, they are not cell cycle specific, and may cause damage even to resting oocytes [55, 59, 60]. As mentioned above, age seems to be a critical factor deciding the extent of the oocyte and chemotherapy induced ovarian failure with older women posing a greater risk group.

In terms of radiotherapy it is useful to mention that the ovarian follicles are very sensitive and vulnerable to DNA damage caused by ionizing radiation. At the same time oocytes exhibit a rapid onset of events such as chromosome condensation and disruption of the nuclear envelope [61, 62]. There is still a necessity for further research in identifying the molecular cascades triggered by radiotherapy.

Mechanisms of action for ovarian protection

1. GnRH molecular mechanism

Gonadotropins are proteins that were first introduced in the 1960s and have since been used in ovarian stimulation cycles, in order to induce multiple follicular development.
The GnRH, is a decapeptide and a member of the GnRH family of proteins. It is a so-called trophic peptide hormone that is responsible for the release of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary [62, 63]. The protein itself is synthesized and released from the hypothalamus.

GnRH binds to its cognate receptor GnRHR that is located in the anterior pituitary. GnRHR belongs to the family of G-coupled receptors [64]. The binding of the GnRH to its receptor triggers the coupling of the receptor to Gq/11 proteins leading to activation of phospholipase C which transmits its signal to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) [65, 66].

Following its activation, DAG activates the intracellular protein kinase C (PKC) pathway. IP3, in turn, stimulates the release of intracellular calcium. Other molecules such as phospholipase D, phospholipase A2 (PLA2), and proteins of the MAP kinase signaling pathway are activated that in turn regulate the expression and secretion of molecules such as FSH and LH [66]. These pathways also stimulate the expression of gonadotropin and they are shown in Figure 1.

In later studies GnRH agonists and antagonists have been employed in order to impair follicle depletion. In humans there are studies that have exhibited a reduction in the rate of amenorrhea in over 50% of the patients that underwent treatment with GnRH agonists compared to controls [67, 68]. GnRH agonists bind onto the GnRH receptor and enhance signaling events via the GnRHR thus increasing production of LH and FSH. They are molecules synthetically

![Figure 1. — The GnRH activating pathways.](image)
modeled after the GnRH decapetide that carry changes in positions within the ten amino acid chain, especially in positions 6 and 10 [69]. The usual advantage of these molecules is their slower degradation compared to the original GnRH molecule [70, 71].

The GnRH is believed to play a protective role in the case of ovarian reserves in patients that are treated for cancer via the use of chemotherapy and radiotherapy. The pathways that are activated upon binding of GnRH onto its receptor are shown in this figure. Guanine nucleotide binding protein alpha 11 (Gq/11), phospholipase C (PLC), phospholipase D (PLD), phospholipase A2 (PLA2), diacylglycerol (DAG), inositol 1,4,5-triphosphate (IP3), calcium/calmodulin-dependent protein kinase II alpha (CaMK), protein kinase C (PKC), v-raf-1 murine leukemia viral oncogene homolog 1 (Raf-1), mitogen-activated protein kinase kinase 1 (MEK 1/2), extracellular signal-regulated kinase 1/2 (Erk 1/2), LHβ polypeptide (LHβ), FSH, beta polypeptide (FSHβ).

In general, further work is necessary to shed more light into whether GnRH and analogues may actually protect the ovary as studies seem to be contradictory with some of them, suggesting no actual protective effect of these molecules [72]. A more clinical review on the actions of GnRH and its analogues will be considered later.

2. Other mechanisms for ovarian follicles apoptosis and apoptosis inhibition

The actual mechanisms by which damage may occur in the ovaries and the relevant biochemical pathways that are triggered after chemotherapy or radiotherapy are still under investigation. Nevertheless recent experimental data has been indicating that various molecules may play significant roles in preventing ovarian follicle premature death and even more, apoptosis due to cancer treatment.

GnRH may act directly onto its receptor GnRHR in order to inhibit follicle apoptosis [73]. GnRH may also be responsible for the upregulation of a protein called sphingosine-1-phosphate (SIP) [74, 75], thus SIP playing an important role as an apoptosis inhibitor. In turn, another protein, acid sphingomyelinase is the enzyme required to produce ceramide, an early messenger of apoptosis in response to stress [75]. It has been shown in experimental, in vivo, mouse models treated with agents, such as doxorubicin, that the lack of acid sphingomyelinase from their ovaries or when wild type mice were treated with SIP did resist oocyte apoptosis [76]. SIP molecules bind onto receptors termed Edg receptors or S1P receptors [77]. Upon binding onto their cognate receptors they elicit intracellular signalling pathways via regulation of diverse G coupled proteins, exerting their effects [76, 77].

Caspases comprise another family of proteins that may play significant roles in oocytes’ apoptosis due to chemotherapy. In mice, it has been shown that in the case of female germ cells and upon an insult in their metabolic status, caspase-2 and caspase-3 molecules are activated and execute the apoptotic signaling cascade. However in the case where DNA damage chemotherapeutic agents are used, caspase signaling pathways are triggered that may involve caspase-12 especially in the case when caspase-2 and caspase-3 are absent [78]. It is also known that caspase-9 may play a role in mouse oocytes’ apoptosis during the meiotic prophase progression [79] but it is still unknown whether it plays a role in the apoptosis of damaged oocytes during chemotherapy.

Except the caspases and SP1 signalling cascades, evidence begins to appear that other molecular pathways may be involved in apoptosis due to chemotherapy. Recent data suggests that platinum damaged oocytes may be rescued via the inactivation of the p53 signalling network [80]. In addition in mice, it has been shown that the thyroid hormone 3,5,3’-triiodothyronine (T3) protects granulosa cells from chemotherapy induced apoptosis [81]. These new discoveries show that there is still much to be considered in terms of the molecular machinery behind oocytes chemotherapy induced apoptosis.

Fertility preservation options for women after cancer

In literature, there are several options for fertility preservation targeted at women after cancer therapy. The use of a fertility preservation method needs individualization and depends on the time of cancer treatment (radiotherapy or chemotherapy), time available, type of cancer, and patient’s age.

Apoptotic inhibitors

The general notion on apoptotic inhibitors is that they constitute molecules that are equipping the doctor’s arsenal in combating causes for ovarian failure and more specifically assist in reducing the damage in ovarian reserves caused by standard chemotherapeutic agents and radiotherapy [82, 83].

Although great progress has been achieved there, is still a necessity for further research to take place in order for targeted action of apoptotic inhibitors to occur. More specifically, further studies are necessary in order to clarify the negative effect on reducing the tumor mass by a concomitant administration of apoptotic inhibitors and cancer therapeutics such as chemotherapy agents, especially at the molecular level. This process may identify novel targets for designing novel anti-apoptotic compounds and also will provide clinicians with more tools in order to fight tumor more effectively without leading oocytes into cell death.

Ovarian suppression (GnRH analogue treatment)

Many studies have evaluated the utility of treatment with GnRH analogues, in order to preserve ovarian function during cytotoxic therapy. Investigators attempted to render the germinal epithelium quiescent by suppression of go-
nadotropins (using GnRH agonist). This search has suggested that receiving GnRH analogues during radiotherapy and/or chemotherapy, may increase the possibility of a woman to maintain her menstruation after therapy [84, 85].

However, conflicting outcomes on the results of GnRH analogues have been presented, intensifying the debate regarding the existence of FSH receptors in the primordial follicles and GnRH analog receptors in the human ovary [85]. The study of Meirow et al., failed to demonstrate a protective effect of GnRH after chemoradiotherapy in patients undergoing bone marrow transplantation [86]. However, in a recent study Li et al., suggest that the combination of GnRH agonists and antagonists protects primordial ovarian follicles in rats [17] but, this remains to be proved in humans. Three other small randomized trials performed in humans, reported that GnRH analogues was not effective in preserving fertility in patients receiving chemotherapy for Hodgkin lymphoma [87, 88] or breast cancer [89]. Ovarian control markers were not different in the control subjects despite the level rates of amenorrhea in the group receiving GnRH analogs.

The treatment with GnRH analogs should begin at least ten days before the first chemotherapy, due to the initial flare-up effect which causes undesirable ovarian stimulation. Administration should continue until the end of chemotherapy, so that the downregulating effect remains for at least two weeks after the end of treatment. However, no safe results can be obtained by the use of GnRH analogs as the available studies are limited by the small sample size, lack of randomized control group, and lack of definitive information regarding actual fertility outcome [1, 90].

Alternative ways for preservation of ovarian reserve

I. Ovarian transposition (oophoropexy)

This method is suitable for patients undergoing gonadotoxic radiotherapy. The ovarian follicles are sensitive to DNA damage from radiation as the exposure can cause atrophy and decreased follicle number. The degree of ovarian damage, depends on the dose of radiation, the patient’s age, and the combination of radiotherapy with chemotherapy [90]. The most common indications for ovarian transposition are cervical and vaginal cancer, pelvic sarcomas, and Hodgkin’s disease.

This method is suitable for patients undergoing gonadotoxic radiotherapy alone without chemotherapy. Transposition can be performed laparoscopically just before the start of radiotherapy. Beginning radiation therapy immediately, decreases the chance of failure from ovarian migration back to the field of treatment [91]. The success rate of this procedure varies between 16% and 90% [92, 93]. The failure of this method and the subsequent low rate of success, is due to variant factors such as scatter radiation, dose, patient’s age, vascular compromise, and whether ovaries are shielded during the procedure [90]. Complications are relative rare and include chronic abdominal pain, infraction of the fallopian tube, and ovarian cysts [94-96].

2. Cryopreservation

Cryopreservation of embryos, is a proven effective method for preserving fertility. Cryopreservation of oocytes and ovarian tissue are promising approaches, but remain under investigation [97]. Successful cryopreservation of an intact whole human ovary has not yet been successful.

- Cryopreservation of embryos

To date, the most effective approach with regards to fertility preservation is embryo cryopreservation. The human embryo, is resistant to damage caused by cryopreservation procedures. The post-thaw survival rates of embryos are between 35-90% while implantation rates are between 8% and 30%. In case that multiple embryos are available for cryopreservation the pregnancy rate can reach up to 60% and delivery rates per embryo are about 18-20% [98].

- Cryopreservation of oocytes

I. Cryopreservation of mature oocytes (after gonadotropin stimulation).

Cryopreservation of oocytes, is more problematic than sperm or embryo cryopreservation. The main obstacle is the sensitivity of oocyte to chilling, probably because of the sensitivity the spindle apparatus and the higher lipid content of the cells. Cooling and exposure to cryoprotective agents (CPAs) may increase the incidence of aneuploidity in human oocyte due to damages in the cytoskeleton [99]. The eligibility of a woman for this method depends on the type of cancer and woman’s age, as a lot of patients may not have more than one opportunity for oocyte harvesting before undergoing chemotherapy or radiotherapy, since one cycle of controlled stimulation requires a few weeks. The success of the method is also dependent on the total number of oocytes harvested as < ten oocytes means very low chances of pregnancy [1].

II. Cryopreservation of immature oocytes after in vitro maturation (IVM) – (without gonadotropin stimulation).

Immature oocytes have been harvested both in situ and excised ovarian tissue [100]. The oocytes can be matured in vitro either before freezing or after thawing. These oocytes are expected to be more resistant to damages from chilling than mature oocytes since they do not contain a metaphase spindle, but few pregnancies from frozen-thawed immature oocytes have been reported [101,102]. IVM, has not been studied extensively in humans and may also have deleterious effects on spindle development and alignment of chromosomes.

- Cryopreservation of ovarian tissue

The idea of cryopreserving ovarian tissue is based on the finding that the ovarian primordial follicles are more resistant to cryo-injury than mature oocytes, because oocytes exhibit a relatively inactive metabolism and also lack a metaphase spindle, zona pellucida, and cortical
granules [103]. Cryopreserving the entire ovary with its vascular supply might help decrease the degree of follicle loss during the initial ischemia period, but at present there is no efficient technique for such a purpose. Although ovarian tissue cryopreservation is not a widely used method, it may be the only acceptable method for any prepubertal or premenarchal female patient receiving chemotherapy or pelvic radiotherapy. The most challenging part of this procedure, is the heterotopic or orthotopic reimplantation of the frozen tissue, a method that is still under investigation.

The first transplantation of cryopreserved ovarian tissue was reported in 2000 [104]. Until today very few pregnancies have been reported using this technique. The risks of ovarian tissue cryopreservation include reimplantation of the primary tumor, malignant transformation, as well as risks related to the invasiveness of the procedure [105]. Limiting factors of this method are its current experimental status, the availability of the procedure in some selected centers and the limited life of the ovarian grafts.

- **IVF in women after cancer treatment**

At present, the vast majority of patients who underwent chemotherapy or radiotherapy resort to in vitro fertilization (IVF), in order to conceive. Especially for the patients who offered a cryopreservation technique, IVF seems to be the only choice.

Modern assisted reproductive technology (ART) methods, allow the transfer of cryopreserved embryos in a woman’s uterine safely, increasing the method’s success rates. The introduction of intracytoplasmic sperm injection (ICSI), is of great importance when cryopreserved mature oocytes are used. Exposure to cryoprotecting agents causes hardening in zona pellucida of the oocytes, so that fertilization has to be carried out about three to five hours after thawing while the oocyte remains fertile. ICSI is used for such a direct fertilization. The overall birth rate per cryopreserved oocyte is about 2% when using IVF techniques [106]. Several stimulation agents are used in IVF. Even women with estrogen–sensitive cancer are not excluded from the IVF techniques, as new stimulating agents are used such as tamoxifen or aromatase inhibitors.

Finally, a reference to anti-Müllerian hormone (AMH) in women after cytotoxic therapy is necessary. AMH is used as a marker for the follicles’ deposits in women. Several reports to the literature indicate that AMH levels decline with age, predict time of menopause, predict pregnancy after IVF, and are associated with fecundity in the general population. It is of utmost interest that mid-reproductive age cancer survivors who received highly gonadotoxic therapy had AMH levels similar to those in women 40–42 years of age [104], thus providing this group of women with the opportunity to conceive after IVF.

**Conclusion**

The gonadotoxic effect of chemotherapy and radiotherapy agents is possibly due to mechanisms that still necessitate to be elucidated in the future. It is obvious that the POF rates that have been reported so far differ enormously and are affected by the therapeutic agent(s) used and the patients’ age range. Despite the effectiveness of therapy, the methods that are reviewed in this paper for preserving ovarian reserves do not guarantee a 100% success rate in achieving fertility for survivors. It is more likely that different methods need to be applied in combination in order to preserve ovarian reserves and achieve higher fertility rates. This notion is further enhanced by the fact that even less invasive and less expensive methods, such as the use of GnRH in preserving ovarian reserves, still need to be investigated as research results are contradictory. As research is expanding in these areas the more likely is that we will achieve such targets in the future.

**References**


Molecular aspects and clinical methods for preserving ovarian reserves in women receiving cancer treatment


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Absence of blood type A or AB may be associated with diminished oocyte reserve

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Summary

Purpose: To determine if blood type A protects against developing diminished oocyte reserve. Materials and Methods: Retrospective evaluation of incidence of blood type A (or AB) in women with normal oocyte reserve (day 3 serum follicle stimulating hormone (FSH) ≤ 11 mIU/ml) vs. diminished oocyte reserve (FSH ≥ 18 mIU/ml). Results: Five hundred forty-seven of 1,232 (44.4%) women with normal reserve had blood type A or AB vs. 33.8% (44/130) with diminished oocyte reserve (p = 0.027, chi-square). Conclusions: Lack of blood type A or AB may link to some other gene that may be responsible for premature depletion of oocytes.

Key words: Blood type; Diminished oocyte reserve.

Discussion

These results are consistent with the possibility that women with blood type A are more protected against developing diminished ovarian reserve. The next step would be to determine if oocyte quality, as manifested by live delivered pregnancy rates, can be related to blood type.

Corroboration by other centers could lead to a possible greater understanding of mechanisms for diminished oocyte reserve by exploring gene linkages with blood type and other genes that may play a role in folliculogenesis or follicular atresia.

If absence of type A is corroborated to be an independent risk factor for developing premature ovarian reserve, this is one additional piece of information that a single woman could use in determining if she wants to freeze oocytes for the future.

References


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Original Articles

Reproductive Biology Section

Introduction

A previous study suggested that the presence of blood group A might be an independent risk factor for early onset ovarian hyperstimulation syndrome [1, 2]. If this is true, there exists the possibility that the women with this blood type may be more protected against diminished oocyte reserve.

The aim of the present study was to test the hypothesis that women with type A or AB blood may have a lower incidence of decreased oocyte reserve compared to women with blood type B or O.

Materials and Methods

A retrospective chart review was performed on women aged ≤ 39 years with day 3 serum follicle stimulating hormone (FSH) ≤ 11 mIU/ml (serum E2 < 50 pg/ml) vs. those whose serum FSH was ≥18 mIU/ml. The frequency of various blood types were determined in each group.

Results

There were 1,232 women evaluated whose serum FSH was ≤ 11 mIU/ml (normal oocyte reserve group). There were 547 (44.4%) with blood type A or AB.

There were 130 women evaluated with serum FSH ≥ 18 mIU/ml and 44 (33.8%) had blood type A or AB (p = 0.027, chi-square analysis).
Mid-luteal phase injection of subcutaneous leuprolide acetate improves live delivered pregnancy and implantation rates in younger women undergoing in vitro fertilization-embryo transfer (IVF-ET)

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3 Philadelphia College of Osteopathic Medicine, Department of Obstetrics and Gynecology, Philadelphia, PA (USA)

Summary

**Purpose:** To see if the single injection of one mg of the gonadotropin releasing hormone agonist (GnRHa) leuprolide acetate given in the mid-luteal phase can increase live delivered pregnancy and implantation rates. Furthermore the purpose was to determine if improvement was found, did the mechanism involve increased secretion of human chorionic gonadotropin (hCG). **Materials and Methods:** A prospective study was conducted in women aged ≤ 35 who were undergoing in vitro fertilization-embryo transfer (IVF-ET). They were advised of data from Tesarik et al. and a previous pilot study conducted in the present IVF center showing improved pregnancy rates with the injection of a GnRHa three days after embryo transfer. They were offered the option of returning for a one-mg injection s.c. of leuprolide acetate or not. Clinical and live delivered pregnancy rates were compared according to those taking or not the leuprolide acetate one-mg injection. Chi-square analysis was used for statistical comparisons. Serum beta-hCG levels were compared between those conceiving with or without the extra injection of leuprolide. **Results:** There was a non-significant trend for higher live delivered pregnancy rates in those taking leuprolide (47.8%, 64/134) vs. those not taking it (38.6%, 76/197). For those pregnant there was no difference in hCG levels according to taking the GnRHa or not. **Conclusions:** The 25% increased live delivered pregnancy rate per transfer was insufficiently powered to detect a significant difference. The results do justify continuing the study. Perhaps the difference could be wider using a slightly older age group whose embryos are frequently less hearty.

Key words: Luteal phase; Gonadotropin releasing hormone agonists; Leuprolide acetate; In vitro fertilization-embryo transfer; Implantation.
The average first serum beta-hCG level from pregnant women taking leuprolide was 285 mIU/mL and 273 mIU/mL for those pregnant not taking it ($p = \text{NS}$).

**Discussion**

There have been a few studies suggesting improved benefit from the use of GnRHa in the mid-luteal phase: Tesarik et al. – triptorelin [1], Picard et al. – buserelin [2].

This is the first study with the GnRHa leuprolide acetate. Although there were no significant differences noted, there was a trend for improved pregnancy outcome by using one-mg of leuprolide acetate three days after ET. The possibility is that the younger group has less likelihood of the need to improve embryo implantation compared to women of more advanced reproductive age. Presently the authors are evaluating women under similar circumstances between age 36-39 where pregnancy rates are lower.

**Conclusion**

It would appear if indeed a GnRHa study with more power shows a significant difference, the mechanism does not seem to be related to increasing the beta-hCG output from the fetal-placental unit.

**References**


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Table 1. — Effect of GnRHα single injection on pregnancy rates following IVF-ET.

<table>
<thead>
<tr>
<th>Leuprolide acetate one mg given</th>
<th>No. transfers</th>
<th>No. clinical pregnancies (%)</th>
<th>No. live delivered pregnancy rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>134</td>
<td>69 (51.5%)</td>
<td>64 (47.8%)</td>
</tr>
<tr>
<td>No</td>
<td>197</td>
<td>97 (44.2%)</td>
<td>76 (38.6%)</td>
</tr>
</tbody>
</table>

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Improved implantation and live delivered pregnancy rates following transfer of embryos derived from donor oocytes by single injection of leuprolide in mid-luteal phase

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Summary

Purpose: To determine if the use of a single injection of one-mg leuprolide acetate in mid-luteal phase can increase pregnancy rates in donor oocyte recipients. Materials and Methods: Prospective study where couples were made aware of a study using the gonadotropin releasing hormone agonist (GnRHa) triptorelin that in the mid-luteal phase found improved pregnancy rates following embryo transfer in donor oocyte recipients. They were given the option of a single one-mg injection of the GnRHa leuprolide acetate. Pregnancy outcome was compared according to whether leuprolide was given or not. Also compared were the average first serum beta-hCG level in those who conceived according to taking leuprolide or not. Results: Chi-square analysis showed a significantly higher clinical and live delivered pregnancy rate (63.9% and 52.8%) in those supplementing with leuprolide than those who did not (39.5% and 32.9%). Similarly implantation rates were significantly higher (44.2% vs. 25.2%). The average first serum beta-hCG level for those conceiving and taking leuprolide was 294 mIU/mL vs. 325 mIU/mL for those who did not. Conclusions: Similar to triptorelin the mid-luteal injection of leuprolide acetate improves pregnancy outcome in donor oocyte recipients.

Key words: Donor oocyte recipients; Luteal phase; Leuprolide acetate; Embryo implantation.

Introduction

Recipients receiving donor oocytes have in general the highest pregnancy rates per cycle. Some studies in women having IVF-ET with their own oocytes have been found to have improved pregnancy rates following injection of gonadotropin releasing hormone agonist (GnRHa), e.g., triptorelin or buserelin [1, 2]. However at least in younger women aged ≤ 35, no significant differences were found using the GnRH agonist leuprolide acetate (although there was a slightly positive trend for improved outcome) [3].

With such high implantation rates with donor oocytes one might expect not to find much of an improvement with luteal phase GnRH agonists. However, improved pregnancy rates were claimed by Tesarik et al. using triptorelin in a donor oocyte model [4].

Materials and Methods

A prospective study with patient option of adding leuprolide acetate one mg three days after embryo transfer or not. They were advised of the generally good pregnancy outcome with donor oocytes without the use of GnRHa supplementation. However they were advised of the study by Tesarik et al. showing higher pregnancy rates with injection of a GnRHa in a donor oocyte model [4].

The average first serum beta-hCG levels were compared in those conceiving with and without the extra injection of leuprolide acetate. All recipients were on a graduated estradiol regimen with subsequent vaginal and intramuscular progesterone.

Results

There were 36 women choosing to use leuprolide acetate and 76 who did or not. Chi-square analysis showed a significant difference in both clinical (p = 0.027) and live delivered (p = 0.009) pregnancy rates.

The implantation rates for those taking leuprolide were 44.2% vs. 25.2% (p = 0.001). The average first serum beta-hCG level for those conceiving in this study was 294 mIU/mL for those taking the GnRHa vs. 325 mIU/mL for those not taking leuprolide.

Discussion

These data confirm the conclusions by Tesarik et al. that a single injection of GnRHa three days after embryo transfer improves the live delivered pregnancy rate and im-
planted rates in donor oocyte recipients [4]. Tesarik et al. found the GnRHs triptorelin to be effective and this study found that same benefit with leuprolide acetate [4].

**Conclusions**

Similar to the present findings with leuprolide with IVF-ET cycles and in contrast to the study by Tesarik et al. [4], there was no increase in the first serum beta-hCG level in those taking vs. not taking luteal phase leuprolide [1, 3].

**References**


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**Table 1.** — The effect of mid-luteal phase injection of leuprolide acetate on pregnancy rates in oocyte recipients.

<table>
<thead>
<tr>
<th>Leuprolide acetate one mg given</th>
<th>No. transfers</th>
<th>No. clinical pregnancies (%)</th>
<th>No. live delivered pregnancies (%)</th>
</tr>
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<td>Yes</td>
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<td>19 (52.8%)</td>
</tr>
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<td>No</td>
<td>76</td>
<td>30 (39.5%)</td>
<td>25 (32.9%)</td>
</tr>
</tbody>
</table>

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Medication exposure and spontaneous abortion: a case-control study using a French medical database

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Summary
Purpose of investigation: Few studies have been conducted to investigate drug effects on spontaneous abortion risk. The objective of the present study was to evaluate the potential association between first trimester drug exposure and spontaneous abortion occurrence.

Materials and Methods: The authors performed a nested case-control study using data from TERAPPEL, a French medical database. Cases were the women who had a spontaneous abortion (before the 22nd week of amenorrhea) and controls were women who gave birth to a child. Analyzed variables were: maternal age, obstetric history, tobacco, and alcohol and drug consumption during the first trimester of pregnancy. For comparison of drug exposures between cases and controls, the authors calculated odds ratios (ORs) by means of multivariate logistic regressions adjusted on age and on other drug exposures. Results: The study included 838 cases and 4,508 controls that were identified in the database. In adjusted analyses, cases were more exposed than controls to “non-selective monoamine reuptake inhibitors” [OR=2.2 (CI 95% 1.5-3.3)], “anti-protozoals” [OR = 1.6 (CI 95% 1.1 - 2.5)] and “centrally acting anti-obesity products” [OR = 3.4 (CI 95% 1.9 - 6.2)]. Conversely, controls were more exposed than cases to H1 antihistamines [OR = 0.6 (CI 95% 0.4 - 0.9)]. Conclusion: This exploratory study highlights some potential associations between first trimester drug exposure and risk of spontaneous abortion. Further studies have to be carried out to investigate these findings.

Key words: Spontaneous abortion; Drug exposure; Case-control study.

Introduction
Spontaneous abortion can be defined as the spontaneous loss of the conceptus before 20 weeks gestation [1]. It is one of the most frequent adverse outcomes in human pregnancy [2]. The incidence of spontaneous abortion reported by several authors among clinical pregnancies is approximately 10-20% [2, 3], however prospective studies on conception and early pregnancy have reported fetal loss rates approaching one-third [4].

Some factors have been associated with spontaneous abortion [2, 5, 6]. However, they are difficult to evaluate because many pregnant women are not closely followed-up during early pregnancy and may never present for care if they experience a spontaneous abortion. Most spontaneous abortions are due to a conceptus with an abnormal number of chromosomes [1, 7, 8]. Uterine anatomic defects are another well-known spontaneous abortion etiologic factor [9]. The rate of spontaneous abortion also seems to increase with immunological factors (autoimmune diseases [7]), endocrine abnormalities (diabetes [10], thyroid diseases [11]), thrombophilia [12], various environmental exposures (tobacco [13], alcohol [14] and moderate-to-heavy caffeine use [15]), and other maternal conditions (age [16, 17], obesity [18], infertility [19-20], and increasing number of previous spontaneous abortions [21]).

Pregnant women are often exposed to medication, sometimes inadvertently, especially during early pregnancy when they do not yet know that they are expecting a child. Various studies have reported that an elevated number of drugs are prescribed to a woman during her pregnancy (an average of six to 16 different drugs) [22-23]. Few studies have been conducted to specifically investigate a relationship between drug exposure and spontaneous abortion risk, consequently little is known about this topic.

The French Regional Pharmacovigilance Centers (CRPVs) are regularly questioned about potential drug exposure risks during pregnancy. Many of these questions relate to early pregnancy exposure. Follow up is performed and pregnancy outcomes are registered in TERAPPEL, a database shared by several CRPVs, enabling the follow up of early drug exposed women. The present study was conducted using this database in order to obtain further data on the potential relationship between first trimester drug exposure and spontaneous abortion.
Materials and Methods

The authors performed a nested case-control study using the French database TERAPPEL. Since 1984, this database has been used to record health professionals' requests from the participating CRPVs concerning women exposed to drugs during pregnancy and breastfeeding. Data concerning the pregnancies leading to questions to the CRPV are collected prospectively and registered in the database (up to December 31, 2010, 31,019 questions had been registered in the database). From the first contact (most of the time, a telephone contact), the health professional informs the CRPV on the pregnancy progress and drug exposures of the pregnant woman. Two paper questionnaires designed to complete data on potential risk factors during pregnancy (such as maternal diseases and drug exposures) are subsequently sent by the CRPV to the correspondent. The first questionnaire is sent just after the health professional’s call and the second when the woman is supposed to deliver. Thanks to these questionnaires, TERAPPEL contains information about the call purpose, woman’s state of health, medical history, drug exposure (including period of exposition) according to the ATC classification, and pregnancy outcome.

Cases were all the women registered in TERAPPEL whose pregnancy outcome was “spontaneous abortion” (before the 22nd week of amenorrhea) and controls were all the women registered in TERAPPEL whose pregnancy outcome was “birth”. For both groups the authors only selected pregnancies whose call purpose was “first trimester risk assessment”, so that cases and controls had the same drug exposure risk during the first trimester of pregnancy. Analyzed variables were: age, obstetric history (births, ectopic pregnancies, intrauterine deaths, medical terminations of pregnancy, and spontaneous and voluntary abortions), tobacco, alcohol, and drug consumption during pregnancy. To avoid an artificial overexposure of the control group, which had a longer mean duration of pregnancy, the authors took into account the same period of drug exposure for both groups, which corresponded to the average gestation length for cases.

Data were analyzed using SAS version 9.1 software. The authors compared quantitative variables (age, number of different drugs taken by women during the study period) between cases and controls using Student tests. Categorical variables (medication use, consumption of tobacco and alcohol and obstetric history) were compared between the two groups using the Chi-square or Fischer’s exact test when appropriate. For drug exposures, whenever possible, relevant confounders significantly associated with spontaneous abortion in univariate analysis were included in regression models.

Results

The present study included 838 cases and 4,508 controls that were registered in the TERAPPEL database. Spontaneous abortions were reported at a mean time of 9.4 (±3.0) weeks since last menstrual period. In the control group, length of gestation was 38.9 (±2.0) weeks since last menstrual period.

Women’s characteristics

The general characteristics for the case and control groups are presented in Table 1. Cases were significantly older than controls (32.4 vs 30.6 years-old, $p < 10^{-4}$). The history of ectopic pregnancy, intrauterine death, voluntary abortion, and medical pregnancy termination was similar in both groups. The case group had experienced significantly more spontaneous abortions ($p = 1.10^{-4}$) and births ($p = 7.10^{-3}$) in the past than the control group.

Medication exposure

The average number of different drugs used during the study period was not significantly different between cases and controls (respectively, 2.25 (±1.84) vs 2.20 (±1.90)). In the two groups, the first intake of drug occurred on average during the first week of pregnancy. Table 2 shows drug exposure for cases and controls according to ATC classification. Regarding both groups, the most commonly taken drugs were for the “nervous system”, “anti-infectives for systemic use”, “alimentary tract and metabolism”, and “musculoskeletal system” ATC classes. After adjusting for age and other drug classes associated with spontaneous abortion risk, the authors found that cases were more exposed than controls to “antiparasitic products” (OR 1.51, 95% CI 1.04 - 2.19) in particular “antiprotozoals” (OR 1.63, 95% CI 1.06 - 2.50), “anti-obesity preparations, excluding diet products” (OR 2.58, 95% CI 1.47 - 4.53) and in particular “centrally acting anti-obesity products” (OR 3.40, 95% CI 1.85 - 6.24) and to “non-selective monoamine re-uptake inhibitors” (OR 2.19, 95% CI 1.46 - 3.29). Conversely, after adjusting for age, the authors found that cases had experienced significantly more spontaneous abortions ($p = 1.10^{-4}$) and births ($p = 7.10^{-3}$) in the past than the control group.
were significantly less exposed than controls to “antihistamines for systemic use” (OR 0.59, 95% CI 0.37 - 0.94).

Discussion

Few studies have been conducted to specifically investigate a relationship between drug exposure and spontaneous abortion risk. The present exploratory study reports that first trimester exposure to “centrally acting anti-obesity products”, “non-selective monoamine reuptake inhibitors”, and “antiprotozoals” are associated with an increase in spontaneous abortion risk. In contrast, the study shows that controls were more exposed to “antihistamines for systemic use” than women who had experienced a spontaneous abortion.

The majority of previous studies which reported findings on medication and spontaneous abortion risk were cohort-designed to investigate the effects of specific drugs and did not study spontaneous abortion as a primary objective. The present authors conducted a first exploratory case-control designed study to generate hypothesis on the potential links between first trimester drug exposure and risk of spontaneous abortion. The case-control approach is a good design for investigating simultaneously the potential effects of different drug exposures on the occurrence of an event. This design, associated with the large population size (more than 5,000 pregnancies registered in the database), allowed the authors to have an overview of the potential effects of a wide variety of drugs on spontaneous abortion risk. Moreover, unlike studies performed on prescription or reimbursement databases, the present methodology (information on drug exposures provided by physicians who question their patients on their drug consumption) enables accounting of over-the-counter drugs.

However, the methodology of this exploratory study presents some limitations, mostly due to the source of the data. Indeed, TERAPPEL database had not initially been implemented for this specific study, and consequently some variables are missing. The first limitation that should be pointed out, is that the authors could not take into account all the potential confounding factors. First, confounding by specific indications which increase spontaneous abortion risk cannot be excluded. Other potential confounding factors such as obstetric history and alcohol or tobacco use could neither be taken into account because of the large proportion of missing data. However maternal age, a well-known risk factor for spontaneous abortion [16-17] that was significantly associated with spontaneous abortion risk in the present crude

<table>
<thead>
<tr>
<th>No. ATC</th>
<th>ATC title</th>
<th>No. of cases and controls exposed</th>
<th>p</th>
<th>Odds ratio for spontaneous abortion (95% CI)</th>
</tr>
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<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Alimentary tract and metabolism</td>
<td>123 (4.68%)</td>
<td>664 (4.73%)</td>
<td></td>
</tr>
<tr>
<td>A02-A02A</td>
<td>Antibiotics and antiseptics, excluding diet products</td>
<td>22 (0.83%)</td>
<td>40 (0.89%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>A02-A02AA</td>
<td>Central anti-inflammatory products</td>
<td>21 (0.81%)</td>
<td>29 (0.64%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>B</td>
<td>Blood and blood forming organs</td>
<td>42 (0.41%)</td>
<td>249 (0.52%)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Cardiovascular system</td>
<td>66 (0.88%)</td>
<td>312 (0.30%)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Dermatological</td>
<td>33 (0.44%)</td>
<td>131 (0.35%)</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Genito-urinary systems and sex hormones</td>
<td>81 (0.67%)</td>
<td>412 (0.14%)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Psychotropic agents</td>
<td>17 (0.23%)</td>
<td>107 (0.27%)</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>Antineoplastic and immunomodulating agents</td>
<td>17 (0.23%)</td>
<td>107 (0.27%)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>Musculo-skeletal system</td>
<td>116 (1.34%)</td>
<td>588 (0.34%)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Nervous system</td>
<td>357 (4.60%)</td>
<td>1830 (4.09%)</td>
<td></td>
</tr>
<tr>
<td>N05</td>
<td>Psychopharmacics</td>
<td>171 (0.81%)</td>
<td>728 (1.15%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>N06</td>
<td>Psychomimetics</td>
<td>172 (0.33%)</td>
<td>742 (0.66%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>N06AA</td>
<td>Antidepressants</td>
<td>210 (0.29%)</td>
<td>726 (0.17%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>P</td>
<td>Anti-infective agents, insecticides and repellents</td>
<td>39 (0.45%)</td>
<td>88 (0.55%)</td>
<td>p&lt;0.104</td>
</tr>
<tr>
<td>P01</td>
<td>Antiparasitics</td>
<td>41 (0.89%)</td>
<td>156 (0.46%)</td>
<td>p&lt;0.04</td>
</tr>
<tr>
<td>P03</td>
<td>Antiprotozoals</td>
<td>32 (0.81%)</td>
<td>110 (0.24%)</td>
<td>p&lt;0.02</td>
</tr>
<tr>
<td>R</td>
<td>Respiratory system</td>
<td>24 (0.70%)</td>
<td>407 (0.14%)</td>
<td></td>
</tr>
<tr>
<td>R06-R06A</td>
<td>Antihistamines for systemic use</td>
<td>24 (0.86%)</td>
<td>201 (0.46%)</td>
<td>p&lt;0.03</td>
</tr>
<tr>
<td>S</td>
<td>Sensory organs</td>
<td>10 (0.13%)</td>
<td>51 (0.13%)</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Various</td>
<td>29 (0.46%)</td>
<td>171 (0.79%)</td>
<td></td>
</tr>
<tr>
<td>V02</td>
<td>Non aspirin NSAIDs</td>
<td>73 (0.71%)</td>
<td>407 (0.03%)</td>
<td></td>
</tr>
</tbody>
</table>

* Adjusted on age and other drug exposures
** Adjusted on age
analysis, was included in regression models. Moreover, the authors studied the differential effects of drugs associated with an increase of spontaneous abortion risk: “centrally acting anti-obesity products”, “non-selective monoamine reuptake inhibitors” or “antiprotozoals”. A second limitation is the possibility of a selection bias: women included had been exposed to at least one drug, which motivated the call of the health professional. However this bias is attenuated by the comparative analysis of cases and controls.

Regarding antidepressants, most of the studies failed to show a significant association between exposure to these drugs during pregnancy and spontaneous abortion [24-31]. A significant increase of spontaneous abortion risk has rarely been associated with this drug class in pharmacoepidemiologic studies [32-34]. Meta-analysis reported significant increase of the spontaneous abortion risk associated with all antidepressants [35], serotonin reuptake inhibitors [36], and specific antidepressants like paroxetine or venlafaxine [37]. Serotonergic mechanism has been suggested, as 5-hydroxytryptamine is abortive in experimental animals [38]. However, potential confounding factors, especially maternal depression, have been inconsistently considered in the relevant studies. In the present study, the association between antidepressants and spontaneous abortion was observed mainly with imipraminic antidepressants (non-selective monoamine reuptake inhibitors), especially with clomipramine. Two prospective studies, which included a total of 378 pregnant women exposed to imipraminic antidepressants, did not report any increased risk of spontaneous abortion compared with the general population rate [27, 31]. However, the interpretation of these findings is limited as no control group was included in these studies. Other studies (case-control or meta-analysis) reported a non-significant increase of spontaneous abortion risk with imipraminic antidepressants exposure [33, 35]. As an indication bias cannot be ruled out in the present study, further analyses are needed to confirm or disprove this potential relationship.

The present results showed a significant association between spontaneous abortion risk and “centrally acting anti-obesity products” or “antiprotozoals” exposures, in crude and in adjusted analyses. For “antiprotozoals”, the difference was observed particularly for “nitroimidazole derivatives” but the possible indication bias, and the small numbers of pregnancies in this subclass (<10 in each group), limit the interpretation of the results. The most encountered drug of “centrally acting antiobesity products” was dexfenfluramine. A minor study evaluating the effects of phentermine/fenfluramine on approximately 100 pregnant women did not report any increase of spontaneous abortion risk with these drugs [39] and the rare literature data available on the topic do not suggest a positive link between spontaneous abortion risk and amphetamine exposure [40]. Some studies reported that spontaneous abortion risk was increased with ecstasy (another amphetamine) [41], but this data is difficult to interpret because women exposed to ecstasy often have a potentially dangerous lifestyle for the fetus. Due to potential indication bias since obesity constitutes a recognized risk factor for both maternal and fetal complications such as spontaneous abortion [42-44], it is necessary to remain cautious concerning the interpretation of the present results.

The present study does not support the conclusion that non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) use is associated with an increased risk of spontaneous abortion. The association between gestational use of non-aspirin NSAIDs and spontaneous abortion remains controversial. Three publications of pharmacoepidemiology studies have reported a positive relationship between NSAIDs use and spontaneous abortion risk [45-47]. The mechanisms suggested by authors involve the role of prostaglandins in implantation and in maintaining placental perfusion. In the Nielsen et al. study [45], protopathic bias was possible since the association between NSAIDs exposure and spontaneous abortion was higher when exposure occurred immediately before spontaneous abortion, and in a subsequent re-examination of results adjusting for gestational age, the observed associations were no longer statistically significant [48]. If, as suggested by the only of these three studies which included non-prescription NSAIDs [46], the relationship between NSAIDs and spontaneous abortion is stronger with exposure closer to conception time, it is possible that the lack of significance observed in the present study could be explained by the authors’ difficulty in collecting information on exposure to drugs around conception time. However, the present study is not the only one that does not suggest a positive association between non-aspirin NSAIDs use and spontaneous abortion. Indeed, a recent cohort study of almost 3,000 pregnancies did not observe any association between early pregnancy exposure to over-the-counter NSAIDs, particularly non-aspirin, and spontaneous abortion [49]. In France, ibuprofen can be dispensed without prescription. The present method has the advantage of providing access to data on exposure to over-the-counter drugs, even if underreporting of self-medication is possible.

Surprisingly, controls were significantly more exposed to “antihistamines for systemic use” (H1 antihistamines) than cases, and the difference was still significant in the present age-adjusted analyses. Literature data on the potential link between H1 antihistamine use and spontaneous abortion risk is very limited. A prospective, controlled, and observational study of 53 pregnant women exposed to hydroxyzine and 39 to cetirizine showed no difference with regards to the risk of spontaneous abortion rate between the hydroxyzine or cetirizine groups and the control groups [50]. Some antihistamines are indicated in the treatment of vomiting and it has been described that women who experience intense nausea and vomiting in early pregnancy have less risk of spontaneous abortion than women with less or no nausea and vomiting [51]. However, in the present study, confusion by...
indication does not explain the observed association. Indeed, most of the antihistamines associated with a decrease in spontaneous abortion risk in the present study are not registered for nausea and vomiting indication: only the “piperazine derivatives” (ATC code: R06AE), represented in majority by cetirizine and levocetirizine (eight out of the nine cases and 97 out of the 98 controls exposed to “piperazine derivatives”), were significantly associated with a decrease in spontaneous abortion risk. In literature, links have been reported between hyper-histaminemia and specific gestational complications such as spontaneous abortion [52, 53]. A histamine injection in pregnant cats and inhibition of diamine oxidase (the main enzyme involved in histamine metabolism at the fetomaternal interface) in pregnant rats was reported to induce spontaneous abortions [54, 55]. Observations of spontaneous abortion in women with diamine oxidase deficits and increased histaminemia levels have also been published [56, 57]. This could be explained by the contractile effect of histamine on uterine musculature or indirectly by an increased production of the uterotonic PGF2 alpha (which is thought to play a key role in the initiation and maintenance of normal labour) [52]. Treatment of threatened abortion with antihistamines has been investigated and it was shown that a combined therapy of antihistamines and antispasmodic had the same protective effect as a tranquilizer and gestagen combined therapy [58].

Conclusion

The authors report the results of the first exploratory case-control designed study performed to investigate potential associations between first trimester drug exposure and spontaneous abortion occurrence. “Non-selective monoamine reuptake inhibitors”, “centrally acting antiobesity products”, and “antiprotozoals” were associated with an increased risk of spontaneous abortion, but an indication bias cannot be ruled out, since the pathologies for which these drugs are used can also be involved in spontaneous abortion. In contrast, antihistamines for systemic use” were significantly associated with a decrease in spontaneous abortion risk. This could be explained by a pathophysiological hypothesis since H1 antihistamines could have potential protective effects on histamine-induced uterine contractions. However, further investigations are needed to specifically study this potential association.

References


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Introduction

The success of embryos implantation in women undergoing in vitro fertilization (IVF) is depended mainly on oocyte quality [1-3]. Numerous local factors promote or inhibit follicular development and affect reproductive outcome [4-7]. Cytokines play a critical role in oocyte maturation, fertilization, and embryo implantation. Among them, the vascular endothelial growth factors (VEGFs) regulate vascular development during embryogenesis and blood vessel formation [8]. There are five VEGF family members, which are dimeric glycoproteins of approximately 40 kDa: VEGFA, B, C, D, and placenta growth factor (PLGF). They exert their actions after binding to specific VEGF receptors (VEGFRs) and co-receptors. The receptors are tyrosine kinases (RTKs), known as VEGFR-1, -2 and -3 (VEGFR1–3), while co-receptors (lacking established VEGF-induced catalytic function) include heparin sulphate proteoglycans (HSPGs) and neuropilins. The VEGFRs have an extracellular portion consisting of seven immunoglobulin-like domains, a single transmembrane spanning region and an intracellular portion containing a split RTK domain. VEGFA, B, and PLGF bind to VEGFR1, VEGFA, and E bind to VEGFR2, and VEGFC and D bind to VEGFR3. Thus, VEGF-A binds to VEGFR-1 and VEGFR-2. The latter seems to participate in almost all of the known cellular responses to VEGF [9]. VEGFR-1 is thought to modulate VEGFR-2 signaling. VEGFA has drawn special attention as a particular feature of the VEGFA ligand is the dramatic upregulation of its expression under hypoxic conditions [10].

The expression of VEGFRs (especially VEGFR1 and VEGFR2) is also induced by hypoxia and from a teleological view this expression contributes to augmentation of oxygen supply to oocytes and implanted embryos. Indeed, oocytes are particularly sensitive to hypoxic dam-

Summary

Purpose: The intracytoplasmic sperm injection (ICSI) outcome is depended mainly on oocyte quality. Cytokines and their receptors play a critical role in oocyte maturation, fertilization, and embryo implantation. The purpose of the study was to study the levels of vascular endothelial growth factors (VEGFA, VEGFR1, VEGFA) in follicular fluids (FF) women participating in ICSI-in vitro fertilization (IVF) cycles in relation to cycle’s outcome. Material and methods: One hundred and fifty three samples of 70 women participating in ICSI cycles were classified in three infertility groups: male factor, female factor, and low responders. For controlled ovarian stimulation in male and female factor group, the long agonist protocol with leuprolide and recombinant follicle stimulating hormone (FSH) was employed, while the antagonist cetorelix was used in low responders. Cytokines levels were evaluated with enzyme-linked immunosorbent assay (ELISA). Results: In a total of 153 samples, the overall pregnancy rate was 51.6%, the higher one observed in female factor group (59% vs 37.5% and 28.6% in male a factor and low responders group, \( p = 0.013 \). VEGFR2 differed statistically significantly between the two groups, being higher in the pregnancy group [median (IQR): 5,630 (4,870 - 6,651) vs 4,938 (4,068 - 6,020)] in the non-pregnancy group, \( p = 0.003 \). There were significant correlations between VEGF receptors, differentiated depending on infertility groups. Conclusions: The VEGFA/VEGFR2 system is important in human reproduction and the association pattern between VEGFA receptors may serve as a marker for ICSI outcome. Examination for spermatozoa functional defects may increase pregnancy rate in male factor group.

Key words: Infertility; VEGFA; VEGF receptors; ICSI IVF; Pregnancy.

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age [11]. Sufficient oxygen supply is crucial for embryo development procedures and adequate vascular network is necessary for adequate blood supply, which in turn is translated into oxygen and nutrients supply [12, 13].

Controlled ovarian hyperstimulation (COH) is used in IVF cycles in order to increase the number of mature oocytes and to maximize conception chances. Improving pregnancy rates is associated with investigation of all possible factors affecting embryo implantation and development and VEGFA among other cytokines has been the target of intense research in the last decade. The research has focused mainly on follicular fluids (FF), as serum concentrations of these factors are low and interesting findings has been presented: VEGF levels were found to be significantly correlated with grade of perifollicular vascularity and the presence of the VEGF-A receptors, particularly in the granulosa cells, suggests that VEGF-A might be involved in proliferation initiation of primordial follicles or play a role in human preantral follicles [14,15]. VEGF levels increase during the first trimester, but FF-VEGF levels have been found significantly elevated in non-pregnant women as compared to pregnant women [3].

The various cytokines involved in fertilization and the rather contradictory reported results ask for further investigation, in attempt to study pregnancy outcomes in women undergoing IVF. Significant questions remain regarding the role of VEGFRs as only recently experimental data have suggested that the VEGF/VEGFR-2 pathway plays a key role in the maintenance of early pregnancy, the temporal and spatial relationships of VEGFA, and its receptor expression in a luteal endometrium, as well as the significance of the research finds for the various groups of women undergoing IVF [16,17].

The present study attempts to shift attention to VEGFRs, rather than VEGF itself, as recent findings suggest interaction between VEGFRs subtypes. The aim of this study was to study the levels of VEGFA, VEGFR1, VEGFA, and leptin in FF of three groups of women participating in intracytoplasmic sperm injection (ICS)-IVF cycles: male factor infertility women, female factor, and low responders. The authors also studied pregnancy outcome in relation to the above cytokines and receptors’ FF concentrations.

Materials and Methods

The studied sample consisted of 70 women who participated in ICSI cycles. Women had never undergone any IVF technique before. One hundred and fifty three samples were collected and they were classified into three infertility categories depending on infertility cause, according to existing literature [1,18]: a) male factor group (N=56), b) female factor group (women with reproductive disorders, except from polycystic ovarian syndrome (PCOS), N=83), and c) low responders (N=14).

