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Sympathomimetic amines are a safe, highly effective therapy for several female chronic disorders that do not respond well to conventional therapy

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Summary

Purpose: To evaluate the efficacy of sympathomimetic amine therapy for women with chronic disorders including, but not limited to, pelvic pain. Materials and Methods: Dextroamphetamine sulfate 15-mg extended release capsules were given to women with a variety of treatment refractory conditions including, but not limited to, pelvic pain. The dosage could be increased to 60 mg depending on tolerance to the medication and degree of improvement of the condition. Results: A very high percentage showed marked amelioration of their symptoms despite previous failure with medical or surgical therapy. Conclusions: The human species, especially women, seem to be more prone to certain specific tissue permeability defects and diminished sympathetic tone, which compounds the problem, since the sympathetic nervous system controls permeability. Thus, besides pelvic pain and interstitial cystitis, dextroamphetamine sulfate, which seems to restore sympathetic tone possibly by increasing dopamine secretion to the nerve fiber, provides gratifying relief to a variety of chronic disorders. These other disorders include: severe headaches, inflammatory bowel disease, gastrointestinal motility disorders, fibromyalgia, and other musculoskeletal pain, chronic fatigue syndrome, and urticaria.

Key words: Dextroamphetamine sulfate; Pelvic pain; Infertility disorders; Miscarriage; Pre-eclampsia; Ovarian hyperstimulation syndrome.

Introduction

As suggested by the title, it is my intent to convince the readers that the sympathomimetic amine, dextroamphetamine sulfate, is the most important single agent of all medications to help provide relief from suffering in women from a variety of medical conditions. It is my hope that if you are convinced as to the efficacy of this type of therapy by this “perspective”, that this manuscript will have a major positive impact on your medical practice.

The reader should be forewarned that most of the evidence that will be provided will be from anecdotal case studies. There will be occasional data from a small series but only one randomized comparative study. However, I think you will agree that based on the dramatic quick response to treatment with dextroamphetamine sulfate in many chronic female-related disorders that previously failed to respond adequately to “conventional” therapy, that there is a high likelihood that this concept is correct and the therapy is quite beneficial.

One can hope that this study will generate interest for some group to initiate a large randomized controlled study. If such a study corroborates the benefits of dextroamphetamine sulfate in treating these disorders, this may convince a much larger body of physicians to try this therapy. However, with this drug available as a generic, and with the difficulty a pharmaceutical company would have in obtaining a unique patent for its use, since there are already many publications concerning its efficacy, it is unlikely a randomized controlled study will be performed since most are supported by pharmaceutical companies. Thus, realistically, my main goal is to share my knowledge with the readership to enable you to provide the same relief to your patients that I have provided to my patients for the past 30 years. Maybe, you will publish some unique case reports in various journals when you see how efficacious this therapy is or publish some case series of your own. Possibly you may write to the Editor of this journal if you have had positive experiences with this therapy but do not hesitate to publish any negative experiences. Credibility will be increased if confirmation of efficacy comes from a variety of different physicians.

At this point, you as the reader must be wondering what kind of female disorders respond to amphetamines. The discussion will emphasize pelvic pain but will not be limited...
to that subject [1-8]. The proposed mechanism is that certain tissues are more susceptible to permeability defects and absorption of chemicals and toxins into these tissue can lead to inflammation and pain [1, 2, 9]. If these noxious factors infiltrate mitochondria, weakness and organ malfunction may occur.

The sympathetic nervous system controls cellular permeability. The hypothesis leading to treating with dextroamphetamine sulfate is that hypofunction of the sympathetic nervous system related to diminished secretion of sympathomimetic amines, can be corrected by either the amphetamine substituting for the defective sympathomimetic amines or by the amphetamine stimulating dopamine [1, 2].

**Chronic pelvic pain syndromes**

*Dysmenorrhea, deep dyspareunia, middleschmertz, chronic pelvic and chronic back pain*

Endometriosis can be associated with pelvic pain syndromes including chronic pelvic pain, dysmenorrhea, dyspareunia, middleschmertz, or premenstrual backaches [10]. Yet, it is well known that some infertile women having no pelvic pain can be found to have extensive endometriosis. On the other hand, some women with excruciating pain are found to have mild or minimal endometriosis or no endometriosis at all [10].