The long agonist protocol for COH in male and female infertility factor group was used as previously described [19]. Briefly, ovarian hyperstimulation performed by administration of recombinant follicle stimulating hormone (FSH) after pituitary suppression with leuprolide started in the midluteal phase of the preceding cycle. In low responders, the antagonist cetrorelix was administered, according to a standard protocol described elsewhere [20]. The dosages of gonadotropins were individualized according to serum estradiol (E2) levels and transvaginal ultrasound measurements of the follicles. When at least three follicles had reached a diameter of 17 mm and serum E2 levels were increased to approximately 300-500 pg/ml per follicle, ovulation was induced with 10,000 IU of human chorionic gonadotropin (hCG). Transvaginal oocyte aspiration with ultrasound guidance was performed under general anesthesia 36 hours later. During oocyte aspiration, FF samples were collected from distinct, mature follicles and they were placed into sterile tubes. Samples with massive blood contamination or flushing fluid were excluded. FF samples were immediately centrifuged for 15 minutes at 1,500 rpm and the supernatants were stored at –75 °C for further analysis.

Two hours after follicular aspiration, the cumulus oophorus and corona radiata were removed mechanically under dissecting microscope, with simultaneous incubation in ICSI cumulus solution (recombinant human hyaluronidase, 80 U/ml for 60 seconds. The incubation in ICSI cumulase was followed by repeated aspirations into denuding pipettes. Only metaphase II (MII) oocytes were used for ICSI that was performed two to three hours later using an inverted microscope with micromanipulators. The injected oocytes were cultured in universal IVF medium, at 37°C, in a humidified atmosphere with 5 % CO2. After 18 hours of incubation, the injected oocytes were examined for the presence of two or more pronuclei as a sign of fertilization. The normally fertilized oocytes, with two pronuclei and two polar bodies (2PN oocytes), were transferred into a fresh medium (ISM1 culture medium) and cultured for 24-30 hours.

Embryos were further cultured in ISM2 medium beyond second day of fertilization, until embryo transfer and blastocyst development, around 5th/6th day after fertilization. The luteal phase was supported daily with 600 mg natural progesterone administered vaginally. Pregnancies were defined by the presence of hCG >150 IU/ml, around 15th day after implantation and verified by positive fetal heart beats.

Measurements of cytokine concentrations

In every FF sample, commercial enzyme immunoassay (ELISA) kits were used to measure the concentrations of free VEGFA, VEGFRs, and total leptin and leptin receptor. All measurements were carried out in duplicate and according to the manufacturers’ instructions.

Statistics

Descriptive and analytic statistics was performed. Data are presented as median and InterQuartileRange (IQR) or mean ±standard deviation (SD), depending on normality of distribution. Normality was checked with Shapiro-Wilk test. Mann-Whitney –U test was used for comparison of two independent samples, if distribution was not normal, whereas in normal distributions t-test was used instead. Rates were compared using x2 test with Yates correction for two-by-two tables, and Spearman correlation was applied for bivariate correlations. A logistic regression model was applied for outcome prediction. Statistical significance was set at p < 0.05, while Bonferroni correction test was used for multiple comparisons. All tests were two–tailed.
Results

Regarding infertility categories, 56 samples were classified in male factor group, 83 in female factor, and 14 in low responders. Women’s age differed statistically significantly between groups, the youngest age (31.20 ± 2.38 years) observed in male factor group and the oldest (40.09 ± 2.52 years) in low responders. Women were overall overweight, as mean body mass index (BMI) values exert 25.00 in all three categories. In a total of 153 samples, the overall pregnancy rate was 51.6%, the higher one observed in female factor group (59% vs 37.5% and 28.6% in male factor and low responders group, \( p = 0.013 \) (Table 1). When male factor and low responder categories were merged, differences between female factor group and the remaining subjects regarding pregnancy was more obvious: 35.7% vs 59.0%, \( p = 0.007 \) (data not shown). When pregnancy and non-pregnancy groups (irrelevant of infertility cause) were comparatively studied, only VEGFR2 differed statistically significantly between the two groups, being higher in the pregnancy group [median (IQR): 5,630 (4,870 - 6,651) vs 4,938 (4,068 - 6,020) in the non-pregnancy group, \( p = 0.003 \)] (Table 2). As infertility group and VEGFR2 were associated with pregnancy, a logistic regression model adjusted for age was applied to study the above factors as predictors of pregnancy (yes/no). In this model subjects were classified in two infertility categories (female factor group against all others). The infertility group emerged as independent predictor of pregnancy, whereas VEGFR2 failed to have a significant component to the equation, due to its zero coefficient. The female factor subjects had approximately 3.4 times greater probability to have a pregnancy (1 / 0.296) (Table 3). Spearman correlations within the three infertility groups revealed positive correlations between VEGFR1 - VEGFR2 (\( p = 0.004 \)), and VEGFR2-VEGFA (\( p < 0.001 \)),
while negative correlation was observed between estradiol and VEGFR1 ($p = 0.034$). In the female factor group, negative correlations between VEGFR1-VEGFR2 ($p = 0.021$) and VEGFR2-VEGFA ($p = 0.025$) were observed. In the low responders and the female factor group, E2 was marginally statistically significantly related to VEGFR2 ($p = 0.059$ and $p = 0.060$, respectively, Table 4).

**Discussion**

The findings of the present study underline the importance of FF study in human reproduction. Infertility due to female factor seem to be an independent predictor of pregnancy in women under ICSI. The results also call for attention in the case of low responders and male factor group, as these groups exhibit a similar pregnancy outcome, despite possibly different underlying mechanisms, implying that serious—and probably difficult to detect—functional defects may be present in spermatozoa and oocytes.

The three groups of the present study exhibit distinct characteristics regarding the concentrations VEGFA, its receptors, and leptin/leptin receptor. Moreover, pattern of associations is significantly differentiated between male and female factor group, being quite opposite in the case of VEGFA receptors. In male factor group, concentrations of VEGFR1 and VEGFR2 were positively associated to one another: VEGFR1 was negatively associated with E2 and VEGFR2 was positively associated with VEGFA. In female factor group, VEGFR1 and VEGFR2 were negatively associated to one another, while VEGFR2 were negatively associated to VEGFA. The pattern of associations in the male factor group reflects previously reported data suggesting that estradiol decreased secreted VEGFR1 and increased secreted VEGFA [4, 21-23]. The strong association between VEGFR1 and VEGFR2 indicates that these two receptors may work hand in hand as the formation of heterodimers and interaction between the two receptors has been documented [23, 24]. The marginally negative correlation between E2 and VEGFR-2 in the female factor and low responders may reflect interaction between the two receptors and indicate common pathophysiological pathways. This association may suggest that VEGFR2 is the most important receptor determining embryo development, representing an endogenous female defect. Previous data support the significance of the VEGFA/VEGFR2 system for pregnancy maintenance in animal studies and VEGFR-2 appears to be the most important receptor in VEGF-induced mitogenesis and permeability [18, 25]. The associations between VEGFA/VEGFR2/VEGFR1 might indicate adaptation to hypoxia, the receptors being upregulated in order to bind as many as possible VEGF molecules.

Regarding pregnancy rates in the present study, these are comparable with previous findings. More specific, in the case of male factor infertility, ICSI pregnancy rates range from 15% (germ cell hypoplasia) to 37% (non-obstructive azoospermia) [26, 27]. In the case of female factors, pregnancy rates ranged from 6% to 49%, depending on woman age, the highest rate achieved in women < 34 years (1). Despite the apparent integrity of VEGFA/VEGFR1/VEGFR2 system in the male factor group, pregnancy rates were lower in this group when compared with the female group. This discrepancy could be attributed to functional defects in morphologically integral spermatozoa. DNA fragmentation examination is not routinely used. Indeed, the proportion of sperm with DNA fragmentation appears to be potentially useful as a predictor of ICSI outcome, whereas embryo quality based on morphological criteria, appeared unaffected by DNA fragmentation, while appropriate semen preparation decreases sperm fragmentation levels [28, 29]. Several tests are available to measure sperm DNA fragmentation levels and the routine assessment of DNA fragmentation is strongly recommended in order to improve ICSI outcomes, at least in the male factor group.

**Limitations**

The small sample size of the low responders group may have obscured some features of this particular infertility subgroup, while the role of the receptors in repetitive ICSI attempts was not studied. Moreover, the role of VEGFR3 and other cytokines was not investigated. Further research should broaden the array of cytokines studied and focus on different subpopulations of women under IVF, as different cytokines profile may well account for the differences observed in fertility rates in these groups.

**Conclusion**

The inclusion of women with female factor infertility (except from PCOS) provided a useful insight in the possible mechanisms related to female infertility and underline the importance of VEGFA/VEGFR2 system in human reproduction. Instead of VEGFA itself, its receptors and especially their association pattern may serve as a marker for differentiating infertility groups, while infertility group may be a predictor of ICSI outcome. The inclusion of larger cohorts may further elucidate the findings of the present study. Moreover, focusing on functional defects of spermatozoa may lead to better sperm preparation and higher pregnancy rates in male factor groups. Enhancing VEGFR2 production in women with reproductive disorders might also be a future promising therapeutic perspective.

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REFERENCES


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Improved neonatal prognosis following restriction in the number of transferred embryos in assisted reproduction – single center yearly comparison from Turkey

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Summary
Purpose: To evaluate the impact of new legislation for assisted reproductive technology (ART) restricting the number of transferred embryos on neonatal prognosis of infants born after infertility treatments. Materials and Methods: Neonatal records of all live born infants in Ege University Maternity Ward were reviewed for 2006 and 2012. Neonatal outcome measures such as birth weight (BW), gestational age (GA), preterm birth (PTB), very low birth weight (VLBW), and neonatal intensive care unit (NICU) admission were evaluated. Results: Compared to 2006 percentage of newborns conceived by medically assisted reproduction (MAR) decreased from 14.6% to 5% in all live births, from 23.8% to 8.2% in NICU patients in 2012. The number of fetuses in the last pregnancy, frequency of intrauterine reductions, spontaneous pregnancy losses, aminatal bleeding, and premature delivery decreased. Percentage of multiples among MAR newborns (31.7% vs. 55.7%), twins from 51.4% to 30.9%, triplets from 4.3% to 0.8% all decreased significantly. Mean BW and gestational age increased resulting in decreased frequency of PTB and VLBW. Consequently Level 3 NICU admission rate significantly decreased from 44.3% to 22%. Conclusion: The new ART legislation in Turkey resulted in decreased rate of multiple births, prematurity and related complications, and NICU admissions in MAR newborns. However the twin rates are still high. Since uncontrolled ovulation stimulation and intrauterine insemination techniques are also associated with multiple births and unfavorable neonatal outcomes, these procedures deserve close monitorization.

Key words: Medically assisted reproduction; Newborn; Multiple; Morbidity; Legislation; Turkey.
intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), sepsis, and mortality for infants admitted to the NICU at Ege University Children’s Hospital. Medically assisted reproduction (MAR) was defined as reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intrauterine, intracervical, and intravaginal insemination with semen of husband/partner or donor [11]. Local ethical committee approval was obtained for the study.

Statistical Package for the Social Sciences 19.0 was used for data analyses. Normally distributed data were summarized as mean ± SD and percentages, whereas non-normally distributed data were given as median (min-max). Chi-square test, Fisher exact test, independent samples t-test (with 95 % confidence interval) and Mann Whitney U tests were performed to determine differences between the study groups as appropriate. All p values < 0.05 were considered significant.

Results

Frequency of infertility treatment

Nine hundred and sixty-one live born infants from 2006 (Group 1) and 2,460 live born infants from 2012 (Group 2) were enrolled to the study (Table 1). Percentage of newborns conceived by MAR decreased from 14.6 % to 5 % in all live births; and from 23.8 % to 8.2 % in infants admitted to the NICU (all p values <0.001). In 2006, 20.1 % of patients cared in the Level 2 NICU and 24.5 % of patients cared in the Level 3 NICU were newborns from MAR pregnancies; whereas in 2012 these percentages decreased to 11 % in Level 2 NICU and 13.1 % in Level 3 NICU (p = 0.009 and 0.002, respectively).

Table 1. — Distribution of study population in two study periods.

<table>
<thead>
<tr>
<th>Group 1 (n=961)</th>
<th>Group 2 (n=2460)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous MAR</td>
<td>p</td>
</tr>
<tr>
<td>All live births*</td>
<td>821 (85.4%)</td>
</tr>
<tr>
<td>Discharged from newborn nursery**†‡</td>
<td>606 (89.2%)</td>
</tr>
<tr>
<td>Admitted to NICU**†‡</td>
<td>215 (76.2%)</td>
</tr>
<tr>
<td>Level 2§</td>
<td>143 (79.9%)</td>
</tr>
<tr>
<td>Level 3§</td>
<td>191 (75.5%)</td>
</tr>
</tbody>
</table>

* p < 0.001 for Group 1 vs. Group 2; † p < 0.001 for spontaneous vs. MAR in group 1; ‡ p < 0.001 for spontaneous vs. MAR in group 2; § p < 0.005 for percentage of MAR in Group 1 vs. Group 2.

Characteristics of the parents and MAR procedures

Maternal and paternal ages were similar in MAR and spontaneous pregnancies (Table 2). In 2012 infertile couples appeared to apply for fertility treatments earlier, with a shorter duration of childless years, but this difference was not statistically significant. The number of fetuses in the last pregnancy decreased in 2012 compared to 2006 (p < 0.001, Table 2). Intrauterine reduction procedures were applied less frequently and also the frequency of spontaneous losses decreased in 2012. IVF and ICSI constituted a higher percentage in fertility treatments, but this trend did not reach statistical significance.

Pregnancy complications

In 2012 maternal follow-up visits for both spontaneous and ART pregnancies increased in number due to continuing improvements in healthcare delivery in Turkey. However, for both study periods; MAR pregnancies had increased risk of complications (such as gestational diabetes, antenatal bleeding, preterm birth, premature rupture of membranes-PROM) when compared to spontaneous pregnancies (Table 3). However the frequency of antenatal bleeding, cerclage application, hospitalization, administration of tocolytics, and antenatal steroids together with preterm delivery decreased in MAR pregnancies in 2012.

Neonatal prognosis

In 2006, infants conceived by MAR had lower mean gestational age and mean birth weights (BW) and lower
### Table 3. — Antenatal complications of spontaneous and MAR pregnancies in study groups.

<table>
<thead>
<tr>
<th>Complications</th>
<th>Group 1 (n=961)</th>
<th>Group 2 (n=2460)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spontaneous</td>
<td>MAR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=821) n (%)</td>
<td>(n=140) n (%)</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia/eclampsia</td>
<td>34 (4.4)</td>
<td>14 (10)</td>
<td>0.012</td>
</tr>
<tr>
<td>Gestational DM</td>
<td>68 (8.3)</td>
<td>31 (22.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abruptio placenta</td>
<td>3 (0.4)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>8 (1)</td>
<td>1 (0.7)</td>
<td>1.000</td>
</tr>
<tr>
<td>Placental insufficiency</td>
<td>5 (0.6)</td>
<td>0 (0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>3 (0.4)</td>
<td>1 (0.7)</td>
<td>0.468</td>
</tr>
<tr>
<td>Antenatal bleeding*</td>
<td>81 (9.9)</td>
<td>34 (24.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Premature birth*</td>
<td>140 (17.1)</td>
<td>83 (59.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PROM</td>
<td>56 (6.8)</td>
<td>17 (12.1)</td>
<td>0.037</td>
</tr>
<tr>
<td>Regular follow up*</td>
<td>750 (91.4)</td>
<td>132 (94.3)</td>
<td>0.317</td>
</tr>
<tr>
<td>Cerclage*</td>
<td>12 (1.5)</td>
<td>11 (7.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tocolysis*</td>
<td>101 (12.3)</td>
<td>69 (49.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antenatal steroids*</td>
<td>89 (10.8)</td>
<td>74 (52.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DM: diabetes mellitus, UTI: urinary tract infection, PROM: premature rupture of membranes

*p < 0.001 for Group 1 vs. Group 2 MAR pregnancies

### Table 4. — Neonatal characteristics of infants born from spontaneous and MAR pregnancies.

<table>
<thead>
<tr>
<th>Neonatal characteristics</th>
<th>2006</th>
<th>2012</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g), Mean (±SD)*</td>
<td>3016.82 (828)</td>
<td>2293.68 (788)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age (week), Mean (±SD)*</td>
<td>37.02 (3.19)</td>
<td>34.11 (3.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apgar score at 1st min, Mean (±SD)</td>
<td>8.50 (1.6)</td>
<td>7.91 (1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apgar score at 5th min, Mean (±SD)</td>
<td>9.72 (0.9)</td>
<td>9.54 (0.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C/S, n (%)*</td>
<td>648 (78.9)</td>
<td>132 (94.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiple births, n (%)*</td>
<td>54 (6.6)</td>
<td>78 (55.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Singleton</td>
<td>767 (93.4)</td>
<td>62 (44.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Twins</td>
<td>49 (6.6)</td>
<td>72 (51.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triplets</td>
<td>5 (0.6)</td>
<td>6 (4.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Premature birth (&lt;37 weeks), n (%)*</td>
<td>211 (25.7)</td>
<td>110 (78.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Premature birth (&lt;32 weeks), n (%)*</td>
<td>67 (8.2)</td>
<td>28 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLBW (&lt;1500 gr), n (%)</td>
<td>57 (6.9)</td>
<td>72 (51.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGA, n (%)</td>
<td>13 (1.6)</td>
<td>3 (2.1)</td>
<td>0.717</td>
</tr>
<tr>
<td>Congenital anomaly, n (%)</td>
<td>51 (6.2)</td>
<td>4 (2.9)</td>
<td>0.165</td>
</tr>
<tr>
<td>Need for neonatal care, n (%)</td>
<td>215 (26.2)</td>
<td>67 (47.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Level 3*</td>
<td>191 (23.3)</td>
<td>62 (44.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Level 2</td>
<td>143 (17.4)</td>
<td>36 (25.7)</td>
<td>0.025</td>
</tr>
<tr>
<td>CPAP/N IMV, n (%)</td>
<td>40 (4.9)</td>
<td>19 (13.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surfactant, n (%)*</td>
<td>59 (7.2)</td>
<td>26 (18.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mechanical ventilation, n (%)*</td>
<td>74 (9.0)</td>
<td>28 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phototherapy, n (%)*</td>
<td>194 (23.6)</td>
<td>65 (46.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RDS, n (%)*</td>
<td>56 (6.8)</td>
<td>25 (17.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TTN, n (%)</td>
<td>33 (4.4)</td>
<td>14 (10)</td>
<td>0.005</td>
</tr>
<tr>
<td>Pneumonia, n (%)</td>
<td>12 (1.2)</td>
<td>4 (2.9)</td>
<td>0.273</td>
</tr>
<tr>
<td>PDA, n (%)*</td>
<td>27 (3.3)</td>
<td>11 (7.9)</td>
<td>0.017</td>
</tr>
<tr>
<td>Sepsis, n (%)</td>
<td>20 (2.4)</td>
<td>5 (3.6)</td>
<td>0.394</td>
</tr>
<tr>
<td>NEC, n (%)</td>
<td>8 (1)</td>
<td>2 (1.4)</td>
<td>0.646</td>
</tr>
<tr>
<td>IVH, n (%)*</td>
<td>31 (3.8)</td>
<td>13 (9.3)</td>
<td>0.008</td>
</tr>
<tr>
<td>BPD, n (%)</td>
<td>8 (1.0)</td>
<td>3 (2.1)</td>
<td>0.207</td>
</tr>
<tr>
<td>Mortality</td>
<td>23 (2.8)</td>
<td>6 (4.3)</td>
<td>0.418</td>
</tr>
</tbody>
</table>

C/S: cesarean section; VLBW: very low birth weight; SGA: small for gestational age; CPAP: continuous positive airway pressure; N-IMV: nasal intermittent ventilation; RDS: respiratory distress syndrome; TTN: transient tachypnea of newborn; PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; IVH: intraventricular hemorrhage; BPD: bronchopulmonary dysplasia; *p<0.005 for comparison of MAR babies in Group 1 vs. Group 2.
Apgar scores and were delivered by cesarean section (C/S) more frequently when compared to spontaneously conceived newborns (Table 3). These babies needed NICU admission and respiratory support therapies more frequently. Patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) were more common, but mortality rates were similar.

In 2012, mean BWs, mean gestational ages, and Apgar scores were lower and frequencies of C/S, multiple births, preterm birth (PTB), very low birth weight (VLBW) and NICU admissions were higher in MAR infants compared to spontaneously conceived infants (Table 4). RDS, TTN, and non-invasive ventilation were more common; but the need for surfactant and invasive mechanical ventilation therapies and mortality rates were similar to spontaneously conceived infants.

Comparing neonatal characteristics of newborns from MAR pregnancies in 2006 vs. 2012; the frequency of PTB and VLBW decreased and accordingly there was a significant increase in mean BWs and mean gestational ages in 2012. In 2012 the percentage of multiples in MAR infants was decreased significantly when compared to 2006 (31.7% vs. 55.7%, p < 0.001). The frequency of twins decreased from 51.4% to 30.9%; triplets from 4.3% to 0.8%. Although the decrease in the need for total NICU hospitalization was not significant, Level 3 NICU admission rates significantly decreased in 2012 (22% vs. 44.3%, p < 0.001). A significant decrease in RDS frequency resulted in a decreased need for surfactant administration and mechanical ventilation. PDA and IVH were also seen less frequently in 2012.

Discussion

The authors have demonstrated that infertility treatments are associated with increased rates of multiple gestations and adverse neonatal outcome. However, in accordance with the new regulations, the number of fetuses in the last pregnancy has decreased, as did the rate of twins and triplets and neonatal outcome parameters improved in 2012.

Frequency of ART

The frequency of ART (ICSI/IVF) and non-ART (ovulation induction [OI] / intrauterine insemination [IUI]) infertility treatment utilization increased steadily throughout the world after the birth of the first successful IVF infant in the late 1970s. According to latest reports, ART contributed to 1% of all births in 2006 and 1.4% of births in 2009 in the United States [12-13]. The percentage of infants conceived through ART is above 3.0% in most of the Nordic countries; 4.9% in Denmark; and between 1.2% and 1.8% in Germany, France, UK, and Italy [14]. In the current study the percentage of newborns conceived through MAR (reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intrauterine, intracervical, and intravaginal insemination with semen of husband/partner or donor [11]) pregnancies among all live births, was significantly lower in 2012 than in 2006, 5% vs. 14.6%. When we exclude ovarian stimulation and intrauterine insemination procedures; the total percentage of ART (IVF and ICSI) among all live births was 4.3% for 2012 and 11.9% for 2006. The decrease in this rate is in parallel with the decreased number of multiple births after regulations restricting the number of transferred embryos in ART.

In 2006, MAR infants constituted 20.1% of Level 2 NICU patients and 24.5% of Level 3 NICU patients in the present center in a similar pattern with the multicenter data giving the ratios as 21% for Level 2 and 25% for Level 3 MAR infants [3]. The percentage of MAR infants among NICU patients decreased significantly to 11% for Level 2 and 13.1% for Level 3 in 2012 in the present center showing the positive effect of new legislation. Similarly, Guzoglu et al. have recently demonstrated that, after the new legislation, NICU utilization decreased in newborns conceived by IVF or ICSI [15].

Characteristics of the parents and MAR procedures

Parental characteristics were similar in the two study periods. However, after the new legislation favoring SET, the median number of fetuses in the last pregnancy was decreased in 2012 when compared to 2006, with median values of one vs. two, respectively. The significant decline seen in intrauterine reduction and spontaneous loss rates is a reflection of the decreased fetus number. United States data for 2009 gives the average number of embryos transferred as 2.1 among women aged < 35 years, 2.5 among women aged 35–40 years, and 3.0 among women aged > 40 years [13]. The 11th European IVF-Monitoring Report of the European Society on Human Reproduction and Embryology (ESHRE), comprising 33 European countries and 1,029 clinics showed the total percentage of SET 21.4%, double embryo transfer (DET) 53.4%, triple embryo 22.7%, ≥ four embryos 2.5%, together with twin birth rate of 21.3%, and triplet rate of 1% [14]. The same ESHRE registry data from infertility clinics of Turkey shows SET ratio of 1.5%, DET 24.1%, three embryos 52.8%, ≥ four embryos 11.4% resulting in higher ART multiple rates as 32.9% for twins and 4.1% for triplets in 2007 before the 2010 legislation [14]. Kutlu et al. have reported significantly decreased multiple pregnancy rates without causing a significant decline in the pregnancy rates after the new legislation [16].

Pregnancy complications

Pregnancy complications were seen more frequently in MAR pregnancies in both study periods. However in the second study period, perinatal care of all mothers improved significantly. Together with better antenatal care decreased rate of multifetal pregnancies, resulted in a decreased frequency
of antenatal bleeding and premature delivery, necessitating C/S, hospitalization, and treatments such as cerclage and tocolytics less frequently in these MAR pregnancies.

**Neonatal outcome for MAR infants**

Mean BW of 2,293 (±788) grams for MAR babies increased to 2,613 (±719) grams in 2013, in accordance with the increased mean gestational ages from 34.1 (3.3) weeks to 35.8 (2.6) weeks in 2012.

The frequency of premature birth (< 37 weeks) decreased from 78.9% to 52.8% and very preterm birth (< 32 weeks) from 20% to 5.7%. In USA, the percentage of preterm(< 37 weeks) and very preterm (<32 weeks) newborns among infants conceived with ART were 33.4% and 6.1%, respectively, whereas in the general birth population these ratios were 12.2% and 2% in 2009 [13]. D’Angelo et al. have recently investigated outcomes of pregnancies among women who used ART, ovulation stimulation, vs. spontaneous pregnancies [17]. They have reported that the prevalence of adverse infant outcomes increased with the use of more intensive treatment, giving the rate of preterm birth (< 37 weeks gestation) as highest among the ART group (16.1%), followed by the medication-only group (11.0%), and lowest in spontaneous pregnancy group (8.0%). The frequency of very preterm infants has reached reported frequencies from developed countries, but the frequency of near term infants is still high in accordance with the still high twin rates.

In the present center, 20% of MAR babies were born as VLBW infants in 2006, whereas in 2012 this percentage decreased to 8.1%. In the United States, 6% of ART infants and 1% of general birth population were VLBW [13].

After the new ART legislation favoring SET, the percentage of MAR babies from multiple gestations decreased from 55.7% to 31.7%. The frequency of twin births was 51.4% vs. 30.9% and triplet rates were 4.3% vs. 0.8%. The percentage of multiple pregnancies varied between 15-40% in ART pregnant [18, 19]. In the United States, among 31,582 ART newborns, twin rate was 44.5% and triplet rate was 9.3% between 1997-2000 [20]. Again in USA in 2006, 1% of all births were ART newborns; but 17% of twins and 38% of triplets or higher order multiples were conceived with ART [12]. In 2009 data of USA, among ART infants 47% were born as multiple-birth infants, compared with only 3% of infants among the general birth population [13].

Yayla and Baytur evaluated the epidemiology of multiple births in Turkey, in 2003-2004; and reported that 76% of twins and 90% of triplets was achieved with ART (for twins; spontaneous: 24.15%, OI-IUI: 31.70%, IVF-ICSI: 44.15%; for triplets; spontaneous: 10%, OI-IUI: 27.50%, IVF-ICSI: 62.5%). They concluded that ART may be responsible in 75% of multiple pregnancies [21]. ESHRE registry data from Turkey shows ART multiple rates as 32.9% for twins and 4.1% for triplets in 2007 [14].

The limitation of this study was to reflect only the pediatricians’ point of view about the effect of new ART legislation on neonatal outcomes. The data recorded for this study was obtained from the medical records of the newborns, reflecting the statements of the family regarding the mode of conception and pregnancy follow-up. Possibility of families to withhold information about details of conception results is a limitation of estimating the real frequency of fertility treatments. The present authors also do not know the number of embryos transferred, but only the number of fetuses in the last pregnancy. They did not have the ART center records available to us to evaluate the number of unsuccessful ART trials. Therefore, determining the change in the pregnancy success rates with the new ART guidelines was beyond the scope of this study.

Since the most important factor being responsible of multiple pregnancies is the number of embryos transferred; it is obvious that limiting the number of embryos to be transferred by the new ART legislation in Turkey resulted in a decreased rate of multiple births, prematurity, and related complications in MAR babies. However, the twin rates are still high and near term deliveries remain to be frequent, compared to European and USA data. These findings indicate ongoing high DET rates and uncontrolled infertility treatments in Turkey. Since twin gestations still cause maternal and neonatal risks, the goal of infertility treatments should be one healthy child. Single-embryo transfer should be promoted more and ovulation stimulation medication use needs to be closely monitored.

**References**


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Introduction

Hysterosalpingography (HSG) is a radiological procedure, performed mainly in the diagnostic protocol of infertility, which allows, through the introduction of a radio-opaque contrast medium through the cervical canal, the accurate assessment of the uterine cavity and fallopian tubes [1].

Other procedures that are used for the evaluation of the uterus are saline-infusion sonography (SIS), optimal for viewing the uterine cavity, magnetic resonance imaging (MRI), especially useful for evaluating the myometrium and ovaries, and laparoscopy with the chromopertubation (or dye) test, accurate to diagnose the causes of tubal occlusion and to study pelvic peritoneum [1].

The introduction of hysterosalpingo-contrast sonography (HyCoSy) has become an increasingly popular alternative, combining the principles of SIS with those of HSG. In fact, HyCoSy has proven to be an acceptable, time efficient, and well-tolerated alternative to HSG with comparable accuracy in the assessment of the uterine cavity and tubal patency. HyCoSy is a simple, safe, and effective outpatient procedure that may add value to a streamlined initial infertility evaluation [2].

Despite the arrival of these newer imaging investigations, HSG still remains today the most used tool to study infertility, due to its ability to optimally evaluate the tubal occlusion which is present in 12-33% of infertile couples [3]. In recent years the number of HSG has increased considerably, probably in relation to the progress of reproductive medicine, the successes of the results of medically assisted reproduction procedures, and the tendency of women to postpone pregnancy always later in their lives [1].

HSG is an examination that allows morphological study but not female genital tract functionality, because physiological integrity is evident only after a patient becomes pregnant. The main indications for HSG include uterine and tubal disease. Uterine pathology is represented by: uterine myoma, endometrial polyps, synechiae, and uterine malformations (uterus septum is the most frequent). Tubal pathology includes: proximal, distal occlusion, perifimbrial adhesions, and hydrosalpinx [4]. However, mild or moderate grade endometriosis is not visible with HSG. Conversely, laparoscopy allows to visualize the uterine anatomy, relationships between tube and ovary, pelvic adhesions, and any endometriosis lesions if present. On the other hand, HSG is less expensive and less invasive than laparoscopy. For the study of morphology of the uterus, and in particular of the uterine malformations, HSG must be combined with other imaging techniques such as transabdominal and transvaginal pelvic ultrasound, and/or MRI.

SIS is a simple, outpatient procedure, using ultrasonography: sterile saline solution (contrast medium) is injected into the uterine cavity through a catheter with balloon endocervical sampling of 5 French, so that the uterine cavity and fallopian tubes can be displayed [5, 6]. SIS can now be...
considered how broadly validated survey in research of the causes of infertility, is able to increase the power of routine transvaginal ultrasound in evaluating uterine cavity diseases and congenital or acquired pathologies associated with infertility.

HyCoSy uses hyperechoic contrast medium (such as Echovist-200) or a mixture of saline and air for evaluating tubal patency [2].

The aim of this retrospective study was to analyze indications of HSG performed in a 17-year period at the Institute of Radiological Sciences at the University of Sassari, Italy.

Materials and Methods

The present case series included 2,845 HSG, performed from January 1997 to March 2014 at the Institute of Radiological Sciences at the University of Sassari, Italy The age of the patients was between 20 and 48 years (mean age ± SD = 34.5 ± 2.5).

HSG was performed with the patient supine on the fluoroscopy table in lithotomy position. After cervical preparation (commonly with povidone-iodine solution), a tenaculum was placed for cervical stabilization and uterine positioning. The HSG catheter (typically 5F) was inserted through the endocervix. The catheter balloon tip was inflated to aid in uterine traction, limit efflux of contrast, or spontaneous expulsion of the catheter. Iodinated hydrodisoluble contrast media was instilled through the catheter into the uterine cavity. Radiograph images were obtained intermittently to document filling of the uterine cavity and fallopian tubes (Figure 1). The authors began the procedure at low pressure through a balloon catheter placed in the uterine cavity. Then, in case of failure or incomplete tubal opacification, other injections were performed, with higher pressure, while also varying the decubitus of the patient. In the presence of proximal tubal occlusion, mono or bilateral, the authors scheduled a second HSG after about a month [7]. Selective catheterization was performed during the execution of the second HSG in case of persistence of the tubal occlusion. The case series was divided for years and for ages to analyze the growth or decrease in the number and percentage of negative or pathological examinations.

Results

The negative exams were 2,039 out of 2,845 (71.67%) (Table 1). The negative results (normal findings and bilateral tubal patency) of examinations performed per year were as follows:

- 1997: 105/121 negative results (86.78%)
- 1998: 104/141 negative results (73.76%)
- 1999: 45/57 negative results (78.95%)
- 2000: 45/62 negative results (72.58%)
- 2001: 50/60 negative results (83.33%)
- 2002: 77/97 negative results (79.38%)
- 2003: 132/167 negative results (79.04%)
- 2004: 141/181 negative results (77.90%)
- 2005: 137/168 negative results (81.55%)
- 2006: 156/202 negative results (77.23%)
- 2007: 148/190 negative results (77.89%)
- 2008: 135/181 negative results (74.59%)
- 2009: 283/377 negative results (75.07%)
- 2010: 163/221 negative results (73.76%)
- 2011: 152/202 negative results (75.25%)
- 2012: 170/223 negative results (76.23%)
- 2013: 123/154 negative results (79.87%)
- 2014 (up to March 31): 32/41 negative results (78.05%)

Fifty-six out of 2,845 (1.97%) patients presenting with bilateral tubal occlusion to normal inflation pressure become patent tubes by injecting contrast medium at high-pressure. 133/2845 (4.67%) patients achieved opacification of a sin-
Table 1. — Results of hysterosalpingographies.

| Negative: normal findings (bilateral tubal patency) | 2,039 |
| Bilateral occlusion | 55 |
| Monolateral occlusion | 138 |
| Proximal monolateral occlusion. Repeat after one month | 15 |
| Monolateral occlusion and hydrosalpinx | 18 |
| Bilateral hydrosalpinx | 90 |
| Monolateral hydrosalpinx | 35 |
| Cervical stenosis | 1 |
| Bilateral occlusion after ESSURE | 2 |
| Bilateral occlusion after tubal ligation | 1 |
| Negative for bilateral tubal patency at high pressure | 56 |
| Negative for monolateral tubal patency at high pressure | 133 |
| Negative after bilateral selective salpingography | 8 |
| Negative after monolateral selective salpingography | 4 |
| Tubal patency after a second HSG | 2 |
| Tubal patency after high pressure; endometrial polyp | 1 |
| Tubal monolateral patency after selective salpingography | 6 |
| Unicorneate uterus; tubal monolateral occlusion | 11 |
| Bicornuate uterus one cervix | 39 |
| Negative; arcuate uterus | 2 |
| Negative; septate uterus | 5 |
| Negative; uterus didelphys with double vagina | 1 |
| Negative; myoma | 59 |
| Negative; endometrial polyp | 41 |
| Myoma; monolateral tubal patency after high pressure | 6 |
| Myoma; hydrosalpinx | 3 |
| Myoma; monolateral occlusion | 3 |
| Myoma; bilateral occlusion | 1 |
| Multiple myomas; negative | 18 |
| | 1,772 |

...g tube after high pressure injection. Twelve out of 2,845 (0.42%) patients underwent a second HSG after one month and, through selective salpingography, both tubes become patent.

The pathological changes observed are as follows: 141 mono (Figure 2) and 55 bilateral proximal occlusions: 12 resolved spontaneously to the next control and 12 resolved after selective salpingography; 35 unilateral and 90 bilateral hydrosalpinx; 58 congenital uterine anomalies; 72 fibromyomas and 41 endometrial polyps (confirmed with subsequent SIS and/or hysteroscopy). In all three patients undergoing bilateral tubal occlusion by hysteroscopic approach (ESSURE), the HSG confirmed the total closure of the tubes. During all diagnostic procedures, significant complications were not reported.

Discussion

The retrospective study carried out at the Institute of Radiological Sciences of Sassari University, showed that requests for the HSG underwent fluctuations over the years: 121 in 1997, 141 in 1998, to achieve a decrease in the years 1999, 2000, 2001, and 2002, respectively, with 57, 62, 60 and 97 requests. An increase once again was observed in recent years: 167 requests in 2003, 181 in 2004, 168 in 2005 up to 211 in 2006, 190 in 2007, 181 in 2008, and a sharp increase in 2009 with 373 requests. This increase in exams noticed in the recent years, agrees with the observations reported by other authors [1] and is presumably linked to the progress of reproductive medicine, the successes of the results of medically assisted reproduction procedures, and the tendency of women to postpone pregnancy always later in their lives [1]. In fact, the age range in which HSG was a major request was that between 30-35 years with 617 procedures, followed by 464 procedures between 35-40 years. HSGs were still performed in women that are rather young of 20-25 years or older with an age of 45-50 years.

Another interesting fact is the high proportion about 70% of negative tests that deviates from the data reported in the literature by some authors [8-9] who mention in their cases a percentage of negativity of 64% and 67%. The present high percentage of HSG negatives could be linked to the fact that, in the present case, HSG did not represent the final diagnostic process investigation of infertility, but often was the first test for evaluating tubal patency.

Withs regard to the treatment of proximal tubal obstruction, the present authors [7] studied whether a second HSG done after one month compared to the previous one, could make it possible to clean the tube, reducing the use of selective salpingography in patients with proximal tubal obstruction due to deposits of mucus. For this purpose, a sample of 360 infertile women with proximal tubal occlusion uni- or bilateral were advised to have a second HSG after about a month from the previous, and of these, 40 patients were subjected to a second HSG after a month from the first HSG performed. In 24 patients out of 40 (60%), the execution of the second HSG obtained the patency of both tubes and only in the remaining patients selective salpingography was necessary. So in conclusion, in the infertile women with proximal tubal obstruction, is better to make a second HSG subjecting the patient to a very low dosage of radiation and also a smaller risk than the selective salpingography that must be carried out only in case of failure after the repetition of the second HSG.

Examination success is related to the choice of the catheter as well as SIS [5]: on the market, there are several catheters, with different features and different terms of use. Before coming to the choice of dedicated two-way balloon catheter, for a certain period of time, Foley catheters, two-way, were used normally: are soft, not stiff, and have the advantage of being inexpensive; variants to the balloon catheter are formed from the catheter by Goldstein (Cook), which does not possess the balloon at the top and is positioned in the uterus with a cup that is slid along the catheter and joins the external uterine orifice. Some authors [4] decided to proceed with the use of two-way balloon catheter to perform the HSG with the aim of ascertaining pain in patients who are subjected to the HSG using three different techniques. Always according to the above-mentioned complications, it is crucial to choose the contrast medium: in spite of the fact that in the present authors...
practice they use just non-ionic water-soluble iodinated contrast media, some authors prefer to use lipid-soluble contrast media; the most used was the Lipiodol contrast medium [4].

The present authors’ choice falls on the water-soluble non-ionic iodinated contrast medium with key features such as: good radio-opacity, the absence of toxicity, good tolerability and easy application; also in this case the contrast medium must be radio-opaque enough to delimit precisely the uterus.

To exclude an active pelvic infection, the present authors used erythrocytocyte sedimentation rate (ESR) assessment; in fact, pelvic inflammatory disease (PID) causes an increase of the ESR, but patients with high ESR and with negative culture for Chlamydia and gonorrhea, are still subjected to examination [1]. The authors also gave a non-steroidal anti-inflammatory medication one hour before the procedure [1].

In the present authors’ practice, they did not observe significant complications, although in the literature several complications have been described as inflammatory reactions resulting from the HSG, which represent a serious complication of the procedure. The presence of a tubal inflammatory reaction in patients with regular tubal patency is a major event and especially damages a woman’s fertility. Antibiotic prophylaxis did not alter significantly the proportion of pelvic peritoneal inflammation [1]. Complications have also been reported in the literature due to perforation of the uterus and fallopian tubes. These are usually caused by improper use of an unsuitable tools, or the presence of uterine anomalies.

Luciano et al. [10] evaluated the benefits and accuracy of HyCoSy in studying the tubes, compared with HSG and laparoscopic chromopertubation. The sensitivity and specificity for HyCoSy in determining tubal patency compared with laparoscopic chromopertubation were 97% and 82%, respectively; the positive predictive value was 88% and the negative predictive value was 95%. The authors [10] concluded that HyCoSy had a similar sensitivity and specificity for tubal patency as HSG, without the inconvenience and potential hazards of HSG, such as possible iodine-based sensitivity to the radiopaque dye and exposure to radiation.

Considering that laparoscopic chromopertubation is the reference test (gold standard), HSG and HyCoSy are equally effective in the diagnosis of tubal patency. HyCoSy is a cheap technique, rapid, well-tolerated, and one of the most important advantages is the ability to obtain information on the status of tube and uterus in one survey time. HyCoSy allows to perform a continuous real-time survey, does not require the use of irritating contrast media as it uses as a mixture of air and a sterile saline solution and this makes it the simplest and most cheap method without affecting the outcome of tubal patency, it does not involve exposure to ionizing radiation (such as HSG) but is a simple ultrasound transvaginal examination and therefore is repeatable.

A technique of diagnostic imaging for the evaluation of infertility should be non-invasive, not expensive, rapid, and of simple execution, and should also be able to provide information regarding tubal patency and pelvic diseases.

HyCoSy should then precede HSG and patients with suspected tubal pathology should be selected [1]. It would therefore be desirable to perform before a HyCoSy as screening for selected patients with tubal pathology and HSG should be applied only in such cases. HyCoSy does not give an image of the whole tube, while the HSG allows a more accurate location of tubal occlusion allowing opacification through selective catheterization. For all the aforementioned reasons, HSG today remains a useful diagnostic investigation tool in the diagnostic work-up of infertile patients.

References


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Neonatal and obstetric outcomes of in vitro fertilization (IVF) and natural conception at a Chinese reproductive unit

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3Reproductive Medicine Center, Weifang People’s Hospital, Shandong (China)

Summary
In vitro fertilization (IVF) has been associated with an increased risk of preterm delivery, caesarean delivery, low and very low birth-weight infants. The authors investigated the possible high risks of adverse health outcomes in infants conceived using IVF and intra-cytoplasmic sperm injection (ICSI). The present study includes 443 infants born to 424 women who conceived naturally and 694 infants born to 536 women that had IVF or ICSI. The study was conducted in the Department of Obstetrics at the Yu Huang Ding Hospital from 2008 to 2009. The main outcome measures were: gestational age, birth weight, mode of delivery, multiple pregnancy rates, and baby gender. The results showed significant differences between the neonatal and obstetric outcomes of IVF/ICSI and natural conception pregnancies. When referred to singletons only, there were no major differences seen in the neonatal and obstetric outcomes between the IVF and the control group. When the IVF group was divided into two sub-groups according to the patient’s age (< 35 and ≥ 35 years), there was no statistically significant difference between the two groups in the observed outcomes.

Key words: Neonatal and obstetric outcomes; In vitro fertilization; Multiple pregnancy; eSET.

Introduction
During the past decades, the development of in vitro fertilization (IVF) has resulted in over four million children being born using IVF and the demand for IVF treatment continues to increase. This constant increase is the result of changes in socio-demographic trends such as the decrease in the fertility rates, advanced maternal age, and changes in social trends such as the offer of treatment to single women and same sex couples [1, 2]. Despite the benefits that assisted reproductive technology (ART) offers to a great number of women and couples worldwide, the safety of the procedures has been questioned particularly in their potential to cause health problems in the offspring [3], as compared to the respective rates of adverse health outcomes in naturally conceived children [4]. Some of the adverse outcomes that IVF has been associated with are: an increased risk of preterm delivery, caesarean delivery, and low and very low birth weight [5-7]. Such adverse outcomes may be associated with the increased incidence of multiple pregnancies following IVF [8]. However, it has been shown that singleton IVF pregnancies can also result in preterm delivery and low and very low birth weight infants when compared with spontaneously conceived single pregnancies [9]. IVF twins have similar obstetric and neonatal outcome as compared with spontaneously conceived deliveries [10]. This has been explained, but not conclusively proved, by the increasing age of subfertile women, a factor, which is a well known high risk factor [11]. Advanced maternal age is an important factor and may depend on differences in the studied population characteristics and/or in the obstetric management approach to pregnancies. Nevertheless, even in case control studies the “matching” criteria were not equal, and also the same obstetric unit was present in only three of them [12-14]. The aim of this study, was to compare the obstetric outcomes between IVF and natural conception in different age (≥ 35 and < 35 years) groups of women, providing a particular focus on the differences between singleton and multiple pregnancies.

Materials and Methods
All patients that were pregnant following an IVF cycle were scheduled for delivery between 2008 and 2009 at the Department of Obstetrics at the Yu Huang Ding Hospital. There was no significant difference between the neonatal and obstetric outcomes for the patients who conceived with intra-cytoplasmic sperm injection (ICSI) or IVF. Patients that underwent oocyte donation, sperm donation, or in vitro maturation, who originally conceived twins, but gave birth to singletons, were excluded from the statistical analysis. Patients who conceived spontaneously at the same obstetric department during the research period were selected randomly. Age was the only factor considered in the analysis. The mean (± standard deviation) patient’s age at delivery was 31.59 ± 3.48 years in the IVF/ICSI group, and 31.31 ± 3.45 years.

*Contributed equally to this work.

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in the natural conception group, which was not statistically significant ($p = 0.224$). All women that participated in the study were non-smokers and had no chronic illnesses (i.e. cardiovascular, pulmonary, renal, diabetes or other metabolic diseases).

The main outcome measures for this study were: gestational age, birth weight, mode of delivery, multiple pregnancy rates, and the baby’s gender. Gestational age was measured in completed weeks. For the spontaneous conception group, gestational age was based on early fetal ultrasound measures or detailed information on the woman’s last menstrual period. For the IVF/ICSI group, gestational age was calculated by adding 14 days to the day of the oocyte retrieval.

Preterm delivery was defined as the birth of the baby whose gestational age was below 37 completed weeks of gestation, and premature delivery was defined as the birth of the baby whose gestational age was below 32 weeks of gestation.

The weight of infants was examined within 24 hours of delivery. Low birth weight (LBW) was defined as < 2,500 g, and very low birth weight (VLBW) as < 1,500g.

Data analysis was performed using the statistical package SPSS (version 15.0). The means were compared using the independent t-test, the chi-squared test or the Fisher’s exact test. Statistical significance was set as $p \leq 0.05$.

This study was approved by the reproductive medicine ethics committee of Weifang People’s Hospital. Written Informed Consent Forms were obtained from the patients.

### Results

The study included 443 infants born to 424 women after natural conception, which was defined as the control group. The other group studied comprised of 694 infants born to 536 women who had IVF/ICSI, and was defined as the IVF group. In the control group, there were 19 sets of twins, and in the IVF group there were 154 sets of twins and two sets of triplets.

#### Table 1. Comparison of neonatal and obstetric outcome data of 424 spontaneously conceived and 536 conceived after IVF.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=424)</th>
<th>IVF group (n=536)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>31.59±3.48</td>
<td>31.31±3.45</td>
<td>0.224</td>
</tr>
<tr>
<td>Delivery modes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>225 (53.1%)</td>
<td>70 (13.1%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Caesarean delivery</td>
<td>199 (46.9%)</td>
<td>466 (86.9%)</td>
<td></td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>38.89±1.92</td>
<td>38.01±1.78</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pre-term labour total</td>
<td>38 (9.0%)</td>
<td>84 (15.7%)</td>
<td>0.002</td>
</tr>
<tr>
<td>GA &lt; 32</td>
<td>5 (1.2%)</td>
<td>3 (0.6%)</td>
<td>0.478</td>
</tr>
<tr>
<td>GA &lt; 34</td>
<td>8 (1.9%)</td>
<td>22 (4.1%)</td>
<td>0.05</td>
</tr>
<tr>
<td>GA &lt; 37</td>
<td>25 (5.9%)</td>
<td>59 (11%)</td>
<td>0.005</td>
</tr>
<tr>
<td>Multiple pregnancy rates</td>
<td>19 (4.5%)</td>
<td>156 (18.2%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Average birth weight</td>
<td>3,337.97±606.97</td>
<td>3,100.87±642.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLBW (&lt; 1,500 g)</td>
<td>4 (0.9%)</td>
<td>6 (1.2%)</td>
<td>0.917</td>
</tr>
<tr>
<td>LBW (&lt; 2,500 g)</td>
<td>29 (6.5%)</td>
<td>101 (14.6%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Baby gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>248 (56%)</td>
<td>349 (50.3%)</td>
<td>0.061</td>
</tr>
<tr>
<td>female</td>
<td>195 (44%)</td>
<td>345 (49.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Neonatal and obstetric outcomes are presented in Table 1. As it can be seen, there are some evident differences between the two groups regarding gestational age, birth weight, multiple pregnancy rates, and preterm labour.