Laparoscopic removal by laser has led to long-lasting relief of pelvic pain from endometriosis in a minority of cases. In most cases, the relief is short-lived or not at all [11]. Recurrence rates of endometriosis of 40-60% within one to two years has been reported [12]. Even laparoscopically lasering endometriotic implants combined with medical therapy do not prevent the return of pain from endometriosis [13]. Some argue that the reason why frequently the pain returns following laser vaporization is because there is the return of endometriosis as the laser does not completely remove the entire endometriotic lesion. Some claim that deeply infiltrating endometriosis is more associated with pelvic pain and this requires surgical excision rather than laparoscopic laser ablation [14]. There are studies claiming lower rates of recurrence of endometriosis with surgical excision in the range of 19-34% [15, 16].

One study by Yeung et al. provided results that were extremely interesting. They performed laparoscopies in teenage girls with severe dysmenorrhea and re-laparoscoped them two years later [17]. Not one showed a return of the endometriosis two years later when surgery was performed by careful excision rather than laser ablation [17]. However 50% had the return of pain [17]. I find this study extremely interesting. The study did claim that 50% showed relief of pain for two years, which may be higher than expected by laser vaporization [17]. However, 50% had return of pain without visual evidence of the return of endometriosis which suggests that the endometriosis, per se, is not the actual cause of the pain. A recent randomized controlled trial found no significant difference in overall visual analogue score for pain at 12 months when comparing excision vs. laparoscopic ablation of endometriosis [18].

Case reports have been described of relief of pelvic pain that was refractory to medical and surgical therapies that has responded to treatment with the sympathomimetic amine dextroamphetamine sulfate. We believe this works, by replacing or stimulating an increase in the defective neurotransmitter with subsequent restoration of normal sympathetic tone and therefore diminishing cellular permeability [2, 3].

One method of controlling pelvic pain, especially dysmenorrhea, is to use a low estrogen oral contraceptive or a low dosage progestin only, e.g., norethindrone 0.35mg, on a daily basis. The low dose estrogen can be used without a break to induce menses. If it works adequately, this therapy will fulfill the criteria of a treatment with low risk and relatively low cost. Unfortunately, the pain relief is not adequate in many women and further therapy is needed [4].

Suppressing estrogen levels by use of long acting gonadotropin releasing hormone agonists (GnRHa), e.g., depo-leuprolide acetate or the nasal inhalant nafarelin, has too many menopausal symptoms along with the possible risk of precipitating subsequent osteoporosis. Impeded androgens, e.g., danazol or ten-mg norethindrone acetate, have too many androgen side effects, e.g., weight gain, hirsutism, and acne and may cause atherosclerotic plaque [4, 19]. Since estrogen has been considered the main stimulator for endometriosis development, theoretically, it could be a target for therapy with aromatase inhibitors, e.g., letrozole. However, these agents were considered for treatment, but again too many menopausal side effects for a non-malignant non-life threatening disorders [19].

As mentioned in the aforementioned editorial, our preference for treating pelvic pain, by far, is the use of sympathomimetic amines specifically dextroamphetamine sulfate [4]. Since writing that editorial, we have completed a prospective series involving women with documented endometriosis with at least one year of severe pelvic pain not relieved adequately with oral contraceptives, surgery, or non-steroidal anti-inflammatory drugs (all three of these were a pre-requisite for inclusion). They could have also been treated with GnRHa’s, but this was not a prerequisite for inclusion in the study. There were 15 women recruited. The average age was 32.5 years. These data were presented at the 2013 meeting of the American Society for Reproductive Medicine in Boston, MA [20]. The results are seen in Table 1. After three months, seven reported marked improvement (46.6%), five moderate, and three mild improvement. By six months, eight (53.3%) reported marked improvement, six (40%) moderate improvement, and one with mild improvement. Even the one with mild improvement said she did not want to consider a laparoscopy. Thus, 80% reported at least moderate im-
A previously unreported case provides extra credibility to support using amphetamines for pelvic pain while illustrating another point. A 35-year-old woman started out with dysmenorrhea at age 28 with a two- to three-day premenstrual component which gradually extended to continual pelvic pain, exacerbated by menses and severe dyspareunia so that intercourse was not possible. When she presented at age 35 for consideration of treatment for her pelvic pain, from her reading, she assumed we were going to try laparoscopic surgery. Instead we recommended dextroamphetamine sulfate. She had her doubts because besides the pelvic pain, she suffered from a severe anxiety problem. Unfortunately, despite a multitude of trials with anti-anxiety medication, she had side effects from them all, and thus was not on any anti-anxiety therapy. She was started on 15 mg amphetamine sulfate extended release capsules. Not only did she tolerate the medication, but it completely corrected the anxiety disorder. Even more important, except for mild dysmenorrhea, all the pelvic pain disappeared. Thus, even the presence of severe anxiety does not preclude amphetamine therapy. It was discovered that, at least in some cases, the drug can help treat anxiety.