The rates of the caesarean sections (CS) were high in both groups, but higher in the IVF group. The difference was statistically significant ($p < 0.001$).

The rate of VLBW and premature delivery was similar in the two groups.

With regards to gender of the baby, the rate of males was 56% in the control group and 50.3% in the IVF group. This difference was not statistically significant, but with reference to the 14 hundred million population of China, it may indicate that the “family’s explicit craving” or “sex selection” plays a prominent role in the reason for the increased rate of males in control group.
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Table 2 represents 405 singletons in the control group and 380 singletons in the IVF group. The mean gestational age was significantly lower ($p < 0.001$) in the IVF group (38.59 ± 1.45 weeks) when compared to the natural conception group (39.02 ± 1.76 weeks).

With regards to the mode of delivery, most women still choose to have caesarean sections. Specifically, 44.9% in the control group and 83.4% in the IVF group had CS a difference which is statistically significant ($p < 0.001$). With regards to the preterm delivery rates ($\leq 32$, $\leq 34$, $< 37$ weeks) and the average birth weight (VLBW, LBW), there was no significant difference between the two groups.

In Table 3, there is a trend showing that the twins in the IVF group had noticeable adverse neonatal and obstetric outcomes when compared with singletons that were naturally conceived. The rate of premature labour children (GA < 32w VLBW) was similar between the twins and singletons. However, it should be noted that the sample size of this study was not large and could account for this. Further research in this area is necessary to investigate the relationship.

The data in Table 4 describe the neonatal and obstetric outcomes in the two groups (IVF and control) between different age groups (≥ 35 and < 35 years). In the < 35 age group, there were more adverse outcomes in the IVF group compared to the control group. All the data observed were statistically different between the two groups ($p < 0.001$). In the ≥ 35 age group, a statistically significant difference was also presented in the rate of caesarean delivery and multiple pregnancies ($p < 0.001$).

Table 5 shows the data for the two different age groups (≥ 35 and < 35 years) and refers to singletons only. There were no major differences seen in the neonatal and obstetric outcomes (including the rates of preterm labour and LBW average birth weight) between the IVF and the control group. The IVF group had a statistically significant higher percentage of caesarean deliveries in both age groups (< 35 and ≥ 35 years).

Table 6. — Comparison of neonatal and obstetric outcome data of 424 aged <35 years and 112 aged ≥ 35 years conceived after IVF.

<table>
<thead>
<tr>
<th></th>
<th>&lt;35 (n=424)</th>
<th>≥35 (n=112)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Delivery modes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>62 (14.6%)</td>
<td>8 (7.1%)</td>
<td>0.037</td>
</tr>
<tr>
<td>Caesarean delivery</td>
<td>362 (85.4%)</td>
<td>104 (92.9%)</td>
<td></td>
</tr>
<tr>
<td>Gestational weeks</td>
<td>38.04±1.79</td>
<td>37.89±1.78</td>
<td>0.425</td>
</tr>
<tr>
<td>Preterm labor total</td>
<td>68 (16%)</td>
<td>16 (14.3%)</td>
<td>0.650</td>
</tr>
<tr>
<td>Average birth weight</td>
<td>3,102.47±629.40</td>
<td>3,094.57±693.10</td>
<td>0.897</td>
</tr>
<tr>
<td>LBW (&lt; 2,500 g)</td>
<td>20 (4.7%)</td>
<td>8 (7.1%)</td>
<td>0.592</td>
</tr>
<tr>
<td>Multiple pregnancy rate</td>
<td>130 (30.7%)</td>
<td>26 (23.2%)</td>
<td>0.123</td>
</tr>
</tbody>
</table>
and ≥ 35 years), when compared to the control group (p < 0.001).

The IVF group was divided into two sub-groups according to the patient’s age. The first group involved patients with age < 35 years, and the second group involved patients with age ≥ 35 years. The outcomes are presented in Table 6. There was no statistically significant difference between the two groups in all observed outcomes.

**Discussion**

IVF has been associated with an increased risk of preterm delivery, caesarean delivery, low and very low birth weight infants. In accordance to other studies, these outcomes have also been confirmed in this study. The increased risk of adverse neonatal and obstetric outcome in IVF could be explained primarily by the high incidence of multiple births. However, this may not be the only factor responsible since the literature has shown advanced age of women seeking infertility treatment, to be a well known high risk factor.

Several previous studies have found that singletons conceived with IVF have a poorer outcome compared with singletons who were conceived naturally, when compared with the general population [9, 15, 16]. However, this study does not support these findings, since most of the neonatal and obstetric outcome studied did not differ between the two groups studied (405 singletons in the control group and 380 singletons in the IVF group). This finding indirectly demonstrates that poor IVF outcome may be associated with the high rate of multiple births. This is confirmed by comparing the 380 singletons and the 115 twins/triplets conceived with IVF, which resulted in a statistically significant difference (p < 0.001). This result also demonstrated that the high rates of multiple births in IVF could influence the obstetric outcomes.

Advanced maternal age is another factor that may influence poorer obstetric outcomes after IVF [17-19]. However, this is not supported by all researchers [20]. Since there were no measurable differences found when comparing the two different age groups (< 35 and ≥ 35 years) after IVF, these findings do not support the fact that age may contribute to adverse IVF outcomes.

It is well known that the chance of conceiving decreases with increasing maternal age and that there is a sharp decline in women > 35 years old. Suzuki *et al.*, have shown that obstetric outcomes in pregnancies conceived after IVF may be attributed to mechanisms other than the advanced maternal age though [20].

In other reports, evidence suggests that there is an increased incidence of preterm labour and low birth weight in IVF singletons, when compared to spontaneous pregnancies [7, 16, 19]. However, in this study when comparing 405 singletons from the natural conception group with 380 singletons from the IVF group, there was no significant difference noted. When the results were stratified by age groups (< 35 and ≥ 35 years), there was still no significant difference found.

A possible explanation for this could be the “vanishing twin theory” [21] as these have been eliminated from the crude data. Specifically, from all IVF singletons born, only 10% originated from a twin gestation in early pregnancy. When these singletons were compared with singletons originating from a single gestation, the survivors of the “vanishing twin pregnancy” have been found to be at higher risk of preterm delivery and LBW [21, 22]. Characteristics related to the patients and their individual reproductive/obstetric management might also play a role in these results.

In addition, the high rate of caesarean delivery in the IVF group, which has been observed in many studies before [12, 20, 23] should not be overlooked. The routine use of CS section in IVF patients is performed for many reasons. Firstly, IVF pregnancies are highly valued by the infertile couples and their doctors, and a CS is chosen even for minor complications. Secondly, the IVF population is characterized by advanced maternal age and a large proportion of multiple births post IVF, which can also contribute to the preference for CS. The use of caesareans in such a routine way could cause an iatrogenic increase in caesarean deliveries.

The ultimate goal in ART treatment is the birth of a healthy child. The incidence of multiple pregnancies caused by the replacement of more than one embryo in an attempt to enhance the pregnancy rates, is the most common complication associated with IVF, which may seriously influence the neonatal and obstetric outcomes [24]. Due to these known adversities, many countries initiated the implementation of elective single embryo transfer (eSET) as an effective strategy to minimize the twinning pregnancy rate associated with IVF. The use of eSET and consequent reduction of the twinning rate results in the improved outcome of children born after IVF and has been shown in several recent studies [25, 26].

The Human Embryology and Fertilisation Authority (HFEA), which regulates IVF in the UK, has recently introduced a twin pregnancy target of no more than 15% of all IVF births (www.hfea.gov.uk), and all registered fertility clinics are guided to comply [26]. However, since there is no such regulation in China, eSET is advocated in many reproductive centres, even if improved outcomes are reported in the literature [27, 28].

A major barrier of selecting eSET among clinicians and women is the worry that the use of eSET will reduce overall live birth rates [29, 30]. The fact that eSET may well reduce the chances of having a live birth or resulting in the need for additional treatment cycles makes women, in particular, to refuse eSET. Therefore, a study with a larger sample and adequate statistical power is needed to analyse his matter further and be used for future guidance for the patients.
Even if the incidence of multiple pregnancies is highly related to adverse obstetric outcomes in IVF pregnancies, it is not the sole factor to explain these adversities. Several other factors such as parity, smoking, BMI, alcohol intake during pregnancy, marital status, and years of education are correlated with specific adverse outcomes and may explain the multi-factorial character of such negative outcomes [31].

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Impact of chorioamnionitis on the development of human fetal lung: an immunohistochemical study

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Summary

Purpose: Current studies suggest that changes of chorioamnionitis are associated with the appearance of bronchial-associated lymphoid tissue (BALT), during fetal development. The aim of this study was to examine and analyse apart from the appearance of BALT, the expression of structural proteins in the lung parenchyma during gestation. Materials and Methods: A series of 149 paraffin-embedded human fetal lung specimens at the second trimester of development were examined by immunohistochemistry using the monoclonal antibodies CD20, CD3, Tenascin-C, Vimentin, and Fibronectin. Results: The results of this study showed that 1) BALT does not develop in fetal period and 2) BALT which develops during fetal period is probably in response to antigenic stimulation where in the present cases occurs to be changes of chorioamnionitis which decreased the expression of filaments proteins in the intermediate cells of lung parenchyma in comparison with the normal ones. Conclusion: The expressions’ pattern of intermediate filaments proteins in the lung parenchyma can be modified by the presence of chorioamnionitis in the fetal membranes.

Key words: Chorioamnionitis; Lung; Vimentin; Tenascin-C; Fibronectin; BALT.

Introduction

It is now generally accepted that histologic chorioamnionitis is the hallmark of ascending intrauterine infection, and inflammation in and between placental villi indicates blood-borne infection. Although chorioamnionitis constitutes the most common form of placental inflammation in humans and occurs in approximately 4% of otherwise non-complicated term births [1-4], very little is known about the biological and clinical pathological significance and consequences of chorioamnionitis in mesenchymal components of fetal lung parenchyma during the development. The expression status of proteins: Tenascin-C - a large glycoprotein synthesized by fibroblasts which is believed to have active functions in fetal lung branching morphogenesis [5], Vimentin - an intermediate filament protein, serving as modulator between extracellular influences governing calcium flux into the cell and stains mesenchymal components [6], Fibronectin - a cell adhesive extracellular matrix protein highly expressed in developing lungs [7], and in correlation with the appearance of bronchus-associated lymphoid tissue (BALT) - refers to the well-organized lymphoid tissue that is located under the respiratory epithelium in the bronchial wall [8] - expressing by the lymphocytic markers CD20, and CD3 have never been assessed.

Accordingly the aims of the present study were: i) to explore the immunohistochemical expression of Tenascin-C, Vimentin, and Fibronectin in fetal lung without changes of chorioamnionitis, and ii) to discover the expression of the above proteins in fetal lung with changes of chorioamnionitis. In the latter case, a concomitant variable degree of lymphocytic infiltrate was seen in the lung parenchyma especially around the bronchial tree.

Materials and Methods

Specimens of 149 post-mortem human fetal lung at the second trimester of development during the years 2005-2012 were obtained from the archives of the Department of Histology – Embryology, Medical Faculty of Democritus, University of Thrace, Alexandroupolis, Greece. 32 out of 149 specimens were with normal histology fetal membranes whereas 18, 25, and 45 were presented with mild, moderate, and severe chorioamnionitis, respectively. In addition, the authors examined 17 fetal membranes with hydropic degeneration of chorion villi, eight coming from embryos diagnosed with Down syndrome, two cases infected with Toxoplasma hominis, and also two infected with cytomegalovirus (CMV).

A series of four-μm thick sections were cut from the specimens. The histological features of all cases were reassessed on the basis of sections stained with haematoxylin and eosin. Representative paraffin blocks were available for immunohistochemical evaluation.

Immunohistochemistry

Immunohistochemical staining was performed on four-μm-thick deparaffinised sections. The sections were pretreated by heating in a pressure cooker containing a citrate buffer (pH 6.0), then washing with distilled water and cooking to room temperature with Tris buffer. Sections were incubated with primary antibodies for 30 min-
utes. The primary monoclonal antibodies that were used were: anti-Vimentin (1:400 dilution in 10% Normal Rabbit Serum (NRS)/PBS), anti-Tenascin-C (1:100 dilution in NRS/PBS), and anti-Fibronectin (1:400 dilution in NRS/PBS).

After washing following primary antibody incubation, the bound antibody was visualized by the alkaline phosphatase anti-alkaline phosphatase (APAAP) method using the alkaline phosphatase detection system.

Diaminobenzidine was used as the chromogen substrate, producing a brown end-product and the sections were counterstained using haematoxylin. The specificity and the pattern of each antibody were tested on positive control tissue samples according the manufacturers' technical data.

For the immunohistochemical expression of lymphoid tissue (BALT), either B-lymphocytes or T-lymphocytes, the authors used the monoclonal pan-B cell antibody CD20, and the polyclonal pan-T cell antibody CD3 (ready to use).

The sections were scored using a semiquantitative system based on the frequency of immunohistochemical reactivity of individual parenchymal elements or vessels as follows: negative (0), weak (1+), moderate (2+), or strong (3+). Evaluation of immunohistochemical expression was based on the frequency of cytoplasmic reactivity.

Results

One hundred and forty nine specimens met inclusion criteria for performing Tenascin-C, Vimentin, Fibronectin immunohistochemistry.

The authors observed that 32 cases showing normal histology of fetal membranes, all 32 cases, showed strong positivity (3+) with Vimentin, Fibronectin, and Tenscin-C in the intermediate cells of the lung parenchyma.

On the contrary, the positive expression 3(+) of all Vimentin, Fibronectin, and Tenscin-C proteins were decreased accordingly to the severity of inflammation.

In more details, Vimentin expression ranged from 16.66% in mild to 8.88% in severe changes of acute chorioamnionitis. Same pattern presented Fibronectin and Tenascin-C expression that fluctuated from 16.66% in mild to 4.44 in severe inflammation and 11.11 to 6.66%, respectively.

Immunohistochemically staining for Vimentin, Fibronectin, and Tenascin-C in 17 samples diagnosed with hydropic degeneration of chorionic villi showed positive expression (3+) in 58.82%, 41.17%, and 58.8%, respectively.

In eight samples coming from embryos with Down Syndrome, diagnosed with severe changes of chorioamnionitis, the positive expression for the structural proteins is about 12.5%. Finally, none of the samples coming from infected by Toxoplasma hominis and CMV embryos presented positive expression for these proteins.

On the other hand, in 32 cases showing normal histology of fetal membranes, all of these presented no staining (0+) with CD20 and CD3 in the peribranchial tissue of the lung parenchyma.

The immunohistochemical reactivity data are summarized in Tables 1-5 and Figures 1 and 2. According to data that provided in Tables 4 and 5 the positive expression of

| Table 1. — Vimentin expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion). |
|---|---|---|---|---|
| Number of incidents | Histological changes of fetal membranes | Vimentin expression in intermediate cells of the lung parenchyma |
| 149 | | 0+ (%) | 1+ (%) | 2+ (%) | 3+ (%) |
| 32 | Normal histology fetal membranes | 32 (100) |
| 18 | Mild changes of acute chorioamnionitis | 10 (55.55) | 5 (27.77) | 3 (16.66) |
| 25 | Moderate changes of acute chorioamnionitis | 13 (52) | 5 (20) | 4 (16) | 3 (12) |
| 45 | Severe changes of acute chorioamnionitis | 26 (57.77) | 8 (17.77) | 7 (15.55) | 4 (8.88) |
| 17 | Hydropic degeneration of chorionic villi | 2 (11.76) | 2 (11.76) | 3 (17.65) | 10 (58.82) |
| 8 | Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis | 4 (50) | 2 (25) | 1 (12.5) | 1 (12.5) |
| 2 | Toxoplasmosis - severe changes of acute chorioamnionitis | 2 (100) |
| 2 | Cytomegalovirus - severe changes of acute chorioamnionitis | 1 (50) | 1 (50) |

| Table 2. — Fibronectin expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion). |
|---|---|---|---|---|
| Number of incidents | Histological changes of fetal membranes | Fibronectin expression in intermediate cells of the lung parenchyma |
| 149 | | 0+ (%) | 1+ (%) | 2+ (%) | 3+ (%) |
| 32 | Normal histology fetal membranes | 32 (100) |
| 18 | Mild changes of acute chorioamnionitis | 5 (27.77) | 6 (33.33) | 4 (22.22) | 3 (16.66) |
| 25 | Moderate changes of acute chorioamnionitis | 10 (40) | 7 (28) | 5 (20) | 3 (12) |
| 45 | Severe changes of acute chorioamnionitis | 28 (62.2) | 10 (22.22) | 5 (11.1) | 2 (4.44) |
| 17 | Hydropic degeneration of chorionic villi | 5 (29.4) | 3 (17.65) | 2 (11.76) | 7 (41.17) |
| 8 | Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis | 4 (50) | 3 (37.5) | 1 (12.5) |
| 2 | Toxoplasmosis - severe changes of acute chorioamnionitis | 2 (100) |
| 2 | Cytomegalovirus - severe changes of acute chorioamnionitis | 1 (50) | 1 (50) |
Table 3. — Tenascin-C expression in the interstitial tissue of lung parenchymal tissue in correlation with the histological changes of fetal membranes (amnion and chorion).

<table>
<thead>
<tr>
<th>Number of incidents</th>
<th>Histological changes of fetal membranes</th>
<th>Tenascin expression in intermediate cells of the lung parenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0+ (%)</td>
</tr>
<tr>
<td>32</td>
<td>Normal histology fetal membranes</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Mild changes of acute chorioamnionitis</td>
<td>7 (38.88)</td>
</tr>
<tr>
<td>25</td>
<td>Moderate changes of acute chorioamnionitis</td>
<td>14 (56)</td>
</tr>
<tr>
<td>45</td>
<td>Severe changes of acute chorioamnionitis</td>
<td>27 (60)</td>
</tr>
<tr>
<td>17</td>
<td>Hydptic degeneration of chorionic villi</td>
<td>2 (11.76)</td>
</tr>
<tr>
<td>8</td>
<td>Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>2</td>
<td>Toxoplasmosis - severe changes of acute chorioamnionitis</td>
<td>2 (100)</td>
</tr>
<tr>
<td>2</td>
<td>Cytomegalovirus - severe changes of acute chorioamnionitis</td>
<td>2 (100)</td>
</tr>
</tbody>
</table>

Table 4. — CD20 expression in the peribranchial tissue of lung parenchyma in correlation with the histological changes of fetal membranes (amnion and chorion).

<table>
<thead>
<tr>
<th>Number of incidents</th>
<th>Histological changes of fetal membranes</th>
<th>CD20 expression in intermediate cells of the lung parenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0+ (%)</td>
</tr>
<tr>
<td>32</td>
<td>Normal histology fetal membranes</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Mild changes of acute chorioamnionitis</td>
<td>1 (5.55)</td>
</tr>
<tr>
<td>25</td>
<td>Moderate changes of acute chorioamnionitis</td>
<td>2 (8)</td>
</tr>
<tr>
<td>45</td>
<td>Severe changes of acute chorioamnionitis</td>
<td>2 (4.44)</td>
</tr>
<tr>
<td>17</td>
<td>Hydptic degeneration of chorionic villi</td>
<td>15 (88.23)</td>
</tr>
<tr>
<td>8</td>
<td>Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>2</td>
<td>Toxoplasmosis - severe changes of acute chorioamnionitis</td>
<td>1 (50)</td>
</tr>
<tr>
<td>2</td>
<td>Cytomegalovirus - severe changes of acute chorioamnionitis</td>
<td>1 (50)</td>
</tr>
</tbody>
</table>

Table 5. — CD3 expression in the peribranchial tissue in correlation with the histological changes of fetal membranes (amnion and chorion).

<table>
<thead>
<tr>
<th>Number of incidents</th>
<th>Histological changes of fetal membranes</th>
<th>CD3 expression in intermediate cells of the lung parenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0+ (%)</td>
</tr>
<tr>
<td>32</td>
<td>Normal histology fetal membranes</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Mild changes of acute chorioamnionitis</td>
<td>2 (11.11)</td>
</tr>
<tr>
<td>25</td>
<td>Moderate changes of acute chorioamnionitis</td>
<td>2 (8)</td>
</tr>
<tr>
<td>45</td>
<td>Severe changes of acute chorioamnionitis</td>
<td>2 (4.44)</td>
</tr>
<tr>
<td>17</td>
<td>Hydptic degeneration of chorionic villi</td>
<td>12 (70.58)</td>
</tr>
<tr>
<td>8</td>
<td>Down Syndrome (trisomy 21) - severe changes of acute chorioamnionitis</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>2</td>
<td>Toxoplasmosis - severe changes of acute chorioamnionitis</td>
<td>1 (50)</td>
</tr>
<tr>
<td>2</td>
<td>Cytomegalovirus - severe changes of acute chorioamnionitis</td>
<td>1 (50)</td>
</tr>
</tbody>
</table>

Figure 1. — Representative micrographs immunostaining for Vimentin (A), Fibronectin (B), and Tenascin-C (C) in intermediate cells of the lung parenchyma coming from embryos with severe changes of acute chorioamnionitis in fetal membranes (original magnification x200).
the development of BALT constitutes a response to antigen associated with potent antigens [14].

The bronchial lamina propria of normal and diseased lungs is present during the prenatal periods and they demonstrated that BALT in the fetal and neonatal lungs that the presence of BALT in 100% of cases is associated with chorioamnionitis [13]. Barman et al. showed in their study that BALT did not develop in comparison with a fetus or an adult [10].

In accordance with this, other studies demonstrated that the inflammation of the fetal membranes can be attributed to the fact that mucosal tissues of an infant, is difficult to be isolated and studied, for ethical reasons. It is also difficult to foresee the whole organization and possible functions of MALT of a newborn child, in comparison with a fetus or an adult [10].

Edward Klein is considered to be the first to describe lymphoid tissue correlating with bronchial mucosa in 1875. Furthermore, he recognized the resemblance between this tissue and MALT in other positions such as Peyer’s patches in small bowel mucosa [11]. A century later, Bienenstock et al. transplanted a study of lymphoid tissue in rabbit lungs, focusing at the level of morphology and functions of this tissue [12].

Tscherning et al. proved in their study of a series of 145 fetal and neonatal lungs that the presence of BALT in 100% of cases is associated with chorioamnionitis [13]. Barman et al. showed in their study that BALT did not develop in prenatal periods and they demonstrated that BALT in the bronchial lamina propria of normal and diseased lungs is associated with potent antigens [14].

In accordance with this, other studies demonstrated that the development of BALT constitutes a response to antigen [15]. In the case of chorioamnionitis, this can be explained by the mix of amniotic fluid with lung fluid by fetal breathing causing lung exposure [16], and three pulmonary outcomes of concern for preterm infants – respiratory distress syndrome (RDS), pneumonia/sepsis, and bronchopulmonary dysplasia (BPD) can be caused [17].

Emery and Dinsdale proved in their study that in the context of unexplained childhood deaths, according to the measurement of the antigenic state, many of those children showed a higher number of lymphoreticular aggregates [18]. They also proved that the presence of lymphoreticular aggregates used as measure of the concentration of biologically derived antigens, first emerged a week after birth and progressively increased in number, entirely presented by the age of five years [19].

Sminia et al. recognised the interaction between antigens and BALT, resulting in local and systemic immune reactions finding a similar role of BALT with Peyer’s patches [20]: Furthermore, Meuwissen et al. demonstrated that BALT was mentioned to be more conspicuous around bronchiole and smaller bronchi, correlating the hyperplasia of BALT with the degree of the inflammation of the respiratory system [21]. Other studies with fetal sheep exposed to intra-amniotic LPS showed persistently activated leucocytes in the airways, CD3 positive lymphocytes to lung tissue, increased expression of toll-like receptors 2 and 4, decreased caveolin-1 expression, and changes in multiple other signalling pathways [22, 23].

Getahun et al. showed the results of a study involving the effects of chorioamnionitis on early childhood asthma. They conducted a retrospective cohort study in children born alive in Kaiser Permanent Southern California (KPSC) health maintenance organization. They included 510,216 live and still births, excluding 8,738 cases of stillbirths, spontaneous and induced abortions, pregnancies delivered at fewer than 23 weeks gestation, children with birth defect, and neonatal mortality. They defined as exposure variable the clinically diagnosed chorioamnionitis and as the outcome variable the physician-diagnosed asthma in children younger than eight years. The results demonstrated that in contrast with children born around 37 and 38 weeks of gestation and not exhibited to chorioamnionitis in utero, the probability of asthma manifestations in children born preterm and exposed to chorioamnionitis in utero were much larger. This conclusion can be explained by the fact that chorioamnionitis releases microorganisms, toxic substances, and inflammation mediators in the growing bronchi. Thus, inflammation, injury, apoptosis, and airway remodelling is created and that results probably in bronchopulmonary dysplasia, suggesting the fetal origin of asthma [24].

According to the provided data of the literature, there is strong evidence that acute chorioamnionitis is associated with fetal lung development. The results of the present study suggest that the inflammation of the fetal membranes clearly affects the expression’s pattern of intermediate fil-
ments proteins in the lung parenchyma and also induces the development of BALT in fetal period. Thus, the association between chorioamnionitis and preterm gestation may result in increased risk of childhood asthma. Elucidation of the effects of inflammation on the fetal lungs and other organs will allow more refined approaches to the care of preterm infants exposed to inflammation in utero. Intratrucine inflammation induces the expression of enzymes responsible for prostaglandin production prevents, at least in part, the effects of inflammation on fetal lungs [25]. Preterm infants are at risk of acute respiratory distress as a result of lung immaturity; evidence of exposure to infection and/or inflammation before birth is associated with a reduced risk of neonatal RDS [26].

Based on the results of this study, the authors conclude that there is positive correlation between exposure of the fetus to a severe inflammatory response and lung development, and the possibility of causing chronic lung disease of prematurity. However, additional research is needed to confirm the findings in order to lead to a better understanding of the pathobiologic mechanism of the disease and its prevention.

References


Detecting coagulability status by thromboelastography in women with the history of preeclampsia and inherited thrombophilia

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Summary

Objective: To assign tendency to thrombosis in patients with preeclampsia and inherited thrombophilia using thromboelastography (TEG), and therefore to evaluate possible relationship between thrombophilia and preeclampsia. Materials and Methods: Kinetics of clot formation was assessed with TEG analyzer in 49 patients with severe preeclampsia, 54 cases with previous diagnosis of inherited thrombophilia, and 31 controls. Results: 'r', 'k', TMA, coagulation index (CI) parameters were found statistically discrete between patients with inherited thrombophilia and controls. The difference between preeclampsia and control groups was not statistically significant. The difference in α angle was statistically significant between thrombophilics and preeclampsics (p = 0.01), and between thrombophils and controls (p = 0.004). CI was found statistically lower in thrombophilia group than control group (p = 0.006). Particularly, clot lysis time (CLT) was measured to shorten in preeclampsia when compared with controls and patients with thrombophilia (p = 0.032, p = 0.028, respectively). Conclusions: Not only the inherited thrombophilia group but also preeclampsia group demonstrated elongated clot initiation patterns when compared to the controls. Moreover, apart from the patients with inherited thrombophilia, preeclampsics exposed shorter CLT values indicating a possible increment in clot turn over, which eventually results in increased depletion of coagulation substrates, and thus, increased frequencies of oxidative cycle injury.

Key words: Blood; Coagulation; Thromboelastography; Preeclampsia; Thrombophilia.

Introduction

Hypertensive disorders which are seen in 5% to 15% of all pregnancies are one of the major causes of fetomaternal morbidity and mortality. It is of great importance to identify patients who are under risk of development of preeclampsia in order to gain advantage, if possible, from close follow-up together with convenient treatment [1].

The pathogenesis of preeclampsia is not yet fully evident. It is supposed that the development of preeclampsia is a consequence of alterations in placental microcirculation. Accordingly, a failure in achieving low resistant uteroplacental blood flow due to inadequate trophoblastic invasion of maternal spiral arteries gives rise to insufficient placentation [1, 2].

On the other hand, increased tendency to development of thrombosis is closely related with abnormal placentation. This condition is seen frequently in the presence of a hereditary or acquired risk factor [2]. This fact is more important for the women because of increased state of thrombosis already in pregnancy [3]. In the recent years, thrombophilia is indicated to be responsible from severe preeclampsia, eclampsia, HELLP syndrome, placental abruption, intrauterine growth restriction (IUGR), intrauterine fetal losses, and recurrent pregnancy losses (RPLs). Kupferminc et al. [4] demonstrated that hereditary and acquired thrombophilic factors are related with pregnancy complications. Correlation of thrombophilia and pregnancy complications could be thought to originate from insufficient fetalplacental circulation in this intuition.

Thromboelastography (TEG) which evaluates viscoelastic characteristics of the blood in vitro was first described by Hartert et al. [5] in 1948. It was used extensively in cardiac surgery and in renal and liver transplantation to monitorize coagulopathy closely and coordinate anticoagulant treatment [1]. Since vascular and endothelial injury accompany preeclampsia, and increased frequencies of coagulation disorders could be encountered in complicated pregnancy states, we aimed to investigate possible abnormal coagulability conditions in patients with inherited thrombophilia and history of severe preeclampsia using TEG, and thus to determine the relationship between thrombophilia and preeclampsia. As it is known, hemostasis is a dynamic, highly complex process which encloses vast interacting factors such as procoagulants, fibrinolytic proteins, activators, inhibitors, and cellular elements. Therefore, whole steps of coagulation cascade could be evaluated with TEG (Figure 1) [6].
Figure 1. — Diagram outlining the relationship between coagulation cascade and TEG, together with the display of TEG parameters.

Reaction time (r): The time elapsed from the beginning to the early clot formation. It is the distance from the beginning of the graph to the point where 2-mm deflection begins, and given in millimeters on the graph. k: The time length needed for a firm and steady clot formation. It is the time between ‘r’ and the point where 20-mm deflection exists. α angle: The parameter that shows the speed and the power of clot formation. It is calculated from the 20-mm deflection point on the graph, and directly related with ‘k’. Maximum amplitude (MA): Represents the formation strength and rigidity of the coagulum. It is the value given in millimeters when the clot reaches its maximum width. Projection of MA (PMA): Gives an opinion about MA before the final measurement of MA itself. It begins on the screen when the amplitude reaches 5 mm and ceases when the clot formation slows down. Time for MA (TMA): Measures the time from the beginning of the survey until the most powerful state of the sample. Amplitude (A): It is the measurement of the extent of the studied sample in any time interval. It represents the function and elasticity of the clot, and its value is given in millimeters. Amplitude can be converted to the real measurement of the clot strength by SEMS (shear elastic modulus strength) which is given in dyne/cm². Absolute SEMS value gives G parameter. Calculation of G is formulated by G=5000A/ (100-A). Amplitude value of normal whole blood is 50 mm. Its SEMS value corresponds to 5000 dyn/cm². A rise in amplitude from 50 mm to 67 mm causes a two-fold increase in SEMS value. Therefore, G value is more convenient in reflecting any changes in clot formation besides measuring clot strength. E: Represents normalized value of G parameter, and it is thought as an elasticity constant. Thrombodynamic potential index (TPI): It is formulated by TPI = E_max/k. E at maximum amplitude (E_max) = (100xMA)/(100-MA). Coagulation index (CI): Consists of most of the TEG parameters including r, k, MA, and α angle. Normal values of CI are between -3.0 and +3.0. Values < -3.0 represent hypocoagulability, whereas > +3.0 indicates hypercoagulability. Reduction in length of amplitude after MA is represented by A30 (at 30 min) and A60 (at 60 min). Similarly, decreased area after MA is defined by LY30 and LY60 which stand for the course of fibrinolysis process, and given in percentages. Clot lysis time (CLT): Displays the time interval after MA until amplitude decreases to 2 mm.

Figure 2. — Working principle of TEG.
Materials and Methods
The study was accomplished in our perinatology clinic between March 2003 and December 2009. Seventy-two patients with recent diagnosis of severe preeclampsia, 73 patients with previous diagnosis of inherited thrombophilia, and 31 healthy multiparous women were enrolled to participate in the study. Participants were enrolled at the earliest fourth month after delivery. All participants were Caucasians with average socio-economic status. Thrombophilia group was consisted of participants who experienced recurrent first-trimester pregnancy losses or unexplained second- or third-trimester fetal losses together with presence of at least one of the factors including deficiencies of antithrombin III, protein-S and protein-C, mutations of protrombin, factor V Leiden and MTHFR genes, or positivity of lupus anticoagulant and antiphospholipid antibody. Eight patients out of 73 patients with thrombophilia rejected participating in the study, five patients were under low molecular weight heparin treatment and medical records of six patients were incomplete. Therefore, we enrolled 54 patients with inherited thrombophilia. Fourteen of 72 patients with severe preeclampsia refused to participate in the study, and nine of them were excluded from the study because of incomplete medical records and communication. Forty-nine women with severe preeclampsia were enrolled in the study. Severe preeclampsia was defined as presence of one of the following criteria in her previous pregnancy: systolic blood pressure measurement above 160 mm Hg or diastolic blood pressure measurement above 110 mm Hg on two occasions at least six hours apart, more than five grams urinary protein excretion in 24 hours or 3+ and greater random urine dipstick testing, less than 500 ml of urinary discharge in 24 hours, cerebral or visual disturbances, pulmonary edema or cyanosis, epigastric or right upper quadrant pain. Control participants were structured from 31 individuals with history of preeclampsia and control individuals; §Statistical significance between patients with inherited thrombophilia and history of preeclampsia; Statistical significance between patients with history of preeclampsia and control individuals; ⋆Statistical significance between patients with inherited thrombophilia and the controls.

Technique
Blood samples of two ml from all participants were taken. Subsequently, one ml of achieved sample was drawn into chaolin containing tubes within 30 seconds, and mixed up. 0.36 ml of the mixture was collected with a straw and was put inside the cup which was placed in the thromboelastography analyzer and processed with TEG analyzer which was calibrated before at 37°C according to the instructions of the manufacturer. When coagulation begins, fibrin particles are formed in between a thermostatically controlled heated cup which turns in a 4°45’ angle and a pin suspended on a torsion wire (Figure 2). Second phase, measures the speed at which the clot forms, and depends on the changes in distension of the clot when it begins to form. It is measured electromagnetically and recorded as graphs [7]. In addition to assessment of the beginning phase of the coagulation, it also evaluates speed and strength of clot formation as well as fibrinolysis of the clot. Therefore, disorders of both hypercoagulability and hypocoagulability could be detected as well [8].

Statistics
Statistics were performed using Statistical Package for the Social Sciences software version 13.0. Continuous variables were given in means ± standard deviations. Comparisons between two groups possessing normally distributed variables were performed with independent samples t test. Comparisons of more than two groups were fulfilled with single factor analysis of variance (ANOVA) test. The differences in two groups and more than two groups which do not show normal distribution were checked with Mann-Whitney U and Kruskal-Wallis tests, respectively. Groups comprising categoric variables were compared with Pearson Chi-square test. The level of statistical significance was defined as p < 0.05. ROC analysis was done among patients with inherited thrombophilia to determine cut of values for r, α angle, CI, TMA, CLT parameters.

Results
Demographic characteristics of the study participants are given in Table 1. There was a statistically significant difference between mean age of the patients with inherited thrombophilia and history of preeclampsia, and between preeclampsia and control groups (28.9 ± 5.9, 33.6 ± 5.8, and 33.6 ± 4.9, respectively; p = 0.002 for both). We thought that the differences in formerly mentioned variable did not influence the outcomes of this study. There was no difference between the groups when they considered the day after last menstrual period which TEG was conducted. Dispersion of TEG parameters according to the groups are given in Table 2. The differences in mean values of MA, G, EPL, A, LY30, A30, CL30, A60, CL60, LY60, TPI, E, SP, and LTE between all three groups were not statistically significant. The elongated “r” value which indicates a defect in the first fibrin formation and a deficit in the coagulation factors, inhibitors and/or activators, and thus, a delay in
thrombin formation, was found to be significantly higher in patients with thrombophilia compared to the control group in this series (10.7 ± 2.8 and 7.8 ± 3.7, respectively; p = 0.003). Although r value was established to lengthen in patients with preeclampsia (9.5 ± 3.0) compared to the controls, no statistically significant differences were found between preeclampsia group and the control group, and preeclampsia and thrombophilia groups.

‘k’ value was found significantly higher in patients with thrombophilia when compared with control group in this series (3.4 ± 1.4 min and 2.6 ± 1.1 min, respectively; p = 0.025). Although we found a distinction between patients with preeclampsia (k = 2.9 ± 1.2 min) and the controls, and between preeclampsia and the thrombophilia groups with regard to k value, the differences did not reached expected statistical significance.

In patients with thrombophilia, α angle which measures the fibrin formation and cross-binding speed (kinetics of clot) was statistically smaller when compared with preeclampsia (49.8 ± 10.2 and 54.6 ± 9.7, respectively; p = 0.01) and control groups (57.4 ± 9.2; p = 0.004), However, the difference between preeclampsia and control groups was not statistically significant.

Mean value of TMA which measures the time period from the beginning of working the blood sample up to the most powerful state of the clot was found significantly longer in thrombophilia group when compared to control group (34.5 ± 4.4 min and 29.4 ± 5.9 min, respectively; p = 0.002). Although, there were differences between preeclampsia (31.4 ± 5.7 min) and the control groups, and preeclampsia and the thrombophilia groups, they did not reach statistical significance.

For the r value, sensitivity and specificity values were 58.1% and 93.1% when cut off value was taken as 7.3, respectively. A r value measured above 7.3 was found to increase thrombophilia risk 3.98 times (Figure 3).

To assess efficiency of TEG in demonstrating thrombophilia ROC analysis was used. For the α angle, sensitivity and specificity values were 54.8% and 86.2%, respectively, when cut off value was taken as 58.4. An α angle measured above 58.4 was found to increase thrombophilia risk 3.98 times (Figure 4).
Detecting coagulability status by thromboelastography in women with the history of preeclampsia and inherited thrombophilia

Discussion

Preeclampsia, eclampsia, HELLP syndrome, abruptio placentae, and IUGR are serious obstetrical complications, and are main causes of perinatal morbidity and mortality. The results of studies on the etiology of these complications are yet equivocal to conclude in treatment methods. Hence, the treatment of pregnancy complications is usually directed to give up with the pregnancy. There are strong evidences suggesting that the uteroplacental insufficiency is the critical factor which provokes those complications. Accordingly, inadequate trophoblastic invasion causes the release of thrombin-antithrombin complexes in the uteroplacental bed, and thus, fibrin accumulates and inadequate degradation of fibrin results in formation of the thrombotic plugs. As a consequence, endothelial damage occurs and eventually uteroplacental insufficiency develops [9].

It is known that coagulation capability is increased in pregnancy. This increases risk of deep venous thrombosis (DVT) and pulmonary embolism in pregnancy. The reason for increased coagulation is increased thrombocyte aggregation, increased concentration of the coagulation factors, a decrease in the concentrations of coagulation inhibitors (antithrombin III, protein-C), a resistance to activated protein-C, and low fibrinolytic capacity [10]. Therefore, changes in the coagulation system in relation to pregnancy may call for the appearance of thrombotic context by increasing the concealed thrombogenic tendency that is already present in a patient with thrombophilia [11].

There are several studies evaluating the association between the inherited thrombophilia and pregnancy complications. This study analyzed the association between thrombophilia and severe preeclampsia, which is an important cause of fetal and maternal morbidity and mortality. TEG, which assesses the coagulation system as a whole, is used in this study instead of the tests that evaluate a single step of the coagulation system [12]. TEG measures the kinetics, integrity, and dissolution (stability) of the thrombus, and thus the functionality and sustainability of the thrombus as the ability of the thrombus to stop bleeding [13].

When compared to the routine coagulation tests, TEG has been used in the diagnosis of dilutional coagulopathy, DIC, and fibrinolysis in transplantations, during which major hemorrhages frequently occur. It can also be used in obstetrical hemorrhages in the same way. In addition, it can be used to define hypercoagulability states, although it has not been used much for this purpose.

Conventional coagulation tests end after the first fibrin formation. However, TEG continues to analyze and measure the kinetics of the thrombus (the rate of formation), and its durability, integrity, and dissolution. Thus, general information about the coagulation process is obtained by a single test [14-16]. TEG provides information about coagulation in general, from hypercoagulation to hypocoagulation and potential fibrinolysis, in addition to normal coagulation. Laboratory coagulation tests measure isolated specific points of the coagulation process. A wide variety of tests are needed to measure the whole cascade, which results in a waste of time, money, and labor.

Hypercoagulability in pregnancy can be demonstrated by TEG parameters [8, 17-19]. This fact is more evident in women in the course of active labor [20]. This hypercoagulable state returns to normal at sixth week after delivery [21]. The ‘r’ and ‘k’ values are decreased and the angle of α and MA are increased during pregnancy [18, 19]. Larger studies
are needed to identify the time in which these changes occur in terms of gestational weeks, which are already unknown.

When we evaluated TEG parameters in this series, the MA value which shows thrombocyte aggregation, the G value that shows the integrity of the thrombus, the A value which shows the function and elasticity of the thrombus, and the thrombo-dynamic potential index (TPI) that shows hypocoagulability and hypercoagulability states, were found to be similar in all three groups. No statistically significant differences were found between the three groups regarding clot lysis parameters, comprising the percentage of lysed clots in a time period after MA (LY30 and LY60), the values indicating decreased amplitude (A30 and A60), and the thrombus dissolving index in a specific time period after MA (CL30 and CL60).

A variety of reports are present in the literature evaluating the association of TEG and thrombophilia. It was suggested that TEG could be used in pregnant women to assess the risk of preeclampsia and thrombocytopenia [16], as well as, it could indicate the risk for recurrent pregnancy loss [14]. In another study, it has been postulated that TEG could contribute to treatment by identifying the recurrent pregnancy loss, IUGR, and congenital and acquired thrombophilia in preeclampsia [22].

Similar to our results, Sharma et al. [23] demonstrated that MA values were significantly higher in pregnant women who were diagnosed to have mild preeclampsia at birth when compared to healthy pregnant women. In addition, all TEG parameters were correlated with hypocoagulability in all pregnant women with severe preeclampsia and thrombocytopenia. The risks of abortus and abruptio placenta were found to be higher in another study when hypercoagulability was assigned with TEG [24].

Miall et al. [25] found significant correlations between PT, aPTT, plasma antithrombin levels, and TEG parameters including r, k, and MA. However, no correlations were identified between the TEG parameters and other thrombophilic factors (protein-C, protein-S, Factor V Leiden mutation, pro-thrombin G20210A mutation, MTHFR C677T mutation, and lupus anticoagulant). In their study, they established a significant correlation between TEG parameters and second trimester losses, nevertheless there was no correlation between TEG and other pregnancy complications.

Regan et al. [26] found MA value to be significantly higher in the patients with a history of recurrent pregnancy losses (RPLs), and the k value to be significantly higher in non-pregnant women with a history of second trimester losses. As Rai et al. [27] mentioned, pre-pregnancy MA value can be used to predict pregnancy complications. According to the serial TEG evaluations in the early weeks of pregnancy, the increments of MA in TGK were suggested to be associated with future pregnancy losses in the following weeks, while pregnancy resulted in live birth in cases with stable MA values with no change between fifth and 12th week of pregnancy [28].

A wide variety of results exist in the literature in studies performed with conventional laboratory tests to define the association of thrombophilia with preeclampsia, and its complications. These variations might be due to population differences, study design, and differences in the definition of preeclampsia. For example, factor V Leiden mutation is frequently seen in Caucasians, while it is extremely rare, almost non-existent in Asian and Japanese societies [29]. Since the rates of venous and arterial thrombosis and placental thrombosis in preeclampsia and other pregnancy complications are not affected from ethnic groups and races, other thrombophilic factors with undefined roles yet might have important influences on the clinical progress. Some thrombophilic women have not experienced thromboembolic complications during their pregnancies [30]. This observation demonstrates that additional factors are needed for the development of preeclampsia.

Management of a patient with a positive result for thrombophilia in thrombophilia survey is an actual clinical dilemma for future pregnancies and for the treatment in the time periods other than pregnancy. Today, there is no evidence to support women with thrombophiloprophylaxis without a history of thromboembolus but with a positive thrombophilia screening test result [31]. However, there are sufficient evidences demonstrating that endothelial damage and activated mononuclear cell-derived tissue injury as the main concerns in pregnancies of them. Though, it is thought that low pressure interstitial blood flow and trophoblastic dysfunction in a maternal hypercoagulability state might trigger pathophysiological mechanisms of the disease through placentral fibrin deposition and placentalfibrin.

On the contrary, Mousa et al. [11] evaluated the association between thrombophilic state and placental histology in 79 women. They found that 70% of thrombophilia-positive women and 78% of thrombophilia-negative women had abnormal placentals histology. Therefore, they concluded that there is a weak correlation existed between the patholog-ical placental changes and thrombophilic state in women with severe pregnancy complications.

The reported recurrence rate of severe preeclampsia is 20% [32], and it is unknown that how high is the risk of recurren-c e in a thrombophilic woman. Kupferminc et al. [4, 33] demonstrated that 57% to 67% of multipara women with recurrent pregnancy complications had one thrombophilic factor. However, the type of complications might differ in one pregnancy to the other. On the other hand, the recurrence rate of severe preeclampsia is high, and even higher in thrombophilic women particularly with factor V Leiden and/or factor II mutations.

In the present series, r, k, and TMA values were found to be significantly higher in the thrombophilia group compared to the control group (p < 0.01), while no statistically significant differences were found between preeclampsia and control groups, and between preeclampsia and thrombophilia groups regarding these variables. CI and α angle were found to be significantly lower in the thrombophilia group compared to the control group (p < 0.05), while no statistically significant differences were found between preeclampsia and control groups, and between preeclampsia and throm-
bophilia groups. On the other hand, CLT was identified to be significantly lower in the preeclampsia group compared to both thrombophilia (p = 0.028) and control groups (p = 0.032). There was no statistically significant difference between thrombophilia group and the control group with regard to CLT. The r, k, angle, CI, and TMA parameters in TEG were significantly different in the thrombophilia group compared to the control group, while no difference was shown between the preeclampsia and control groups. Only CLT was statistically significantly lower in the preeclampsia group compared to the other two groups.

Thrombophilia emerges as a result of the deficiency of non-homogeneous factors responsible in different steps of the coagulation cascade. In each woman in whom a thrombophilia was identified in the laboratory, it is well known that the tendency to thrombosis differs according to the homozygosity/heterozygosity of the defect, to the factor activity, and to the type of the mutation. Randomized controlled studies in larger populations are needed, including the subgroups, since the defects in the different steps of the coagulation cascade might be reflected in TEG as various different results.

We esteem from this study that facts which provoke abnormal clot formation and fibrinolysis processes could be related with preeclampsia pathogenesis. This phenomenon could be due to presence of insensible consumption of coagulation parameters in preeclampsia, which is already experienced in the states of thrombophilia.

References

Microwave endometrial ablation after endometrial curettage for the management of heavy menstrual bleeding

Department of Obstetrics and Gynecology, University of Patras, Medical School, Rion (Greece)

Summary
Objectives: The aim of the present study was to evaluate the efficacy of microwave endometrial ablation after endometrial curettage, in selected patients with heavy menstrual bleeding. Material and Methods: Thirty-two premenopausal women with heavy menstrual bleeding underwent microwave endometrial ablation at the Department of Obstetrics and Gynecology of the University of Patras Medical School. All patients did not respond to previous medical treatment, had completed their childbearing, and did not desire future fertility. Post-treatment follow up protocol included physical and ultrasonographic evaluation at three, six, nine, and 12 months for the first year and yearly after. Results: The authors had no cases of uterine perforation, thermal injury to adjacent organs, and infection or sepsis. During follow up, there was a gradual decrease in amenorrhea rate (90.6% - 68.8%) and in satisfaction rate (90.6% - 71.9%). Moreover during follow up, eight women underwent total abdominal hysterectomy. Among them, seven women had uterine myomas and one woman had adenomyosis. Conclusions: Endometrial preparation with endometrial curettage seems to be a good alternative to hormonal pre-treatment. It has the advantage of avoiding delays, side effects, and cost of hormonal pre-treatment. Moreover, microwave endometrial ablation after endometrial curettage is successful and highly acceptable.

Key words: Microwave endometrial ablation; Endometrial curettage; Heavy menstrual bleeding.
length. They performed endometrial curettage and sent specimen for pathologic evaluation. Immediately after, the authors performed microwave endometrial ablation. Microwaves generated at a frequency of 9.2 GHz using magnetron. The microwave probe was inserted into the uterine cavity until the tip reached the fundus and then activated. Once a temperature of 95°C was achieved, the probe was moved from side to side to uterine walls with gradual withdrawal at a rate that allowed the temperature to be maintained within the therapeutic range of 70-80°C. Care was taken to avoid treating the cervical canal.

Postoperatively, all women received a single dose of intravenous antibiotics. Also, they received non-steroidal anti-inflammatory drugs for postoperative analgesia.

Post-treatment follow up protocol included physical and ultrasonographic evaluation at three, six, nine, and 12 months for the first year and yearly after.

The study was approved by the Ethical Committee of the Hospital. Informed consent was obtained from each woman included in the study.