**Interstitial cystitis**

We reported a small series on the use of dextroamphetamine sulfate for interstitial cystitis [6]. The small series required for entry that the subject was a woman with painful bladder for over a year who had failed to have adequate improvement from “standard therapies”. The study required the triad of bladder pain, urgency, and frequency despite negative urine culture. Furthermore, cystoscopy findings had to be consistent with the diagnosis of interstitial cystitis [6]. Six cases were enlisted with four of the six having such severe symptoms that they could not function in daily society. Five of six women had nocturia also. Within one month of therapy on 15 mg dextroamphetamine sulfate extended release capsules, all six showed significant relief of dysuria, urgency, frequency, and nocturia. Five of six women increased the dosage of the amphetamine to 25 or 30 mg, whereas one had total relief at 15 mg and preferred no increase. Within two to six months the urinary symptoms were either completely gone or so mild as to be considered very tolerable [6]. The drug was continued for a year and at the end of the year the symptoms remained gone or nearly gone [6].

One unreported case illustrates the continuum between pelvic pain possibly “associated” with endometriosis and/or adenomyosis, and interstitial cystitis. A physician’s daughter for several years had such severe dysmenorrhea that about eight/nine times per year she would go to the emergency room for parenteral narcotics to ease the pain. We placed her on amphetamine salts, which includes dextroamphetamine sulfate, and on 20 mg extended release capsules she had almost complete relief. This beneficial
effect persisted for several years from age 28-34. One cycle she had an anovulatory cycle and when menses ensued, she was passing large clots. For the first time she experienced fairly severe dysmenorrhea again.

She was assured that probably the pain was related to the clots, and with the control of her bleeding, her next menstrual cycle would not be painful. However, she was concerned that her insurance was about to be dropped, and if a laparoscopy was needed, she would have to pay out of her pocket. Her father strongly suggested a laparoscopy. Though a laparoscopy was not considered by our team, we were willing to perform it, if they desired. In our consultation, we discussed that we usually use laser vaporization and did discuss the surgical extirpation procedure for deep infiltrating lesions favored by some other gynecologists. Her father, a dermatologist, did a literature search and concluded that it would be best performed by a surgeon using the non-laser technique. His computer investigation concluded that the best surgeon for his daughter was in a city 965 km away with a world renowned endometriosis surgeon skilled in removing deep infiltrating endometriotic implants.

She only had stage I endometriosis with only a couple small implants, which were removed. The surgeon advised her that now with the endometriosis removed she can stop the amphetamine. She complied. She had no dysmenorrhea for three months, but on the fourth month it was as severe as ever, requiring a trip to the emergency room. She called the surgeon and he explained that she most likely has adenomyosis and that the only treatment would be hysterectomy. She did not ask our opinion, but proceeded with simple hysterectomy. Within a few months she developed classic symptoms of interstitial cystitis. She came back to our office and she was retreated with dextroamphetamine sulfate and her bladder symptoms completely disappeared. If this single woman marries and wants a child, she will need an extremely expensive gestational carrier. This case is an example of why sympathomimetic amine therapy should be chosen before any surgical procedure [4, 11]. It is interesting that without a uterus, the permeability defect shifted to the urinary bladder. She never had symptoms of interstitial cystitis before. The case supports the concept that endometriosis may be associated with the sympathetic neural hyperalgesia syndrome causing severe pelvic pain, but not the actual cause of it, since the removal of her one endometrial implant by one of the world’s leading endometriosis surgeons failed to improve her pain, whereas amphetamine did relieve the pain. Furthermore, the surgeon’s presumptive reason for failure was that the pain was probably from adenomyosis (explained the lack of success of laparoscopic excision). However, the pathology report on the uterus removed by hysterectomy showed no evidence of adenomyosis. Finally, it was extremely interesting that without a uterus the syndrome shifted to her bladder, and thus illustrates a similar mechanism for dysmenorrhea and pelvic pain of bladder origin [5, 6].

Other pelvic pain syndromes – vulvovaginitis, backache

One interesting case report involved a six-year-old girl with premature pubarche who developed a severe chronic vulvovaginitis that responded completely and very quickly to treatment with dextroamphetamine sulfate [8]. Most of the multiple pediatric endocrinologists and pediatric gynecologists that she consulted focused on some occult connection to her three hydroxysteroid dehydrogenase type of congenital adrenal hyperplasia playing an etiologic role when the problem was related to increased permeability of the vulva related to sympathetic hypofunction [8].

Another woman with severe backaches that were attributed to herniated disks wanted to avoid orthopedic surgery. She showed dramatic improvement with dextroamphetamine therapy and the pain has been eradicated for years while on therapy [7]. Her dysmenorrhea that had been present long before the backache actually started, also dissipated [7].

Prevention of miscarriage

There is still controversy as to what treatments are beneficial for the prevention of recurrent miscarriage. I am a strong proponent of progesterone therapy [29-33]. What therapies are available for women who continue to have first trimester pregnancy losses despite progesterone therapy?

We considered that a miscarriage could possibly be related to inability to preclude chemicals and toxic factors from invading the placenta related to sympathetic nervous system hypofunction. Indeed, two cases were reported suggesting that successful completion of the first trimester could have been aided by sympathomimetic amine therapy [23]. One case related to severe oligoasthenozoospermia in her male partner had in vitro fertilization-embryo transfer (IVF-ET) at another reproductive center and conceived three times, but despite progesterone supplementation, had three first trimester miscarriages. She also conceived three more times with donor sperm insemination and progesterone support. However, all three ended in first trimester miscarriages. This 36.8-year-old woman successfully delivered a live baby full-term with her first IVF-ET cycle in our practice by also treating her with dextroamphetamine sulfate 15 mg extended release capsules [23].

Another woman conceived two times with IVF-ET and one frozen ET and miscarried all three, including a documented normal male fetus. She was successful in cycle 4 with the addition of dextroamphetamine sulfate [23].

Another unreported woman in her mid-thirties had primary recurrent miscarriages three times in natural cycles. I was very optimistic that as long as her follicle was ma-
ture (which our testing concluded was mature) that taking progesterone supplementation in the luteal phase and throughout the first trimester would be all that was needed for a successful outcome. She conceived quickly, but despite aggressive progesterone supplementation, she had miscarriage number four. A gestational sac was evident but no fetal pole, so chromosome analysis on the products of conception was not performed. A fifth pregnancy also resulted in miscarriage and chromosome analysis showed a normal female. A sixth and seventh miscarriage found normal male fetuses. We discussed the possibility of treatment with dextroamphetamine sulfate. We had her talk with one of our employees who had failure to conceive at another reproductive center at age 35 despite several cycles of IVF-ET and many cycles of intrauterine insemination. Though we were successful with IVF-ET in achieving pregnancies in all three IVF-ET cycles (40 years old for cycle 1), she miscarried all 3. She was however, successful with her fourth cycle with the addition of amphetamine and completed her second trimester. Unfortunately our employee developed pre-eclampsia and had neo-natal death of a premature baby. Meanwhile, the primary aborter with seven first-trimester miscarriages on our advice, and after talking to our employee when she was late in the second trimester, decided to take the dextroamphetamine sulfate 15 mg extended release capsule. She delivered a full-term healthy baby. Subsequent to this, our employee now 42 years of age decided to try IVF again. Two weeks before her expected start date for her stimulation drugs, she visited her hair dresser. She remembered that her head had been yanked back fairly hard when she was having her hair washed. Four days later, she had a stroke. It was found to be secondary to embolism from a dissected right carotid artery which may have occurred during the hair-washing. We were now faced with a dilemma. Though she had 99% recovery, we believed that she could be at more risk from emboli during the hypercoaguable pregnancy state. The safest solution would be the use of a gestational carrier. However, our employee could not afford one. The primary aborter who had seven losses in a row before using dextroamphetamine sulfate offered to be a free gestational carrier. Only two embryos were transferred but both implanted. The gestational carrier stayed on the dextroamphetamine sulfate until the 27th gestational day and this was the only agent that provided relief [34].