Results

The median age of women was 46.4 years (range 35-53). The median uterine cavity length was 87 mm (range 60-120). The median endometrial thickness was ten mm (range 8-15). The median operating time for microwave endometrial ablation was 89 seconds (range 47-180). All tissue specimens from endometrial curettage were negative for malignancy.

All patients did not respond to previous medical treatment for heavy menstrual bleeding. Moreover they had completed their childbearing and they did not desire future fertility.

Most patients had little or no postoperative discomfort. All patients received non-steroidal anti-inflammatory drugs for postoperative analgesia. All of them, returned home at the same day.

Postoperatively, almost all patients had a mild vaginal discharge for three to four weeks. The discharge was usually watery and occasionally blood-stained. In the present study population, there were no cases of uterine perforation, thermal injury to adjacent organs, infection or sepsis.

During follow up, there was a gradual decrease in amenorrhea rate (90.6% - 68.8%) and in patient satisfaction rate (90.6% - 71.9%) (Table 1).

Moreover during follow up, eight women underwent total abdominal hysterectomy. Among them, seven women had uterine myomas and one woman had adenomyosis.

Discussion

Microwave endometrial ablation is a minimally invasive surgical technique for patients with heavy menstrual bleeding [11]. It is a second generation endometrial ablative technique and introduced in clinical practice in 1995 [11, 12]. Microwave endometrial ablation is a non-hysteroscopic technique that is easily learned and does not require irrigation fluid [13]. It is used in patients that completed their childbearing and they do not desire future fertility [14, 15].

<table>
<thead>
<tr>
<th>Follow up</th>
<th>Amenorrhea rate</th>
<th>Patient satisfaction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>90.6%</td>
<td>90.6%</td>
</tr>
<tr>
<td>6 months</td>
<td>87.5%</td>
<td>90.6%</td>
</tr>
<tr>
<td>9 months</td>
<td>78.1%</td>
<td>84.4%</td>
</tr>
<tr>
<td>12 months</td>
<td>75%</td>
<td>81.2%</td>
</tr>
<tr>
<td>24 months</td>
<td>68.8%</td>
<td>71.9%</td>
</tr>
<tr>
<td>36 months</td>
<td>68.8%</td>
<td>71.9%</td>
</tr>
</tbody>
</table>

Such patients have experienced failure or were intolerant to medical therapy for heavy menstrual bleeding [15]. They should be willing to accept normalization of menstrual flow, not necessarily amenorrhea, as an outcome [15].

The device uses microwave energy at a fixed frequency of 9.2 GHz [11, 12, 16]. At the wavelength chosen, microwaves cause direct tissue heating to a depth of three mm (close to the applicator tip) [11, 13, 17]. Moreover they cause conductive heating to adjacent tissue for an additional depth of two to three mm [13, 17]. At therapeutic temperatures, the total depth of penetration (five to six mm) coagulates and destroys the basal layer of endometrium and glands, while spares myometrium [11-14, 16]. The mean treatment time is approximately 3.5 min and determined by the size of the endometrial cavity and endometrial thickness [11, 13, 15]. Especially in women with large and severely distorted uterine cavity, endometrial ablation tends to be incomplete [18].

Postoperative, endometrium and superficial myometrium undergoes necrosis with various degrees of acute inflammation lasting three months [18, 19]. This may be followed by a phase of repair and regeneration [19]. In many cases that phase results in endometrial scarring and fibrosis [19]. The degree of intrauterine adhesions may become progressively more severe [19]. Moreover, intrauterine adhesions can obstruct any bleeding from residual or regenerated endometrium [19, 20].

Generally, endometrial ablation is more effective when performed in relatively thin or atrophic endometrium [14, 21]. This can be achieved in three ways: scheduling procedure to the immediate postmenstrual phase, using hormonal pre-treatment (GnRH analogs, danazol) for four to six weeks or performing preoperative endometrial curettage [14 21-23].

Scheduling procedure to the immediate postmenstrual phase or inducing a withdrawal bleed with progestogen, is an acceptable and efficacious alternative without detriment to long term outcome [23].

Hormonal pre-treatment especially with GnRH analogs, associated with shorter operating time, lower rate of postoperative dysmenorrhea, and increased rate of postoperative amenorrhea [15, 21, 22, 24]. However, it has additional cost and unpleasant side effects [12, 23, 25]. Moreover increases significantly the cervical resistance and the risk for cervical trauma and false passage formation [12, 23, 25].
Preoperative endometrial curettage has the advantage of avoiding delays, side effects, and cost of hormonal pre-treatment [14,26]. In most cases, microwave endometrial ablation without hormonal pre-treatment is successful and highly acceptable [22]. In the present study population, the authors used preoperative endometrial curettage for endometrial preparation.

The most common postoperative side effects are: cramping/pelvic pain, nausea and vomiting, vaginal discharge, and vaginal bleeding/spotting [11,17].

Cervical manipulation and microwave endometrial ablation release prostaglandins that cause postoperative discomfort and pelvic pain [12]. The use of non-steroidal anti-inflammatory drugs reduce that symptoms significantly [12, 16, 27]. In the present study population, most patients had little or no postoperative discomfort. All patients received non-steroidal anti-inflammatory drugs for postoperative analgesia and returned home at the same day.

Postoperative, almost all patients have a mild vaginal discharge for three to four weeks [11, 27]. It is usually watery and occasionally blood stained [27]. In the present study population, most patients had a mild vaginal discharge for three to four weeks.

Although rare, the most severe postoperative complications are: uterine perforation, thermal injury to adjacent organs, infection or sepsis [12, 17, 28-30]. Most of them occur due to unrecognized uterine perforation at the time of dilation [12, 17, 20, 29, 30]. It is obvious that preoperative diagnostic hysteroscopy is necessary to recognize false passage or uterine perforation [12, 13]. In the present study, although the authors did not use preoperative diagnostic hysteroscopy, they had no severe postoperative complications.

Pregnancy and its associated complications (miscarriage, preterm labor, intrauterine growth retardation, intrauterine fetal demise, abnormal placental adherence, and caesarean hysterectomy), are well recognized after endometrial ablation [31]. For that reason premenopausal patients undergoing microwave endometrial ablation should be counseled to use an appropriate contraception method [15, 31, 32].

The success rate of microwave endometrial ablation depends on definition of success (amenorrhea, oligomenorrhea or normal menstrual flow), patient satisfaction (adequate counseling, realistic goals), and length of follow up [24]. Most patients have a reduction in menstrual loss within three months after treatment [33]. However, the maximum reduction observed six months after treatment [33] As the reduction is gradual, six months should be allowed before considering the treatment as a failure [33].

Microwave endometrial ablation results in amenorrhea in 50-65% of women [13, 15]. Residual or regenerated endometrium can be present in those patients [18, 19]. However, intrauterine adhesions can obstruct any bleeding from that residual or regenerated endometrium [19, 20]. The main reason for treatment failure is incomplete endometrial ablation [18, 34]. In the present study, there was a gradual decrease in amenorrhea rate (90.6% - 68.8%) during follow up.

Especially in patients with preoperative endometrial curettage, it seems that there is a lower amenorrhea rate after microwave endometrial ablation [26]. This may be due to inadequate endometrial curettage [26]. Moreover, intrauterine blood clots after endometrial curettage may decrease the transmission of microwave energy [26]. Although hormonal pre-treatment has a global effect on endometrium, endometrial curettage may miss some areas [26].

There is an additional effect on dysmenorrhea, that improved in most cases [16]. A possible explanation is that microwave energy destroys endometrium and adenomyotic foci in myometrium [26]. Therefore patients with adenomyosis and severe dysmenorrhea should have pre-treatment consultation regarding treatment effects on dysmenorrhea [26].

Moreover, microwave endometrial ablation results in satisfaction in 70-98.5% of women [15, 16, 24, 33, 35]. In the present study, there was a gradual decrease in patient satisfaction rate (90.6% - 71.9%) during follow up.

It is obvious that microwave endometrial ablation is a safe non-hysteroscopic endometrial ablative technique that offers distinct advantages for both patients and surgeons. According to the present results, endometrial preparation with endometrial curettage seems to be a good alternative to hormonal pre-treatment. It has the advantage of avoiding delays, side effects, and cost of hormonal pre-treatment. Also, it provides tissue specimen for further pathologic evaluation. Moreover, microwave endometrial ablation after endometrial curettage is successful and highly acceptable.

References


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Introduction

Preeclampsia is a serious cardiovascular complication of pregnancy characterized by hypertension, proteinuria, and generalized systemic vasoconstriction [1]. The disorder is diagnosed in the latter half of pregnancy, affects about 5% of pregnancies, and accounts for considerable mortality and morbidity [2]. Although several models have been proposed for the pathogenesis of preeclampsia, how this process occurs is not fully understood. Body mass index (BMI) is a classic obesity parameter and obesity is defined as BMI > 30 kg/m². It has already been shown that obesity leads to the development of insulin resistance and consequently to hyperinsulinenia. Clinical trials confirmed hyperinsulinenia as an important risk factor for the development of hypertension [3]. Although pathogenic mechanisms responsible for the coexistence of hypertension and obesity have not been completely explained, some authors indicate that hormonal and adipokine activity may play a role in the pathogenesis of both diseases [4]. Adipokines might play a role in the pathogenesis of preeclampsia. Increased concentrations of the appetite-suppressive adipokine leptin have been found to precede the clinical onset of preeclampsia. Moreover, leptin affects lipid metabolism, regulates food intake, modulates taste perception and the feeling of satisfaction after consumption, stimulates the sympathetic nervous system, and regulates the metabolism of insulin, glucose, and triglycerides [4]. Adipokine ghrelin, peptide hormone secreted by the stomach and duodenum, is involved in short-term regulation of appetite and reduces sympathetic activity [3, 4]. Adipokine chemerin is linked to facets of the metabolic syndrome in vitro and in vivo. Thus, chemerin mRNA expression is elevated in adipose tissue of mice on high-fat diet [5]. Adipokine nesfatin-1 is a recently discovered hormone that is derived from the previously described protein nucleobindin-2 [6]. Fasting nesfatin-1 levels were significantly lower in type 2 diabetic patients but its effects on gestational diabetes mellitus are unknown. Free fatty acids (FFAs) which also called non-esterified fatty acids are fatty acids that are not esterified to glycerol or another alcohol such as choline or cholesterol. In blood plasma or serum, FFAs are not free but bound to plasma albumin. Circulating FFAs are key regulators of glucose metabolism and have been shown to be increased in preeclamptic patients during and before the clinical onset of the disease [7].

Summary

Purpose of investigation: To investigate the roles of adipokines, free fatty acid (FFA), and oxidative stress in obese and non-obese preeclamptic patients. Materials and Methods: Gestational age-matched obese preeclamptic (n=32), non-obese preeclamptic (n=32), and non-obese normotensive healthy (n=32) pregnant women were included in the study. Serum insulin, insulin resistance, leptin, nesfatin, ghrelin, chemerin, FFA levels, total antioxidant status, total oxidant status, and oxidative stress index were determined. Results: Leptin and nesfatin levels were significantly lower and ghrelin levels were significantly higher in the normotensive group as compared to the preeclamptic groups, while no difference was observed between obese and non-obese preeclamptic groups. Chemerin and FFA levels were significantly higher in obese preeclamptics as compared to non-obese preeclamptics and normotensive group. Total antioxidant status (TAS) levels were significantly higher in the normotensive group as compared to the preeclamptic groups, while no difference was observed between obese and non-obese preeclamptics. Total oxidant status (TOS) and oxidative stress index (OSI) levels were significantly lower in the normotensive group as compared to the preeclamptic groups, while no difference was observed between obese and non-obese preeclamptics. Conclusion: Serum levels of adipokines, TOS, and FFAs were significantly higher in pregnant with preeclampsia as compared to non-obese normotensive controls. Chemerin and FFA levels were significantly higher in obese preeclamptics as compared to non-obese preeclamptic controls. Key words: Preeclampsia; Obesity; Free fatty acid; Adipokines.
Maternal endothelial dysfunction is widespread and may explain all clinical signs of preeclampsia [8]. In the past several years, evidences have been accumulating that there is a second important biochemical imbalance in preeclampsia; that is, women with preeclampsia have an increased oxidative stress and lipid peroxidation and at the same time have a deficiency in several important antioxidants [9].

Adipokines were studied both in preeclampsia and in obesity; however, there has not yet been a study comparing obese and non-obese preeclamptic patients in the literature. In the present study, the authors aimed to investigate the roles of adipokine and FFA levels on the development of preeclampsia and on the development of obesity in preeclampsia. In addition, they aimed to investigate total antioxidant and oxidant status in the study groups.

Table 1. — Baseline characteristics of the study population. Age, BMI, SBP, DBP, gestational age, fasting glucose, fasting insulin, HOMA-IR, CRP, creatinine, HDL, and cholesterol.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1 (obese preeclamptic)</th>
<th>Group 2 (non-obese preeclamptic)</th>
<th>Group 3 (non-obese normotensive)</th>
<th>p value (1-2)</th>
<th>p value (1-3)</th>
<th>p value (2-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.28 ± 6.00</td>
<td>31.28 ± 8.05</td>
<td>31.03 ± 7.12</td>
<td>0.987</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.2 (30.4 - 57.6)</td>
<td>24.6 (20.8 - 24.9)</td>
<td>24.5 (21.2 - 24.9)</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>165 (140 - 240)</td>
<td>160 (140 - 220)</td>
<td>120 (100 - 130)</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>100 (80 - 160)</td>
<td>100 (80 - 120)</td>
<td>73 (60 - 80)</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at blood sampling (days)</td>
<td>35.0 (25 - 39)</td>
<td>34.5 (25 - 40)</td>
<td>35.5 (26 - 38)</td>
<td>0.837</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>80.31 ± 13.17</td>
<td>75.63 ± 13.65</td>
<td>74.75 ± 11.55</td>
<td>0.181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (microU/ml)</td>
<td>10.02 (3.77 - 58.00)</td>
<td>6.31 (1.27 - 47.25)</td>
<td>6.56 (1.20 - 43.00)</td>
<td>0.070</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.15 (0.37 - 13.18)</td>
<td>1.35 (0.19 - 11.43)</td>
<td>1.06 (0.18 - 10.30)</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>1.08 ± 0.37</td>
<td>0.79 ± 0.33</td>
<td>0.29 ± 0.21</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.63 (0.47 - 1.62)</td>
<td>0.61 (0.41 - 0.95)</td>
<td>0.55 (0.44 - 1.46)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>55.22 ± 18.81</td>
<td>59.00 ± 20.08</td>
<td>53.88 ± 8.92</td>
<td>0.448</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>208.66 ± 40.69</td>
<td>179.56 ± 29.99</td>
<td>156.81 ± 17.49</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>215.88 ± 32.82</td>
<td>189.22 ± 47.43</td>
<td>167.22 ± 38.82</td>
<td>0.0001</td>
<td></td>
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</tr>
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</table>

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein.
Values for median (range) or mean ± SD are shown.

Materials and Methods

Study design

This prospective study was approved by the local Institutional Review Board and confirmed written consent forms were obtained from all the participants. Obese preeclamptic, non-obese preeclamptic and non-obese normotensive healthy pregnant women in their third trimester, admitted to the Department of Obstetrics and Gynecology of the present tertiary center, between March 2012 and July 2012 constituted the two study groups and the control group, respectively. Cases with multiple pregnancy, diabetes mellitus, gestational diabetes, gestational hypertension, psychiatric disorders, cancer, stroke, severe hepatic or renal disease, acute cardiovascular events, platelet disorders, endocrine diseases, hyperlipidemia, fetal infections, fetal anomalies, rupture of membranes, and autoimmune diseases were excluded from the study.

Preeclampsia diagnosis was done as to criteria defined by the report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [10]. BMI was calculated as weight before pregnancy divided by squared height. Obesity was defined as BMI > 30 kg/m². Maternal ages, gravity, parity, abortions, height, weight, systolic blood pressures
A venous blood sample was obtained after an overnight fast. Each collected blood sample was immediately centrifuged at 4,000 rpm +4 °C for ten minutes and then transferred into an Eppendorf tube. Samples were transferred on ice and kept in -70 °C deep freeze until the end of the study, which was completed in four months. Insulin resistance was calculated with the use of homeostasis model of insulin resistance (HOMA-IR) = (fasting insulin (μU/ml) × fasting glucose (mmol/l))/22.5 [11]. The plasma leptin, nesfatin, ghrelin, chemerin, and FFAs levels were determined using the enzyme-linked immunosorbent assay (ELISA) method. Plasma insulin was measured by a chemiluminescence method. C-reactive protein (CRP) was assessed by immunonephelometry. Total antioxidant status (TAS) was measured by Erel’s methods [12]. Total oxidant status (TOS) was measured by Erel’s methods [13]. The TOS level to TAS level ratio was regarded as the oxidative stress index (OSI) [14].

Routine biochemistry parameters (serum creatinine, glucose, cholesterol, and triglycerides) were determined by photometric method.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 19.0 for Windows. A normal distribution of the quantitative data was checked using Kolmogorov-Smirnov and Levene tests. Parametric tests used in the study were One Way Anova for independent groups, Fisher’s LSD test for the homogeneous variances and Games-Howell test for the non-homogeneous variances. Non-parametric tests used in the study were Kruskal-Wallis H test for independent groups, Bonferroni-corrected Mann-Whitney U test for post hoc comparisons. The distribution of categorical variables in both groups was compared using Pearson chi-square test. The relative importance of independent variables was assessed by stepwise binary logistic regression analysis using the forward Wald method. The cut-off points were calculated by the MedCalc software as the points with the best sensitivity-specificity balance. Data are expressed as mean ± SD or median (interquartile range), as appropriate. Statistical significance was assumed for p < 0.05.

### Results

Gestational age-matched obese preeclamptic (n=32), non-obese preeclamptic (n=32), and non-obese normotensive healthy (n=32) pregnant women were included in the study. Table 1 summarizes the clinical characteristics of the groups studied during pregnancy. Age, gestational age at blood sampling, fasting glucose levels, and high density lipoprotein (HDL) levels were not significantly different between groups. SBP, DBP and creatinine levels were significantly lower in the normotensive group as compared to both obese and non-obese preeclamptic groups. Measures of insulin sensitivity (fasting insulin and HOMA-IR) in the obese group was significantly higher than both non-obese preeclamptic and non-obese normotensive groups. CRP, cholesterol, and triglyceride levels were significantly different among groups, each parameter being highest in the obese preeclampsics and lowest in the non-obese normotensive patients.

Leptin and nesfatin levels were significantly lower and ghrelin levels were significantly higher in the normotensive group as compared to the preeclamptic groups, while no difference was observed between obese and non-obese preeclamptic groups (Table 2). Chemerin and FFAs levels were significantly different between each group, each parameter being highest in the obese preeclampsics and lowest in the non-obese normotensive patients (Figure 1).

TAS levels were significantly higher in the normotensive group as compared to the preeclampsics, while no difference was observed between obese and non-obese preeclamptic groups (Table 3). TOS and OSI levels were significantly lower in the normotensive group as compared to the preeclampsics, while no difference was observed between obese and non-obese preeclampsic groups (Figure 2).

Estimates of preeclampsia risk according to the risk factors were calculated by logistic regression. Preeclampsia

### Table 2. — Comparison of chemerin, leptin, nesfatin, ghrelin, and FFA between study groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (obese preeclamptic)</th>
<th>Group 2 (non-obese preeclamptic)</th>
<th>Group 3 (non-obese normotensive)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemerin (µg/l)</td>
<td>234.0 ± 26.41</td>
<td>218.8 ± 17.40</td>
<td>163.3 ± 13.77</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>11.12 ± 4.48</td>
<td>8.58 ± 4.36</td>
<td>5.84 ± 2.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>Nesfatin (ng/ml)</td>
<td>0.32 (0.15 - 1.17)</td>
<td>0.30 (0.13 - 0.65)</td>
<td>0.21 (0.13 - 0.46)</td>
<td>0.001</td>
</tr>
<tr>
<td>Ghrelin (ng/ml)</td>
<td>0.53 ± 0.22</td>
<td>0.55 ± 0.19</td>
<td>0.79 ± 0.26</td>
<td>0.0001</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.80 (0.35 - 1.87)</td>
<td>0.68 (0.38 - 1.93)</td>
<td>0.57 (0.31 - 0.89)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

---

**Outcome parameters**

A venous blood sample was obtained after an overnight fast. Each collected blood sample was immediately centrifuged at 4,000 rpm +4 °C for ten minutes and then transferred into an Eppendorf tube. Samples were transferred on ice and kept in -70 °C deep freeze until the end of the study, which was completed in four months. Insulin resistance was calculated with the use of homeostasis model of insulin resistance (HOMA-IR) = (fasting insulin (µU/ml) × fasting glucose (mmol/l))/22.5 [11]. The plasma leptin, nesfatin, ghrelin, chemerin, and FFAs levels were determined using the enzyme-linked immunosorbent assay (ELISA) method. Plasma insulin was measured by a chemiluminescence method. C-reactive protein (CRP) was assessed by immunonephelometry. Total antioxidant status (TAS) was measured by Erel’s methods [12]. Total oxidant status (TOS) was measured by Erel’s methods [13]. The TOS level to TAS level ratio was regarded as the oxidative stress index (OSI) [14].

Routine biochemistry parameters (serum creatinine, glucose, cholesterol, and triglycerides) were determined by photometric method.

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 19.0 for Windows. A normal distribution of the quantitative data was checked using Kolmogorov-Smirnov and Levene tests. Parametric tests used in the study were One Way Anova for independent groups, Fisher’s LSD test for the homogeneous variances and Games-Howell test for the non-homogeneous variances. Non-parametric tests used in the study were Kruskal-Wallis H test for independent groups, Bonferroni-corrected Mann-Whitney U test for post hoc comparisons. The distribution of categorical variables in both groups was compared using Pearson chi-square test. The relative importance of independent variables was assessed by stepwise binary logistic regression analysis using the forward Wald method. The cut-off points were calculated by the MedCalc software as the points with the best sensitivity-specificity balance. Data are expressed as mean ± SD or median (interquartile range), as appropriate. Statistical significance was assumed for p < 0.05.
Figure 1. — Chemerin, leptin, nesfatin, and ghrelin levels among the study groups.

Figure 2. — Total antioxidant status, total oxidant status, and oxidative stress index among the study groups.
Table 3. — *Comparison of total antioxidant status, total oxidant status, and oxidative stress index between study groups.*

|                          | Group 1 (obese preeclamptic) | Group 2 (non-obese preeclamptic) | Group 3 (non-obese normotensive) | p value  
|--------------------------|-----------------------------|----------------------------------|----------------------------------|---------  
| Total antioxidant status (mmol Trolox Equiv/L) | 0.21 ± 0.18 | 0.24 ± 0.13 | 0.41 ± 0.19 | 0.0001  
| Total oxidant status (µmol H2O2 Equiv/L) | 11.04 (4.90 - 37.14) | 10.86 (3.49 - 38.91) | 8.05 (5.48 - 13.83) | 0.008  
| Oxidative stress index | 73.88 (13.16 - 1187.67) | 54.78 (16.34 - 590.00) | 26.80 (7.21 - 53.62) | 0.0001  

Table 4. — *Estimates of preeclampsia risk according to the risk factors (Binary Logistic Regression, Forward Wald Model; CI = Confidence Interval).*

|                          | p value | Odds Ratio (95% CI)  
|--------------------------|---------|---------------------  
| C-reactive protein       | 0.048   | 6.297 (1.014 - 39.125)  
| Cholesterol              | 0.007   | 9.800 (1.840 - 52.630)  
| Creatinine               | 0.001   | 21.280 (3.530 - 125.000)  
| Chemerin                 | 0.0001  | 701.022 (53.373 - 9207.522)  

Table 5. — *Estimates of obesity risk in preeclamptic patients according to the risk factors (Binary Logistic Regression, Forward Wald Model; CI = Confidence Interval).*

|                          | p value | Odds Ratio (95% CI)  
|--------------------------|---------|---------------------  
| HOMA-IR                  | 0.016   | 12.344 (1.597 - 95.416)  
| Cholesterol              | 0.012   | 16.651 (1.871 - 148.192)  
| Triglycerides            | 0.012   | 62.706 (2.438 - 1612.828)  

development ratio was 6.297 times higher when CRP blood level was over 0.48 mg/dl (p = 0.048); 9.800 times higher when cholesterol blood level was over 180 mg/dl (p = 0.007); 21.280 times higher when creatinine blood level was over 0.62 mg/dl (p = 0.001); 701.022 times higher when chemerin level was over 183.98 μg/l (p = 0.0001) (Table 4). Obesity development ratio in preeclamptic patients was 12.344 times higher when HOMA-IR index was over 0.95 (p = 0.016); 16.651 times higher when cholesterol blood level was over 184 mg/dl (p = 0.012); 62.706 times higher when triglyceride blood level was over 180 mg/dl (p = 0.012) (Table 5).

Discussion

In the current study, the authors demonstrated that maternal serum levels of chemerin, leptin, nesfatin, and FFAs were significantly higher, while ghrelin levels were significantly lower in preeclampsia patients as compared to non-obese normotensive controls during pregnancy. They also demonstrated that maternal serum levels of leptin, nesfatin, and ghrelin levels did not differ between obese and non-obese preeclamptic groups, while chemerin and FFAs levels were significantly higher in obese preeclampsics as compared to non-obese preeclamptic patients. TAS levels were significantly lower, while TOS levels were significantly higher in preeclampsia patients as compared to non-obese normotensive controls. TAS and TOS levels did not differ between obese and non-obese preeclamptic groups.

A growing body of evidence strongly supports the association between preeclampsia and common metabolic complications: 1) obesity is an independent risk factor for preeclampsia [15]; 2) patients with insulin resistance are more likely to develop preeclampsia [16]; 3) preeclampsia is also associated with hypertriglyceridemia, hypercholesterolemia, increased concentrations of FFAs, and reduced HDL concentrations [17]; and 4) women who had preeclampsia have an increased risk for metabolic syndrome-related morbidity and mortality later in life [18]. In the present study, patients with preeclampsia and the normotensive control group were compared in terms of factors predisposing to preeclampsia. Preeclampsia development ratio was 6.297 times higher when CRP blood level was over 0.48 mg/dl; 9.800 times higher when cholesterol blood level was over 180 mg/dl; 21.280 times higher when creatinine blood level was over 0.62 mg/dl. Despite the compelling evidence for the association between obesity-related complications and preeclampsia, the mechanism by which excess adipose tissue exerts its deleterious effect and predisposes pregnant women to develop preeclampsia remains unknown.

Development of insulin resistance in the third trimester of pregnancy, together with adipose tissue accumulation, is a possible adaptation of the maternal metabolism to optimize fetal nutrition. Obesity is associated mainly with insulin receptor resistance, resulting from the impairment of insulin binding to tissue receptors, especially in adipose tissue and muscles [19]. Hyperinsulinemia, like hyperleptinemia, contributes to activation of the sympathetic nervous system, and subsequently to the increase in blood pressure [20, 21]. The authors found significantly higher HOMA-IR in obese preeclamptic patients than both non-obese preeclamptic and non-obese normotensive healthy subjects. This differ-
ence could result from both hypertension and the obesity in this group. Furthermore, in the present study, obese and non-obese preeclamptic patients were compared in terms of factors predisposing to obesity. Obesity development ratio was 12.344 times higher when HOMA-IR index was over 0.95; 16.651 times higher when cholesterol blood level was over 184 mg/dl; 62.706 times higher when triglyceride blood level was over 180 mg/dl.

The present findings are in accordance with the hypothesis that fat-secreted factors play a role in the pathogenesis of preeclampsia. Adipose tissue has an endocrine function, secreting several metabolically active proteins, termed adipokines [22]. During pregnancy, the placenta is an additional source of adipokines [23]. The physiological significance of adipokine upregulation in preeclampsia remains unclear so far. Chemerin induces insulin resistance in human skeletal muscle cells [24]. In agreement with these in vitro findings, experiments in rodents demonstrate convincingly that administration of chemerin impairs glucose tolerance, lowers serum insulin levels, and decreases basal glucose uptake in diabetic mice in vivo [25]. Leptin may play an important role during pregnancy. Studies have shown that leptin levels rise between the first and the last two trimesters of pregnancy and return to pre-pregnancy levels within the first days postpartum [26]. In agreement with the present study, it has previously been shown that circulating leptin is increased in preeclamptic women [27]. Normal human pregnancy results in a pronounced physiologic hyperlipidemia involving a gestational rise in blood triglycerides and cholesterol. Women with preeclampsia display additional alterations in blood lipids, reflecting a disordered lipid and lipoprotein metabolism [28]. In the majority of these studies, however, preeclampsia either was not defined or was combined with non-proteinuric gestational hypertension or included women with superimposed chronic hypertension. Lorentzen et al. and Endresen et al. analyzed sera obtained from women in late pregnancy after an eight- to ten-hour fast [29, 30]. Serum triglyceride and FFAs concentrations in women with preeclampsia were higher than those in women with uncomplicated pregnancy. In agreement with other investigators, the present authors found that serum levels of chemerin, leptin, nesfatin, and FFAs levels were significantly higher in preeclampsia patients as compared to non-obese normotensive controls. Adipokine levels did not differ between obese and non-obese preeclamptic groups, while chemerin and FFAs levels were significantly higher in obese preeclampsia as compared to non-obese preeclamptic patients. The present findings support the hypothesis that adipokine and FFAs play a role in the pathogenesis of preeclampsia and Chemerin and FFAs are also associated with obesity in preeclamptic patients. TAS levels were significantly lower, while TOS levels were significantly higher in preeclampsia patients as compared to non-obese normotensive controls. TAS and TOS levels did not differ between obese and non-obese preeclamptic groups and these findings suggest that preeclampsia is associated with increased oxidative stress and decreased antioxidants.

Pregnancy itself is a condition of increased oxidative stress due to increased mitochondrial activity and reduced scavenging potential [34]. Elevated levels of oxidative stress status in pregnancy were shown in many studies. This status is aggravated in pregnancies with preeclampsia. Preeclampsia is characterized by increased oxidative stress and decreased antioxidants [35]. In preeclamptic women, maternal circulating levels, placental tissue levels and production rate of lipid peroxides are increased and several antioxidants are markedly decreased [36]. In the present study, antioxidants were higher and oxidative stress markers were lower in the normotensive group as compared to the preeclamptic groups, while no difference was observed between obese and non-obese preeclamptic groups.

Some limitations of the study need to be discussed: the patient groups were small and some results could not reach or were bordering on statistical significance. Therefore, further detailed studies based on a larger population are needed for a more comprehensive evaluation.

Conclusions

The authors demonstrated that maternal serum levels of adipokine and FFAs were significantly higher in preeclampsia patients as compared to non-obese normotensive controls. Adipokine levels did not differ between obese and non-obese preeclamptic groups, while chemerin and FFAs levels were significantly higher in obese preeclampsia as compared to non-obese preeclamptic patients. The present findings support the hypothesis that adipokine and FFAs play a role in the pathogenesis of preeclampsia and Chemerin and FFAs are also associated with obesity in preeclamptic patients. TAS levels were significantly lower, while TOS levels were significantly higher in preeclampsia patients as compared to non-obese normotensive controls. TAS and TOS levels did not differ between obese and non-obese preeclamptic groups and these findings suggest that preeclampsia is associated with increased oxidative stress and decreased antioxidants.

References

Chinese IUD removal techniques in a Chinese population in central Italy

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Summary
Objective: To describe routine techniques and a newly developed approach to the removal of Chinese intrauterine devices (IUDs).
Methods: Office records regarding women of Chinese nationality who presented to a tertiary care hospital for IUD removal between January 2007 and March 2012 were retrieved. Their demographic data were reviewed and menstrual/obstetric history, IUD type, and reasons given for removal were recorded. All underwent pelvic transvaginal ultrasound scanning. Results: Of 134 Chinese IUDs, 18 (13.4%) were removed successfully in an office setting using a hook or uterine curette without general anesthesia or cervical dilation. Extraction under brief general anesthesia was performed in 55 (41.0%) cases. A further 61 (45.5%) Chinese IUDs were successfully removed in an office setting using a miniature resectoscope. Four types of Chinese IUDs were removed, the most common being the stainless steel ring (55.7%). Conclusions: All removal procedures were effective and safe. The mini-resectoscope appears to be a safe and effective tool enabling minimally invasive surgery.

Key words: Intrauterine device; Contraceptives; Extraction; Resectoscope.

Introduction
The intrauterine device (IUD) is the most widely used reversible birth control method, currently worn by about 160 million women worldwide; over-two thirds of these are Chinese [1]. The government of the People’s Republic of China (PRC) adopted the so-called “one child policy” in 1979 to stem the nation’s population surge.

The most common IUD in the PRC is the stainless steel ring (SSR), which has been available since the 1950s [2, 3]. Despite having the largest number of IUD users in the world, the PRC has been demonstrated to have one of the highest failure rates, ~10% in the first year [4]. This is second only to Brazil’s 13% in developing countries; in developed countries the first year failure rate is only 2% [5, 6]. Some studies suggest that the high failure rate is related to the quality of IUDs made in PRC.

In 1993 various types of rings were banned by the Chinese government (China State Family Planning Commission - SFPC) because of excessive pregnancy rates and other side effects compared with more modern copper IUDs [7]. However, even though they are beginning to be replaced by copper devices – whether imported or of Chinese manufacture (TCu220C from Tianjin, TCu380 from Xiping, and MLCu375 from Wuxi) – ring devices are still widely used [2]. Their main advantage is an almost unlimited lifespan and their greatest drawback is removal, which, when necessary, requires specific instruments.

Lack of a visible string requires cervix dilation using a uterine probe. When required, stabilization is achieved with a single-tooth tenaculum applied to the anterior lip of the cervix with the help of a plastic os finder. Once the uterine cavity is entered, its length and direction are noted and a removal hook is inserted [8]. If routine removal fails, partially embedded or retained parts can generally be removed by hysteroscopy under direct visualization [9, 10]. However, hysteroscopy entails complications, most frequently uterine perforation [11]. Gas embolism with air or carbon dioxide is a rare (three per 17,000 procedures) but often fatal outcome [12, 13]. The authors describe the routine techniques applied to remove Chinese IUDs and a newly devised method.

Materials and Methods
The present study involved 134 patients of Chinese nationality who presented to the Gynecology and Obstetrics Outpatient Department of “Val Vibrata” University Hospital (Sant’Omero, Italy) from January 2007 to March 2012 for IUD removal. They were attended by three Italian gynecologists with a ten-year experience in office hysteroscopy.

Demographic data were reviewed and specific information including menstrual and obstetric history (pregnancies, live births), type of IUD worn, and reasons for removal was recorded.

Because a new removal method began to be applied in January 2010, patients were divided into a group treated from 2007 to 2009 (group 1) and another treated from 2010 to 2012 (group 2). The relationships between IUD type, obstetric history, age at IUD removal,
reasons for removal, and technique used were investigated in each group. The patients were then divided in another two groups: group A including patients asking for IUD removal due to gynecological problems (spotting, recurrent infection, pelvic pain, menstrual disorders, IUD + pregnancy) and group B including patients asking for IUD removal in order to conceive. IUD type and location were established by gynecological exploration, examination with a speculum, and pelvic transvaginal ultrasound. All 134 IUDs lacked a string.

The techniques used to remove the device are described below. Each patient gave her written informed consent to be included in this study. With the patient in the dorsal lithotomy position, a speculum was gently introduced to expose the cervix. Occasionally, a single-tooth tenaculum was applied to the anterior lip of the cervix for stabilization. Device removal without sedation was achieved by use of a small curette or an IUD removal hook inserted blindly or under ultrasound guidance, as appropriate. In case of unsuccessful removal (because the cervix was stenotic, the patient complained of excessive pain, or the IUD could not be reached with the hook) removal in hospital under general anesthesia was offered.

To visualize the IUD and confirm the diagnosis, office hysteroscopy was performed with a hysteroscope, which is endowed with a five Fr (1.67 mm) operating channel; 200 ml saline (0.9% sodium chloride) was used for distension at a pressure ranging from 14 to 17 kPa (105-130 mmHg). Anesthesia was induced using a mask (fentanyl 50 µg and isopropylphenol 180 mg). The cervix was dilated to size 5, then a Foerster-Ballenger curved ring forceps (18 cm, 7 in) was introduced into the cavity and used to remove the IUD under the guide of the earlier hysteroscopy. The whole procedure lasted about 15 minutes.

Beginning in 2010 the present authors investigated the feasibility and acceptability of surgical IUD removal without general anesthesia using a miniature resectoscope (16 Fr / 5.3 mm outer sheath and 0° grade optics). Images were viewed on a high-resolution color monitor using a one-chip camera and recorded.

The procedure consisted of four steps: 1) vaginoscopic approach and cavity distension with saline (0.9% sodium chloride) infused via a flexible 500 ml bag wrapped in a pressure cuff connected to a manometer and pumped up to 80-120 mmHg); 2) endoscopic evaluation of device type and location; 3) grasping of the IUD with the resectoscope hook (angled 90°) under vision; 4) IUD removal together with the mini-resectoscope under dynamic vision. During the procedure the endometrium was inspected and the tubal ostia were identified. The hysteroscope was then withdrawn toward the internal uterine orifice to obtain a panoramic view of the cavity.

No pharmacological preparations or local anesthetics were administered before examination. Adverse intraoperative events were recorded. Records were reviewed after 14 days for postoperative complications. All procedures were performed in the follicular phase of the menstrual cycle, usually within seven days of the end of menstruation. Prophylactic antibiotics were not routinely given for either office or IUD removals.

Statistical analysis
The χ² test was used to estimate the association between categorical variables; Wilcoxon’s test was applied to interval and ordinal variables. Continuous variables were compared using Student’s t-test. A p-value < 0.05 was considered significant. SAS software was used for statistical analyses.

Results
The population comprised of 134 Chinese women wearing an IUD; their mean age was 30.3 years ± standard deviation (sd) 4.0 (28.9 ± sd 3.9 in group 1; n=73 and 32.0 years ± sd 3.5 in group 2, n=61).

A total of 11/134 (8.2%) women were nulliparous and 27 (20.1%) had had at least one cesarean delivery. In particular, nine (12.3%) group 1 subjects were nulliparous and 16 (21.9%) had had at least one cesarean delivery; two (3.3%) group 2 patients were nulliparous, and 11 (18.0%) had had at least one cesarean delivery. Finally, 48 (65.8%) group 1 and 48 (78.7%) group 2 women had had at least one vaginal delivery.

IUDs had been fitted during the puerperium or within 12 months of delivery. The main reasons for removal were a desire to conceive (n=102; 76.2%); gynecological problems (e.g. spotting, recurrent infection, pelvic pain or menstrual disorders like hyperpolymenorrhea: n=29; 21.6%), and having become pregnant while wearing the IUD (n=3; 2.2%) (Table 1). In group 1 (n=73), 49 (67.1%) women were planning a pregnancy, five (6.9%) had menstrual disorders, two (2.7%) had become pregnant while wearing the device, three (4.1%) had pelvic pain, eight (11.0%) had spotting, and six (8.2%) had recurrent infection. In group 2 (n=61), 53 (87.0%) women desired a pregnancy, three (4.9%) had hyperpolymenorrhea, one (1.6%) had become pregnant while wearing the IUD, three (4.9%) had lower abdominal pain, and one (1.6%) suffered from irregular vaginal bleeding. Group 2 contained more women aged ≥ 30 years (n=45; 74%) than younger patients (n=16; 26%) (p = 0.002).

The IUDs removed more frequently were round-shaped SSRs (known as “Chinese rings”) and isosceles triangle, uterine cavity-shaped devices (UCDs) (Figure 1). On ultrasound the SSR had a distinct echogenic ring-like appearance and the UCD was easily distinguishable as a uterus-shaped echogenic area (Figure 2). Other rings consisted of a stainless steel spiral spring enveloping a thin inner ring made in flexible steel (double ring); this was more difficult to identify by ultrasound since it often appeared as a blurred ring-like echogenic area.

The only medicated IUD we removed was the γ Cu-380 (Figures 3, 4), consisting of a γ-shaped stainless steel wire frame with a copper wire in the middle layer. A silicone elastomer bead containing indomethacin is attached at either end of the horizontal arms and a silicone elastomer ring is placed.

<table>
<thead>
<tr>
<th>Reason for Removal</th>
<th>SSR n (%)</th>
<th>UCD n (%)</th>
<th>Double Ring n (%)</th>
<th>γ Cu 380 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual cycle disorders</td>
<td>7 (8.6)</td>
<td>5 (15.2)</td>
<td>1 (20.0)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Pain</td>
<td>6 (7.4)</td>
<td>3 (9.1)</td>
<td>0 (0)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>IUD + pregnancy</td>
<td>2 (2.5)</td>
<td>1 (3.0)</td>
<td>0 (0)</td>
<td>0</td>
</tr>
<tr>
<td>Desire to conceive</td>
<td>66 (81.5)</td>
<td>24 (72.7)</td>
<td>4 (80.0)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>81</td>
<td>33</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>

SSR: stainless steel ring; UCD: uterine cavity-shaped device.
From January 2007 to December 2009 (group 1), 73 Chinese IUDs were removed; 18 (13.4%) were removed in an office setting using the hook or the uterine curette without general anesthesia or cervical dilation from women who had had at least one vaginal delivery. Of these, 14 (77.8%) were SSR and four (22.2%) were γ Cu-380 devices. Extraction under brief general anesthesia was carried out in 55 (41.0%) women, nine (16.4%) nulliparous and 16 (29.1%) subjects with at least one previous cesarean delivery. False passage through the cervix during cervical dilation occurred in 2/55 patients (one nulliparous and one menopausal).

Overall 33 (60%) SSR, 15 (27.3%) UCD, five (9.1%) γ Cu-380, and two (3.6%) double ring devices were removed.

Adoption of the mini-resectoscope approach in January 2010 involved that all group 2 procedures were performed in an office setting. Two patients experienced a vagal reaction that resolved spontaneously; in all the others removal was...
completed successfully and was well-tolerated, with pain described as inferior or equal to menstrual pain. The devices removed were 34 (55.7%) SSRs; 18 (29.6%) UCDs; three (4.9%) double ring and six (9.8%) γ Cu-380s. SSRs were 58% in group 1 and 42% in group 2. The difference was not significant (p = 0.231). No other complications occurred. No postoperative fever was noted. The difference between IUD type and GROUP was not significant (p = 0.55) (data not shown). The technique used and the reasons for IUD removal were not significantly different between the groups (p = 0.36) (Table 2). The technique used and patient age were not significantly different between the groups (p = 0.846) (Table 3).

To evaluate the association between the reasons for IUD removal and age before 2010, the 134 patients were divided into two age groups: < 30 years (n = 60; 44.8%) and ≥ 30 years (n = 74; 55.2%). The two groups were comparable in frequency (p = 0.23). A significant association was found between reasons for IUD removal and age (p = 0.01) (Table 4). More women aged < 30 years (n = 52; 86.7%) underwent IUD removal because they sought a pregnancy compared with women aged ≥ 30 years (n = 50; 67.6%). IUD type, years wearing the device, and obstetric history did not affect the removal technique.

Average operating time with the resectoscope, from the beginning of vaginoscopy to IUD removal, was 2.3 minutes.

Discussion

This study reports on three techniques adopted to remove various types of Chinese IUDs in Italy. The present findings showed that the SSR was the IUD worn by the majority of our Chinese patients (60.5%), followed by UCD (24.6%), γ Cu-380 (11.2%), and double ring (3.7%) devices.

The SSR (Shanghai ring) was first produced in Shanghai in 1970. The ring is one-inch (2.54 cm) in diameter, flexible, springy, string-free, and designed not to be removed easily, due to China’s “one child” policy. It was used for immediate post-placental insertion. The rings are fitted using a “fitting fork” or “fitting pliers”. To date the technique used most frequently to remove them has been the hook. If the ring cannot be released, it is cut in two using two hemostat forceps before taking it out. This is preceded by cervical dilation.

A review of the literature disclosed no relevant information about the removal of these devices in Faculty of Family Planning and Reproductive Health Care (FFPRHC) and Royal College of Obstetricians and Gynecologists (RCOG) guidance documents, National Guidelines Clearing House or the WHO publication Improving Access to Quality Care in Family Planning - Selected Practice Recommendations for Contraceptive Use, 2002 [15].

One study recommends using three-dimensional ultrasound to locate and identify the IUD type and reports successful removal in 26-28 cases by hysteroscopy, laparoscopy or laparotomy [16].

The present authors noted that γ Cu-380s, UCDs, and double rings were removed more frequently without sedation using the mini-resectoscope (γ Cu-380s removed with the mini-resectoscope, six; with hook or uterine curette, four; with curved ring forceps, five; UCDs removed with the mini-resectoscope, 18; with hook or uterine curette, 15; with curved ring forceps, zero; double rings removed with the mini-resectoscope, three; with hook or uterine curette, two; with curved ring forceps, zero).

A number of reasons may explain why IUDs fitted in the PRC tend to be more difficult to remove than those commonly used in America and Europe. Poor familiarity with the many different types of Chinese IUDs can make physicians uncomfortable about attempting removal in an office setting [8]. In addition, all 134 IUDs removed in the present study had no string, thus requiring some degree of intrauterine manipulation for removal.

Previous vaginal deliveries did not affect the removal technique, even though the mini-resectoscope was applied more frequently in women who had already had a child (48%) than in nulliparous women or patients who had had a cesarean delivery (18.1% and 40.7%, respectively).

The mini-resectoscope was used more often in women aged ≥ 30 (n = 45; 74%) than in younger patients (n = 16; 26%) (p = 0.002); instead patient age did not significantly correlate with the technique applied before 2010 (p = 0.846).
Currently, IUDs are used by almost 50% of women of reproductive age in China. Many ask to have their IUD removed following menopause. In general, an IUD should be removed within 12 months, preferably at six months from the last menses, when removal is usually simple because the cervix is still soft. Declining estrogen levels more than one year into menopause typically leads to atrophy of the vagina, cervix, and uterine body, and the cervix may become stenotic. In one study only 56.1% of women had their IUD removed successfully two years after menopause [17], Zhang et al. [18] reported that uterine atrophy, cervical adhesions, cervical hardness, and IUD deformation were the major factors hampering removal.

In China a variety of drugs such as mifepristone and misoprostol are administered to post-menopausal women prior to IUD removal to induce cervical ripening and facilitate the procedure, reducing the risk of complications. Estrogen regimens may be used when neither drug can be used [19]. These medical agents were never used in the present authors procedures.

The mini-resectoscope appears to be an acceptable tool for hysteroscopic surgery and can be used for IUD removal without general anesthesia [20]. Thanks to its small diameter, dilation of the cervical canal is not required, resulting in shorter operating time and preventing unnecessary tissue damage. The diameter narrow shaft also minimizes the risk of intra- and post-operative complications.

The vaginoscopic approach without a speculum and tenaculum avoids discomfort to patients and ensures complete compliance. Other small hystoscopes (total diameter < five mm) used in office settings can be introduced in the internal cervical os without a speculum or tenaculum by the vaginoscopic approach, but only the resectoscope hook can catch or grasp the IUD and it pull out. Extracting a ring is more problematic than extracting an IUD with a tail. Hysteroscopy appears to be useful to remove a missed or retained IUD, since it offers direct visualization of the endometrial cavity [21] and allows surgical removal of foreign bodies [22].

None of the procedures using the mini-resectoscope had to be interrupted due to severe pelvic pain [23], uterine bleeding obscuring visualization, extended operating times or inadequate wall distension, nor were major complications such as uterine perforation, hemorrhage or postoperative infection reported either intraoperatively or after patient discharge. Two patients experienced a vaginal reaction that resolved spontaneously. The hysteroscopic approach is therefore especially useful to treat the most numerous patient group, i.e. women asking for IUD removal to become pregnant.

In conclusion, all Chinese IUD removal procedures described were effective and safe. The mini-resectoscope, recently introduced on the market, appears to be a useful and minimally invasive tool.

References

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Effect of maternal cervical bacterial colonization on neonatal outcome in high-risk pregnancies: results from a tertiary maternity center in Turkey

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Summary
Purpose: To evaluate and compare the morbidity and mortality of neonates born to pregnant women with positive and negative cervical cultures. Materials and Methods: The demographic and clinical features of mothers included in this study, along with details of the microorganisms isolated on maternal cervical cultures and the number of days between a positive cervical culture and delivery were recorded. Neonates were stratified into two groups based on cervical culture results of their mothers - Group 1, positive cervical culture; Group 2, negative cervical culture. Results: A total of 216 women who delivered 242 infants were included in the study. Group 1 consisted of 90 neonates while Group 2 had 152 newborns. The difference between the groups with demographic characteristics was statistically insignificant. Mean levels of the acute phase reactants, CRP, and IL-6, obtained six hours after delivery were significantly higher in Group 1 compared to Group 2 (p < 0.05 for C-reactive protein (CRP) and p < 0.001 for IL-6). Although there was no difference between groups in terms of duration of respiratory support, mean duration of hospitalization, as well as mortality rate were significantly higher in Group 1 (p < 0.001, p < 0.05, respectively). Conclusions: Women diagnosed with a high-risk pregnancy should be treated with antibiotics immediately after a positive cervical culture result, and delivery should be delayed until the success of antibiotic treatment can be evaluated. Early initiation of maternal antibiotic therapy is associated with shorter durations of hospital stay for newborns. Close follow-up of mothers with high-risk pregnancies and extension of treatment duration are critical for determining prognosis in newborn infants.

Key words: Cervical bacterial colonization; Maternal antibiotic therapy; Neonatal outcome.

Introduction
Cervical bacterial colonization in pregnancy is a pre-disposing factor for maternal serious infections such as vaginitis, cervicitis, intra-amniotic infection, endometritis, or septicemia [1]. Fetus is at greater risk of cervical bacterial colonization and short cervix related morbidity and mortality than the mother [2]. Fetal infections may appear as early neonatal infections such as pneumonia, meningitis, and sepsis and are associated with a serious increase in mortality and morbidity in preterm neonates [3]. Preterm neonates who are developing early infections, commonly have subtle and non-specific clinical symptoms. Increasing use of antenatal and intrapartum antibiotics for the prevention of neonatal infection may result in false negative cultures of blood and cerebrospinal fluid, making the diagnosis of sepsis difficult [4]. Neonatal infections should be diagnosed as soon as possible by laboratory tests rather than cultures that result in longer period of time. Cervical culture positivity may have an effect on neonatal morbidity and mortality. When the duration between culture positivity and delivery increases and antibiotic therapy prolongs, neonatal morbidity may be decreased or affected [5].

This retrospective study was aimed to evaluate and compare the morbidity and mortality of the neonates of cervical culture positive and negative pregnant women and to determine the predictive value of cervical cultures for the prognosis of newborns.

Materials and Methods
Study patients
This retrospective study included 216 pregnant women who were admitted to the high-risk pregnancy service of the present tertiary maternity teaching hospital from June 2010 to February 2011 with symptoms of preterm delivery, early membrane rupture or vaginal discharge. This retrospective study was undertaken at Zekai Tahir Burak Maternity Teaching Hospital with the approval of the local ethics committee.

The demographic and clinical features of mothers included in this study, along with details of the microorganisms isolated on maternal cervical cultures, and the number of days between a pos-
itive cervical culture and delivery were recorded. The cervical cultures of those women were obtained during hospitalization. Neonates were stratified into two groups based on cervical culture results of their mothers - Group 1, positive cervical culture; Group 2, negative cervical culture. If the culture was positive antibiotic therapy was begun.

With the multipregnancies a total of 242 newborns (22 twin, two triplet pregnancies) delivered by those mothers. The demographic features, laboratory evaluation for sepsis, duration of respiratory support, duration of hospitalization, sepsis and prematurity related complications, like respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH) ≥ grade III, and mortality were noted.

Suggested diagnostic criteria for sepsis in neonates (two or more of the following clinical features) were used to identify patients for sepsis evaluations [6]: (1) respiratory compromise includes following tachypnea, apnea, increased ventilatory support, or desaturation; (2) cardiovascular compromise, including bradycardia, pallor, decreased perfusion, or hypotension; (3) metabolic changes including hypothermia, hyper-thermia, feeding intolerance; glucose instability, or metabolic acidosis; or (4) neurologic changes consisting in lethargy, hypotonia, or decreased activity. In addition to laboratory results showing elevated levels of C-reactive protein (CRP) or interleukin-6 (IL-6). Patients with culture positivity were accepted as proven sepsis [6]. Patients diagnosed within the first 72 hours of life were considered to have early onset neonatal sepsis (EOS) [7]. RDS diagnosis made by typical clinical (grunting, cyanosis, tachypnea), and radiologic finding [8]. NEC was defined according to modified Bell’s criteria (≥ Grade 2), and PDA was defined as clinical diagnosis plus treatment with ibuprofen, surgical ligation, or both [9, 10]. BPD was defined as requirement for oxygen at 36 weeks' corrected gestational age or at discharge from the participating unit [11]. ROP was diagnosed according to the international classification of retinopathy of prematurity [12]. Diagnosis and severity of IVH were based on the criteria of Papile [13]. Clinical chorioamnionitis diagnosis was made by maternal fever (>38°C), leukocytosis (>15,000/mm³), CRP positivity, vaginal discharge, and abdominal tenderness [14].

Laboratory analyses

Routine venous blood sampling was performed within the first 24 hours after delivery. Blood for complete blood counts was obtained either by venipuncture, by arterial puncture or through a central catheter. Complete blood counts determinations were performed using a twice-daily calibrated automated hemocytometer. Serum concentrations of CRP were measured by a Tinaquant CRP high sensitive immune turbidimetric assay on an analyzer according to manufacturer instructions. Plasma levels of IL-6 were determined by IL-6 solid phase, enzyme labeled, chemiluminescent sequential immunometric assay on an analyzer, as per manufacturer instructions.

Blood cultures

Blood cultures were performed in newborns when neonatal sepsis was suspected in first 72 hours. Blood culture depended on clinical condition and increase in acute phase reactants (IL-6 and CRP), not taken from healthy controls. A microbial detection system was used to detect positive blood cultures.

Cervical culture

Vaginal discharge was collected from the posterior vaginal fornix with a sterilized cotton wool swab. Immediately after collection, the swab specimen was suspended and transported to the laboratory. Collected samples were immediately inoculated on 5% sheep blood agar and eosin-methylene blue agar plates. After 24-48 hours of aerobic incubation at 37°C, the plates were interpreted. Identification and antimicrobial susceptibility testing of microorganisms was performed according to the Clinical and Laboratory Standards Institute Guidelines with conventional microbiological methods and confirmed by an automated microbiology system.

Statistical analysis

Statistical analyses were performed using the SPSS for windows (ver. 17.0) statistical package. Chi-square test was used to compare categorical variables between groups. Difference between two groups was examined by independent samples t-test for normally distributed variables and Mann Whitney U test for non-normally distributed variables. Correlation made by Spearman test. A p-value <0.05 was considered statistically significant.

Results

Of all the medical records reviewed, the records of 216 pregnant women fulfilled the criteria necessary for inclusion in the final analysis (Figure 1). Group 1 (positive cervical culture) consisted of 90 neonates while the remaining...
152 preterm infants made up Group 2 (negative cervical culture).

The demographic, maternal, and clinical characteristics as well as the laboratory findings of the study population are summarized in Table 1. There was no statistically significant difference between groups in terms of maternal age, gestational age, birthweight, gender, and mode of delivery. Although the frequency of premature rupture of membranes (PROM) was higher in Group 1, the difference was not statistically significant (p > 0.05). Cervical culture were taken a median of five days [2-14] prior to delivery. The parameters of complete blood count as hemoglobin, white blood cell, and platelets were not different between groups, but the values of acute phase reactants CRP and IL-6 were higher in Group 1, and the difference was statistically significant for CRP (p < 0.001) and for IL-6 (p < 0.001, Table 1).

Of the 25 (27.7%) newborns in Group 1 who developed EOS, only 11 (12.2%) had positive blood cultures. In Group 2, 38 (25%) of the newborns were diagnosed with EOS, 15 (9.8%) of which had a positive blood culture. The difference between groups in this regard was statistically insignificant (p > 0.05).

Prematurity associated complications were also evaluated. Duration of hospitalization and mortality ratio were statistically higher in Group 1; 23.4 ± 16.5 vs 16.5 ± 13.7 days and 20 (22.2%) vs 13 (8.6%), respectively (p < 0.001, p < 0.05, respectively, Table 1).

Ten of the patients who had positive cervical culture showed histological signs of chorioamnionitis. The subgroup analysis of the patients with positive cervical culture with chorioamnionitis or without chorioamnionitis showed that the complications as BPD, ROP, NEC, and prematurity associated complications were also higher in Group 1 vs Group 2 (p < 0.05).

The microorganisms that were isolated from cervical cultures of mother were Escherichia coli (n. 51, 56.6%), Klebsiella pneumonia (n. 12, 13.3%), Staphylococcus aureus (n. 7, 7.7%), Streptococcus agalactiae (n. 7, 7.7%), Enterobacter aerogenes (n. 6, 6.6%), and the others Enterococcus spp, Pseudomonas aeruginosa and Serratia fonticola (Table 2).

In five of the infants in Group 1, the bacteria detected in blood cultures were the same pathogens which were isolated in the respective maternal cervical cultures, namely Escherichia coli in three infants, Klebsiella pneumonia in two infants, and Streptococcus agalactiae in one infant. In the remaining five infants with a positive blood culture, the microorganism isolated was different from that observed in the respective maternal cervical culture.

The mean duration of maternal antibiotic therapy up to the time of delivery was 7.35 ± 5.2 days. The most frequently preferred antibiotics for mothers with positive cervical cultures were ampicillin-sulbactam (85%), clarithromycin (8.5%), and cefamezin (6.5%).
The duration of the cervical cultures obtained before the delivery were noted. The authors noticed that as the period between the day of positive cervical culture and the delivery shortened, the hospitalization duration was prolonged ($p = 0.017$, $r = -0.285$).

**Discussion**

The lower genital tract is a source of ascending infections in pregnancy and in most cases with positive amniotic fluid cultures, the same organisms are recovered from vaginal swabs. Preterm delivery was shown to have a relationship with bacterial vaginosis [15-18]. McDonald *et al.* pointed out that the women with bacterial vaginosis have increased risk of PROM and preterm labor [19]. The causative microorganisms shown in cervical colonization were different in different countries, *Group B streptococcus* is the predominant microorganism in western countries whereas *E. Coli* is more prevalent in others. Lajos *et al.* evaluated 212 pregnant women with preterm labor or PROM and reported the prevalence of endocervical colonization as 14.2% [20]. *Group B streptococcus* was the most common organism and endocervical colonization was associated with a higher incidence of EOS and neonatal mortality compared with negative cultures. In the present report, *E. coli* was the most common organism of cervical colonization with 56.6%; the others were *Klebsiella pneumonia* (13.3%), *S. aureus* (7.7%), *S. agalactiae* (7.7%), and *E. aerogenes* (6.6%).

In the present study, sepsis was more prevalent in cervical culture positive group but this was not statistically significant. The remarkable feature was as the period between the day of positive cervical culture taken and the delivery shortened, the hospitalization duration of the neonate was prolonged ($p = 0.017$, $r = -0.285$). Ovalle *et al.* reported that delivery in culture positivity and high-risk pregnancy should be as soon as possible [21]. On the other hand, King *et al.* showed that waiting for antibiotic therapy for mother and infant would be better than earlier delivery [5]. The present authors think that antibiotic therapy was begun to the mothers immediately when positive cervical cultures had been detected and as the period of antibiotic therapy prolonged and the risk of neonatal complications decreased.

The low rates of postpartum blood culture growths in neonates from Group 1, who were born to mothers with known cervical bacterial colonization, could be attributable to maternal antibiotic use and low growth rates of the causative microorganisms. Therefore neonates born from culture positively mothers may have more morbidities and mortality than other infants.

Chorioamnionitis, a common complication of pregnancy, is not only associated with adverse maternal outcomes, such as postpartum infections and sepsis, but has also been implicated in the development of severe fetal and neonatal complications, including still birth, premature birth, neonatal sepsis, and BPD [22]. As would be expected, the presence of chorioamnionitis in the present study population was associated with a higher rate of fetal complications such as BPD, ROP, and NEC, as well as with longer durations of hospital stay.

Since the mothers that cervical cultures taken were in high-risk group of pregnancies, the associated risk factors other than cervical colonization might lead to premature birth. To make a better comparison, the pregnant women who had no known medical risk factors for preterm delivery should be studied and this was the limitation of the present study. Further prospective studies will shed light on this subject.

Fichorova *et al.* reported that their data clearly demonstrated that maternal microorganisms associated with systemic inflammatory patterns detectable after birth placent al colonization with vaginal microorganisms can induce a systemic inflammatory response in the fetus and newborn [23]. As a result of this important finding, independently from microorganism isolated from maternal cervix, neonates are affected from intrauterine inflammation. Although pregnancy is widely considered to be state of “relative immune compromise”, several studies have reported on the contrary. Beigi *et al.* managed to demonstrate up to to two-fold elevations in levels of endocervical cytokines in pregnant women compared to non-pregnant ones [24]. They suggested that the systemic inflammatory response associated with pregnancy itself, as well as with cervical colonization leads to elevations in levels of proinflammatory cytokines which have a negative impact on neonatal morbidity and mortality. In the present study, the authors observed that babies born to mothers in Group 1 had higher baseline (within first six hours) levels of the acute phase reactants CRP and IL-6 compared to those in Group 2 ($p < 0.05$ and $p < 0.001$, respectively). Furthermore, the mean duration of hospital stay and mortality rate in Group 1 were significant lower than those observed in Group 2 ($p < 0.001$, $p < 0.05$, respectively). These findings could be attributed to maternal systemic inflammation and to the “cytokine storm” associated with pregnancy.

In the present study, the authors observed a higher albeit insignificant rate of sepsis in infants born to mothers with positive cervical cultures compared to culture-negative mothers. The authors recommend that all mothers with high-risk pregnancies be screened with cervical cultures, and that antibiotic treatment be initiated immediately after a positive culture result. Delivery should be delayed until the success of antibiotic treatment can be evaluated. Early initiation of maternal antibiotic therapy is associated with shorter durations of hospital stay for newborns. Close follow-up of mothers with high-risk pregnancies and extension of disease duration is critical for determining prognosis in newborn infants.
References


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The frequencies of the presence of embryonic pole and cardiac activity in early miscarriages with abnormal karyotypes

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Summary
Aims: The objective of this study was to compare the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal embryonic karyotypes. Materials and Methods: From January 2008 to December 2012, 405 patients with early miscarriage were evaluated during pregnancy by regular ultrasound, and karyotyping was performed on chorionic villus tissue after curettage. The frequencies of the presence of an embryonic pole and cardiac activity were compared between patients with a normal embryonic karyotype and patients with an abnormal embryonic karyotype. Results: Of the 405 samples, 224 cases (55.3%) had an abnormal karyotype, and 181 cases (44.7%) had a normal karyotype. The frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal embryonic chromosomes (71.8% and 57.5%, respectively) were similar to those of miscarriages with abnormal embryonic chromosomes (74.1% and 62.1%, respectively). The frequencies of the presence of an embryonic pole and cardiac activity were higher in miscarriages with viable autosomal trisomies (trisomies 21, 13, and 18), monosomy X, and triploidy than in miscarriages with a normal karyotype or other abnormal karyotypes. Conclusions: The frequencies of the presence of an embryonic pole and cardiac activity are higher in miscarriages with viable autosomal trisomies, monosomy X, and triploidy than in miscarriages with a normal karyotype or other abnormal karyotypes.

Key words: Miscarriage; Chorionic villus; Karyotype analysis; Ultrasound.

Introduction
Miscarriage affects approximately 10-15% of clinically recognized pregnancies and is the most common complication of pregnancy [1]. Eighty percent of miscarriages occur in the first 12 weeks of pregnancy [2]. Anatomic, genetic, endocrinological, immunological, and thrombophilic factors are associated with miscarriage [3]. Miscarriage due to various underlying pathologies may occur at different stages of the pregnancy [4]. More than half of miscarriages are related to chromosome abnormalities [5]. Accordingly, there is a question of whether miscarriages with an abnormal embryonic karyotype occur at different gestational stages compared with normal embryonic karyotype miscarriages. The presence of an embryonic pole and cardiac activity are the primary landmarks of embryo development. The rate of miscarriage decreases significantly after embryonic cardiac activity is demonstrated [6]. Thus, the differences in the frequencies of the presence of an embryonic pole and cardiac activity are important to investigate in miscarriages with normal and abnormal karyotypes. Reports indicate that chromosomal abnormalities are associated with up to 90% of losses before the development of an embryo. Between eight and 11 weeks of gestation, approximately 50% of losses are related to chromosome abnormalities. Between 16 and 19 weeks of gestation, this proportion decreases to 30% of losses. After 20 weeks of gestation, the rate of aneuploidy in stillbirths is less than 15% [7]. Previous studies have indicated that compared to losses occurring later in gestation, preembryonic losses are more likely to be associated with fetal aneuploidy [8,9]. However, there are some conflicting reports. Munoz et al. reported that the chromosomal abnormality rate is similar in cases of miscarriage with an absent or present embryo [10]. In a report by Coulam et al., the frequency of an empty gestational sac and the presence of cardiac activity prior to pregnancy loss was the same in chromosomally abnormal and normal conceptuses [11]. Given the conflicting results of existing studies, the purpose of the current study of 405 patients with early miscarriage was to explore possible associations between an abnormal embryonic karyotype, the frequency of the presence of an embryonic pole, and cardiac activity at the time of diagnosis.

Materials and Methods
Population
In this retrospective study, data were collected from January 2008 to December 2012 among 405 patients who were admitted to Sun Yat-Sen Memorial Hospital and received dilation and curettage for miscarriage in the first trimester. According to our protocol, patients who are admitted for threatened abortion or who have a history of recurrent miscarriage receive serial human chorionic gonadotropin (HCG) level measurements and transvaginal ultrasound examinations at six to seven weeks of gestation. If no fetal cardiac activity is observed, ultrasound is repeated one week later. If
fetal cardiac activity is observed, ultrasound is repeated every two weeks until ten to twelve weeks of gestation.

Miscarriage was diagnosed based on the following criteria: (1) gestational sac diameter of greater than 20 mm without a yolk sac, (2) an embryo with a crown-rump length (CRL) of greater than six mm without cardiac activity and (3) loss of cardiac activity that was previously identified. If there was doubt, a scan was repeated after one week. The gestational age was calculated from the last menstrual period, records of basal body temperatures, ultrasound measurements or the date of embryo transfer. If miscarriage was diagnosed, dilation and curettage were performed, and karyotyping of chorionic villi was performed after obtaining the patient’s consent.

The patient records noted observations of an embryonic pole or cardiac activity prior to miscarriage. Patient characteristics were also collected, including maternal age at the time of miscarriage and pregnancy history. Recurrent miscarriage was defined as three or more clinical pregnancies that ended in a first-trimester loss (including the current miscarriage). Patients were excluded for the following reasons: (1) a serial ultrasound examination was not performed before the miscarriage, (2) karyotyping of chorionic villi either failed or was not performed, (3) the patient had multiple pregnancies, (4) miscarriage occurred at more than twelve weeks of gestation or (5) biochemical pregnancy or ectopic pregnancy was present.

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Karyotype analysis

The production of conception was obtained by dilation and curettage under sterile conditions after obtaining the patient’s informed consent. Chorionic villi were transported in RPMI culture medium and subsequently inspected under a dissecting microscope to release the villi from the maternal deciduas and blood clots. The chorionic villi were then minced and digested in dispase and cultured in the medium. The laboratory at the present hospital performed the karyotype analysis using standard tissue culture techniques and the G-banding technique.

Statistical Analysis

The statistical analysis was performed using the software SPSS 16.0. Continuous data were reported as means and standard deviations and were analyzed with t-tests. Categorical data were reported as percentages and were analyzed with chi-square tests and p-values of less than 0.05 were considered to be statistically significant.

The ethics review board at Sun Yat-Sen Memorial Hospital at Sun Yat-Sen University approved this study, and all of the patients provided written informed consent.

Results

A total of 405 subjects were included in the analysis. The mean maternal age was 32.2 ± 4.9 years (20-45), and the mean gestational age was 8.8 ± 1.4 weeks (5.9-12). In total, 129 (31.9%) of the subjects were 35 years or older. The number of previous spontaneous abortions ranged from 0 to 10. A total of 237 patients presented with recurrent miscarriage (including the current miscarriage). The distribution of gestational age at the time of miscarriage is displayed in Figure 1.

The karyotype results of the 405 subjects are shown in Table 1. In total, 181 (44.7%) of the 405 specimens exhibited a normal karyotype. Of the normal karyotypes, 106 were 46, XX and 75 were 46, XY. The ratio of 46, XX to 46, XY was 1.4:1. In total, 224 (55.3%) specimens exhibited abnormal karyotypes. A total of 132 of the abnormal karyotypes were trisomic, including 21 viable autosomal trisomies (trisomies 21, 18, and 13) and 111 other autosomal trisomies. The most frequent trisomy was 16 (40 cases), followed by trisomies 22, 15, and 21. Other abnormal karyotypes included monosomies (5.4%), triploidies (5.4%), tetraploidies (0.7%), structural abnormalities (3.7%), double abnormalities (4.0%), and mosaicisms (3.5%).

The clinical characteristics of the abnormal and normal embryonic karyotype groups are shown in Table 2. Maternal age, history of recurrent miscarriage, and gestational age were significantly different between the miscarriages with abnormal and normal karyotypes (all, p < 0.05). The distribution of gestational age at the time of miscarriage by abnormal and normal karyotype is shown in Figure 2.

The frequencies of the presence of an embryonic pole and cardiac activity in the abnormal karyotype group were higher in miscarriages with viable aut...
The frequencies of the presence of embryonic pole and cardiac activity in early miscarriages with abnormal karyotypes

### Table 1. Karyotype results in 405 miscarriages.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>181</td>
<td>44.7</td>
</tr>
<tr>
<td>46, XX</td>
<td>106</td>
<td>26.2</td>
</tr>
<tr>
<td>46, XY</td>
<td>75</td>
<td>18.5</td>
</tr>
<tr>
<td>Viable autosomal trisomies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+21</td>
<td>21</td>
<td>5.2</td>
</tr>
<tr>
<td>+21</td>
<td>8</td>
<td>2.0</td>
</tr>
<tr>
<td>+18</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>+13</td>
<td>7</td>
<td>1.7</td>
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<tr>
<td>Other autosomal trisomies</td>
<td>111</td>
<td>27.4</td>
</tr>
<tr>
<td>+2</td>
<td>7</td>
<td>1.7</td>
</tr>
<tr>
<td>+3</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>+4</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>+5</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>+7</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>+8</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>+9</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>+10</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>+11</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>+12</td>
<td>3</td>
<td>0.7</td>
</tr>
<tr>
<td>+14</td>
<td>5</td>
<td>1.2</td>
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<tr>
<td>+15</td>
<td>9</td>
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<tr>
<td>+16</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>+17</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>+20</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>+22</td>
<td>16</td>
<td>4.0</td>
</tr>
<tr>
<td>Monosomies</td>
<td>22</td>
<td>5.4</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>17</td>
<td>4.2</td>
</tr>
<tr>
<td>-21</td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td>-18</td>
<td>1</td>
<td>0.2</td>
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<tr>
<td>Triploidy</td>
<td>22</td>
<td>5.4</td>
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<tr>
<td>Tetraploidy</td>
<td>3</td>
<td>0.7</td>
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<tr>
<td>Structural abnormalities</td>
<td>15</td>
<td>3.7</td>
</tr>
<tr>
<td>Double abnormalities</td>
<td>16</td>
<td>4.0</td>
</tr>
<tr>
<td>Mosaicisms</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>Total</td>
<td>405</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Table 2. Clinical characteristics of the abnormal and normal karyotype groups.

<table>
<thead>
<tr>
<th>Karyotype Group</th>
<th>Number</th>
<th>Maternal age, y</th>
<th>Gestational age, wk</th>
<th>Gravidity</th>
<th>History of live birth</th>
<th>Maternal age ≥ 35 y</th>
<th>History of miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype (n=181)</td>
<td>181</td>
<td>30.9 ± 4.5</td>
<td>8.6 ± 1.4</td>
<td>3.8 ± 1.9</td>
<td>32 (14.3%)</td>
<td>36 (19.9%)</td>
<td>117 (64.6%)</td>
</tr>
<tr>
<td>Abnormal karyotype (n=224)</td>
<td>224</td>
<td>33.3 ± 4.9</td>
<td>8.9 ± 1.4</td>
<td>3.4 ± 1.8</td>
<td>32 (14.3%)</td>
<td>93 (41.5%)</td>
<td>120 (53.6%)</td>
</tr>
</tbody>
</table>

### Table 3. The frequencies of the presence of an embryonic pole and cardiac activity in the abnormal karyotype and normal karyotype groups.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>N</th>
<th>Presence of embryonic pole n (%)</th>
<th>Presence of cardiac activity n (%)</th>
<th>Gestational age (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>181</td>
<td>130 (71.8%)</td>
<td>104 (57.5%)</td>
<td>8.6±1.4</td>
</tr>
<tr>
<td>Viable autosomal trisomies</td>
<td>21</td>
<td>20 (95.2%)</td>
<td>18 (85.7%)</td>
<td>9.5±1.6</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>17</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td>Triploidy</td>
<td>22</td>
<td>22 (100%)</td>
<td>21 (95.5%)</td>
<td>9.3±1.1</td>
</tr>
<tr>
<td>Other abnormal karyotype</td>
<td>164</td>
<td>107 (65.2%)</td>
<td>83 (50.6%)</td>
<td>8.6±1.3</td>
</tr>
</tbody>
</table>

### Table 4. The frequencies of the presence of an embryonic pole and cardiac activity and the gestational age among different abnormal karyotypes.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>N</th>
<th>Presence of embryonic pole n (%)</th>
<th>Presence of cardiac activity n (%)</th>
<th>Gestational age (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal karyotype</td>
<td>181</td>
<td>130 (71.8%)</td>
<td>104 (57.5%)</td>
<td>8.6±1.4</td>
</tr>
<tr>
<td>Viable autosomal trisomies</td>
<td>21</td>
<td>20 (95.2%)</td>
<td>18 (85.7%)</td>
<td>9.5±1.6</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>17</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
<td>10.3±1.1</td>
</tr>
<tr>
<td>Triploidy</td>
<td>22</td>
<td>22 (100%)</td>
<td>21 (95.5%)</td>
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</tr>
</tbody>
</table>

a: viable autosomal trisomies vs. normal karyotype (p < 0.05), vs. other abnormal karyotypes (p < 0.05). b: monosomy X vs. normal karyotype (p < 0.05), vs. other abnormal karyotypes (p < 0.05). c: triploidy vs. normal karyotype (p < 0.05), vs. other abnormal karyotype (p < 0.05). d: other abnormal karyotype vs. normal karyotype (p > 0.05).
Miscarriage is a heterogeneous condition that is associated with several underlying causes. Miscarriage due to various underlying pathologies may occur at different gestational stages. Previous studies have indicated that pre-embryonic losses are more likely to be associated with fetal aneuploidy than losses occurring later in gestation [8,9]. However, in the present study, the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with an abnormal karyotype were 74.1% and 62.1%, respectively, which were not significantly different from the normal karyotype frequencies (71.8% and 57.5%, respectively). The present findings are consistent with a number of chorionic villi sampling studies. A study by Munoz et al. revealed similar abnormal karyotype rates in anembryonic and embryonic groups (61% vs. 68%, respectively) [10]. Lathi et al. reported that the abnormality rate in anembryonic gestations was 58%, which was not significantly different from the 68% rate that was observed in pregnancies with embryonic poles. In a comparison of miscarriages with and without a history of documented cardiac activity, a previous study found no significant difference in the rate of abnormal karyotype [21]. Studies in patients presenting with miscarriage have not reached the same conclusions. Therefore, the risk of an abnormal embryonic karyotype cannot be predicted by whether an embryonic pole or cardiac activity is present prior to miscarriage.

Most embryos with chromosomal numerical abnormalities will miscarry, but a few can live until term, such as those with trisomies 21, 18, and 13 (called viable autosomal trisomy), monosomy X and triploidy [20,22]. Miscarriage with these viable abnormal karyotypes may occur in different gestational stages compared with other abnormal karyotypes. The present data indicate that the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with monosomy X, triploidy, and viable autosomal trisomies are higher than the frequencies in miscarriages with other abnormal and normal embryonic karyotypes. Moreover, the gestational ages of miscarriage with these viable aneuploidy karyotypes are older than the gestational ages of the other groups. The Munoz et al. study found that viable aneuploidy in miscarriage with an embryonic pole was more common than viable aneuploidy in miscarriage without an embryonic pole (19% vs. 2%) [10]. Bessho et al. found that the CRLs of monosomy X, trisomy 21, and triploidy were longer than the CRLs of normal karyotype or other trisomies [9]. This indicates that when miscarriage occurs, there is a greater degree of embryonic development in conceptuses with triploidy, monosomy X and trisomy 21 compared to other karyotypes. The chronological differences of embryo lifespans with abnormal karyotypes may be associated with programmed death that is activated by a particular lethal gene.

The present study has several limitations. The authors compared the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal karyotypes. Other ultrasound-observable miscarriage-related appearance findings, such as small gestational

tosomal trisomies (95.2% and 85.7%, respectively), monosomy X (100% and 100%, respectively), and triploidy (100% and 95.5%, respectively) than in miscarriages with a normal karyotype (71.8% and 57.5%, respectively; all p < 0.05) and other abnormal karyotypes (65.2% and 50.6%, respectively, all p < 0.05). There was no difference in the frequency of miscarriage between the normal karyotype and other abnormal karyotype groups (Table 4). The gestational ages of the viable autosomal trisomies, monosomy X, and triploidy groups were higher than those of the normal karyotype and other abnormal karyotype groups (all, p < 0.05).

**Discussion**

Approximately 10-15% of clinically recognized pregnancies result in miscarriage, and the majority (60-70%) of these miscarriages can be attributed to detectable chromosomal abnormalities. Cytogenetic analysis of the production of conception contributes to the discovery of the causes of miscarriage, and patients can be counseled about further testing and the prognosis for future pregnancies. Aneuploidy in miscarriage may also reduce the need for testing for other causes, such as immunological issues and thrombophilia, which can lower the financial cost [12]. The present data revealed that 55.3% of miscarriages had an abnormal karyotype, which is consistent with previous reports [13]. However, chromosomal analysis is unavailable at some hospitals and cannot always be performed. An exploration of the clinical and ultrasound characteristics of miscarriages with abnormal and normal karyotypes would aid in the counseling of these patients.

The present data revealed that the percentage of recurrent miscarriage in the abnormal karyotype group (53.6%) was lower than the percentage in the normal karyotype group (64.6%), which is consistent with previous studies [14,15]. Stephenson et al. revealed that the rate of abnormal karyotypes in recurrent miscarriage was 46%, which was lower than the 63% in the control group [16]. These findings suggest that a recurring cause other than abnormal karyotype likely leads to recurrent pregnancy loss [14]. The risk of miscarriage increases as the woman’s age increases. The risk of miscarriage between six and 12 weeks of gestation in women less than 35 years of age is 9-12%. The risk increases to 40-50% in women older than 40 years of age, as there is a markedly increased incidence of trisomic embryos [17-19]. The present data indicated that in the normal karyotype group, the mean age, and percentage of women with advanced age was higher than in the normal karyotype group. Previous studies have also demonstrated that advanced age was the greatest risk factor for an abnormal embryonic karyotype in miscarriage [3,20].

Miscarriage is a heterogeneous condition that is associated with several underlying causes. Miscarriage due to various underlying pathologies may occur at different pregnancy stages. Previous studies have indicated that pre-embryonic losses are more likely to be associated with fetal aneuploidy than losses occurring later in gestation [8,9]. However, in the present study, the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with an abnormal karyotype were 74.1% and 62.1%, respectively, which were not significantly different from the normal karyotype frequencies (71.8% and 57.5%, respectively). The present findings are consistent with a number of chorionic villi sampling studies. A study by Munoz et al. revealed similar abnormal karyotype rates in anembryonic and embryonic groups (61% vs. 68%, respectively) [10]. Lathi et al. reported that the abnormality rate in anembryonic gestations was 58%, which was not significantly different from the 68% rate that was observed in pregnancies with embryonic poles. In a comparison of miscarriages with and without a history of documented cardiac activity, a previous study found no significant difference in the rate of abnormal karyotype [21]. Studies in patients presenting with miscarriage have not reached the same conclusions. Therefore, the risk of an abnormal embryonic karyotype cannot be predicted by whether an embryonic pole or cardiac activity is present prior to miscarriage.

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The present study has several limitations. The authors compared the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal karyotypes. Other ultrasound-observable miscarriage-related appearance findings, such as small gestational
sacs, small fetus size, and enlarged yolk sacs, were not included in this study. Other abnormal ultrasound findings have been found to be associated with abnormal karyotypes. Compared with cases with normal ultrasounds, the frequency of chromosomal anomaly is significantly higher in the presence of abnormal morphological features (33.8% vs. 85.2%, respectively), such as early symmetrical arrested growth, small gestational sac, small fetus, enlarged yolk sac, and empty sac [23]. Ljunger et al. found that small embryonic poles were significantly more likely to be associated with aneuploidy than normal-sized embryonic poles (70.1% vs. 50.0%, respectively) [24]. An investigation of ultrasounds in miscarriages with different abnormal embryonic karyotypes would contribute to identifying the association between abnormal karyotypes and specific morphological types in early miscarriage.

In conclusion, embryonic karyotype anomaly is a major reason for early miscarriage. There are no differences in the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal karyotypes. However, the presence of an embryonic pole and cardiac activity in miscarriages with monosomy X, triploidy and viable trisomy is more common than in miscarriages with a normal karyotype or other abnormal karyotypes.

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References


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Introduction

Endometriosis is a gynecological disease that affects approximately 2%-48% of women during their reproductive years [1]. It is characterized by the presence and proliferation of endometrial glands and stroma outside the uterus. The patients mainly complain of pelvic pain, dysmenorrhea, and infertility. Laparoscopy offers the most specific and sensitive technique for evaluating and monitoring endometriosis. However, microscopic or occult endometriosis may be misdiagnosed due to the inability to visualize the lesions. Moreover, invasive procedures have been linked to the accidental development of endometriosis [2]. Furthermore, persistence or recurrence of endometriosis mostly occurs after regular therapy. Therefore, the benefits of non-invasive, biochemical diagnostic markers, especially in serum, for detection of endometriosis are evident. However, microscopic or occult endometriosis may be misdiagnosed due to the inability to visualize the lesions. Moreover, invasive procedures have been linked to the accidental development of endometriosis [2]. Furthermore, persistence or recurrence of endometriosis mostly occurs after regular therapy. Therefore, the benefits of non-invasive, biochemical diagnostic markers, especially in serum, for detection of endometriosis are evident. 

YKL-40 is a 40 kDa heparin- and chitin-binding glycoprotein also known as human cartilage glycoprotein 39 (Hcgp39), 38-kDa heparin-binding glycoprotein or chitinase-3-like protein 1 (CHI3L1) [3]. The abbreviation YKL-40 is based on the one letter code for the first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L) and the apparent molecular weight of YKL-40 [4]. YKL-40 is normally expressed by a number of different cell types such as chondrocytes, synoviocytes, vascular smooth muscle cells, macrophages, and neutrophils. It has been recognized as a growth factor capable of stimulating connective tissue cell growth and endothelial cell migration, while inhibiting mammary epithelial cell differentiation [5].

Growing evidence has indicated that expression levels of YKL-40 are elevated in multiple human diseases including type 2 diabetes, obesity and insulin resistance in children, Alzheimers’ diseases, and heart failure [6]. Elevated YKL-40 levels were observed in a vast array of inflammatory diseases that contain bacterial infections, rheumatoid arthritis, osteoarthritis, hepatitis, asthma and chronic obstructive pulmonary diseases, neuroinflammation, and bowel lesions [7]. In the chronic inflammatory diseases, YKL-40 is supposed to mediate infiltration, differentiation, and maturaion of macrophages, the primary leukocytes in response to inflammation [8].

The present study was designed to establish serum YKL-40 concentrations in patients with endometriosis compared to age-matched healthy subjects. To the authors’ knowledge, this is the first study evaluating serum YKL-40 levels in endometriosis. The present results indicate that YKL-40 levels were increased in patients with endometriosis compared to controls. The authors propose that circulating YKL-40 levels could be a novel biomarker for diagnosis and follow-up of endometriosis.

Materials and Methods

Study design

This study was approved by the local Institutional Review Board. Written informed consent was obtained from all subjects. A total of 63 women of reproductive age with regular menstrual
cycles (25-32 days) admitted to the present tertiary center between February and September 2013 constituted the study group. Of these participants, 33 patients with endometriosis (patient group) and 30 fertile women (control group) without endometriosis were included in the study. The diagnosis of endometriosis was confirmed histopathologically in all cases in patient group. The decision of the operation was verified by high CA-125 levels combined with at least four-months of ultrasound examination.

**Serum YKL-40 analysis**

YKL-40 levels were determined using a YKL-40 enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer’s protocol. Absorbance was measured using a microplate reader.

**Statistical analyses**

Data were analyzed using the Statistical Package for Social Sciences 19.0 for Windows. Parametric tests were applied to data of normal distribution and non-parametric tests were applied to data of questionably normal distribution. Independent-samples t-test and Mann–Whitney U-test were used to compare independent groups. Data are expressed as mean ± SD or median (interquartile range), as appropriate. All differences associated with a chance probability of 0.05 or less were considered statistically significant.

**Results**

A total of 63 cases consisting of 33 endometriosis patients and 30 healthy controls met the eligibility criteria for the study. The mean age of the patient group was 32.06 ± 0.86 (range 30 to 34) years, while the mean age of the controls was 31.53 ± 1.43 (range 26 to 34) years (p = 0.087). The mean body mass index (BMI) of the patient group was 26.21 ± 2.29 (range 20.3 to 31.9) kg/m², while the mean BMI of the controls was 25.63 ± 2.68 (range 21.2 to 32.3) kg/m² (p = 0.358). The mean CA-125 level of the controls was 59.88 ± 5.79 (range 49.63 to 75.08) U/ml, while the mean CA-125 level of the controls was 19.6 ± 3.54 (range 10.16 to 28.17) U/ml (p < 0.001). Dysmenorrhea and chronic pelvic pain were found to be more frequent in endometriosis group (p < 0.001, Figure 1).

![Figure 1](image-url) — Figure comparing serum YKL-40 values in patients with endometriosis and controls. Serum YKL-40 was significantly higher in patients with endometriosis.

### Table 1. — Demographic and clinical variables of the study group. Data are expressed as mean ± SD or number (percentage).

<table>
<thead>
<tr>
<th></th>
<th>Patient group (n = 33)</th>
<th>Control group (n = 30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>32.06 ± 0.86</td>
<td>31.53 ± 1.43</td>
<td>0.087</td>
</tr>
<tr>
<td>BMI</td>
<td>26.21 ± 2.29</td>
<td>25.63 ± 2.68</td>
<td>0.358</td>
</tr>
<tr>
<td>CA-125 level (U/ml)</td>
<td>39.88 ± 5.79</td>
<td>19.6 ± 3.54</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>25 (76%)</td>
<td>9 (30%)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Chronic pelvic pain</td>
<td>22 (66.7%)</td>
<td>5 (16.7%)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Marital status (Married/single)</td>
<td>22/11</td>
<td>13/17</td>
<td>0.108</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, the authors attempted to demonstrate whether there was a relationship in serum YKL-40 concentrations between patients with endometriosis and age-matched healthy subjects. This is an original and unique study for evaluating serum YKL-40 levels in endometriosis and the authors found elevated YKL-40 levels in patients with endometriosis compared with control subjects.

Endometriosis is a multifactorial disease and angiogenesis plays an important role in pathophysiology [9]. The angiogenic potentials of both the endometrium and the peritoneal environment influence lesion establishment [10]. Indeed, endometriotic lesions require an adequate blood supply to survive in their ectopic sites. In normal eutopic (intrauterine) endometrium, it has been suggested that vessel elongation, rather than branch point sprouting, is the primary mechanism for rapid vessel growth during the proliferative phase [11]. The precise mechanism in endometriosis lesions has not been evaluated up to now. Recruitment of new capillaries from existing, adjacent peritoneal microvessels was postulated [10]. Derivation of new blood vessels from circulating endothelial progenitor cells, the so-called “vasculogenesis,” also seems to be important in the pathogenesis of endometriosis [12]. The initiation, extent, and suppression of angiogenesis are highly dependent on the balance between pro- and anti-angiogenic factors. Hanahan and Folkman described this phenomenon as the angiogenic switch in tumors [13]. Recently, molecules with anti-angiogenic potential appear to be suppressed in patients with endometriosis compared to controls. For example, interferon gamma-induced protein 10 concentrations are reduced in peritoneal fluid and adiponectin levels are reduced both in the peritoneal fluid and serum of endometriosis patients compared to controls [14]. Vascular endothelial growth factor (VEGF) is one of the most prominent and well-studied proangiogenic growth factors in endometriosis and it is widely believed that VEGF is the main stimulus for angiogenesis and increased
vessel permeability in this disease [15]. Significantly increased VEGF levels have been detected in the peritoneal fluid and lesions of endometriosis patients compared to controls [16]. Similarly, YKL-40 was recently identified as a potent angiogenic factor capable of inducing endothelial cell angiogenesis in breast cancer [17]. In the tumor microenvironment, a significant amount of angiogenic factors are secreted from tumor cells and activate adjacent vascular endothelial cells to induce angiogenic responses [18]. YKL-40 and VEGF are believed to be mainly derived from tumor cells and both have strong angiogenic activities in tumor development.

Dupont et al. suggested that YKL-40 may be a novel marker for the detection of early stage ovarian cancer compared to CA 125 and CA 15-3 [19]. The usefulness of YKL-40 in the management of recurrent ovarian cancer has been reported. Patients with high plasma YKL-40 at the time of relapse had significantly shorter survival rates than patients with normal levels the plasma YKL-40 was demonstrated to be an independent prognostic factor [20]. Therefore, an elevated level of serum YKL-40 can serve as a novel marker for the detection of endometrial cancer, and identify a high risk subset of patients at risk for poor clinical outcomes. To date, only one study has reported the expression and distribution of the YKL-40 in peritoneal endometriosis [21]. The results of this study yielded that YKL-40 was associated with the severity of peritoneal endometriosis. However, it is not clear whether the expression of YKL-40 was caused by the proliferation and turnover of ectopic endothelial tissue or extracellular matrix remodeling and fibrosis. In the present study, serum YKL-40 concentrations in patients with endometriosis were significantly higher than age-matched healthy subjects. The authors think that inflammatory process during endometriosis results in elevated YKL-40 levels. The exact mechanism through which YKL-40 levels are elevated remain to be elucidated via further in vitro and in vivo trials.

Limitations of the present study are relatively small size of this series and lack of definite criteria for selection of patients. In addition, some details of history and factors that may influence the outcome may not be completely documented. Due to these restrictions, associations should be interpreted with caution. However, the authors hope that this study will pioneer further studies on this method.

Conclusion

The present data demonstrate that YKL-40 is an effective tool for identification of women with and without endometriosis. The authors anticipate that further studies of the proteins identified herein will expand our understanding of the nature of endometriosis and assist in the eventual development of new diagnostic and therapeutic approaches for endometriosis.

References


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Introduction

Obesity is defined as a pathological state, characterized by excessive fat accumulation until that level when it begins to be a health risk. Today, obesity is a serious health problem in many countries, especially in North America, as in many European countries [1].

Body mass index (BMI) is the ratio between weight in kilograms and the square of the height in meters and according to the National Health and Nutrition Examination Survey (NHANES) is usually used as parameter to define nutritional status [2]. Normal weight is with a BMI from 18.5 to 24.9. BMI between 25 and 29.9 is considered overweight and BMI over 30 indicates obesity. BMI over 35 is defined as malignant or pathological obesity.

Over the last decade, nutritional status has been recognized as one of the most important factors of the reproductive health [3]. Therefore, excessive body weight and obesity become one of the most challenging issues in the field of gynecology and obstetrics [3]. The results of numerous studies have shown that this group of women were at higher risk for the development of wide range of gynecological diseases such as: polycystic ovary syndrome (PCOS), irregular menstrual cycle, endometrial cancer, etc [4, 5]. Furthermore, it was also noticed that females with increased body weight are the risk group for developing a number of complications during pregnancy and childbirth (hypertensive syndrome in pregnancy, gestational diabetes, thrombosis, prolonged labor, stimulation/induction of labor, feto-pelvic disproportion, and cesarean section [4, 5].

In modern obstetrics era, cesarean section has become one of the most common modes of delivery, and gradually tends to take the leading role worldwide. The prevalence of cesarean section in different countries varies from 5% to 30% of the total number of deliveries [6]. The results from the studies that have examined the factors associated with this mode of delivery showed that overweight pregnant women appeared as an important factor which are mostly included in the high-rate of cesarean sections.

The aim of this study was to investigate the effect of overweight and obesity in pregnancy on the obstetrician’s decision to complete delivery by cesarean section in the representative sample of females in the population of Belgrade (Serbia).

Materials and Methods

The study was undertaken at the Institute of Gynecology and Obstetrics, Clinical Center Serbia (IGA KCS) in Belgrade, and in the Hospital for Gynecology and Obstetrics, Clinical Hospital Centre “Zvezdara” (KBC) in Belgrade.

The study was designed as a cross-sectional study and included 200 patients. The sample was created using consecutive sampling design comprising women who had cesarean section during the period from the January 1st, 2008 until July 30th, 2008. This study used a specially formed, structured questionnaire, composed on the basis of literature data [7]. BMI was calculated according to...
method proposed in 1985 by Garrow (originally called the Quetelet’s index (QI)), and was determined by the formula: BMI = TM (kg) / TV² (m²) [8].

For checking the hypothesis of the existence of differences in findings between the presence of certain groups, the methods of descriptive statistics: χ² test and student - T- test were used. If it was not possible to apply χ² test, the Fisher’s exact test was used.

The relationship between BMI and caesarean section, as well as between BMI and other investigated variables pregnancy-induced hypertension (PIH), gestational diabetes, urinary tract infections, and neonatal birth weight) was assessed by Spearman’s rank correlation and Pearson’s coefficient of linear correlation.

Results

The mean age of all women participating in the present study was 32.9 years (range 19 up to 44). Of all the women, 67 (33.5%) were older than 35 years. Most of them were nulliparas: 111 (55.5%), and educated: 117 (58.5%). The majority of women had an adequate prenatal care in their pregnancy: 179 (89.5%), while 21 (10.5%) were not subjected to any health-related check-ups during the entire period of pregnancy.

Of the total sample of 200 women who underwent caesarean section during the period of observation, 67 (33.5%) of them were overweight and obese. Based on the results obtained from the questionnaire, the authors noted that the highest number of pregnant women who reported changes in feeding regime had the normal body weight at the beginning of pregnancy: 45 (57.7%) and had a BMI between 18.50 and 24.99 kg/m².

There was a statistically significant difference in the nutritional status of pregnant women and the way of delivery (χ² = 69.141; p < 0.001). Emergency caesarean section was completed in 86 (43%) patients versus planned caesarean section which was completed in 114 (57%) patients. Emergency caesarean section was the most common in patients who were overweight: 29 (33.7%), with BMI between 25.00 and 29.99 kg/m² (Table 1).

There was a statistically significant association between BMI and all the studied pregnancy complications: hypertension (PIH), gestational diabetes (GD), and urinary tract infections (τ(PIH) = 0.637; p < 0.001; τ(GH) = 0.538; p < 0.001; τ(urinary tract infection) = 0.289; p < 0.001). All complications were most common in patients who were obese class I- BMI between 30.00 and 34.99 kg/m².

The average body weight of infants born by caesarean section in the sample was 3,524 ± 606.48 grams. There was a highly significant correlation between the nutritional status of pregnant women during early pregnancy as measured by BMI and birth weight of newborns (τ = 0.224, p < 0.001). The average BMI at the beginning of pregnancy was 23.88 ± 5.12 kg/m², which meant that most of women at the beginning of pregnancy were of normal weight with a BMI between 18.5 and 24.99 kg/m².

In the present study the authors observed gestational increase of body weight with 17.24 ± 5.32 kg. Gestational increase of body weight in pregnancy has proven to be one of the factors that have influenced the weight of newborns, as the authors proved statistically significant association between fetal weight and gestational weight gain (τ = 0.198, p < 0.005).

Out of 200 women (100%) in this study, deliveries by caesarean section were 178; of them (89%) gained 12 kg or more in weight, which significantly deviates from the recommendations of the World Health Organization (WHO).

Discussion

During the past decades it has been recognized that excessive body weight and obesity become one of the most challenging issues in the field of perinatology and obstetrics [9]. Namely, it was observed that body weight during the pregnancy could be a significant predictive factor for delivery mode, as well as for development of various pregnancy-related complications. In the present study, 66.5% of patients were younger than 35 years and the majority of them were educated nulliparous. The majority of women, 89.5%, had adequate prenatal care in pregnancy.

The majority of pregnant women included in the present investigation had normal body weight at the beginning of pregnancy which were in accordance with results obtained in a sample of Australian obstetric population [10]. Furthermore, the most prominent increase in body weight of pregnant women have been detected in the third trimester.

The correlation of obesity in pregnancy and emergency of cesarean sections supports the hypothesis of this study; the influence of obesity on the mode of delivery. As obesity is not classified in the absolute and relative indications for the abdominal delivery in Serbia, the majority of patients with excessive body weight have entered the active phase of vaginal delivery, and in the course of the delivery stalled further progress, because of the possible complications in mother, fetus or both, which of course, influenced the deci-
sion of the obstetrician to complete the delivery by cesarean section. Emergency cesarean section was the most common in patients who were overweight in 33.7% [11, 12].

In this study, the authors observed that in obese pregnant women have often the following complications: preeclampsia, gestational diabetes, and urinary tract infections. These complications were an important factor in the decision for cesarean section [13, 14]. Preeclampsia is most often observed in patients that were registered obese class I, in 78.57%. Gestational diabetes is also commonly diagnosed in women, that were registered obese class I, in 35.71%, and urinary tract infections were also most frequent in the group of patients that were registered obese class I, in 21.43%.