Another woman failed to conceive despite several IVF cycles but was able to conceive the only two times she took dextroamphetamine sulfate [35]. Though one can argue that the conception may have merely been fortuitous, one cannot deny that the drug markedly relieved chronic complex regional pain syndrome (reflex sympathetic dystrophy), making the argument more credible that it helped implantation also [35]. All other therapies had failed to help her severe wrist pain.

Other disorders specific to women

**Vasomotor symptoms**

The first case report of improvement of vasomotor flushing by dextroamphetamine sulfate was in a 29-year-old normal estrogenic woman with no evidence of diminished oocyte reserve [36]. She described typical flushing and night sweats. Her day 3 serum FSH was only four mIU/mL. She was 99% improved within two weeks of taking dextroamphetamine sulfate 20 mg daily. She had failed to improve with oral contraceptives [36].

The benefits of sympathomimetic amine therapy for vasomotor symptoms extend to women with estrogen deficiency and perimenopausal women also [37, 38]. The sympathetic nervous system controls the temperature regulation system of the hypothalamus. It is not clear if this problem is related to simply low sympathetic tone as a direct effect on the temperature regulation system, or do chemicals infiltrate this area of the brain related to increased cellular permeability and cause the vasomotor instability?
Post-partum depression

One suspected cause of post-partum depression is estrogen deficiency related to a delay in restoring the hypothalamic pituitary ovarian axis related to suppression of LH and FSH from prolonged sex steroid exposure during pregnancy, compounded by persistent high prolactin levels [39]. Indeed, estrogen therapy had helped relieve post-partum depression in some women [40, 41]. Some believe that estrogen followed by progesterone supplementation is more effective than estrogen alone [42].

Pregnant women may be more prone to transient hypothyroidism with lymphocytic thyroiditis [43, 44]. Because the gland is injured, in the post-partum period, transient hypothyroidism may occur until the injured thyroid can heal and start making thyroid hormone again [45, 46]. Thyroid hormone replacement has been found to help depression in some cases [43-45]. Because the post-partum period may be associated with lowering of certain biogenic amines, e.g., momamine oxidases and serotonin, anti-depressants are frequently prescribed, especially the newer serotonin re-uptake inhibitors and bupropion [46].

Another unreported case will provide an example once again of a woman failing to gain relief from standard therapy, yet quickly responding to dextroamphetamine sulfate. A woman at age 33 developed post-partum unipolar depression shortly after the successful birth of twins. The depression was so severe one month after delivery that she was committed for hospitalization. The hospitalization did not result in much improvement. She had received both psychosocial treatment and psychotherapy and was started on antidepressant medication.

Though she was discharged from the hospital on fluoxetine Hcl and bupropion, she was still so depressed that she could not return to work. She was also taking topiramate for migraine headaches, but this drug provided her no relief. She had resumed regular spontaneous menses, so there was no evidence of estrogen deficiency. Her free thyroxin levels and thyroid stimulating hormone levels were obtained and they were normal. She was started on dextroamphetamine sulfate extended release capsule 20 mg once daily. When she returned in one month, she stated that within one week her depression completely lifted, her fatigue markedly improved, and she had not had a migraine headache all month. Her insomnia also dissipated.

Other medical conditions more prevalent but not restricted to women

Inflammatory bowel disease

Women seem to be more prone to autoimmune disorders which include inflammatory bowel disease. A 39-year-old woman with Crohn’s disease Stage IV of 12 years duration had failed to respond to mesalamine, prednisone, cyclophosphamide, infliximab, and adalimumab. Colonoscopy showed involvement of the entire colon [47]. She was ad-

vised to have a partial colectomy and diverting ileostomy. Instead, she tried dextroamphetamine sulfate 20 mg per day and was 90% improved in one week and 100% improved by one month (eight to 12 very painful bowel movement per day reduced to one painless bowel movement). A repeat colonoscopy found no evidence of Crohn’s disease [47].

Subsequent to that publication, the woman had remained in clinical remission for three years. She sought help to get pregnant at age 42. She was found to have diminished oocyte reserve as evidenced by elevated day 3 serum FSH and a short follicular phase. She was treated with ethinyl estradiol to lower FSH and lengthen the follicular phase and luteal phase support with supplemental progesterone similar to the technique that we successfully used for a 45-year-old woman with a similar problem [48]. She conceived on her second treatment cycle. She was discharged after completing the first trimester, and she was advised to continue with the amphetamine salts that she had taken during the first trimester until delivery. She was also advised to continue with progesterone until at least 36 weeks. Her obstetrician referred her to a maternal-fetal medicine specialist at a major university hospital. She was told to stop the amphetamine because they were not aware of its use for Crohn’s disease (they made no effort to contact our office for an explanation). They advised her that most of these autoimmune disorders go into remission anyhow during pregnancy. They also stopped the progesterone because this was her first pregnancy and they usually only prescribe it beyond the first trimester for those with previous pre-term delivery.