As recommended by WHO, the allowed increase in body weight in singleton pregnancies should be from 11 kg to 12.5 kg by the end of pregnancy or from 16 kg to 20 kg in twin pregnancies. Obese and overweight pregnant women usually give birth to children over 4,000 g. Overweight newborn, ones with birth weight over 4,000 g could be one of the causes of dystocia (abnormal labor), vaginal delivery failure, and frequent cesarean sections.

In this study, it was observed that patients with high BMI often delivered overweight newborns, and that the average weight gain during pregnancy was 17.24 ± 5.32 kg, which roughly corresponds to the results derived from similar studies [15]. The authors proved that there is a statistically significant correlation between gestational weight gain of the mothers and newborns’ body weight, which supports the theory that glucose is an essential nutritive element in intrauterine fetal nutrition, and that maternal hyperglycemia stimulates high levels of glucose in the fetal circulation and reactive hyperinsulinemia which consequently leads to increased synthesis of triglycerides and increase of fetal fat, leading to fetal macrosomia [16].

About 89% of the patients in this study gained 12 kg or more in weight during pregnancy, that is alarming and an indication for a possible adverse perinatal outcome.

Conclusion

Nutritional status both at the beginning and at the end of pregnancy significantly affects both the flow of the pregnancy and the way of delivery, as well as significantly affects the weight of newborns. Based on the above correlations, the authors conclude that obesity at the beginning and at the end of pregnancy is a significant marker of pregnancies outcome which should be seriously taken into consideration. One of the most important measures to prevent obesity in pregnancy is to increase the activity of existing nutrition guidelines for pregnant women.

References


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Low cord blood serum levels of vitamin D: cause or effect of fetal macrosomia?

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Summary
Aim: The aim of the study was to compare cord blood vitamin D levels of macrosomic large for gestational age (LGA) and appropriate gestational age (AGA) newborns. Materials and Methods: Seventy-nine healthy, normal term newborns were included in the study. They were divided by birth weight into two groups: 37 in the LGA group above 4,000 g, and 42 newborns in the AGA group birth weight between 3,000 g and 4,000 g. Cord blood samples from groups were collected. Circulating 25(OH)D was measured as 25-hydroxyvitamin D3 (25(OH)D3) by high-performance liquid chromatography (HPLC) in serum using a kit. Results: Maternal characteristics (age, body mass index [BMI], and gestational age) did not differ between the AGA and LGA groups. Cord blood 25 OH vitamin D levels were significantly low in neonates with LGA (p = 0.02). Conclusions: The authors found that macrosomic infants had low levels of vitamin D. Providing vitamin D supplements to pregnant women may prevent macrosomia. Randomized controlled trials are needed to prove this assertion.

Key words: Vitamin D; Fetal macrosomia; Large for gestational age (LGA).

Introduction
Fetal macrosomia, commonly defined as a birth weight above the 90th centile for gestational age (GA), is associated with increased risks for the mother, including cesarean section and trauma to the birth canal, and for the baby, including shoulder dystocia and consequent brachial plexus or facial nerve injuries, fractures of the humerus or clavicle, and birth asphyxia [1–6]. The prevalence of macrosomia in developed countries is between 5% and 20%; however, an increase of 15–25% has been reported in the past two to three decades [7-9].

Vitamin D deficiency is an increasing public health concern [10]. Though most commonly associated with rickets in childhood or osteomalacia in later adult life, but also a lack of vitamin D has potential health consequences that reach far beyond disordered calcium regulation and bone mineralization [11]. Vitamin D receptors are distributed in a variety of tissues throughout the body [12]. In pregnancy, vitamin D deficiency has been shown to be associated with complications such as preeclampsia, gestational diabetes mellitus, and primary caesarian section, and it has been hypothesized to also induce increased risk of multiple sclerosis, heart disease, and cancer later in life [13-19]. Low serum levels of 25(OH)D have been linked through observational studies to the pathophysiology of obesity, diabetes mellitus, and metabolic syndrome. Vitamin D receptor is highly expressed in adipocytes and is responsive to activation by 1,25-(OH)2D [20-22]. Also vitamin D is fat soluble and can be stored in adipose tissues [23, 24]. Large cohort studies have shown that an increased percentage body fat and high body mass index (BMI) are strongly and inversely correlated with serum 25(OH)D concentrations, particularly in Caucasians [25, 26]. There is also strong evidence that 1,25-(OH)2D modulates intracellular ionized calcium signaling in the adipocyte, which in turn promotes increased lipogenesis and decreased lipolysis, possibly through the inhibition of uncoupling protein-2 (UCP2) [27]. Although some studies have shown a relationship between vitamin D deficiency and small for gestational age (SGA) infants. All of the studies compared with SGA and appropriate for gestational age (AGA) infants for vitamin D levels. Until now, no firm data have answered the question of whether fetal macrosomia is related to vitamin D deficiency. The aim of the study was to compare cord blood vitamin D levels of macrosomic (large for gestational age – LGA) and AGA infants.

Materials and Methods
This case control cross-sectional study was conducted between December and February 2013 in the Department of Perinatology at Zekai Tahir Burak Women’s Teaching and Research Hospital in Ankara, Turkey. In this hospital there are approxi-
mately 1,000 births in a month. Cases were consecutive births between this period. Seventy-nine healthy, normal term newborns were included in the study. They were divided by birth weight into two groups: 37 in the LGA group above 4,000 g, and 42 newborns in the AGA group birth weight between 3,000 g and 4,000 g. Cord blood samples from groups were collected. The umbilical cord was double clamped and blood samples were collected from the umbilical vein by needle puncture just after delivery. All blood samples were taken from mothers with low-risk, term (37 weeks or more gestation) pregnancies without a history of hypertension, diabetes mellitus, chronic respiratory or cardiovascular diseases, pre-eclampsia/eclampsia, oligohydramnios, intrauterine growth restriction, poor biophysical profile, smoking or vitamin D using. Age, BMI, gestational age, gravidity, parity, maternal fasting blood glucose, levels of vitamin D in newborn cord blood, and birth weight were recorded. Because of seasonal variation of vitamin D levels, study were conducted in winter time. Gestational age at delivery was calculated according to the last menstrual period and confirmed by ultrasound examination during the first trimester or early second trimester. Gestational diabetes was ruled out by normal glucose tolerance test (GCT) or normal oral glucose tolerance test (OGTT) in cases when GCT was abnormally high. These tests were performed between 25 to 29 gestation weeks.

Laboratory methods
Umbilical cord blood samples were taken into vacutainer tubes and centrifuged. Serum samples were then stored in aliquots at -80°C until the testing period. Circulating 25(OH)D was measured as 25 hydroxyvitamin D3 (25(OH)D3) by high-performance liquid chromatography (HPLC) in serum using a kit. The intra-assay coefficients of variation for serum vitamin D was 2.6% and the inter-assay coefficients of variation for serum Vitamin D was 4%. The reference interval was 25-150 nmol/l (10-60 μg/l) for winter time.

Statistical analysis
When the macrosomic birth weight prevalence of 20% among term deliveries [7-9] 95% confidence interval, a = 0.05 and 85% power, the sample size was calculated as 36 cases and 36 controls. A total of 37 cases and 42 controls were evaluated in the present study. Statistical analysis was performed using the Statistical Package for Social Sciences software (SPSS). Vitamin D data was log transformed before statistical analysis by the independent samples t-test. A Mann–Whitney U-test was used to compare the other variables. Statistical significance was set at p < 0.05. The study was approved by the local ethics committee and all of the patients gave informed consent for participation in the study.

Results
All neonates were normal and did not suffer from any complication. Forty (50.6%) neonates were born by cesarean section (22 of AGA group and 18 of LGA group), and 39 (49.4%) by vaginal delivery (20 of AGA group and 19 of LGA group). Forty-four of the neonates were males (55.7%) and 32 (40.5%) were females. Maternal characteristics (age, BMI, and gestational age) did not differ between the AGA and LGA groups (Table 1). Cord blood 25 OH Vitamin D levels were significantly low in neonates with LGA (p = 0.02). Cord blood 25 OH vitamin D3 levels of newborns are shown in Figure 1.

Discussion
This case–control study was undertaken to compare cord blood 25OH vitamin D values of LGA infants and AGA infants. The results showed that LGA infants had a significantly lower 25OH vitamin D levels compared to AGA infants.

The effects of vitamin D deficiency on birth size are inconsistent. The present results add to the evidence that cord blood 25(OH)D concentrations are negatively correlated with high birth weight as reported by other studies [28-30]. In contrast, some studies reported no such correlation [31] or even a positive association [32-33] between birth size and 25(OH)D level. These conflicting findings may be related to the varied populations studied or methods used. Variants in the vitamin D receptor gene may influence the associations between maternal 25(OH)D concentrations and birth size measurements.

Table 1. — Comparison between AGA and LGA newborns cord blood vitamin D, birth weight and, maternal data.

<table>
<thead>
<tr>
<th>Variables</th>
<th>AGA newborns</th>
<th>LGA newborns</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>27.2 (±4.2)</td>
<td>27.6 (±6.5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Gravida</td>
<td>1.8 (±0.89)</td>
<td>2 (±1.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>275 (±9.1)</td>
<td>278 (±6.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.8 (±4.3)</td>
<td>31.2 (±2.5)</td>
<td>0.16</td>
</tr>
<tr>
<td>25OH vitamin D₃ (µg/l)</td>
<td>26.4 (±17.6)</td>
<td>15.1 (±6.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Log 25OH vitamin D₃</td>
<td>1.3 (±0.33)</td>
<td>1.1 (±0.18)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Figure 1. — Cord blood 25 OH vitamin D₃ levels of newborns.
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It is now known that vitamin D has potent antiproliferative properties. The presence of vitamin D dampens proliferation and induces cells to exit the cell cycle via differentiation and, in certain circumstances, induces apoptosis [34, 35]. Therefore, its absence in the prenatal period could lead to inappropriately high cell numbers, which could subsequently influence the size of the offspring. Animal experiments indicate that these mechanisms do have an impact on fetal growth. For instance, the newborn offspring of normocalcemic rats deprived of vitamin D were significantly heavier than those of control animals [29]. Guinea pig fetuses exposed to low levels of vitamin D had expanded growth plates in their long bones [30]. If similar mechanisms operated in humans, the newborns with hypovitaminosis D should be heavier (due to increased cell number) and longer (due to wider growth plates in the lower limb bones).

Recent studies showed that adiposity, the expansion and growth of white adipose tissue (WAT) caused by hyperplasia and hypertrophy of adipocytes [36], is dependent on the neovascularization and dilatation of existing capillaries, respectively [36, 37]. Hence, hypertrophic adipocytes are typically found to possess low-oxygen microenvironments-hypoxia [38]. Similar to tumor growth, the inhibition of adipose tissue angiogenesis inhibits WAT growth and ultimately, the development of adiposity. Numerous studies showed 1α,25(OH)2D3 inhibited the proliferation of cultured endothelial cells and anti-angiogenesis in animal models [39, 40]. The hormone can also interrupt the angiogenic factor interleukin 8 signaling, leading to the inhibition of endothelial cell migration and tube formation [41]. It can be speculated that macrosomia may be related the low level of vitamin D and its antiangiogenic effects.

Environmental factors that have regular seasonal fluctuation influence both the size and shape of neonates. Animal experiments suggest that prenatal hypovitaminosis D may underlie greater limb length [30]. Fetal vitamin D requirements increase during pregnancy (related to the increased need for fetal calcium), thus maternal vitamin D levels tend to fall during the third trimester, especially if this occurs during winter [42]. Seasonal variation of vitamin D levels may be the cause of seasonal change of fetal weight.

There are some limitations of this study. First of all the authors did not evaluated levels of vitamin D in maternal serum at birth. For this reason they can not conclude that this lower levels of vitamin D in macrosomic infants only related with fetal vitamin D levels. In addition this limited number of patients included the study. In the next step, this study should be repeated with a larger number of patients and also maternal serum levels of vitamin D should be evaluated.

In conclusion, the authors found that macrosomic infants had low levels of vitamin D. Providing vitamin D supplements to pregnant women may prevent macrosomia. Randomized controlled trials are needed to prove this assertion.

References


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Preliminary results of tubal surgery with pregnancy outcome

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Summary

Purpose of investigation: To assess the preliminary results of tubal surgery and its effect on pregnancy outcome. Materials and Methods: The study included 440 patients with unilateral or bilateral tubal disease as the only cause of the infertility. All patients undergoing a laparoscopy for infertility were studied in reproductive surgery centre. The fallopian tube was classified into class I-IV. The studied outcomes were live birth, ectopic pregnancy, and miscarriage. After 12 months, cumulative conception rate was calculated. Results: In the 440 patients, 172 patients with mild salpinx abnormality (class I) had a 34% cumulative pregnancy rate, 151 patients with moderate salpinx abnormality (class II) had a 16% cumulative pregnancy rate, and 77 patients with severe salpinx abnormality (class III) had a 10% cumulative pregnancy rate. No intrauterine pregnancies were observed in the severe group of 40 patients (class IV). Conclusion: Surgical laparoscopy is helpful for class I and II tubal abnormality, while it is not for class III and IV abnormalities.

Key words: Infertility; Laparoscopy; Pregnancy outcome.

Introduction

Of couples with infertility, 25% have complete or partial blockage of the fallopian tube [1]. Treatment of tubal infertility is an important and current problem. The standard modality of treatment of tubal factor infertility is either tubal surgery or in-vitro fertilization and embryo transfer (IVF-ET). Since the advent of IVF, the role of tubal surgery has diminished. However, in China, IVF-ET is not subsidized by the government health service. Therefore the cost of IVF is beyond the ability of most people to afford. Surgical laparoscopy costs only one fifth of IVF-ET and provides natural intrauterine pregnancy opportunity for tubal infertility. Because of the increasing prevalence of infertility and the cost-effectiveness of its treatment, surgical laparoscopy is widely performed in China. The American Fertility Society (AFS) score has been used widely in China for predicting pregnancy outcome and guiding infertility surgery. After finishing the first step of a hysterosalpingography check, the clinical management of tubal infertility remains confusing.

Salpingoscopy is an endoscopic technique that allows the direct visualization of the tubal mucosa. The status of the tubal mucosa is the best prognostic factor when evaluating patients with tubal infertility. The prognostic value of salpingoscopy during operative laparoscopy for tubal factor infertility in terms of reproductive outcome has been confirmed [2-4]. A prognostic classification system of the health of the fallopian tube based on salpingoscopy has previously been proposed [5]. However, salpingoscopy, performed during laparoscopy, has not reached wide acceptance in China due to the costly, non-user-friendly, dedicated instrumentation needed. Moreover, it is reported that direct visualization of the ampullary mucosa by salpingoscopy can allow the detection of intraluminal adhesions that place the patient at increased risk for a repeat ectopic pregnancy [6] and hydrosalpinx [7].

In this study, the authors evaluated fallopian tubal status by chromopertubation and AFS. The aim of the present study was to observe the effect of surgical intervention on pregnancy outcomes for different tubal statuses.

Materials and Methods

The study design was approved by the Medical Research Review Board of Women’s Hospital (School of Medicine, Zhejiang University, Hangzhou, People’s Republic of China; RRBNO: 20120009). The study included 440 infertile patients diagnosed by preoperative hysterosalpingography undergoing tubal surgery that were available for analysis between January 2012 and December 2012. Patients’ characters are shown in Table 1. Inclusion criteria: infertility history was longer than one year; husband semen was normal; inpatient for tubal disease; no more than moderate endometriosis; normal uterus; normal ovarian function; with follow-up conditions. Exclusion criteria: infertility history was shorter than one year; husband semen was abnormal; abnormal uterus; moderate or severe endometriosis; ovarian dysfunction; without follow-up conditions. Patients signed the informed consent before operation. Doctors decided to retain or remove diseased fallopian tube according to the wishes of patients and AFS scores (Table 2). Fallopian tubal patency was tested by chromopertubation using methylene blue during operative laparoscopy. All primary infertilities had hysteroscopy performed during laparoscopy. Tubal surgery included pelvic fimbrioplasty,
salpingolysis, single salpingectomy, and bilateral salpingectomy. Tubal status was divided into class I-IV.

The cumulative pregnancy follow-up period was 12 months after surgery. All those who conceived were booked for antenatal care and delivery at the Women's hospital, School of Medicine, Zhejiang University.

**Statistical analysis**

The studied outcomes were live birth, ectopic pregnancy, and miscarriage. Statistical analysis was performed using SPSS Version 13.0 statistical package software. Results were analyzed with one-way ANOVA for categorical variables. Cumulative conception rate (CCR) was calculated with the use of Kaplan-Meier survival analysis. There was significant difference if \( p \) was less than 0.05.

**Results**

**Patients’ characters and pregnancy outcomes**

According to the AFS score, 440 patients were divided into four groups: class I, II, III, and IV. As shown in Table 1, there were no statistical differences among the four groups in the basic characters, including: age, number in each group, primary infertility, second infertility, duration of infertility, husband sperm status, and endometriosis (\( p > 0.05 \)). The authors did reconstructive surgery for all class I-II patients, retaining fallopian tube for class III-IV according to patients’ desires. In the class I group, fimbrioplasty was the main surgical method, accounting for 74.4%. Salpingolysis accounted for 20.3%. The remaining nine patients were normal pelvic. In the class II group, fimbrioplasty and salpingolysis accounted for a similar ratio; they were 50% and 49%, respectively. The remaining nine patients were normal pelvic. In the class III group, salpingolysis and single salpingectomy accounted for the highest ratios; they were 46.8% and 35%, respectively. In the class IV group, bilateral salpingectomy accounted for 50% while salpingolysis and single salpingectomy accounted for 20% and 22%, respectively. Pregnancy outcomes showed that 62 (35.5%) patients got pregnant including one abortion in 172 class I patients. Twenty-three (15.2%) patients got pregnant after reconstructive surgery except for three abortions in 151 class II patients. Six (8%) patients got pregnant in 77 class III patients. No intrauterine pregnancy was observed in 40 class IV patients except one ectopic pregnancy.

**Relationship between follow-up and pregnancy outcomes**

Figure 1 reveals the cumulative pregnancy rate in 12 month follow-up among different groups. The curve shows that the pregnancy rates increased gradually and did not reach the top in classes I and II at the end of the 12th month.
Cumulative pregnancy rates in class III increased gradually also, but at the end of the 12th month, it reached a plateau. There was no increased rate in the class IV group. At the end of the 12th month, the cumulative pregnancy rate in classes I, II, III, and IV was 35%, 15%, 8%, and zero, respectively. After surgical laparoscopy, cumulative natural pregnancy rates in the class I-II groups were significantly higher than in the class III-IV groups ($p < 0.05$).

**Discussion**

Pregnancy outcomes after reconstructive tubal surgery for adnexal adhesions and distal tubal occlusions are thought to be related to the extent of pelvic adhesion and to tubal disease. Based on various parameters, several classification systems have been proposed to assess the extent of tubal disease in order to predict pregnancy outcomes (5, 8-13). However, none of the classifications can be a one-step solution to predicting pregnancy outcomes and avoiding tubal surgeries.

The AFS score system has been used nearly 30 years since 1988. The AFS scoring system is a measurement tool to standardize the measurement of pelvic endometriosis so that doctors working at different hospitals will classify patients in a similar way. It is widely used in Chinese hospitals during laparoscopic subfertility surgery. AFS provides effective evaluation criterion for judging the degree of tubal lesions. However, because the AFS score focuses on the pelvic adhesion, and not on the tubal interluminal mucosal lesions and stenosis, this leads to insufficient or excessive assessments for tubal lesions, which then may lead to removal of normally functioning fallopian tubes or to the retention of non-functioning fallopian tubes.

Since the first report on the value of salpingoscopy at the time of microsurgery [14], there has been increasing interest in salpingoscopy and improved salpingoscopic techniques to detect intraluminal lesions [15, 16]. Now, salpingoscopy is an important tool for detecting mucosal abnormalities, and for eventually referring patients for assisted reproductive technology. Due to the great influence of severity of tubal intraluminal mucosal, authors stated that “there was no correlation between the presence or extent of pelvic adhesion and the presence or extent of intraluminal adhesion.” However, although salpingoscopy accurately assesses tubal mucosa lesions, it allowed improved selection of patients who are candidate for tubal surgery but it is not a substitute for tubal surgery.

Manna *et al.* [13] affirmed the prognostic role of salpingoscopy in infertility patients and suggested that patients with tubal infertility should be offered operative laparoscopy with salpingoscopy as the first step of treatment. Salpingoscopy combined with operative laparoscopy may be the best procedure for infertility patients [3]. However, in China, because of the high costs and lack of skilled technicians associated with salpingoscopy, application of salpingoscopy is limited.

In contrast, testing fallopian tubal patency using chromopertubation using methylene blue is cheap. Chromopertubation plays a prognostic role as the other study stated that when there is a discordant patency the pregnancy rates could be somewhat reduced [17]. The present results showed chromopertubation combined with operative laparoscopy can effectively evaluate fallopian tubal internal patency and external mechanism adhesion. The score combination connected the chromoputation (nature of the mucosal pattern, diameter of the hydrosalpinx, expandability of the ampulla) and AFS score. Egbert *et al.* analyzed the importance of three factors derived from the hysterosalpingography (nature of the mucosal pattern, diameter of the hydrosalpinx, expandability of the ampulla) and of four factors from the findings at laparoscopy (extent of adhesions, nature of adhesions, thickness of tubal wall, and diameter of the hydrosalpinx), and they indicated that a favorable score on the nature of mucosal pattern in one or both tubes concurs with good pregnancy prospects. In contrast, the presence of an unfavorable score for most of the factors in

![Graph showing cumulative pregnancy rates](image-url)

Figure 1. — Cumulative rate was the union factor. Cumulative conception rate (CCR) was calculated with the use of Kaplan-Meier survival analysis. ANOVA was used to analyze variable groups including class I, II, III, and IV, $p < 0.05$. Horizontal axis represents the month. Vertical axis represents the cumulative rate. Student–Newman–Keuls (SNK) was used for comparing the difference between two groups. Class I vs II, $p > 0.05$. Class I vs III, $p < 0.05$. Class I vs IV, $p < 0.05$. Class II vs III, $p < 0.05$. Class II vs IV, $p < 0.05$. Class III vs IV, $p < 0.05$. Cumulative pregnancy rates in class III increased gradually also, but at the end of the 12th month, it reached a plateau. There was no increased rate in the class IV group. At the end of the 12th month, the cumulative pregnancy rate in classes I, II, III, and IV was 35%, 15%, 8%, and zero, respectively. After surgical laparoscopy, cumulative natural pregnancy rates in the class I-II groups were significantly higher than in the class III-IV groups ($p < 0.05$).
at least one tube is associated with a poor fertility prognosis, regardless of the condition of the other tube [18]. According to the present authors’ classification, class I-II patients can benefit from reconstructive tubal surgery, as studies showed that after three years follow-up, mild tubal lesion and unilateral hydrosalpinx patients with tubal disease in the removal of water side, the cumulative pregnancy rate was 55% [19]. All of the hydrosalpinx patients were classified into class III or IV. Based on the cumulative pregnancy life-table, patients in class III-IV were recommended to undertake salpingectomy. It is not helpful for these patients to undergo prolonged observation periods. Salpingectomy or hysteroscopic tubal occlusion of functionless hydrosalpinx has the advantage of adding a valuable evaluation of the endometrial cavity prior to IVF/ICSI. It should be an option for treatment protocol in cases of functionless hydrosalpinx [20, 21]. They have little chance of conceiving naturally from reconstructive salpinx surgery [22, 23]. Operative laparoscopy is expected to become the last resort for guiding natural pregnancy or IVF-ET. Postoperative procedures following female pelvic reproductive surgery had no significant impact on the odds of pregnancy, live birth, ectopic pregnancy or miscarriage [24].

The present results were consistent with the management of hydrosalpinx among Society for Reproduction Endocrinology and Infertility (SREI) / Society of Reproductive Surgeons, which recommend removing a unilateral hydrosalpinx before controlled ovarian hyperstimulation [25]. Recurrent hydrosalpinx may cause failed uterine pregnancy [26, 27]. The fact that no pregnancy was observed in patients that underwent bilateral salpingostomy in the severe degree group further proved a high score-combination score is a poor fertility prognosis for patients, which was consistent with previous evidence supported that only unilateral salpingostomy for a unilateral hydrosalpinx (bilateral salpingostomy for bilateral hydrosalpinx) [28].

Conclusions

The authors suggest a longer observation period for class I-II group patients after surgical laparoscopy, while no longer than one year for class III-IV group patients.

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References

Preliminary results of tubal surgery with pregnancy outcome


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The expression of Forkhead transcription factors in decidua and placenta in patients with missed abortion

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Summary
Background: Forkhead transcription factors 3a (FOXO3a) has pleiotropic biological functions in the female reproductive tract. FOXO3a has a function in decidualization, in placental development, and also in inhibition of apoptosis. This study aims to investigate a possible role of FOXO3a in missed abortion. Materials and Methods: Decidual and placental tissue samples were obtained from the women with unwanted pregnancy as the control group and with missed abortion as the patient group. Immunohistochemistry technique was utilized to compare FOXO3a expression of the decidual cells in uterine decidual stroma and cytotrophoblast-syncytiotrophoblast cells in placental villous stroma. Immunohistochemistry was evaluated semi-quantitatively utilizing the H-score technique. Results: It was demonstrated that H-Scores of FOXO3a expression in both uterine decidual stroma were increased in the missed abortion group (255.83±12.41) than in the normal pregnancy group (133.33±17.43). It was also shown that there was no difference between non-decidual area of the endometrium of the normal pregnancy and the missed abortion group (30.33±4.32; 39.66±14.30, respectively) and placental villous stroma (13.00±1.89; 13.00±1.67, respectively). However, the immunoreactivity of cytotrophoblast and syncytiotrophoblast cells significantly increased in the missed abortion group (18.83±1.47; 322.00±6.06, respectively) than in the normal pregnancy group (11.00±1.26; 254.00±8.17, respectively) (p < 0.05). Conclusion: These data support the hypothesis that increased FOXO3a expression in missed abortion may prevent the discharge of dead fetus to maintain decidualization, prevention of oxidative stress, immunomodulation, and inhibition of apoptosis.

Key words: Forkhead transcription factors 3a; Decidua; Placenta; Missed abortion.

Introduction
Missed abortion is a pregnancy in which there is a fetal demise (usually for a number of weeks), but with no uterine activity to expel the products of conception. The prevalence of missed abortion is about 2% in singleton pregnancies at 10–14 weeks of gestation [1]. Although studies have mainly focused on diagnosis and treatment of missed abortions, attention has not been paid to the underlying causal factor of missed abortion [2].

It is known that both intertwined and coordinated endometrial and embryonic adequate expression is required for a normal implantation and gestation [3]. The number of differentially expressed genes reveals the extent of changes within the endometrium during conception. One of the them is the FOXO subfamily of Forkhead transcription factors (FOXO) proteins comprises three functionally related members, FOXO1, FOXO3a, and FOXO4, which are identified as the important downstream molecules of phosphoinositide-3-OH kinase (PI3K)/Akt pathway, and that are known to play important roles in adaptation to cellular stress, immunomodulation, and the regulation of apoptosis [4].

FOXO3 has been causally linked to multiple cellular processes, which are activated during human parturition [4, 5]. FOXO3 induces matrix metalloproteinase (MMP)-2 and -9 which actively participates the remodeling process. MMP-2 and MMP-9 have the ability to break down several proteins of the extracellular matrix (ECM) to maintain tightly controlled trophoblastic invasion and remodeling of the endometrium [6].

Decidualization is a series of proliferation and differentiation process of the endometrial stromal cells into the decidual cells. Decidual cells are thought to be involved in embryo implantation and in the maintenance of pregnancy through the regulation of trophoblastic invasion, the development of the blastocyst, hormonal secretions, and the provision of protection for the embryo from maternal immune rejection. The decidualization process is poorly understood at the molecular level; a key stimulus is progesterone action on estrogen-primed endometrial stromal cells, which leads to dramatic transcriptional reprogramming [7]. Kajihara et al. reported that FOXO transcription factors upon endometrial decidualization favor tissue preservation and in-
tegrity over apoptotic clearance of defective cells when faced with prolonged oxidative insult during pregnancy [8].

During normal pregnancy, major fluctuations in oxygen concentrations occur at the feto-maternal interface; the dramatic changes in oxygen tension at the utero-placental interface lead to oxidative stress and induce a burst of intracellular reactive oxygen species [9]. The overexpression of a constitutive active FOXO3a mutant in decasualizing cells causes apoptosis, and the silencing of endogenous FOXO3a confers resistance to oxidative apoptosis in undifferentiated cells [8].

Thus, the aim of this work was to analyze the localization and distribution of FOXO3a expression in decidual and placental tissue both in normal pregnancy and missed abortion, to evaluate pathogenesis of missed abortion.

Materials and Methods

The endometrial tissue samples of 15 unwanted pregnancies (five to ten weeks gestational age) and 19 missed abortions (six to 11 weeks gestational age) were obtained with informed consent and in accordance with the requirements of the Ethics Committee of Celal Bayar University. The mean age of women was 27.53 years; the range 21-37 years for normal pregnancy group and mean 28.74 years; range 18-41 years for the missed abortion group.

The abortions were diagnosed by transvaginal ultrasound and were confirmed by repeat ultrasound prior to the dilation and curettage procedure. Chorionic villi and maternal decidua were separated and cleaned. Placental and decidual tissues were fixed in 10% buffered formalin solution and embedded in paraffin. The tissue samples were stored at -20℃ for five minutes, the sections were stained with a substrate system containing diaminobenzidine to detect the immunoreactivity, and then were stained with Mayer’s hematoxylin for counterstaining. They were covered with mounting medium and observed with light microscopy.

Immunostaining for FOXO3a was evaluated semi-quantitatively by means of FOXO3a analysis. Immunostaining intensity was categorized into the following scores: 0 (no staining), 1 (weak, but detectable, staining), 2 (moderate staining), and 3 (intense staining). A HSCORE value was derived for each specimen by calculating the sum of the percentage of cells for fibroblast and decidual cells in uterine decidual stroma; and fibroblasts and mesenchymal cells in placental villous stroma that stained at each intensity category multiplied by its respective score, by means of the formula H-score=∑Pi(i+1), where i=intensity of staining with a value of 1, 2 or 3 (weak, moderate or strong, respectively) and Pi is the percentage of stained epithelial cells for each intensity category.

Statistical analysis

Immunohistochemical values (mean ± SD) data are summarized in Table 1. The comparisons between the two groups were performed by means of Mann-Whitney U test. A p-value < 0.05 was considered significant. The statistical analysis was performed via SPSS statistical software, version 10.0. Error bars of H-Score of HBEGF expression is shown in Figure 1.

Results

In the examination of the deciduas of the samples stained with FOXO3A antibody, the decidua of the group of missed was determined to have a denser immunoreactivity (255.83±12.41) than the control group (133.33±17.43) and a statistical difference between them (p <0.05) was found. When examining the non-decidual regions, the immunoreactivity of both groups was observed to be relatively weaker than decidual region, and a statistically significant difference was not determined between the non-decidua of the control group (39.66±14.30) and the missed group (39.66±14.30) (Table 1, Figures 1, 2).
Moreover, in the examination of FOXO3A immunoreactivities of syncytiotrophoblast and cytotrophoblast forming chorionic villi, the syncytiotrophoblast immunoreactivity of the missed group (18.83±1.47) was observed to be stronger than the control group (11.00±1.26). When examining the cytotrophoblasts, unlike syncytiotrophoblasts, the FOXO3A immunoreactivity was determined to be rather stronger, and the missed group was observed to have a stronger immunoreactivity than the control group (respectively, 322.00±6.06; 254.00±8.17), and a statistically significant difference ($p < 0.05$) between them was as also determined (Table 1, Figures 1, 2).

In the comparison of the immunoreactivity of the stromas of the control group and the missed abortion group, it has been determined that both groups have showed a weak immunoreactivity (13.00±1.89; 13.00±1.67) and there has not been a statistically significant difference between them (Table 1) (Figures 1, 2).

**Discussion**

This is the first study to report on the immunolocalization of FOXO3 in human decidua and placenta of missed abortion. The authors observed that FOXO3 is found in decidual and placental cells of the normal pregnancy and missed abortion groups. FOXO3 expressions are higher in decidual cells, cytotrophoblast, and syncytiotrophoblast in missed abortion group compared to normal pregnancy. The localization of FOXO3 in decidua and placenta of both groups suggests that it has several potential functions during pregnancy and missed abortion pathogenesis.

The authors observed that strong FOXO3a immunoreactivity both in decidua and in missed abortion and normal pregnant endometrium and have supposed that FOXO3a has an important role in maintaining the decidualization during pregnancy. FOXO proteins are not constitutively expressed in human endometrium. Kajihara et al. also reported that prolonged exposure to H$_2$O$_2$ induced FOXO3a expression in undifferentiated cells [8]. Christian et al. have shown that Forkhead homologue in rhabdomyosarcoma is induced in decidualizing endometrium and participates in PKA signal transduction through its ability to interact and transcriptionally cooperate with C/EBP β [10]. The decidua, that is, undergoes noticeable differentiation into the deciduum, a specialized and neovascularized tissue that encapsulates the developing embryo to provide nutrients and control trophoblastic invasion [11].

It is known that FOXO proteins regulate ECM remodeling enzymes and also FOXO3 induces MMP-2 and MMP-9 enzymatic activities [5]. The present authors also considered that FOXO3a may be responsible for pregnancy process and for maintaining decidual remodeling via MMP-2 and MMP-9. Fontana et al. evaluated in vivo and in vitro decidual MMP-2 and -9 activities on 10th day of gestation in CF-1 mouse and suggested specific roles for MMP-2 and MMP-9 in decidual tissues [12]. Matsumoto et al. reported
that the activity of MMP-2 and MMP-9 might increase during decidualization without a corresponding increase of the expression of these genes [13]. Nissi et al. also reported that increase in serum levels of MMP-2/TIMP-2 complex and MMP-9 as well as their inhibitors TIMP-1 and TIMP-2 could reflect the altered architecture of the extracellular matrix during pregnancy [14].

MMP-2 and MMP-9 are not essential for the decidualization, but also they are essential for invasion of EVT cells into endometrial stroma. Invasion of trophoblastic cells into the maternal endometrium is an important step in human embryo implantation and placentation. Cochem et al. reported that this process requires MMP-2 and -9 [15], which are considered key enzymes in degradation of basement membrane, that mainly consist of type IV collagen [16]. Staun-Ram et al. reported that MMP-2 immunoreactivity has been detected in decidual cells but MMP-9 is dominant on the trophoblasts of six to eight weeks of gestation and claimed that MMP-2 is the key regulator of trophoblast invasion in early pregnancy expression and in importance of MMP-2 and -9 in human trophoblast invasion [16].

The present authors have found that FOXO3a immunoreactivity is higher in missed abortion decidua than control group decidua and have supposed that FOXO3a has played an important role in missed abortion pathogenesis. High FOXO3A immunoreactivity in the missed abortion cases of this study may be in order to provide the increase in resistance of decidual cells to mild oxidative stress beside the decidualization. It is known that oxidative stress induces FOXO3a and apoptosis in undifferentiated but not in decidualized HESCs. They have claimed that the FOXO3a expression in the missed abortion group during endometrial decidualization favors tissue preservation and integrity over apoptotic clearance of defective cells when faced with prolonged oxidative insult during pregnancy. Despite the low levels of oxidative stress, the cells might continue to survive due to the effect of FOXO3 on cell survival at the same time, and this may obstruct the excretion of embryo. It is known that excessive oxidative stress in endometrial tissue brings about pregnancy losses and fetal growth retardation through pre-eclampsia by causing cell death [17], and such cases are given antioxidants during pregnancy [8]. Therefore, randomized studies have shown that giving vitamins C and E reduces the incidence of pre-eclampsia in hazardous preeclampsia cases [18].

The increase of FOXO3 immunoreactivity in the missed abortion group may be related with immunoregulation, and thus it hinders the prevention of discharge of the embryo. FOXO3 is a trigger for apoptosis through upregulation of genes necessary for cell death [19]. It is known that Fas-L expression has been reported in human endometrium as well as in the immune-privileged tissues, including testis, cornea, trophoblast, and cancer cells [20]. Fas-FasL system plays an important role in the mechanism underlying this immune-privileged status [21]. Fas-L and C-FLIP are expressed in decidual cells during pregnancy, and this increase is not linked to the elimination of decidual cells but it could be associated with the elimination of activated T cells in order to provide maternal immunotolerance [22].

In the examination of the placental samples of the control group and missed abortion group, the immunoreactivity of FOXO3 has been observed to be rather low in both groups but it has also been determined to increase slightly in the missed abortion group. Syncytiotrophoblasts are known to be in direct contact with maternal blood and to have a critical role in fulfilling the placental functions. It has been reported that FOXO3A plays an important role in the complications of pregnancy, and the increase in syncytiotrophoblasts causes intrauterine growth restriction and preeclampsia by bringing about apoptosis [23]. The disturbances in syncytiotrophoblast are known to obstruct the transmission of nutrients from mother to fetus [24], and the activation of FOXO proteins are also known to disrupt glucose metabolism [25]. Therefore, the moderate expression of FOXO3A in syncytiotrophoblast has been suggested to inhibit apoptosis and to have a role in the continuation of pregnancy. Furthermore, the overexpression of FOXO3 in missed abortion has a critical role in the pathogenesis missed abortion and leads to the continuation of pregnancy triggered by increased decidualization and prevents apoptosis in both decidual and syncytiotrophoblast cells.

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The expression of Forkhead transcription factors in decidua and placenta in patients with missed abortion


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Ultrasonography versus laparoscopy in transcervical resection of septa: a randomized clinical trial

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Summary
Objective: To compare the effects of ultrasonography with laparoscopy on transcervical resection of septa (TCRS). Materials and Methods: The study included 126 patients with uterine septum at the present hospital between January 2010 and August 2012 that were randomly divided into two groups. Seventy patients had TCRS monitored by ultrasound (ultrasound group) while 56 patients were monitored by laparoscopy (laparoscope group). Both groups were followed up for six to 24 months. The intraoperative status, short-term and long-term complications after operation, and pregnancy outcome of two groups were compared. Results: The operations of both groups were successfully completed. The operating time, the first time to get out of bed, postoperative 24hNRS (numeric rating scale) values, postoperative hospital stay, and the incidence of postoperative septum residue of ultrasound group were significantly less than laparoscope group (p < 0.05). No statistical differences were observed in intraoperative complications and pregnancy ratio between the two groups. Conclusion: Both ultrasound and laparoscope monitored TCRS were safe and effective in the treatment of uterine septum. Ultrasound monitored TCRS was more simple, economical, accurate, and non-invasive. For patients without abnormal lesions in pelvic cavity, the present authors tend to choose the ultrasound monitored TCRS.

Key words: Uterine septum; Ultrasound; Laparoscope; Transcervical resection of septa.

Introduction
Uterine septum is the most common type of uterine malformations and accounts for approximately 80% to 90% of uterine malformations [1]. Uterine septum is related to infertility, miscarriage, premature birth, and restriction of intrauterine fetal growth [2]. The purpose of the uterine operation is to restore the normal shape of uterine cavity which is necessary for pregnancy. Traditional abdominal uteroplasty includes abdominal incision of uterus, uterine septum resection, and suture with disadvantages of major trauma, long recovery time, and influence on pregnancy. Transcervical resection of septa (TCRS) with advantages such as safety, less surgical trauma, and shorter operating time has become increasingly popular in clinical application since 1981 [3]. Besides good hysteroscopic surgical skills, appropriate monitoring method is the key to ensure successful operation, decrease operating time, and reduce the postoperative complications.

Both ultrasonography and laparoscopy are used clinically with respective advantages and disadvantages. The present work compared the effects of ultrasonography with laparoscopy on TCRS operation and to choose the appropriate monitoring method during TCRS operation.

Materials and Methods
Clinical data
The study included 126 patients with uterine septum diagnosed by 3D ultrasound and hysteroscopy that were selected from the First Affiliated Hospital of Zhengzhou University from January 2010 to August 2012. The patients were randomly divided into two groups. Seventy patients were monitored by ultrasound (ultrasound group) while 56 patients were monitored by laparoscopy (laparoscope group) during the TCRS operation. As shown in Table 1, the general information of the patients in two groups had no significant difference (p > 0.05). All the patients had no complications. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Operative procedure
All patients underwent surgery by experienced physicians and 400 μg of misoprostol was placed in the posterior vaginal fornix on the night before surgery. The partial uterine septum was cut using needle or ring electrode from the transverse tip to the septum base. The complete uterine septum was first made an incision in 0.5 cm above the internal orifice of uterus and then the septum was cut using needle or ring electrode from the transverse tip to the septum base. The electrotomy mirror direction and cutting depth were monitored by abdominal ultrasonography in ultrasound group. When the distance between cutting end and serosa in cervical bottom was one cm and the thickness of the cervical bottom was coherent, the cutting was stopped. In laparoscopy
group, the hysteroscope was placed in the uterine horn recess and the operation was stopped when the uterus showed even light transmission. The uterine cavity of all patients was injected with biomedical fibrin glue and the metal ring was placed in the uterus after surgery. Three artificial menstrual cycles were induced (dydrogesterone ten mg/day for five days + estradiol valerate one mg/day for 21 days) to promote endometrial repair. Hysteroscopy and ring removal were performed three months after surgery. The operating time, complications such as uterine perforation and water intoxication, the first time to get out of bed, postoperative 24hNRS values, and postoperative hospital stay were recorded.

Follow-up

Ultrasonography combined with hysteroscopy was performed in all patients for diagnosis of uterine septum and measurement of the residue three months after surgery. Pregnancy and delivery follow-up for six to 24 months was done using single blind method.

Statistical analysis

All the data were analyzed by SPSS17.0 software and were expressed as the mean ± standard deviation. Differences in measurement data were compared using t inspection and count data were compared using Fisher’s Exact Test or χ² inspection. A p < 0.05 was considered statistically significant.

Results

Perioperative status

All patients in two groups underwent surgery successfully. As shown in Table 2, the operating time, the first time to get out of bed, postoperative 24hNRS values, and postoperative hospital stay of ultrasound group were significantly less than laparoscope group (p < 0.05).

Complications

All patients had no complications of uterine perforation, water intoxication, and uterine rupture. Three months after surgery, five cases lost of follow up and eight cases had intrauterine adhesions in ultrasound group. In laparoscope group, two cases lost of follow up and eight cases had intrauterine adhesions. Five cases had septum residue with the length of 1.0 - 2.3 cm in which three cases had TCRS twice. As shown in Table 3, there was significant difference between the two groups in the ratio of septum residue (p < 0.05). The ratio of short-term and long-term complications in two groups had no significant difference.

Followed up between three months and two years, eight cases lost to follow up, and 44 cases became pregnant including eight cases of spontaneous abortion, three cases of premature birth, two cases of tubal pregnancy, and 31 cases of term birth in ultrasound group. In laparoscope group, five cases lost to follow up and 39 cases became pregnant, including nine cases of spontaneous abortion, two cases of premature birth, one cases of tubal pregnancy, and 27 cases of term birth. There was no statistically significant difference between the two groups in the pregnancy outcome (p > 0.05).

Discussion

Uterine embryogenesis derived from Mullerian duct. From four to six weeks of embryonic development, paravesical ducts fused and the middle septum began to degenerate which gradually formed the vagina and uterus. When the septum did not disappear or disappear incompletely, it became varying degrees of uterine septum. Uterine septum destroyed the normal shape of uterine cavity and induced irregular differentiation of endometrial gland which might lead uncomfortable nidation or incomplete decidualization. Therefore, the patients with uterine septum often presents with infertility, recurrent spontaneous abortion and premature birth [4].

To resect the uterine septum and correct the uterine cavity shape is the most important way to treat uterine septum. Not all patients with uterine septum require surgery except those have abnormal gestation and birth or patients with long term infertility [5]. TCRS by hysteroscopy is a minimally invasive surgery to uterine cavity [6, 7]. Among all the uterine malformations, uterine septum is the only one which can be treated and corrected by hysteroscopic sur-

Table 1. — General information of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average age (years)</th>
<th>Septum type</th>
<th>Gestation and birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>25.6 ± 3.4</td>
<td>18</td>
<td>52</td>
</tr>
<tr>
<td>Laparoscope</td>
<td>27.5 ± 2.8</td>
<td>10</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 2. — Perioperative status (x ± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cases</th>
<th>Operating time (min)</th>
<th>First time to get out of bed (h)</th>
<th>24hNRS values</th>
<th>Postoperative hospital stay (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>70</td>
<td>19.26±2.65</td>
<td>7.76±1.37</td>
<td>3.11±1.11</td>
<td>3.39±0.77</td>
</tr>
<tr>
<td>Laparoscope</td>
<td>56</td>
<td>48.91±6.79*</td>
<td>10.59±1.58*</td>
<td>4.55±1.09*</td>
<td>4.14±0.77*</td>
</tr>
</tbody>
</table>

* p < 0.05.

Table 3. — Comparison of short-term and long-term complications (cases).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Intraoperative complications</th>
<th>Short-term complications after surgery</th>
<th>Long-term complications after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound</td>
<td>0</td>
<td>0</td>
<td>8 (8/65)</td>
</tr>
<tr>
<td>Laparoscope</td>
<td></td>
<td>5 (5/54)*</td>
<td>8 (8/54)</td>
</tr>
</tbody>
</table>

* p < 0.05.
Ultrasoundography versus laparoscopy in transcervical resection of septa: a randomized clinical trial

Both ultrasound and laparoscope monitored TCRS in pregnancy outcome. There was no significant difference between the two groups in pregnancy outcome. The ultimate aim of TCRS is to improve pregnancy outcome. There was no significant difference that monitored by laparoscope. The ultimate aim of TCRS could effectively treat uterine septum. Ultrasound monitored TCRS is more simple, economical, accurate, and non-invasive. For patients without abnormal lesions in pelvic cavity, the present authors tend to choose the ultrasound monitored TCRS.

References


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Ovarian mature teratoma: a ten year experience in our institution

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Summary
Purpose of investigation: To describe the surgical management and diagnoses of mature ovarian teratomas and ovarian strumas in the present centre. Materials and Methods: Descriptive retrospective analysis of cases of mature ovarian teratoma at the present university-associated hospital over ten years. Results: The mean age was 29 years and in 17 patients the diagnosis was made during other surgery. When surgery was planned, the approach was 80.2% laparoscopic and 16.1% laparotomic. In the laparoscopy group more cases had been diagnosed previously as dermoid cyst by ultrasound and fewer days of hospital admission. In the laparotomy group the authors found higher ultrasound size and the size in the gross pathology description. With regards to treatment, 45.3% of cases underwent ovariectomy and 49.3% a cystectomy. Comparing these two groups, the authors found larger pelvic mass size in the group of ovariectomies. Healthy ovarian tissue in the removed specimen was found more frequently in the ovariectomies group (29.1%) but also in some cystectomies (7.5%). Conclusions: The surgical treatment of the ovarian mature teratoma in the present center was directed on the basis of ultrasound diagnosis, ultrasound tumor size, and the existence of associated gynecologic pathology. The authors strongly recommend a laparoscopic approach and a cystectomy in order to preserve fertility especially in young women.

Key words: Mature ovarian teratoma; Ovarian dermoid cyst; Ovarian masses; Surgical approach; Benign ovarian masses.

Introduction
Ovarian mature teratomas or dermoid cysts are some of the more frequent benign ovarian tumors representing 15% to 20% [1]. Teratomas can occur at any age but the incidence is highest from 20 to 40 years of age [2], with a low rate of malignant transformation [3]. Dermoid cysts arise from pluripotent stem cells with elements of the three germ layers [4, 5], the pathogenesis is thought to be related to a genetic factor [6] and one of the current hypotheses is the parthenogenetic origin of it [7]. The diagnosis of ovarian teratoma is commonly made by ultrasound with a high sensitivity and positive predictive value [8, 9]. The common treatment of this condition is the cystectomy with a laparoscopic approach [10, 11]. The authors retrospectively analyzed trends in management at the present institution over a period of ten years.

Materials and Methods
The authors retrospectively selected cases from the histological reports of mature ovarian teratoma operated on at the Hospital Sant Joan de Deu from 2001 to the beginning of 2011. They found 160 cases with a histological diagnosis of mature teratoma after surgery. They collected epidemiologic data and outcome and compared the group of patients with a laparoscopic surgical approach against the patients with laparotomy surgical approach. They also compared the surgical techniques: cystectomy versus ovariectomy.

The analysis was performed with SPSS version 20. Groups were compared using student t-test, non-parametric Mann-Whitney U test and chi square, each with a significance level of p < 0.05.

Results
Age range was between four and 69 years with a median and mean of 29 and 29.4 years, respectively. (Figure 1). Taking the gynecologic history into account, 100 patients (62.5%) were nulliparous while 60 patients (37.5%) had one or more children, 157 cases (98.1%) had no cancer history, two patients had a history of breast cancer, and one case of endometrial cancer. Whereas 131 (81.9%) had no previous abdominal surgery, 29 cases (18.1%), had at least one previous abdominal surgery and 17 women (6.2%) had at least one previous ovarian surgery from other causes than ovarian mature teratoma.

Diagnosis
First stage diagnoses were made in the clinic, 21 patients (13%) had pain or menstrual disturbances, and 41 cases (25.6%) had complaints of abdominal mass or swelling sensation; on the other hand 83 cases (51.9%) had no symptoms and the pelvic mass was diagnosed during a routine pelvic examination or in abdominal surgery performed for other reasons. In the remaining cases, this variable was not mentioned in the reports.

The diagnosis of ovarian teratoma was highly suspected by pelvic ultrasound in 93 cases (58.1%); another seven cases (4.3%) were reported to have a complex ovarian mass and 40 cases (26.3%) were thought to have a benign ovarian condition other than teratoma. Out of these 140 cases,
24 (25%) required a complimentary computerized tomography (CT) and nine (5.6%) a magnetic resonance imaging (MRI). There were another two cases that the authors diagnosed by CT without having had a previous pelvic ultrasound. There were 17 cases (10.6%) that were a coincidental finding during abdominal surgery, 15 in a cesarean section and two during an appendectomy.

**Surgical approach**

Laparoscopic approach was used in 114 cases (80.2%) and laparotomy in 23 cases (16.1%). In six other cases (3.75%) laparoscopy was converted to laparotomy. These numbers were made from a total of 143 cases, as the authors ruled out the dermoid cysts that were found as part of other surgeries. Laparoscopy and laparotomy groups were homogeneous for age, parity, and surgical history with no significant differences (Table 1).

There was no significant difference in the clinical symptomatology between the two groups: 42 women (36.8%) had pain or swelling in the group of laparoscopies and eight women (34.7%) in the group of laparotomies (Chi square, $p = 0.85$).

Dermoid cyst was suspected by ultrasound more frequently in the laparoscopy group (75 women or 65.7%) than in the laparotomy group (nine women or 39%) with significant differences (Chi-square, $p = 0.01$).

In seven women where the ultrasound was suspected to be malignant, three of the cases required additional medical imaging procedures such as CT or MRI. In four women the approach was laparoscopic and in two women laparotomic (two had associated gynecological pathology); the remaining case was begun with a laparoscopy and converted to laparotomy, and neither of them confirmed malignancy in the posterior histological analysis.

The group with the laparoscopic approach had a mean of 2.88 days of hospital admission (one to ten days). The group with the laparotomies had a mean of 5.97 days of hospital admission (three to 12 days); the two groups did not follow a normal distribution and the authors found significant differences between them (Mann-Whitney U, $p = 0.00$).

On the one hand the mean size measured by ultrasound in the laparoscopy group was 65.8, with a non-normal distribution; on the other hand the size measured by ultrasound in the laparotomies followed a normal distribution with a mean of 96.0 mm, the non-parametric test for comparison of means found significant differences between those two groups (Mann-Whitney U, $p = 0.00$) (Figure 2). Also there were differences between the two groups with regards to the size measured in the gross pathologic description, laparoscopies (mean of 56.08 mm) and laparotomies (mean of 78.53 mm). Aside from these there was less associated gynecological
pathology in the group with laparoscopies (12 cases or 10.5%) than in the group with laparotomies (12 cases or 52.1%), finding significant differences between those groups (Chi square, \( p = 0.00 \)).

There were also seven cases of ovarian torsion in the group of laparoscopies (6.1%) and four patients in the group of laparotomies (17.4%), with no significant differences between them (Chi-square, \( p = 0.07 \)). One case of ovarian torsion was in the group of the laparoscopy converted to a laparotomy.

There were nine major complications in total (5.7%), five (4.3%) in the laparoscopic group and three (13%) in the laparotomies group, and no differences were found between them (Chi-square, \( p = 0.76 \)). These complications were hemorrhages, hemoperitoneum, abdominal wall hematoma, and abdominal bound herniation. The remaining case with a complication was approached by laparoscopy and converted to laparotomy (Table 1).

**Ovarian torsions**

The authors had 12 cases with ovarian torsions associated with mature ovarian teratoma; sonographic size of torsioned cysts had a mean of 92 mm and a median of 100 mm and sonographic size of cysts that were non-torted had a mean of 72.3 mm and a median of 60 mm - both with a non-normal distribution and statistical differences (Mann Whitney U, \( p = 0.01 \)). Also the means of the size of the gross pathological description between torted (103.8 mm) and non-torted cysts (66.9 mm) were statistically different (Mann-Whitney U, \( p = 0.00 \)).

**Type of surgery**

Surgical techniques used were in 79 cases (49.3%) a cystectomy and in 73 cases (45.6%) an ovariectomy (Table 2). Comparing the mean age of the women in the group of ovariectomies (mean age of 29.7, SD: 13.6) with the cystectomies (mean age of 28.9, SD: 12.3), both groups followed a normal distribution and no statistically significant differences were found (\( t \)-test, \( p = 0.70 \)).

Six women in the ovariectomy group had a hysterectomy and ovarian removal; all were indicated by coexisting uterine pathology such as uterine fibroids. Their mean age was 46.8 years with a median of 49 without following a normal distribution.

A cystectomy was performed in 79 women of which 51 (64.5%) had not had children and 28 (35.4%) had one or more children. An ovariectomy was performed in 73 women, of which 46 (58.3%) had not had children and 26 patients (36.1%) had one or more children. The variables surgery and parity were independent and were distributed equally regardless of the other variable (Chi square, \( p = 0.51 \)).

The authors compared the ultrasound size in the group of ovariectomies (mean size of 83.3 mm, SD: 47.4, and normal distribution) with the ultrasound size in the group of cystectomies (mean of 63.6 mm and median of 60 mm, and non-normal distribution), and there were statistically significant differences (Mann-Whitney U, \( p = 0.02 \)). The same analysis for macroscopic size revealed significant differences between the two groups (Mann-Whitney U, \( p = 0.00 \), with a mean macroscopic size of 87.7 mm in the women with ovariectomies and a mean macroscopic size of 55.4 mm in the group of cystectomies; both followed a non-normal distribution.

In the histological analysis of surgical specimens, 158 were diagnosed as ovarian mature teratomas and two as ovarian strumas without finding malignancy in any of them.

In most of the cases, ovariectomies were performed assuming that the dermoid cysts were so large that there was no healthy ovarian tissue remaining and also cystectomies were performed in order to preserve as much healthy ovarian tissue as possible. Nevertheless, in 29 women, healthy ovarian tissue was found on histological examination, 21
of those cases (29.16%) in the group of the ovariectomies and six cases (7.59%) in the group of the cystectomies. The remaining cases were in the group of ovarian mass resection. (Table 3 and Figure 3).

The mean age of women where healthy ovarian tissue was found was 27.73 (SD: 12.67) and the mean age of women where not healthy ovarian tissue was found was 30.11 (SD: 13.10); both had a normal distribution and no significant differences were found ($t$-test, $p = 0.55$).

Discussion

In the present study the range of the women’s ages was between four and 69 years, with a mean of 29, which is consistent with most of the literature [9, 12, 13]. Regarding the clinical diagnosis, different series found an asymptomatic tumors rate ranging from 21.1% to 77% [3, 12, 13]. As reported in the present case study, 51.9% women were without any symptoms and 38.6% women had pain or abdominal swelling.

Diagnosis was made by ultrasound except in 17 cases in which dermoid cyst was coincidentally diagnosed during other surgery and few cases diagnosed at first instance by CT. In the present study the ultrasound sensibility was 58.1% which differs from some literature where the ultrasound sensibilities for dermoid cyst ranged from 85% to 93% [8, 11]. Although the present authors had taken into account characteristic ultrasound features such as shadowing echodensity and regional bright echoes, their low sensibility could have been due to a mistaken diagnosis as ovarian endometrioma which is known to mimic the ultrasound appearance of dermoid cyst [8].

As at many other institutions the laparoscopic approach was in most cases mandatory, but that continues to change from the late nineties where abdominal open surgery was recommended to avoid spillage [3]. Nowadays some institutions still recommend the abdominal approach in those cases where giant teratomas of more than ten to15 cm are suspected, in order to prevent spillage and to have better management in case of malignancy [12, 13]. On the other hand other groups did not find cases of granulomatous peritonitis after spillage where copious lavage of the abdominal cavity was made [14] and most of them avoided spillage when an endobag was used and meticulous surgical technique took place [15-17], hence they recommend the laparoscopic approach despite the size of the mass.

Although the present authors did not report in their cases studied rupture or spillage of cysts, they used the laparoscopic approach more frequently and have also noticed the increasing tendency in the number of laparoscopy in recent years in the present center (Figure 4).

In the present case study the authors had a more frequent laparoscopic approach but the sizes of the mass suspected by ultrasound were larger in diameter in the group of patients that had open abdominal surgery. Also the existence of additional gynecological pathology was also more common in the laparotomy approach. When a dermoid cyst was suspected by clinic and ultrasound features, the laparoscopic approach was more often selected. Nevertheless, no differences were made in the approach if the authors suspected a complex ovarian mass by ultrasound. They also realized that the laparoscopic group had better results in terms of days of admission as data reported [13,17] but did not find any differences in terms of postoperative complications.

The present authors found 12 women with torted cysts, a 7.5% of the total cases which is a little higher than in the lit-
tement where the rate was 4.9% [3]. Most of them were managed laparoscopically as literature recommends [18].

In other reports the decision of cystectomy or ovariectomy is related to age, gravity, and parity [3] but in the present study the authors saw no differences in terms of age and parity.

The parameters that suggest whether to have conservative surgery or not were the larger diameter of the mass assessed by ultrasound or by gross pathologic description and they were significantly larger in the group where an ovariectomy was performed.

The authors had no malignant result in the present case study, but others revealed rates of malignancy from 1.4% to 1.52% [3, 12], however those had larger sample groups and most of the malignant transformation cases were found in women with a median age of 50 years with a mean diameter of 92 mm.

Zupi et al. [11] describe in their report how in 55 cystectomies with the laparoscopic approach they found a functional ovarian volume greater than 1.5 cm³ in the ultrasound performed three to six months after the surgery, even if in the first ultrasound no surrounding ovarian tissue had been detected. These are consistent with the fact that a 29.16% of the present patients had healthy ovarian tissue in the retrieved ovarian mass. This is important for young patients for whom preservation of reproductive capacity is the first priority. The authors also had some adolescents or infant patients, in their cases according to other cases reported [19] in which it is possible to be less aggressive in surgery using a laparoscopic approach.

Conclusion

Analyzing the present case study and comparing it with other case reports made previously, the authors can conclude that teratomas are more frequently associated with young women for which conservative procedures are recommended. The laparoscopic approach is thought to be safer and a cystectomy is preferred rather than removing the complete ovary in order to preserve as much ovarian tissue as possible.

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References


Chronic action of lamivudine and ritonavir on maternal and fetal liver and kidney of albino pregnant rats (Rattus norvegicus albinus, Rodentia, Mammalia): morphological and biochemical aspects

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Summary

Purpose: To investigate the morphological and biochemical effects of lamivudine associated with ritonavir on maternal and fetal livers and kidneys throughout the pregnancy of albino rats. Materials and Methods: Forty pregnant rats were divided into four numerically equal groups: control (C), experiment 1 (E1), experiment 2 (E2), and experiment 3 (E3). Only distilled water was given to the control group, while groups E1, E2, and E3 received, respectively, 5, 15 and 45 mg/kg of lamivudine associated with 20, 60, and 180 mg/kg of ritonavir, per day, throughout the pregnancy. On the 20th day of the pregnancy, the histological structure of the maternal and fetal livers and kidneys was analyzed by means of optical microscopy, along with the blood concentrations of AST, ALT, urea, and matrix creatinine. The numerical variables were analyzed using the Kruskal-Wallis test and Dunn’s multiple comparison test. Results: The histological alterations occurred in both the maternal livers and the maternal kidneys, particularly in group E3, which received the greatest therapeutic dosage (nine times). The blood levels of ALT in group E3 were significantly lower than in the other groups ($p = 0.0037$). The urea and creatinine levels in the blood were significantly lower in group E1 ($p = 0.0420$ and $p = 0.0108$, respectively). Conclusions: The association of lamivudine and ritonavir affected the histological structure of the kidneys of the matrices of group E3. There was a significant decrease in the blood values of urea e creatinine in group E1.

Key words: Lamivudine; Ritonavir; Histology; Biochemical; Teratology; Pregnant rat.

Introduction

The acquired immunodeficiency syndrome (AIDS) is a severe multisystemic disorder that affects the immune system, causing severe immunosuppression and, consequently, the appearance of opportunistic infections and neoplasia [1]. The liver and the kidneys can be affected in this syndrome by opportunistic infections, neoplasia, use of drugs for treatment of infections and, particularly, by use of antiretroviral drugs [2, 3]. Drug-induced hepatitis associated with use of antiretroviral drugs as protease inhibitors, particularly ritonavir, seems to be more frequent among patients with previous infection by the hepatitis B or C viruses [4, 5].

Although monotherapy using zidovudine was initially recommended, pregnant women should receive combined regimens of more potent drugs, because this measure has been correlated with a smaller rate of vertical transmission [6]. The current recommendation for use of antiretroviral drugs is an aggressive combination of drugs that suppresses viral replication as much as possible, preserves immunological function, and minimizes development of viral resistance [7].

Antiretroviral therapy during pregnancy using multiple antiretroviral medications, in comparison with absence of treatment or with treatment using only one drug, has not been associated with increased rates of premature labor, low birth weight, low Apgar scores or stillbirth [8]. A combination of three or more antiretroviral agents is recommended during pregnancy, but it is worth emphasizing that studies evaluating the safety of these drugs during pregnancy, regarding their toxicity and teratogenic potential, are necessary [9, 10].

The antiretroviral drugs administered to pregnant women generally cross the placenta, with consequent exposure of...
the developing embryo and fetus to their pharmacological and teratogenic effects. The critical factors that affect placental transfer of drugs and their effects on the fetus include: the physical-chemical properties of the drug, the speed at which it crosses the placenta, and the amount that reaches the fetus; the duration of exposure to the drug, the distribution characteristics of the drug in different tissues of the fetus, the development stage of the placenta and fetus at the time of exposure to the drug, and the effects of drugs used in association.

Following the notable success in reducing vertical transmission, through combined antiretroviral therapy, attention is now focused on the safety of these drugs for the mother-fetus pair. Not always have studies shown agreement regarding the pharmacokinetics and pharmacodynamics of antiretroviral drugs during pregnancy. Placental transference to the fetus is variable, and the characteristics of the receptors and the responses produced from the associations of drugs are not always elucidated. The potential for toxicity in the mother and in the fetus is always a concern. Although the benefits surpass the potential risks for the fetus, with regard to prevention of vertical transmission, there are legitimate concerns about the use of antiretroviral drugs during pregnancy.

With the aim of better observing the effects of associations of antiretroviral drugs during pregnancy, the authors decided to carry out this experiment among pregnant albino rats, with the objective of evaluating the morphology and histology of the maternal and fetal livers and kidneys and the maternal hepatic and renal biochemical parameters, with use of lamivudine in association with ritonavir throughout the pregnancy.

Materials and Methods

Albino Wistar rats (Rattus norvegicus albinus; Rodentia; Mammalia) of the EPM–1 lineage were used. They were virgin adults of approximately 90 days of age, weighing close to 200 g, from the Central Vivarium of the Federal University of São Paulo (UNIFESP). This experiment was approved by the Institution’s Research Ethics Committee, under the number 1078/05. The animals were raised and maintained at the Central Vivarium of UNIFESP, where they remained confined in metal cages of dimensions 45 x 30 x 15 cm (length, width, and height, respectively). Five animals were kept in each cage, with ad libitum food and water acidified at pH 2.7-3.1, for a 15-day adaptation period. The environmental temperature was 22°C ± 2°C, and artificial lighting produced by fluorescent 40-watt lamps was used, with photoperiods of 12 hours of light and 12 hours of dark. The cages were cleaned and the sawdust was changed daily. The air was filtered with 95% efficiency for five μm particles.

After the adaptation period of approximately 15 days, the animals were mated in the proportions of one male for every two females, and the beginning of the pregnancy was determined through the Hamil-0n and Wolf technique [11], i.e. detection of the presence of spermatozoids in the vagina of the rat on the morning after mating. This was considered to be day zero of the pregnancy. The pregnant rats were confined in individual cages measuring 30 x 15 x 12 cm (length, width, and height, respectively), and underwent random distribution into four groups of ten animals each.

The animals were divided into four groups: control (C), experiment 1 (E1), experiment 2 (E2), and experiment 3 (E3). Group C did not receive any drug, but received two ml of distilled water, by means of gavage, in a single daily dose, from day zero to the 20th day of the pregnancy. Group E1 received five mg/kg/day of lamivudine in association with 20 mg/kg/day of ritonavir, in a single daily dose, orally means of gavage, from day zero to the 20th day of the pregnancy. Group E2 received 15 mg/kg/day of lamivudine in association with 60 mg/kg/day of ritonavir, in a single daily dose, orally by means of gavage, from day zero to the 20th day of the pregnancy. Group E3 received 45 mg/kg/day of lamivudine in association with 180 mg/kg/day of ritonavir, in a single daily dose, orally by means of gavage, from day zero to the 20th day of the pregnancy.

On the 20th day of the pregnancy, all the rats were anesthetized with xylazine and ketamine, at dosages of two mg/kg and 100 mg/kg, respectively, intraperitoneally. Immediately after anesthesia, a longitudinal thoracic-abdominal incision was made on the median line, thereby exposing the internal organs (liver, kidneys, and heart). Then, three ml of blood was collected using a disposable syringe of three ml in total volume, with a needle of 25 x 07 mm (22G), to aspirate the ventricular cavity. The blood volume obtained was transferred to dry tubes, which were non-heparinized. This material was sent to a laboratory in order to determine the values of the transaminases aspartate aminotransferase (AST) and alanine aminotransferase (ALT), in order to evaluate hepatic function, and to determine the urea and creatinine values, for renal function. These were done respectively by means of the kinetic colorimetric and enzymatic colorimetric methods, analyzed using an automated chemistry system equipment. Following this, hysterectomy was carried out to remove the fetuses. Rapidly, from each rat and its offspring, the livers and kidneys were extracted for macroscopic analysis. This material was immersed in 10% formol, buffered using phosphate buffer and was then processed for embedding in paraffin, to enable analysis under an optical microscope.

The mothers, still under anesthesia, were sacrificed by using scissors to perforate the myocardium, and the offspring were decapitated. After these specimens had been obtained for optical microscopy, they were rapidly dissected and, using a steel blade, fragments of thickness three to four mm were removed from the mothers’ and their offspring’s livers and kidneys, by means of a sagittal cut. The fragments for use in the optical microscopy evaluation were immersed in a 10% formol solution for a period of 12 hours. Subsequently, they were subjected to dehydration using ethyl alcohol at progressively greater concentrations until reaching absolute alcohol. After dehydration, the specimens were cleared using xylene and impregnated with liquid paraffin in a laboratory oven-regulated at a temperature of 60°C. The blocks were then cut using a microtome, adjusted to a thickness of five μm. The sections were placed on slides that had previously been greased with Mayer’s albumin and were then kept in an oven regulated at a temperature of 37°C for 24 hours, for the sections to dry and glue on. Afterwards, hematoxylin-eosin (HE) staining was performed. The optical microscopy evaluation was done using a microscope, with an eyepiece lens of 10x magnification and objective lenses of between 4x and 100x magnification. The histological results were demonstrated through photomicrographs of the maternal and fetal hepatic lobes, and of the cortical and medullary regions of the maternal and fetal kidneys, and a comparative analysis was carried out between group C and the E groups.

The mean quantitative AST, ALT, urea and creatinine values measured in the different groups on the 20th day of pregnancy were analyzed statistically using the Kruskal-Wallis test, which was complemented by Dunn’s multiple comparison test when there was statistical significance, in order to locate the groups in which these differences occurred [12]. The histological results
Chronic action of lamivudine and ritonavir on maternal and fetal liver and kidney of albino pregnant rats (Rattus norvegicus albinus, etc.

from the maternal and fetal livers and kidneys were evaluated by means of comparative analysis between group C and the E groups. In all the analyses, the significance level used was \( p < 0.05 \).

**Results**

*Morphological analysis*

1) Morphological analysis on the maternal liver

There were no differences among groups C, E1, and E2. The hepatic parenchyma consisted of a great concentration of hepatocytes, which were organized forming lines, which, in turn, converged to the central-lobular vein. Between the lines of hepatocytes, there were hepatic sinusoids and nuclei of several shapes, which were generally heterochromatic. The hepatocytes were polyhedral voluminous cells, with one or two centrally positioned spherical nuclei that were rich in chromatin, with evident nucleoli. The cytoplasm was not homogeneous, and basophilic and eosinophilic areas were seen to be present. For group E3, the only difference was the alteration of some hepatocytes, which presented hyperchromic nuclei, while others were binucleated and closer to the central-lobular vein (Figure 1).

2) Morphological analysis on the fetal liver

There were no differences among the C, E1, E2, and E3 groups. The fetal liver consisted of a great concentration of hepatocytes that were organized resembling lines. These cells were small and polyhedral, with spherical nuclei that occupied most of the cytoplasm. A nucleolus could clearly be seen in the nucleus and, in the cytoplasm, there were areas with intense acidophilia. In addition, many mitosis figures were observed, indicating cell proliferation. Among the hepatocytes there were capillary blood vessels, a large concentration of nucleated red blood cells, and megakaryocytes.

3) Morphological analysis on the maternal kidney

There were no differences between groups C and E2. The kidneys were well preserved, with the presence of renal corpuscles and proximal and distal convoluted tubules. The glomeruli were composed of capillaries, podocytes, endothelial cells, and mesangial cells. Capsular space and a parietal layer were observed in Bowman’s capsule. It should also be noted that most of the parenchyma and cortical region consisted of proximal tubules, which were formed by cubic or polyhedral cells, containing eosinophilic cytoplasm and a rounded nucleus. For group E2, the morphology was similar, with the exception of some areas with capillary dilation. On the other hand, group E3 presented a narrower cortex, with a reduction of the area of glomeruli, considering that among these the endothelial cells showed pyknotic nuclei (Figure 2).

4) Morphological analysis on the fetal kidney

There were no differences among groups C, E1, E2, and E3. On the 20th day of pregnancy, the kidneys were not completely formed, although the cortical and medullary regions were identifiable. In the cortical region, there were some glomeruli and convoluted tubules, of which the proximal ones were more eosinophilic. In the medullary region there were collector ducts and some loops of Henle.

**Biochemical analysis**

The mean quantitative values measured for AST in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 1. There were no statistically significant differences among the groups studied regarding AST values (\( p = 0.5341 \)).

The mean quantitative values measured for ALT in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 2. There were no statistically significant differences among the groups studied regarding ALT values (\( p = 0.5341 \)).

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pregnancy are reported in Table 2. There was a statistically significant difference among the groups studied regarding ALT values ($p = 0.0037$). Dunn’s multiple comparison test showed that group E3 presented significantly lower ALT values than those of group C, and that group C did not differ from groups E1 and E2.

The mean quantitative values measured for urea in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 3. There was a statistically significant difference in urea values ($p = 0.042$) among the groups studied. Dunn’s multiple comparison test showed that group E1 presented urea values that were significantly lower than those of group C, and that group C did not differ from groups E2 and E3.

The mean quantitative values measured for creatinine in the blood of the rats of each study group on the 20th day of pregnancy are reported in Table 4. There was a statistically significant difference in creatinine values ($p = 0.0108$) among the groups studied. Dunn’s multiple comparison test showed that group E1 presented significantly lower creatinine values than those of group C, and that group C did not differ from groups E2 and E3.

Table 1. — Descriptive statistics on AST in the samples studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>118.00</td>
<td>37.19</td>
<td>109.50</td>
<td>75.00</td>
<td>184.00</td>
</tr>
<tr>
<td>E1</td>
<td>10</td>
<td>126.60</td>
<td>31.45</td>
<td>125.00</td>
<td>75.00</td>
<td>164.00</td>
</tr>
<tr>
<td>E2</td>
<td>09</td>
<td>134.22</td>
<td>31.94</td>
<td>128.00</td>
<td>92.00</td>
<td>195.00</td>
</tr>
<tr>
<td>E3</td>
<td>09</td>
<td>125.44</td>
<td>19.86</td>
<td>122.00</td>
<td>96.00</td>
<td>158.00</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: $p = 0.5341$ (C = E1 = E2 = E3).

Table 2. — Descriptive statistics on ALT in the samples studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>74.20</td>
<td>17.67</td>
<td>76.00</td>
<td>49.00</td>
<td>90.00</td>
</tr>
<tr>
<td>E1</td>
<td>10</td>
<td>61.40</td>
<td>7.32</td>
<td>61.50</td>
<td>51.00</td>
<td>74.00</td>
</tr>
<tr>
<td>E2</td>
<td>09</td>
<td>67.88</td>
<td>9.13</td>
<td>70.00</td>
<td>54.00</td>
<td>80.00</td>
</tr>
<tr>
<td>E3</td>
<td>09</td>
<td>54.77</td>
<td>10.95</td>
<td>53.00</td>
<td>36.00</td>
<td>67.00</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: $p = 0.0037$ * E3 < (C = E1 = E2).

Table 3. — Descriptive statistics on the urea in the samples studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>65.50</td>
<td>6.48</td>
<td>66.50</td>
<td>58.00</td>
<td>74.00</td>
</tr>
<tr>
<td>E1</td>
<td>10</td>
<td>53.90</td>
<td>6.85</td>
<td>54.00</td>
<td>41.00</td>
<td>62.00</td>
</tr>
<tr>
<td>E2</td>
<td>09</td>
<td>61.00</td>
<td>9.87</td>
<td>61.00</td>
<td>46.00</td>
<td>77.00</td>
</tr>
<tr>
<td>E3</td>
<td>09</td>
<td>60.44</td>
<td>7.82</td>
<td>64.00</td>
<td>43.00</td>
<td>66.00</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: $p = 0.0420$ * E1 < (C = E2 = E3).

Table 4. — Descriptive statistics on the creatinine in the samples studied.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>0.51</td>
<td>0.03</td>
<td>0.50</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>E1</td>
<td>10</td>
<td>0.43</td>
<td>0.04</td>
<td>0.40</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>E2</td>
<td>09</td>
<td>0.47</td>
<td>0.04</td>
<td>0.50</td>
<td>0.40</td>
<td>0.50</td>
</tr>
<tr>
<td>E3</td>
<td>09</td>
<td>0.50</td>
<td>0.19</td>
<td>0.40</td>
<td>0.40</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test: $p = 0.0108$ * E1 < (C = E2 = E3).

Discussion

The doses of lamivudine and ritonavir that were applied to group E1 were determined based on calculations of equivalence to the therapeutic dose for humans, according to the mean weights of the rats on day zero of the pregnancy. The doses calculated for groups E2 and E3 were, respectively, equivalent to three and nine times the therapeutic dose for humans, following the research protocol used in the present service, which allowed greater veracity in the comparative analysis on the results. The objective of studying this association in high dosages was to evaluate maternal-fetal toxicity, considering that the drugs are metabolized much faster in rats than in humans [13], and that their bioavailability may be 50% to 90% lower [14].

The period over which the drugs were administered, from day zero until the 20th day of pregnancy, had the objective of covering the implantation phase, embryogenesis (until the 15th day), and fetal development (beginning on the 15th day). Therefore, a wide-ranging period was covered in order to analyze possible deleterious effects on the fetuses [15].

It was decided to study the hepatic and renal morphology and biochemistry of the pregnant rats because of the way in which lamivudine and ritonavir are metabolized and eliminated. At the therapeutic dose, less than 10% of lamivudine is metabolized by the liver, and it is predominantly eliminated through the renal system, by means of glomerular filtration and active tubule secretion [16]. Ritonavir is metabolized by the hepatic system and up to 86% is eliminated by the hepatobiliary system and, to a smaller extent, by the renal system. At high dosages, use of these drugs can lead not only to hepatotoxicity but, in some cases, to nephrotoxicity [17]. In previous studies carried out by the present group, lamivudine and ritonavir were used, at the same doses used in the present study, but separately [18, 19].

In the control group of the present study, the hepatic and renal morphological and biochemical analysis of the mothers and their offspring did not reveal any alteration, thus demonstrating that administering the drugs and feeding and handling the animals did not interfere with the re-
sults from the experiment. This result was also observed with other antiretroviral drugs researched by the present group [18-22].

Regarding the maternal livers, the present histological examinations demonstrated that when the combination of lamivudine and ritonavir was given to the pregnant rats at a dose equivalent to the dose used for humans or equivalent to three times the therapeutic dose, there were no morphological and biochemical alterations. However, alterations were present when the equivalent of nine times the therapeutic dose (E3) was given. The condensation of the cell nucleus or pyknosis that was demonstrated by the presence of hyperchromic nuclei in the hepatocytes of group E3 is an indirect sign of apoptosis, which may indicate the presence of a histological lesion with no biochemical repercussions. The finding of binucleated nuclei is a probable sign of cell multiplication. Thus, the association of lamivudine and ritonavir (at nine times the therapeutic dose) caused slight hepatic cytotoxicity, shown by signs of cell reactivity, in accordance with the observations of Goldman et al. [23].

A statistically significant decrease in the levels of ALT in group E3 was observed, in comparison with the other groups evaluated. Alteration of the ALT levels would be expected, because if the liver has been injured, this enzyme would increase at an earlier stage. One hypothesis, although not very likely, would be that the aggression may initially have occurred more intensely, thereby causing an increase in the ALT levels. Following this, with exhaustion of ALT, a subsequent fall in its levels would have occurred in group E3. Since exhaustion of ALT only occurs in the presence of intense hepatic alterations, this hypothesis would have very little basis for explaining this decrease [24]. The most likely explanation is that although the decrease in the level of this enzyme in group E3 was statistically significant, it did not have any biological significance. The injury to the maternal livers of group E3 was probably induced by the action of ritonavir, due to its high rate of hepatic metabolization and hepatobiliary elimination. This may, to some extent, suggest that hepatotoxicity occurs when the drugs are administered at high doses, either as monotherapy or in associations, for humans [5] or rodents [19].

Regarding lamivudine, some studies have shown that it is safe when administered during pregnancy [25, 26]. In a study carried out by the present group, Pontes et al. [18] used lamivudine in albino rats, separately, at dosages of 5, 15, and 45 mg/kg/day, from day zero until the 20th day of pregnancy, and demonstrated that the drug had no effect that could cause fetal malformations, at those dosages. Unlike other analogous nucleoside reverse-transcriptase inhibitors (zidovudine, didanosine, and stavudine), lamivudine has little action on the DNA polymerase of mammals and is not incorporated into mitochondrial DNA. Incorporation of the nucleoside analogue into the mitochondrial DNA is implied in several toxic effects, among which is liver failure. Clinically, lamivudine produces minimal adverse effects, in comparison with other nucleoside analogues, and it does not show any capacity to cause mitochondrial toxicity [26]. It is believed that it does not induce any important adverse hepatic affects [25] and, because of this, if used in combination with ritonavir, it might attenuate the hepatotoxicity frequently observed with the use of the latter drug.

In the present study, the hepatic histological alterations caused by the association of these two drugs were less pronounced than in studies that used lamivudine and ritonavir separately [18, 19]. The present authors observed that hepatic alterations could already be demonstrated at smaller doses of ritonavir [19], which reinforces the idea that lamivudine may attenuate the maternal toxic effects caused by ritonavir [19]. These alterations to the maternal liver were also observed when these drugs were administered separately, starting at 45 mg/kg for lamivudine (nine times the therapeutic dose) and starting at 60 mg/kg for ritonavir (three times the therapeutic dose). However, when these two drugs were used in association, these alterations were only observed beginning at higher doses of ritonavir (45 mg/kg of lamivudine in association with 180 mg/kg of ritonavir) and at lower intensity. In another study, the adverse effects of ritonavir were 37% more intense when used separately than when used in association with another protease inhibitor, in this case saquinavir (16%) [27]. Thus, this demonstrated that the effect of ritonavir was attenuated by another antiretroviral.

To evaluate renal function, serum urea and creatinine were assayed. Considering the aggression towards the kidneys in group E3, it was expected that these parameters would increase, but there was no increase in the urea levels, which became higher in the more initial phases of aggression. Moreover, there was no increase in creatinine level, which became higher later on. These findings suggest that, despite the occurrence of some degree of structural injury in this group, the reserve functional capacity of the kidneys was sufficient to tolerate the aggression promoted by the combination of these drugs.

In the same way that in other studies carried out by the present group, using separate or combined antiretroviral drugs, even at high dosages, i.e. nine times greater than the equivalent therapeutic dose for humans, there were no structural alterations in the fetal livers and kidneys, thus corroborating the present findings [18-22]. The most adequate scientific foundation for this condition probably relates to the protection that the placental P-glycoprotein (a transmembrane transport protein that exists in great concentration on the maternal surface of the placenta) exerted when stopping appreciable amounts of ritonavir and lamivudine from going through the placental barrier [28].
Conclusion

In summary, the present study showed that the association of lamivudine and ritonavir did not cause fetal liver and kidney alterations, although it was harmful to the maternal livers and kidneys. The lesions observed were less intense than when these drugs were used separately during the pregnancy of albino rats. Although no direct correlation can be made regarding the toxicity of a drug between rats and humans, the findings from the present study suggest that the association of lamivudine and ritonavir promoted alterations to the pharmacokinetics of the drugs. Finally, it needs to be emphasized that with the various antiretroviral drugs available, especially considering their use in associations, research needs to be accelerated with the aim of improving anti-HIV therapy, so as to promote maximum effectiveness in impeding vertical transmission, with minimal deleterious effects to the mother and fetus.

References

Failed surgical therapy for chronic back pain and sciatica may be due to hypofunction of the sympathetic nervous system

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Introduction

Hypofunction of the sympathetic nervous system has been found to be an etiologic factor in a wide variety of chronic treatment refractory pathologic disorders [1]. Many of these disorders involve pain mainly related to failure of the sympathetic nervous system to inhibit cellular permeability allowing the absorption of caustic chemicals and toxins into tissues leading to pain and inflammation [1].

The sympathetic nervous system is also responsible for diminishing capillary permeability when a person stands to prevent leakage from the intravascular to the extravascular spaces directly related to the increase in hydrostatic pressure leading to edema [2]. The edema itself may contribute to pain besides the cellular permeability defect. Thus, the condition has been termed the sympathetic neural hyperalgiesia edema syndrome [3, 4].

The interesting fact about this syndrome is how such a wide variety of symptoms in various parts of the body respond so quickly and effectively to one single agent – the sympathomimetic amine dextroamphetamine sulfate [1].

Pelvic pain is one of the chronic disorders that respond so well to dextroamphetamine sulfate when surgery fails [5, 6]. The first case described with severe pelvic pain who responded to dextroamphetamine sulfate was published in 2007 [7]. Eventually, the woman decided to just tolerate the pain and stopped the sympathomimetic amines because the state she lived in (New Jersey) did not allow off-label use of class II drugs and she found it inconvenient to travel to the state where it could be obtained (Pennsylvania). However, this 44-year-old woman developed severe, excruciating lower backache with pain radiating to her hips and legs after shoveling snow and was diagnosed by radiographic procedures and her orthopedist as having three herniated disks at L4, L5, and S1. Analgesics and anti-inflammatory drugs failed to relieve her pain and surgery was suggested [7]. In lieu of surgery, she returned for treatment with dextroamphetamine sulfate. She had marked improvement of the pain after two doses of dextroamphetamine sulfate and within a week the pain had completely dissipated [7].

The case presented here is another example of severe backache with sciatica that responded quite well to dextroamphetamine sulfate, but in this case in a woman who failed to have relief from back surgery.

Case Report

A 53-year-old patient came to us with a history of pelvic pain and sciatica of eight years duration. She also complained of fluid retention and edema, fibromyalgia, nocturia, and attention...
deficit hyperactivity disorder (ADHD). Additionally, she had a history of interstitial cystitis, depression, and multiple other complaints. Due to all of the ailments, specifically the chronic pain and sciatica, she was out of work upon arrival to the present office in July, 2010. She described her pain as right posterolateral thigh and calf pain that was so intense she wanted to cut her leg off. Her nocturia occurred nightly about two to three times which interrupted her sleep and caused her to be exhausted the next day.

One year prior to her initial visit, she had spinal surgery with a laminectomy and spinal fusion due to a ruptured disc which was discovered upon workup for her chronic pain and sciatica. After the surgery, she reported mild relief; however, her pain returned. She has tried ibuprofen, acetaminophen, pregabalin, ultram, and botox, all of which have provided minimal relief of her constant pain.

When she first came to the office in July of 2010, she was started on 15 mg of dextroamphetamine sulfate extended release once daily. She also completed the water load test [1, 2] previously where she failed the erect portion. The medication provided some relief, especially decreasing her constipation and providing improvement of her ADHD. Her sciatica was also improved. However, after 30 days, this dose was not eliminating her pain. Her dose was therefore increased to 25 mg once a day. Further evaluation a month later revealed more stability of her ADHD. Also, her pain was better in the morning but worse with movement by six o’clock at night, especially in her right lower buttocks and leg. Again her dose was adjusted to 20 mg in the morning and at noon. A month later she stated that she did not have as much pain; however by six o’clock at night she was still constantly in pain. Additionally, she still showed signs of edema. She did not experience any side effects with the current dosage and therefore, her dose was again increased to 15 mg three times a day at 6:30 in the morning, 8:30 in the morning and 3:00 in the afternoon, with a total of 45 mg daily.

Re-evaluation a month later revealed her ability to concentrate and focus had significantly improved. Additionally, her pain had drastically decreased to average a five out of ten from a ten out of ten. Although her pain has dramatically decreased, she still was not pain free and she still experienced bloating. Consequently, spironolactone 100 mg was added to be taken when she began to feel bloated while keeping her dextroamphetamine sulfate the same. Upon evaluation a month later in December 2010, she still was not at a comfortable pain level. Consequently, her dosage was increased again to 25mg twice a day. In January 2011, she expressed a tremendous decrease in pain. Furthermore, she was back to work four times a week which she was never able to do. Additionally, her ADHD has markedly improved and she is now able to focus better and retain more information. She states, however, that she occasionally gets breakthrough pain and she still complains of bloating.

One month later, she still complained of this breakthrough pain about one week a month. The authors thus added 15 mg dextroamphetamine sulfate to her 25 mg twice a day to be used as needed for this week of excessive pain. Three months later, she stated that her pain had tremendously improved. After adjusting the dosage of dextroamphetamine sulfate, she has been provided relief from her chronic pelvic pain and sciatica. Additionally, she is able to work as a nurse on her feet again, four days a week. Her pain went from a constant level of ten out of ten to a two or three. Additionally, she is able to play tennis and do yard work. Through the use of this sympathomimetic amine and without the use of narcotics, the patient was able to get her life back and live relatively pain-free for the first time in years.

Discussion

In the first case described of relief of sciatica from sympathomimetic amine therapy the onset of symptoms were so acute the possibility existed that there was spontaneous improvement rather than from therapy [7]. The present case was so chronic and failed to gain relief even from back surgery indicating little question that the improvement in the backache and sciatica of the woman described was related to the sympathomimetic amine therapy.

The present case also had some of the other conditions associated with this defect: fibromyalgia, chronic fatigue, constipation, weight gain and edema, and bloating [8-12]. All of these symptoms improved in addition to the backache with treatment with dextroamphetamine sulfate.

Similar to pelvic pain, sympathomimetic amine therapy should be given consideration as first line therapy for chronic backache with sciatica.

References


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Removal of an incarcerated intrauterine device in the sigmoid colon under the assistance of hysteroscope and laparoscope: a case report

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Summary

Background/Aims: To explore the value of hysteroscope and laparoscope in removing an incarcerated or ectopic intrauterine device (IUD). Materials and Methods: A 33-year-old woman was admitted to the present hospital on May 22nd, 2013. An incarcerated IUD was proven by ultrasonography. An IUD had been implanted in October 2011. Clinical case report of an incarcerated IUD in the sigmoid colon. Results: An IUD was successfully removed with the assistance of hysteroscope and laparoscope. Conclusion: Ultrasonography should be performed in the follow-up of the patients after IUD implantation. Ectopic or incarcerated IUD can be successfully removed with the assistance of hysteroscope and laparoscope with minimal trauma.

Key words: Intrauterine device; Ectopia; Hysteroscope; Laparoscope.

Introduction

Intrauterine device (IUD) is most commonly used in Chinese women of child-bearing age for contraception. [1] IUD has been proven to be a safe, effective, and cost-effective contraceptive method. [2] However, as a greater number of IUDs have been used for longer periods of time, the incidence of ectopic IUD has increased over the past few years. The incidence of uterine perforation is about 0.05 to 0.13%. [3, 4] Invasive surgical procedures are generally required to remove the ectopic IUD. As a newly developed surgical method, laparoscopic surgery is widely used in clinical practice, particularly gynecologic surgery. Therefore, the removal of ectopic IUD can be completed with the assistance of a laparoscope. The authors report a case of ectopic IUD that has been successfully removed under the assistance of a laparoscope.

Case Report

Medical history

A 33-year-old woman was admitted to the present hospital on May 22nd, 2013 with vaginal bleeding of 25 days duration. An incarcerated IUD was proven by ultrasonography. In this patient, an IUD had been implanted in October 2011 and regular menstrual cycles were reported thereafter (4-5 / 24-25). The date of the last menstrual period was April 27th, 2013. The patient presented to the affiliated Shenzhen Nanshan People’s Hospital of Guangdong Medical University on May 20th, 2013 with continuous vaginal bleeding after the last menstrual period. Outpatient ultrasonography revealed the abnormal position of the IUD. A strong echo of the IUD was found in utero and the myometrium of the fundus, a strong echo of 8 x 5 mm in size was also found at the outer serosa layer of the uterus at the left side of the fundus. The patient was hospitalized for incarcerated IUD. The patient exhibited a normal appetite and control over urinary and fecal discharge as usual. No other serious disease was noted prior to hospitalization. The woman had conceived three times and had given birth to two children in total, her youngest child was born via cesarean delivery in 2010.

Clinical examination

On admission, the woman’s temperature was 36.4°C, heart rate (P) was 76 beats/minute, respiration (R) was 20 times/minute, and blood pressure (BP) was 130/56 mmHg. No other symptoms were noted. Apart from all over the body yellow skin, no other abnormalities were noted on presentation. All cardiopulmonary investigations were normal. The abdomen of the woman was flat and soft and no pressing or rebound pain was noted. A scar of approximately ten cm in length was found in the lower abdomen. The liver and spleen were not examined, and no shifting dullness was detected. Gynecological examination revealed normally developed vulva. The vagina was normal vagina for a women having given birth to child, with a small amount of bloody discharge. The cervix was smooth and soft without lifting pain and no IUD tail was found. The posterior fornix was not full and without tenderness. Reposition of the uterus of normal size and range of motion was detected with slight tenderness. Slight tenderness was also detected in the right adnexal region, with no obvious masses. In contrast, masses without pressing pain were detected in the left adnexal region. Plain abdominal radiograph on May 23rd, 2013 showed the IUD in the pelvic cavity (Figure 1).
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Clinical management

Clinical examination with the assistance of hysteroscope and laparoscope were performed on May 24th, 2013. The results of hysteroscopic examination revealed a U-shaped IUD incarcerated in the myometrium of the left fundus. The uterus was a normal size and shape, and the bilateral openings of the fallopian tubes could be clearly seen (Figure 2). Examinations during the operation revealed a posterial uterus of normal size, and dense adhesions between the sigmoid colon and the left posterior uterine wall which filled the rectouterine fossa (Figure 3). No abnormalities were found for the bilateral adnexa. The patient’s family requested that the operation be performed after the enteroscopic examination for the adhesion between the posterior uterine wall and the lower rectum. Enteroscopy examination was performed on May 28th, 2013, and the results revealed a metallic material in the enteric cavity (Figure 4). The operation to remove the incarcerated IUD with the assistance of hysteroscope and laparoscope was performed on June 4th, 2013; rectal repair, lysis of pelvic adhesions, and bilateral tubal ligation were also performed. An ultrasonic knife was used to carefully divide the adhesion between the uterus and the rectum. Part of the IUD could be seen during the division, and the IUD was then removed by vessel forceps by part and then pieced together (Figure 5). Monopolar electrocoagulation was performed to stop any bleeding of the uterus. Damage to the rectum was identified and repaired with full-thickness continuous suture using size 1-0 Vicryl suture. Bilateral tubal ligation was also performed. Oral food intake was resumed after the anal exsufflation at day 2 after the operation. Plain abdominal radiograph was taken for the reexamination and no IUD was found. The patient was discharged on June 10th, 2013.

Discussion

Ectopic IUD is a very rare condition in clinical practice. Ectopic IUD may occur for a number of reasons. The uterus wall is very soft and easily perforated in breastfeeding and pregnant women. Ectopic IUD is more common in women with hyperflexion or scarring of the uterus, and also in postmenopausal women with metratrophia. In some cases, severe abdominal pain during IUD implantation may be the sign of perforation of the uterus. Inexperienced clinicians may not identify the position of the uterus correctly, resulting in incorrect placement of the IUD. In some women, only X-ray but not ultrasonography is used to re-examine the implantation of IUD.

A number measures could prevent the development of ectopic IUD. Clinicians should strictly comply with the recommended surgical procedures, and correctly identify the position and size of the uterus. An IUD of appropriate type and size should be used. For breast-feeding women, the uterus is very soft and tiny perforations may not be easily identified, therefore clinicians should implant the IUD carefully and gently. For postmenopausal women with metratrophia, incarcerated IUD occurs more easily when the uterus is smaller, therefore the IUD should be removed within one year after the onset of menopause. Using estrogen before the removal of the IUD may improve atrophy of the cervix, thus the authors recommend that misoprostol be used be-

Figure 1. — Plain abdominal radiograph shows the IUD located in the pelvic cavity.

Figure 2. — A U-shaped IUD incarcerated in the myometrium of the left fundus.

Figure 3. — Close adhesions between the sigmoid colon and the posterior uterine wall.
fore removal of the IUD to soften the cervix and relax the uterine orifice. This practice may increase the success rate and decrease the risk of complications. Removal of the IUD under the assistance of hysteroscope should be performed for some cases with ectopic IUD. Ultrasonography should be performed regularly after the implantation of the IUD to clearly identify the position of the IUD and help choosing the treatment methods correspondingly. [5]

For most cases of ectopic IUD, no obvious symptoms are reported. Migration of the IUD through a perforation into the intestine could cause abscess formation, intestinal ischemia, or volvulus. [6, 7] Several methods including ultrasonography, X-ray, hysterosalpinography, and hysteroscopic examination are currently used for the diagnosis of ectopic IUD. Ultrasonography can display the uterine cavity and uterine profile, thereby identifying the location of the IUD in the uterus. However, ultrasonography cannot clearly identify ectopic IUDs made of plastic or silica gel, or IUD that have migrated a long distance from the uterus. X-ray examination is more suitable for identifying IUD made of metallic materials. CT and MRI examinations could also facilitate the diagnosis of ectopic IUD by accurately positioning the IUD. However, for patients with suspected ectopic or incarcerated IUD, hysteroscopic examinations should be performed to identify the position and shape of the IUD in the uterine cavity, help diagnose the adhesion of the IUD in the endometrium, and incarceration of the IUD in the myometrium. These examinations can further identify the position of the adhesion, the depth of the invasion of the IUD into the uterus wall, the size of the adhesion, and the location of the broken IUD, and whether there is IUD in the uterus before the removal of the IUD. Ectopic IUD can also be diagnosed if no IUD is found in the uterus by hysteroscopy examination. A imaging system is used in laparoscopic surgery to obtain a wider-exposed surgical field than conventional surgical procedures, which could facilitate the identification of migrated IUD and determine consequent treatment. Operation with the assistance of hysteroscope and laparoscope were successfully used in the present report to identify and remove the ectopic IUD in the sigmoid colon.

The IUD can migrate to any position within the pelvic cavity, thus it is very difficult to identify the migrated IUD. Ultrasonography or X-ray examination should be performed before the removal of the IUD to identify the position of the IUD and to determine whether the IUD has migrated beyond the myometrium. The clinicians should carefully search for the IUD in clockwise or counterclockwise direction with the assistance of hysteroscope and laparoscope. X-ray images should also be used to help identify the IUD. In some cases, the IUD could migrate into the greater omentum, which makes it more difficult to iden-
tify the exact location of the IUD. In these cases, the patients should be placed in a head-low feet-high position, which could allow the greater omentum to move upward into the abdominal cavity and cause the upward movement of the IUD. Then dynamic X-ray images should be used to help identify the IUD.

There has been debate about whether an ectopic IUD must be removed. Most researchers believe that the migrated IUD could cause adhesion of the surrounding tissues and should be removed as early as possible after diagnosis. However, other researchers believe that ectopic IUDs do not need to be removed for patients without obvious symptoms [8-11]. In the present case report, although no obvious symptoms were found, the patient feared that dry stools could aggravate the effects of the migrated IUD on the rectum, and requested that the IUD be removed. Traditionally, laparotomy or operation through the posterior vaginal fornix is generally used. However, these operative methods only provide a limited surgical field, and increase the risk of intestinal adhesion, require a longer hospital stay, and lead to severe damage to the patients. In recent years, the development of laparoscopic technique has made it possible to remove the migrated IUD with the assistance of a laparoscope. Several researchers have performed a temporary colostomy following the removal of the incarcerated IUD in the sigmoid colon [12, 13], resulting in significant damage. In the present case report, part of the IUD in the peritoneum was removed first, then the adhesive serous layer of intestines was resected, and the remaining portion of the IUD was carefully removed. The patient recovered well after the incision was sutured.

In summary, as a minimally invasive technology which can help examination, diagnosis and treatment, surgery under the assistance of hysteroscope and laparoscope can facilitate the diagnosis and removal of ectopic IUD. It provides a wide and clear surgical field, helps with the division of adhesions and treatment of other complications, shortens operating time, reducing blood loss, accelerating postoperative recovery, and reduces the risk of developing complications with minimal trauma.

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Sexual infantilism in a normal karotypic female related to ovarian agenesis associated with Müllerian agenesis – Case report

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Summary

Purpose: To describe an unusual case of Müllerian agenesis associated with gonadal agenesis and thus sexual infantilism. Materials and Methods: Pelvic magnetic resonance imaging (MRI) and sonography were performed and MRI of the kidneys. Pelvic sonography and serum follicle stimulating hormone (FSH) were also obtained. Results: The only pelvic organ that this 15-year-old girl had was the distal portion of the vaginal canal. The kidneys were normal. Conclusions: This case suggests that at least in some cases some possible viral damaging process may lead to damage to both the ovaries and the Müllerian system. If there was a problem with the anti-Müllerian hormone (AMH), the kidney may be affected. Furthermore, AMH has nothing to do with the ovaries and a chance association of these two entities though possible, seems less likely than a common factor causing both problems.

Key words: Müllerian agenesis; Ovarian agenesis; Sexual infantilism; Premature ovarian failure.

Introduction

The most common cause of primary amenorrhea is Turner’s syndrome (gonadal dysgenesis) with an incidence between one in 2,500 to one in 5,000 live born girls [1]. Turner’s syndrome is generally associated with sexual infantilism in 80-90% of cases [2].

A far less common cause of sexual infantilism is gonadal agenesis where instead of having a missing or defective X chromosome as in Turner’s syndrome, the karyotype is XX and there are no physical stigmata, e.g., short stature as in gonadal dysgenesis. There are no definite causes of gonadal agenesis but possibilities include damage to the early gonad from viruses, metabolic issues, or possible undiscovered genetic mutation.

Isolated gonadotropin deficiency or hypopituitary or hypothalamic abnormalities, e.g., craniohypophyngioma, can also be a cause of primary amenorrhea with sexual infantilism. In gonadal dysgenesis or agenesis the serum FSH and luteinizing hormone (LH) are elevated at the age of puberty but of course it is low in hypothalamic-pituitary conditions.

Müllerian agenesis (Mayer-Rokitansky-Kuster-Hauser syndrome) signifies a woman with primary amenorrhea and congenital absence of the upper two-thirds of the vagina [3]. It is the second most common cause of primary amenorrhea next to gonadal dysgenesis with an established incidence of one in 5,000 newborn girls [4]. Patients with Müllerian agenesis have the absence or hypoplasia of the internal vagina and usually the absence of the uterus and fallopian tubes. Since the ovaries are not Müllerian structures, in contrast to gonadal dysgenesis, these patients with primary amenorrhea can easily be distinguished from girls with Turner’s syndrome because of their normal growth and appropriate sexual development at the typical age of puberty.

Primary amenorrhea and sexual infantilism have been previously reported occurring with Müllerian agenesis [5]. The sexual infantilism was related to the fortuitous and independent presence of isolated gonadotropin deficiency [5].

The authors present another case of Müllerian agenesis associated with primary amenorrhea and sexual infantilism. However, in this case, the cause of the sexual infantilism was not hypothalamus-pituitary related, but was from the much rarer co-existence of gonadal agenesis and Müllerian agenesis.

Case Report

The young woman presented at age 15 with a history of primary amenorrhea and sexual infantilism. A transabdominal ultrasound failed to detect any ovaries but a hypoechoic structure measuring 3.6 x 0.7 x 1.8 cm was seen in the midline of the pelvis which did not look like a uterus but the study suggested that a magnetic resonance imaging (MRI) be performed. The MRI
failed to identify the uterus, cervix, and the majority of the proximal vagina. However, the MRI did identify a portion of the very distal vagina.

Subsequent chromosome analysis found a normal female karyotype (46xx). Her serum testosterone level was only ten ng/dL. A subsequent kidney MRI showed normal sized kidneys without hydronephrosis or nephrolithiasis. The teenager was placed on conjugated estrogen 0.9 mg daily. Despite the estrogen replacement, her serum FSH at age 16.5 years was very elevated at 27 mIU/mL.

**Discussion**

If the frequency of gonadal dysgenesis is one in 2,500 and Müllerian agenesis is one in 5,000, one might expect this combination in about one in 12 million female births. Cases reported to date of bilateral or unilateral gonadal agenesis with normal female karyotype did not have typical phenotypic features of Turner’s syndrome as in the present patient [6-10].

Gonadal agenesis or “pure gonadal” agenesis is rare. The fortuitous association of these two entities would occur much less frequently than the estimated one in 12 million for the combination of Müllerian agenesis and gonadal dysgenesis.

Back in 1979, a study was published showing that the inappropriate retention of the Müllerian structures in a male in some instances could be related to a mutation of the anti-Müllerian hormone (AMH) receptor causing insensitivity to the AMH [11]. Although AMH is mostly secreted by Sertoli cells in the testes beginning at seven weeks, very small amounts of AMH mRNA are present early in life in the ovary. Thus, based on the 1979 study by Imbeaud et al. [11] many researchers believe that mutations for the gene for AMH or genes from the AMH hormone receptor would be found that would explain this phenomenon. Theoretically, some mutation would allow the increased expression of ovary derived AMH, or persistence of secretion or a mutation that would increase the sensitivity of the AMH receptor on the Müllerian structure making them susceptible to regression of the Müllerian structures to the small amount of ovarian AMH. A defect in AMH or its receptor would explain why about one-third of females with congenital absence of the uterus and proximal vagina also have urinary tract abnormalities since the kidneys, and the collection system are Müllerian structures.

However, despite 30 years since the publication by Imbeaud et al. [11] no mutations causing the absence of Müllerian structures have been identified. As mentioned, one of the proposed mechanisms for ovarian agenesis involves destruction of the fetal ovaries by a virus. Theoretically, a virus effecting the fetal pelvis could destroy not only the ovaries but the nearby structure of uterus, upper vagina, and fallopian tubes. However, similar to this case, the kidney’s would already be in another anatomical position and thus protected from the virus. This would explain normal kidney and collection system as seen in this case. Thus, the present authors favor this viral destructive scenario to explain the findings of Müllerian agenesis associated with sexual infantilism which was related to ovarian agenesis in a young lady with a normal female karyotype.

**References**


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Sirenomelia with upper limb malformation: a case report and review of the literature

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Summary
Sirenomelia sequence is a rare lethal pattern of congenital anomalies characterized by fusion of the lower extremities and a variable combination of visceral abnormalities. Some cases accompanied with rare malformations have been reported. In this article, the authors report a case of sirenomelia with upper limb malformations and a review of the literature.

Key words: Sirenomelia; Upper limb malformation; Prenatal diagnosis.

Introduction
Sirenomelia is a rare congenital malformation characterized by different degrees of fusion of the lower extremities in association with sacral agenesis, imperforate anus, colonic or rectal atresia, renal agenesis, absent bladder, absent internal genitalia, and a single umbilical artery, with a reported incidence ranging between 1/24,000 and 1/67,000 births [1]. Survival is extremely rare. Prenatal diagnosis of this condition can be accomplished by ultrasound and relies on the inability to demonstrate separate lower limbs or the identification of a single femur. All human cases of sirenomelia analyzed thus far show a variable degree of renal and urethral dysplasia and gastrointestinal anomalies. There are a few documented cases in which sirenomelia have upper extremities defects. In this article, the authors describe and discuss the association of upper limbs malformations along with craniofacial defects in a sirenomelia, rarely reported in mainland China.

Case Report
A 30-year-old woman, gravida 3, para 0, was referred to the present hospital for nuchal translucency screening at 12 weeks’ gestation. She reported two spontaneous miscarriages during the first trimester, and the karyotype of the abortus tissue in the second miscarriage identified no chromosomal anomalies. She and her husband were non-consanguineous and healthy. She had no history of drugs and alcoholism. There was no history of maternal diabetes or exposure to teratogens. One of her husband’s cousins has sexual deformity. A detailed ultrasound examination was performed. The CRL was 54.5 mm and the NT measurement was 2.1 mm. However, the image of the fetal lower limbs was obscure. She underwent another ultrasound examination at 15 weeks’ gestation. A singleton fetus was revealed with the lower extremity completely fused and no foot. Only one femur about 14 mm was found. Single umbilical artery was showed, and a great intra-abdominal vessel continued in the umbilical cord without branching into the normal left and right common iliac arteries (Figure 1-Aa). The amniotic fluid volume was normal. Owing to the gestational age, the assessment of the fetal organs was difficult. These sonographic findings favored the diagnosis of sirenomelia. In addition, there was a cleft lip and palate, as well as lumbosacral kyphoscoliosis with a cyst about 9 x 9 x 9 mm. Most importantly, the ultrasound demonstrated the presence of the abnormal upper limbs with only the ulna in left forearm, and only the humerus in the right upper limb, both hands were fixed with ulnar deviation (Figure 1-Ab).

The patient and her husband were informed of the findings and received genetic counseling and testing. Fetal karyotyping was performed by cordocentesis, which revealed a female karyotype (46, XX, 9qh+). The parents opted for termination of pregnancy. The abortus was delivered weighing 89 g and measuring 17 cm length.

The diagnosis of sirenomelia was confirmed by X-ray at postmortem examination. The ribs, scapulae, and clavicles were normal. Lower limbs were fused and only one femur was noted. It was also found that only the ulna in left forearm and only the humerus in right upper limb with both hypoplastic hands having ulnar deviation (Figure 1-B).

Postmortem studies revealed hemipelvis, single femur, no foot, and single umbilical artery in the umbilical cord originated high from the abdominal aorta (Figure 1-Ca). The external genitalia were absent and the anus was imperforate. The lumbosacral neural tube was defected with myelomeningocele (Figure 1-Cb). Other malformations included absent bladder, and cleft lip and palate (Figure 1-Cc). The upper limbs were hypoplastic and contracted (Figure 1-Cd).

Discussion
Sirenomelia derives its name from the physical resemblance to the mythic mermaid (siren), with lower extremity fusion and abnormal or absent foot structures [1]. ICD-10 lacks a specific code for sirenomelia, which may be coded as...
Q87.2 “congenital malformation syndromes predominantly affecting limbs”[2]. There are variable associated anomalies of the lower body, including sacral agenesis, imperforate anus, colonic or rectal atresia, renal agenesis, absent bladder, and absent internal genitalia (but not the gonads). Sometimes it also accompanies some rare malformations such as facial dysmorphism, agenesis of corpus callosum, costo-vertebral segmentation defects, anal atresia/stenosis, cardiac malformation, tracheo-esophageal fistula and/or esophageal atresia, renal, and limb anomalies (VACTERL) [3]. Albeit at a much lower frequency, sirenomelia is also associated with malformations of the upper part of the body, including cleft palate, upper thoracic and cervical vertebral abnormalities, pulmonary hypoplasia, and cardiac defects [4]. Till now there are few detailed cases of sirenomelia with upper limbs malformations reported in mainland China. Here the authors found a sirenomelia fetus accompanied with upper limbs malformations with only the ulna in left forearm and only the humerus in right upper limb with both hypoplastic hands. Lynch and Wright once reported a sirenomelia with limb reduction defects, cardiovascular malformation, and renal agenesis in an infant [5]; the present case overlapped with other malformations, such as a cleft lip and palate, myelomeningocele, et al.

Two separate systems of classifications of sirenomelia have been proposed. One is classified into three types according to the number of feet: symphus dipus, both feet present; symphus unipus, only one foot present; symphus apus,
both feet absent [6]. The other is based on the presence of skeletal elements in the thigh and leg proposed by Stocker and Heifetz [7]: classification I, paired femora, tibiae and fibulae; II, a single fused fibula; III, absent fibula; IV, partially fused femora and single fibula; V, partially fused femora and absent fibulae; VI, single femur and tibia; VII, single femur, and absent tibiae and fibulae (Figure 1-D). The present case belonged to type VII according to Stocker’s classification.

The present authors also found single umbilical artery in the umbilical cord arising from the high abdominal aorta which was one of the pathogenic hypotheses of sirenomelia. Stevenson et al. proposed the vascular steal theory [8]. Fetuses with sirenomelia almost invariably exhibit single umbilical artery instead of the normal two. Moreover, this artery has an abnormal origin, arising from the high abdominal aorta, usually immediately below the celiac branch, where it always branches into the common iliac artery. Blood is shunted to the placenta by the single umbilical artery, and the vessels distal to this aberrant umbilical artery were underdeveloped and malformed [9] (Figure 1-E). The single umbilical artery has also been referred to as the persistent vitelline artery. In the present case color Doppler was used to prenatal diagnosis of the vitelline artery. Postmortem examination also confirmed its existence.

Another major hypothesis for sirenomelia is direct damage to the caudal mesoderm of the embryo. This damage may occur from extrinsic pressure or overdistension of the neural tube. Other theories propose an influence of teratogenic agents like cocaine or retinoic acid abuse during the first trimester of pregnancy. Genetic factors, such as HLXB9, sonic hedgehog, patched, brachyury, receptor of retinoic acid, CYP26A1, BMP7, and twisted gastrulation (tsg), appear to play another role [9].

In the karyotyping test the abortus showed a female karyotype (46, XX, 9qh+). It was reported that the sex ratio of male to female was 2.7:1 in sirenomelia [1]. X-linked oligogenes and X-linked mutations are suggested to explain the excess of males. Moreover, 7% of cases are associated with monozygotic twinning, higher than in dizygotic twins or singletons [10].

Since sirenomelia is uniformly lethal, early prenatal diagnosis is required in order to allow termination of pregnancy at an early stage. Both color and power Doppler have been reported as useful to diagnosis this malformation. However, there is only a narrow window, between weeks 8 and 16 of gestation, when the amniotic fluid still depends mainly from maternal production, when visualization of sirenomelia to ultrasound is possible. During the late second trimester, severe oligohydramnios, secondary to renal agenesis or dysgenesis, may hamper a proper evaluation of fetal lower extremities. In those situations, magnetic resonance imaging provides a substitute [10].

In conclusion, sirenomelia is a multisystemic human malformation without explicit etiology. The early diagnosis of this lethal malformation during suitable gestation week is crucial to the treatment plan and termination of pregnancy. Although sporadic case reports and animal models have provided some important insights into the problem and given rise to the pathogenesis, much more work should be done to explore the mechanism and the prevention of sirenomelia in the future.

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References


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Recurrence ectopic pregnancy after ipsilateral partial salpingectomy: a case report

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Summary

Ectopic pregnancy is associated with maternal morbidity and mortality during early pregnancy. Ectopic pregnancy occurs in approximately 2% of all pregnancies, and the risk of ectopic pregnancy is increased by eight-fold in women with a history of ectopic pregnancy. However, recurrent ectopic pregnancy after ipsilateral partial salpingectomy is quite rare. The authors experienced a case of recurrent ectopic pregnancy in the distal remnant after right partial salpingectomy. In this case report, they discuss this unusual case and provide a brief review of the literature.

Key words: Ectopic pregnancy; Recurrent; Partial salpingectomy.

Introduction

Ectopic pregnancy occurs in approximately 2% of all pregnancies, and the risk of recurrence is increased by eight-fold in women with a history of ectopic pregnancy[1]. Over 95% of ectopic pregnancies involve the fallopian tube [2]. Recurrent ectopic pregnancy after ipsilateral partial salpingectomy is very rare, and no exact information is available in the literature. Here, the authors report a case of recurrent ectopic pregnancy after ipsilateral partial salpingectomy.

Case Report

A 28-year-old, gravid 1, para 0 woman presented to the emergency department complaining of irregular vaginal spotting and lower abdominal pain; she had undergone right partial salpingectomy eight years ago because of ectopic pregnancy. The pain was dull in nature and had been present for two days. Her right lower quadrant was tender with no rebound tenderness. Her blood pressure was 130/80 mmHg, and her pulse was 80 beats/minute. Before presentation, transvaginal ultrasonography was performed at a local clinic, which showed a right tubal gestational-sac (G-sac)-like shadow and no fluid collection. The urine pregnancy test performed at the emergency department yielded positive results. Her initial white blood count, hemoglobin, hematocrit, and platelet count were 7.93 K/μl, 11.9 g/dl, 35.4%, and 256 K/μl, respectively. Her serum β-human chorionic gonadotropin (β-hCG) level was 810.4 mIU/ml and 1105 mIU/ml on the day of admission and on day 2 after admission, respectively. A couple of days later, transvaginal ultrasonography showed an empty uterus, a right tubal G-sac-like small cystic lesion, and no fluid collection (Figure 1). The authors recommended a diagnostic laparoscopic surgery with D&C. However, the patient stridently refused their suggestion, requested a hospital discharge, and promised to visit the outpatient department in one week. She returned to the hospital five days after being discharged because of lower abdominal pain and vaginal bleeding. Transvaginal ultrasonography showed no changes from previous findings. She complained of persistent lower abdominal pain. Pelvic computed tomography (CT) was performed, which showed an irregularly enhanced abnormal lesion at the right adnexa and fluid collection in the pelvic cavity (Figure 2). Her serum β-hCG was 768.4 mIU/ml on the day after admission. She underwent therapeutic laparoscopic surgery because of severe lower abdominal pain. The operative findings showed hemoperitoneum and a bulging four-cm mass at the distal remnant of the previous partial salpingectomy (Figure 3). The distal remnant, proximal remnant, and the products of conception were removed. Histopathological examination of the laparoscopic specimen showed a fallopian tube with ectopic pregnancy. Her serum β-hCG on postoperative day 1 was 416.8 mIU/ml. She was discharged on postoperative day 3 and was doing well at the follow-up one week later.

Figure 1. — Ultrasonography showing a right tubal complex cystic lesion and normal ovary.
Ectopic pregnancy is associated with maternal morbidity and mortality during early pregnancy. Hemorrhage due to ectopic pregnancy is the leading cause of pregnancy-related maternal death in the first trimester [1, 2]. Risk factors for ectopic pregnancy include previous ectopic pregnancy, previous tubal surgery, tubal pathology, current use of an intrauterine device (IUD), and in utero diethylstilbestrol (DES) exposure [3, 4]. Ectopic pregnancy occurs in approximately 1.3–2% of all pregnancies, and the risk of recurrence is increased by eight-fold in women with a history of ectopic pregnancy [5]. However, recurrent ectopic pregnancy in the distal remnant of partial salpingectomy is very rare. The present authors searched the literature for cases similar to this case. There have been seven reported cases of recurrent ectopic pregnancy after salpingectomy, including the present case. Of these, three cases showed recurrence at the proximal remnant of a partial salpingectomy, three showed recurrence at the distal remnant of a partial salpingectomy, and only one showed recurrence at the cornual site (Table 1).

Table 1. — Recurrent ectopic pregnancy after salpingectomy.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Age</th>
<th>Previous surgery</th>
<th>Recurrent site</th>
</tr>
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<tr>
<td>Rizos et al.</td>
<td>2003</td>
<td>33</td>
<td>Laparoscopic left partial salpingectomy</td>
<td>Left cornual site</td>
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<tr>
<td>Zuzarte et al.</td>
<td>2005</td>
<td>32</td>
<td>Left partial salpingectomy</td>
<td>Distal remnant of left fallopian tube</td>
</tr>
<tr>
<td>Tan et al.</td>
<td>2007</td>
<td>27</td>
<td>Left partial salpingectomy</td>
<td>Proximal remnant of left fallopian tube</td>
</tr>
<tr>
<td>Chou et al.</td>
<td>2008</td>
<td>23</td>
<td>Segmental resection of right fallopian tube</td>
<td>Distal remnant of right fallopian tube</td>
</tr>
<tr>
<td>Liu et al.</td>
<td>2009</td>
<td>28</td>
<td>Laparoscopic left partial salpingectomy</td>
<td>Proximal remnant of left fallopian tube</td>
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<tr>
<td>Chou et al.</td>
<td>2009</td>
<td>38</td>
<td>Laparoscopic right partial salpingectomy</td>
<td>Proximal remnant of right fallopian tube</td>
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<td>Lee et al.</td>
<td>2011</td>
<td>28</td>
<td>Right partial salpingectomy</td>
<td>Distal remnant of right fallopian tube</td>
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<tr>
<td>Present case</td>
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</table>

Discussion

Ectopic pregnancy is associated with maternal morbidity and mortality during early pregnancy. Hemorrhage due to ectopic pregnancy is the leading cause of pregnancy-related maternal death in the first trimester [1, 2]. Risk factors for ectopic pregnancy include previous ectopic pregnancy, previous tubal surgery, tubal pathology, current use of an intrauterine device (IUD), and in utero diethylstilbestrol (DES) exposure [3, 4]. Ectopic pregnancy occurs in approximately 1.3–2% of all pregnancies, and the risk of recurrence is increased by eight-fold in women with a history of ectopic pregnancy [5]. However, recurrent ectopic pregnancy in the distal remnant of partial salpingectomy is very rare. The present authors searched the literature for cases similar to this case. There have been seven reported cases of recurrent ectopic pregnancy after salpingectomy, including the present case. Of these, three cases showed recurrence at the proximal remnant of a partial salpingectomy, three showed recurrence at the distal remnant of a partial salpingectomy, and only one showed recurrence at the cornual site (Table 1).

These rare cases are caused by the transperitoneal migration of embryos or sperms. It is possible that human gametes transmigrate to the transperitoneum [11]. The sperm approaches the intact fallopian tube through the endometrial cavity. Next, the sperm goes to the opposite fallopian tube through the transperitoneal cavity. When a sperm meets an ovum in the destroyed fallopian tube, fertilization occurs, but the embryo cannot migrate to the endometrial cavity. Embryo implantation thus occurs in the distal remnant of the destroyed fallopian tube [7, 9].

Recurrent ectopic pregnancy after ipsilateral partial salpingectomy is rare but possible. Therefore, physicians should carefully monitor for recurrent ipsilateral ectopic pregnancy.
Acknowledgments

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Endometriosis of episiotomy scar: a case report

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Summary

Endometriosis is characterized by the presence of histologically normal endometrial glands and stroma outside the uterine cavity. Endometriosis predominantly locates on peritoneal surfaces, but it also affects the vagina, vulva, and perineum, usually secondary to surgical or obstetric trauma. Endometriosis in an episiotomy scar is a fairly rare phenomenon. The authors present a case of endometriosis in an episiotomy scar.

Key words: Endometriosis; Episiotomy; Scar.

Introduction

Endometriosis is characterized by the presence of histologically normal endometrial glands and stroma outside the uterine cavity. Ectopic endometrial foci are most commonly found in the pelvis but can occur in many other sites [1]. The authors present an extremely rare case of endometriosis in an episiotomy scar.

Case Report

A 32-year-old patient complaining of a painful mass in the vulva was admitted for surgical excision of the mass. The pain was cyclic in accordance with her menstrual periods. The size of the mass appeared to be larger during menstruation. She had been having this pain for three years and the intensity of the pain seemed to be increasing. She had a history of two normal deliveries with medio-lateral episiotomies; first was 14 years ago and the second was 10 years ago. She had uterine curettage after her second delivery due to postpartum bleeding. The mass was on the episiotomy site, palpable as a firm nodule two cm in diameter. The nodule was wide-excised with a safety margin under general anesthesia (Figure 1). The histopathology report confirmed a diagnosis of endometriosis (Figure 2).

Discussion

Von Rokitansky first mentioned endometriosis in 1860, but Sampson provided the first detailed description in 1921. The etiology and pathogenesis of endometriosis are still controversial [2]. Many theories have been proposed to explain this condition; the endometrium implantation theory, the coelomic metaplasia theory, the lymphatic and vascular metastasis theories, the mechanical transplant theory, the embryonic rests theory, and a recent hypothesis based on the relationship of local immune factors [3]. Perineal endometriosis is an infrequent lesion. The position of the lesions can be explained by mechanical transplantation of endometrial cells to open episiotomy scars, which supports the transport theory of this extrapelvic endometriosis. It is likely that by the direct implantation of endometrial cells during vaginal delivery, viable endometrial cells are implanted into the episiotomy wound and subsequent cell growth occurs at the healing phase of the wound [4]. In the present case, endometriosis in the episiotomy scar may have resulted due to transplantation and implantation of endometrium during the postpartum uterine curettage after her second delivery.

Figure 1. — Introperative status with endometriosis in the episiotomy scar.
Diagnosis of the scar endometriosis is usually highly suggestive from the history and examination alone. The typical clinical presentation is a palpable firm nodule near a surgical scar accompanied by cyclic pain and swelling during menses attributable to the fact that endometrial implants behave like normal endometrium. The late onset of symptoms after surgery is the usual reason for misdiagnosis. The mean period between the procedure and symptoms are 5.72 years [5]. The present patient’s complaints commenced seven years after her second delivery.

Treatment of scar endometriosis is surgical excision. It is recommended that the excision should include five mm of surrounding normal tissue at a surgical margin [6].

In conclusion, when a mass showing symptoms in accordance with the menstrual cycle is present in the episiotomy scar, endometriosis should be considered primarily and surgical excision should be planned.

References


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Transient azoospermia following rosvastatin medication for hypercholesterolemia

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Summary
The authors report a case of transient azoospermia following hydroxymethylglutaryl-coenzyme A reductase (HMGCR) inhibitor rosvastatin medication for hypercholesterolemia. While a primary infertile couple with oligoasthenospermia was preparing for an in vitro fertilization program, the male partner had been diagnosed with hypercholesterolemia in a medical check-up and prescribed four-week oral administration of rosvastatin. No motile spermatozoa were found in the ejaculated semen and urine on the day of follicular aspiration. Azoospermia was confirmed by re-examination in weeks 3 and 7. Spermatozoa appeared in the ejaculated semen in two weeks of drug withdrawal. In week 16, the sperm count and motility increased to the level where intracytoplasmic sperm injection was available.

Key words: Azoospermia; Rosuvastatin; Hypercholesterolemia; In vitro fertilization.

Introduction
Rosuvastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme in cholesterol biosynthesis. The adverse events associated with rosuvastatin are uncommon and widely used for treatment of hypercholesterolemia, dyslipidemia, and hypertriglyceridemia. In addition, rosuvastatin was found to lower the relative risk of heart attack and stroke uniquely and therefore also utilized for prevention of cardiovascular diseases in the high risk cohort [1, 2].

As cholesterol is a substrate for steroid biosynthesis, administration of HMGCR inhibitor may possibly trigger some endocrine disorders including hypogonadism. However, to the authors' best knowledge, there are only one article that reported a case of oligospermia in a secondary infertile man who was taking a HMGCR inhibitor lovastatin for hypercholesterolemia and its recovery after drug withdrawal [3]. Meanwhile, there are no reports regarding the relationship between rosuvastatin and male infertility. The authors here report a case of primary infertile man who developed transient azoospermia following four-week administration of rosuvastatin for hypercholesterolemia and its recovery after drug withdrawal.

Case Report
A primary infertile couple of 46-year-old man and 40-year-old woman with infertility period for nine months visited the infertility care unit. Screening examinations revealed that the male partner had oligoasthenospermia (sperm count 8 × 10⁶ per ml semen; motility 12 %) with elevated serum follicle-stimulating hormone level (14.6 IU/L). Serum luteinizing hormone (5.3 IU/L), free testosterone (8.8 pg/ml), and prolactin (5.0 IU/L) levels measured within the normal ranges. The estimated bilateral testicular volumes were normal (the left testis 15 ml and the right testis 12 ml with orchidometer). Physical examinations and ultrasonogram did not show any pathological findings including varicocele, testicular tumors, and pelvic/urinary/seminal tract inflammation. The couple had seven failed cycles of in vitro fertilization (IVF) cycles using short gonadotropin releasing hormone agonist protocol [4, 5], intracytoplasmic sperm injection, and blastocyst transfer.

On the day of oocyte pickup in the eighth IVF cycle, motile spermatozoa were not found in the fresh ejaculated semen as well as in the urine. IVF was cancelled and the retrieved oocytes were immediately subjected to vitrification. The results of the physical and endocrinological examinations were similar to those at the first visit.

No spermatozoa were found in the ejaculated semen and urine in following three weeks. A detailed interview disclosed that the male partner was diagnosed with hypercholesterolemia in a periodic medical check-up and prescribed oral rosvastatin (2.5 mg/tablet, one tablet per day) for approximately four weeks. Magnetic resonance and physical reexamination did not demonstrate marked abnormal findings regarding testis, seminal tract, seminal vesicle, prostate, and penis. In reference to previous report [3], the authors suggested the consultation with the physician in charge regarding withdrawal or change of medication if possible. The male partner immediately stopped taking medicine following consultation, but semen analysis in weeks 4, 6, and 8 proved azoospermia.

Spermatozoa, however, appeared in the ejaculated semen in week 12 after drug withdrawal. In week 16, the sperm count (73 × 10⁶ per ml semen) and motility (17 %) increased to the level where intracytoplasmic sperm injection was available. In week 18, intracytoplasmic sperm injection was performed using thawed oocytes and fresh spermatozoa.
Discussion

Several reports documented the cases of drug-associated azoospermia/cryptozoospermia including colchicine, cyclophosphamide, clomiphene citrate, hormone/steroids, antibiotics, α-blockers, 5α-reductase inhibitors, pesticides, and recreational drugs [6, 7]. The authors here present a case of transient azoospermia developed in the male partner of an infertile couple following weeks of oral HMGCR inhibitor rosuvastatin administration.

As androgens are synthesized from cholesterol and play an essential role in spermatogenesis, it is conceivable that inhibition of cholesterol biosynthesis by rosuvastatin potentially deteriorated the sperm quantity and quality. Indeed, recent investigations demonstrated that treatment of hypercholesterolemia with HMGCR inhibitors reduced testicular volume and serum total and free testosterone concentration and increased serum follicle-stimulating hormone concentration and the onset of hypogonadism-related symptoms [8]. Following 16 weeks of drug withdrawal, the sperm count and motility improved to the level where intracytoplasmic sperm injection was available, suggesting a reversible cause-effect relationship between rosuvastatin administration and azoospermia in this patient.

References


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A case of idiopathic intracranial hypertension associated with PCOS

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Summary
Idiopathic intracranial hypertension (IIH) is a rare neurologic disorder. It is also known as pseudotumor cerebri. The incidence of IIH is one to two per 100,000 population annually. The higher incidence is in obese women from 15 to 44 years. The main symptoms are headache and visual loss. It mostly affects women of childbearing age who are overweight or obese. There are many theories of pathogenesis of IIH, but precise pathogenesis is unknown. One of the causes of IIH is intracranial venous sinus thrombosis. It can cause increased cerebrospinal fluid (CSF) pressure by obstruction of venous outflow and blocking of CSF absorption. In polycystic ovary syndrome (PCOS) patients, thrombogenic tendency is increased due to increased aromatization of testosterone to estradiol which could induce estrogen-mediated thrombophilia. The authors present a 14-year-old girl with PCOS stigma who presented with a severe headache and papilledema. These symptoms were not improved by standard medical therapy of IIH and PCOS, but improved after laparoscopic ovarian drilling. The authors report it with a review of the literature.

Key words: PCOS; Idiopathic intracranial hypertension; Laparoscopic ovarian drilling.

Introduction
Idiopathic intracranial hypertension (IIH) sometimes called benign intracranial hypertension (BIH) or pseudotumor cerebri (PTC), is a neurological disorder that is characterized by increased intracranial pressure in the absence of a tumor or other neurologic disease. Symptoms are headache, nausea, vomiting, pulsatile tinnitus, double vision, and other visual symptoms [1]. If untreated, it may lead to swelling of the optic disc in the eye, which can progress to loss of vision [2]. Therefore, the main treatment goal of IIH is to prevent or arrest progressive visual loss [3].

IIH is diagnosed with a lumbar puncture and brain scan to rule out other causes [4]. Some patients respond to lumbar puncture or medication, while others require surgery to relieve the pressure. This condition may occur in all age groups, but is most common in young women, especially those with obesity [5].

The cause of IIH is unknown, but, three theories exist as to why the pressure might be raised in IIH: an excess of cerebrospinal fluid (CSF) production, increased volume of blood or brain tissue, or obstruction of the veins that drain blood from the brain. The venous obstruction might cause blood flow from the brain to be impaired or congested. Congestion of venous blood may result from a generally increased venous pressure, which has been linked to obesity and intracranial venous sinus thrombosis. PCOS may be associated with facilitated development of IIH through an increased tendency to thrombosis [6]. The authors report a case of IIH associated with PCOS.

Case Report
A 14-year-old girl whose body mass index (BMI) was 20.61 kg/m² within normal range visited Department of Ophthalmology with complaint of dry eye and visual disturbance. Magnetic resonance (MR) orbit image showed no remarkable abnormal finding. Under the impression of optic neuritis, steroid therapy was started (methylprednisolone one g/day for three days, and then predinisolone 60 mg/day), however, her symptoms got worse and one month later, new symptoms, headache, and nausea appeared.

On further evaluations, slit-lamp biomicroscopy confirmed papilledema (Figure 1). Lumbar puncture revealed that the CSF opening pressure was increased to 34 cmH₂O, cerebrospinal fluid (CSF) composition was normal range, and no bacteria and virus was found in CSF culture. Radiologic imaging studies such as brain magnetic resonance imaging (MRI) with magnetic resonance angiography (MRA) and venography showed no abnormal finding. Through these findings, IIH was diagnosed [4].

General IIH therapy was begun. CSF drainage was performed and medication (acetazolamide, 750mg a day) was commenced. After treatment, headache and eye ball pain were improved slightly but papilledema did not improve. She was hospitalized and discharged repeatedly due to headache, eye ball pain, and high intracranial pressure. Due to her gynecologic symptom, irregular menstrual interval (35 to 70 days), gynecologic evaluation was requested. Her menarche occurred at 11 years of age. On transrectal ultrasound examination, both ovaries were polycystic with more...
than 14 follicles on each ovary (Figure 2) and modified Ferriman-Gallway (mFG) score was eight. On laboratory tests, testosterone, 17α-OH progesterone, free cortisol, DHEA-sulfate, and HbA1c, random glucose level were in normal range. Prolactin was slightly increased to 28.54 ng/ml. LH was 14.7 mIU/ml and FSH was 3.42 mIU/ml; LH/FSH ratio was increased to 4.29 and estradiol was 7.40 pg/ml on the second day of menstruation. Androstenedione was increased to 3.05 pg/ml. Under diagnosis of polycystic ovary syndrome (PCOS), metformin (1,500 mg a day) was used for improving hormonal imbalance, but there was no improvement in her symptoms, so the authors performed laparoscopic ovarian drilling. After general anesthesia, electrosurgical needle puncture was made on each ovary about ten times (Figure 3).

After 1 month of operation, her symptoms were improved with reduced medication (metformin one gram a day) and slit-lamp biomicroscopy test showed no papilledema. LH was 1.05 mIU/ml and FSH was 2.69 mIU/ml on the second day of menstruation. Finally all medication was stopped at 12 months after surgery. At 24 months after surgery, she had regular menstrual cycle and no recurrence sign of IIH.

Discussion

IIH is uncommon disorder in normal population, however, in young overweight women, the annual incidence is 20 per 100,000 persons [4]. The IIH has several clinical findings as follows: 1) increased intracranial pressure or papilledema, 2) normal CSF composition, 3) no imaging evidence of ventriculomegaly or mass lesion, and 4) no other cause was identified such as medication [4].

For a decade, several authors had mentioned that IIH might be a thrombotic disorder, because its findings were closely related with certain sites of intracranial thrombosis, and this thrombosis can cause impairing of CSF resorption.
PCOS is the most common endocrinopathy which incidence is 4%–10% of reproductive age women [7]. There is statistical association of PCOS and increased risk of deep venous thrombosis [5]. Increased aromatization of testosterone to estradiol could induce estrogen-mediated thrombophilia [7].

Glueck et al. studied 65 (consecutive) women referred due to IIH [6]. In this study, 37 of the 65 women had polycystic ovaries. All of the 37 were obese: 16 had a BMI between 30 and 40 and 19 had a BMI >40. There were also some findings related to more conventional predictors of thrombotic risk. Thirty-eight percent of the women with IIH were homozygous for the thrombophilic C677T mutation in the methylene tetrahydrofolate reductase gene: a finding present in 14% of 102 controls. Nine of the patients also had baseline Factor VIII levels greater than 150% percent, 29 had elevations of lipoprotein, and roughly half had symptoms appear during a high-estrogen state (oral contraceptive use, estrogen replacement therapy, or pregnancy) [6]. This study shows the possibility that PCOS can cause thrombophilia and hypofibrinolysis and may facilitate development of IIH.

Diagnosis of PCOS in adolescents demands more attention because characteristics of menstruation in adolescent are very similar with symptoms of PCOS [8]. However, in this case the patient’s menarche occurred at three years and six months before the diagnosis of IIH. Also, ultrasound finding of polycystic ovaries, increased LH/FSH ratio, increased mFG score, and irregular menstruation suggest that she had PCOS. Acetazolamide medication and lumbar puncture for decreasing of intracranial pressure are standard IIH treatment and metformin is widely used to ameliorate hormonal imbalance in PCOS patients. However, her neuro-ophthalmologic symptoms were not improved by these treatments. After laparoscopic ovarian drilling, her symptoms were improved. Laparoscopic ovarian drilling in adolescent girls has a risk for diminishing ovarian reserve so it demands utmost attention. However, in this case, medical treatment was not effective and the patient could not live daily life due to the symptoms of IIH.

From this case, the authors can make a conclusion that PCOS might be associated with development of IIH and treatment of PCOS might stabilize or reverse IIH. If initial medical treatment of IIH and PCOS is not effective, surgery such as ovarian drilling may become an optional treatment. Understanding of the contributions of PCOS to the development of IIH should facilitate development of new approaches to treat this disabling neuro-ophthalmologic disorder.

References


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Struma ovarii complicating pregnancy: a case report

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Summary

Struma ovarii represents an ovarian mature teratoma with thyroid tissue comprising more than 50% of the ovarian tumor. It is a rare condition, representing approximately 1% of all ovarian tumors with a potential malignant transformation 5% to 10%. When it is combined with pregnancy, it renders its management in this circumstance is extremely challenging. The authors present a case of benign struma ovarii diagnosed as a right adnexal mass during first trimester of pregnancy with an uneventful clinical course.

Key words: Pregnancy; Struma ovarii; Adnexal mass; Ovarian cyst.

Introduction

Struma ovarii is a monodermal ovarian teratoma composed mainly of differentiated thyroid tissue. It occurs rarely, representing less than 1% of all ovarian tumors. Generally benign, although the malignant cases count approximately 5-10% [1], it is most common in ages between 40 and 60 years. The symptoms are similar to other ovarian tumors and rarely hyperthyroidism is the presenting symptom, seen in 5-8% of patients with struma ovarii [2]. It can be rarely diagnosed in mucinous cystadenoma or combined with Brenner tumor [3].

During pregnancy, with the increased use of imaging studies, the detection rate of adnexal masses is approximately 2.5% [4]. Consequently, despite that most of them are benign, great attention regarding the diagnostic and therapeutic approach must be given. Thus, although the optimal management of an adnexal mass during pregnancy is laparotomy to exclude malignancy, serial imaging studies as transvaginal ultrasonography and serum tumor markers can distinguish it and may early recognize malignant transformation.

The authors present a case of benign struma ovarii first diagnosed as a right adnexal mass during the first trimester of pregnancy with an uneventful delivery and clinical course.

Case Report

A 36-year-old Caucasian female (para 1, gravida 0) with free family, obstetrical, and gynecologic history presented to the present department complaining of amenorrhea for a period of two months and severe hypogastric pain.

A physical examination revealed an enlarged and painful right adnexa during palpation and an enlarged size of the uterus. The transvaginal ultrasound and the increased B-HCG certified the progressive pregnancy with the presence of one endometrial gestational sac and embryonic heartbeat (8w5d). Additionally, corpus luteum of the left ovary and a 8 × 10 cm right adnexal mass were diagnosed (Figure 1). The adnexal mass revealed no signs of malignancy such as septal spaces and papillary protrusions and there was no free peritoneal fluid. The tumor markers such as Ca-125 were negative. A complete blood count revealed mild leukocytosis (WBC 14,000, neutrophils 90%). A regular consultation was scheduled. During the follow up at the 12th week of gestation, the patient had no clinical symptoms and the transvaginal ultrasound revealed a decreased size of right adnexa (5 × 9 cm) (Figure 2). The size and structure of the mass remained stable throughout pregnancy and there were no clinical signs or symptoms indicating torsion or rupture of the cyst. The tumor markers remained free of malignancy. The patient had a normal delivery of a healthy baby boy (38w5d), weight 3,450 gr, and Apgar score of 9 in the first minute and 10 in the fifth minute.

Figure 1. — Right adnexal mass in first trimester of pregnancy (8w5d).
Post-delivery, the transvaginal ultrasound revealed an increased size of the adnexal mass (5 x 11 cm) with mucus like appearance with normal laboratory tests and serum tumor markers (Figure 3A). The patient underwent an exploratory laparotomy where a 12.5 x 7.5 x 6 cm tumor of the right adnexa was recognized. The left adnexa appeared normal. Patient undergone en bloc resection of the right adnexa with the tumor. Tissue biopsy from the left ovary and peritoneal lavage of Douglas space for cytological examination was performed. The frozen section of the right adnexa was negative for malignancy. The histological examination revealed the presence of ovarian monodermal teratoma covered with thyroid follicles filled with colloid (Figure 3B) representing right struma ovarii. The cytological examination was free of malignancy.

The postoperative course was uneventful and the patient was discharged from the hospital on the third postoperative day. Making the diagnosis of the struma ovarii postoperatively, she underwent thyroid function tests as well as ultrasonography of the thyroid gland which were normal. Four months after the exploratory laparotomy the patient became pregnant again and delivered a full-term healthy baby girl.

Discussion

Adnexal masses correlated with pregnancy vary with regards to the imaging findings and clinical symptoms. Between 1% and 2% of pregnant women will develop an ovarian mass which will be diagnosed sonographically and 1% to 3% of these will be malignantly transformed. [5, 6] Some adnexal masses were diagnosed accidentally during pregnancy and others due to the clinical symptoms they present. The sign of pain indicates in most cases torsion or rupture of the ovarian mass. Torsion can lead to venous and lymphatic blockade which can develop stasis, venous congestion, and necrosis. The incidence is five per 10,000 pregnancies. [7]

The most often types of adnexal masses correlated with pregnancy are cystic teratomas, para-ovarian cysts, serous cystadenomas, and luteomas. [8] Struma ovarii is a monodermal ovarian teratoma, composed mainly of differentiated thyroid tissue, while benign and malignant stumps
Struma ovarii complicating pregnancy: a case report

A struma ovarii is a rare benign tumor that contains thyroid tissue. It can occur in any age group but is more common in reproductive age women. It is usually asymptomatic and is often discovered incidentally during sonography. However, malignant struma ovarii is a rare entity that can be associated with thyroid carcinoma.

Clinical symptoms and clinical characteristics of an adnexal mass are largely nonspecific. In the present case, the mass was clinically diagnosed as an adnexal cyst during pregnancy. The patient had no signs of malignancy and was asymptomatic. The mass was initially diagnosed as benign and managed conservatively.

Surgical resection of struma ovarii continues to be the treatment of choice. However, the management of malignant struma ovarii is more complex. The patient underwent surgical resection, which revealed malignant struma ovarii associated with papillary thyroid carcinoma.

Conclusion

The incidence of struma ovarii during pregnancy is very rare and most of the time is diagnosed incidentally with sonograph. It should be considered in differential diagnosis of an adnexal mass especially during pregnancy. For the vast majority of patients, as in the present case, it is benign and the prognosis is excellent.

References


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Successful management of discordant gastroschisis in monochorionic diamniotic twin: a case report

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Summary

Background: Monochorionic diamniotic (MCDA) twin pregnancy with gastroschisis carries a poor prognosis. Live birth and well-development of both twins are extremely rare. Case: The authors report a rare case of discordant gastroschisis in MCDA twin. Both twins were followed up nine months after intrapartum fetal operation, and both are in good health until now. Conclusion: This report expands successful management of discordant gastroschisis in MCDA twins. Early diagnosis, intensive prenatal care, and multidisciplinary consultation are recommended in management of discordant gastroschisis in MCDA twin.

Key words: Monochorionic diamniotic twin; Gastroschisis; Intrapartum fetal operation.

Introduction

Gastroschisis is a congenital anterior abdominal wall defect with the uncovered abdominal contents protruding through the defect. The cause is not yet clear. The incidence reported is between four and five per 10,000 live births [1]. Prenatal diagnosis of gastroschisis relies on sonography. Early closure can prevent heat and water loss, infection, and bowel edema. Immediate primary fascial closure should be done when possible. Gastroschisis delivery and immediate repair are important in the operating room. Monochorionic diamniotic (MCDA) twin pregnancy with gastroschisis is rare. This report expands successful management of discordant gastroschisis in MCDA twins.

Case Report

A 24-year-old woman (gravida 1, para 0) conceived MCDA twins naturally. At 15 weeks of gestation, twin B was diagnosed with anterior abdominal wall defects with 2.0 × 2.4 × 2.0 cm bowel herniated by ultrasonography while twin A was normal. Second trimester Down’s syndrome screening for both twins was normal. The maternal history was unremarkable for smoking, drug or alcohol abuse. No significant family or obstetric history was elicited. Close follow-up ultrasound evaluation every two weeks was maintained with multidisciplinary consultation. At 20 weeks ultrasound, evaluation still showed anterior abdominal wall defects of twin B other than twin A (Figure 1). At 32 weeks, ultrasound showed a 7.1 × 4.3 × 5.0 cm bowel-like echo floating in the amniotic cavity, while at 35 weeks it increased to 8.9 × 5.6 × 6.5 cm. Electronic fetal heart rate monitoring performed biweekly from 32 weeks gestation. Twins were delivered by emergency cesarean section at 35 weeks and five days of gestation due to suspected fetal distress of the twin with gastroschisis. They were female, twin A weighted 1,950 g with Apgar scores of 10 at one minute and 10 at five minutes; twin B (Figure 2) weighted 1,890 g who had a three-cm abdominal wall defect to the right of the umbilicus with the uncovered abdominal contents (most of the small intestine, part of the colon and stomach, ileocecal, and appendix) protruding through the defect. Twin B was given endotracheal intubation, nasogastric tube, placement of peripheral intravenous line for anesthetic and paralysis, and immediate surgical repair in the operation room. Temperature, electrocardiogram, pulse, blood pressure, and oxygen saturation were continuously monitored. After intrapartum fetal operation, twin B was transferred to neonatal intensive-care unit (NICU). She stayed in NICU for three weeks before discharged, and the abdominal wall healed well at 16 days (Figure 3). Karyotype test of both newborns’ cord blood was normal (46XX). Monochorionic diamniotic placenta was identified with pathological examination. Both twins were followed up by nine months and are in good health until now.

Discussion

The etiology of gastroschisis remains elusive. Discordant gastroschisis in MCDA twin pairs is extremely rare. Reports from the literature only showed one pair of female and two pairs of male discordant gastroschisis monozygous twins [2, 3], but the placenta was not available for examination, and the management and follow-up were unclear. As monozygotic twins are essentially genetically identical (with the exception of post-zygotic somatic mutations), this case may suggest environmental factors that play a greater role than genetic factors in the development of gastroschisis as reported before [2].

Progressive evolution of the treatment modalities has led to increased survival in patients with gastroschisis. This is due to improvement in the prenatal diagnosis and post-operative treatment for respiratory compromise, treatment of sepsis, and the use of parenteral nutrition until enteral feeds. This case also showed that immediate surgical repair after delivery is very important.
The mean gestational age at spontaneous delivery in gastroschisis is approximately 36 to 37 weeks [4]. The risk of in utero fetal death is higher in fetuses with gastroschisis. The recommended mode and timing of delivery is still debated, because labor may be deleterious to bowel loops (by compression and twisting) and ruptured membranes may contribute to neonatal infectious complications. A major controversy in the perinatal management of these conditions is whether cesarean delivery leads to an improved neonatal outcome. However, most authors have found no significant benefit to cesarean section. A systematic review of 27 peer-reviewed observational studies similarly found insufficient evidence to support induction of labor in gastroschisis. Additionally, there was no significant relationship between mode of delivery and time until enteral feeding or length of hospital stay [5]. A randomized controlled trial (RCT) also showed the same results [6]. A retrospective study showed that immediate vs traditional surgery resulted in neonates that had less edematous bowel with less fibrous peel, were more likely to be closed primarily, spent less time on a ventilator, seemed to be fed sooner, and were discharged home earlier [7].

Early diagnosis, intensive prenatal care, and multidisciplinary consultation are recommended in management of this case. The present team included obstetricians, sonographer pediatricians, anesthesiologists, pediatric surgeons, and nurses. As survival rates following live birth of an infant with gastroschisis continue to improve, better evaluation would seem to be required, both to long-term nutritional and neuro-developmental outcomes.

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References


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