During her second trimester, her Crohn’s disease severely flared up. The gastrointestinal team at this university hospital was consulted and they strongly suggested partial bowel resection and diverting ileostomy. This procedure was performed. She delivered at 32 weeks but the baby survived and is doing well. Following the delivery, her bowel was re-connected. Shortly after surgery, her frequent bowel movements with severe dyschezia returned. The gastrointestinal specialist recommended total colectomy and permanent ileostomy. Instead, she returned to our office and she was re-started on the amphetamine salts. Her symptoms quickly abated again and she has been well controlled now for 1.5 years post-partum on dextroamphetamine sulfate.

Another unreported case of Crohn’s disease is very interesting. A 41-year-old nurse married to a specialist in internal medicine had a 25-year history of Crohn’s disease and had failed to respond to any of the standard medications used for Crohn’s disease, similar to the other case just previously mentioned, and also failed to improve following two partial bowel resections. She finally had a total colectomy and a permanent ileostomy. Unfortunately, her abdominal pain was still so severe she needed to take hydromorphone hydrochloride every two to four hours.

She decided to try to have her first baby. She went to a reproductive endocrinologist at a major university medical...
center who advised her that because of her age and diminished oocyte reserve, as evidenced by an elevation in day 3 serum FSH, that she would require donor oocytes with the embryos transferred to a gestational carrier because of her need to take such a high dosage of hydromorphone with the highly likely addiction of the baby. She agreed to the gestational carrier but wanted to try IVF with her own oocytes.

She failed to achieve a pregnancy twice despite transfer of embryos to gestational carrier. The reproductive endocrinologist refused to try again with her own oocytes insisting on donor oocytes.

The woman sought a second opinion still wanting to use her own oocytes and consulted our clinic. We explained that the higher FSH regimen used by the other IVF center would likely create chromosomally abnormal embryos related to meiosis II errors [49]. A better chance of success would be achieved by mild FSH stimulation [50]. We also advised her that it may be possible to improve the pain using a safe drug in pregnancy, dextroamphetamine sulfate, and eliminate the hydromorphone hydrochloride. Within one month, her pain was completely eradicated. She conceived on her first embryo transfer to her own uterus with her own oocytes and delivered a healthy baby. For three years she has remained pain free just taking dextroamphetamine sulfate.

Another woman (unreported case) had right lower quadrant pain for 22 years. The pain was unexplained but hypothetical causes that were entertained included chronic appendicitis, chronic salpingitis (though laparoscopy showed normal fallopian tubes), and pelvic congestion syndrome. Her daughter was a patient taking dextroamphetamine sulfate for pelvic pain and edema. The mother requested to try the drug to help her edema and weight increase despite dieting. She never dreamed that the treatment would eliminate the constant daily right lower quadrant pain of 22 years duration within one month of treatment. In fact she had never even mentioned it during her initial consult when we took her medical history. She never thought that anything would relieve the discomfort.

I chose two inflammatory bowel cases with pregnancy implications. Space limitation does not allow any in depth presentation of other cases with gastrointestinal issues where there was chronic severe symptoms refractory to standard therapy but who responded dramatically to amphetamine therapy. These included other types of inflammatory bowel disease, e.g., ulcerative colitis [51], lymphocytic colitis (accepted for publication but not in press yet), and gastrointestinal motility disorders including achalasia [52], gastroparesis [53], pseudointestinal obstruction [54], and pathological constipation [55].

Severe headaches

Women seem to be more prone to headaches than men. We have published unique case reports of women suffering from long term headaches not relieved by conventional treatment that responded very well to amphetamines [56, 57]. Dextroamphetamine sulfate completely abrogated severe headaches related to intracranial hypertension that failed to respond to acetazolamide [58]. The papilledema completely disappeared [58]. Which reminds me of a woman with 25 years of migraine headaches which a neurologist told her 25 years before was probably the start of multiple sclerosis (unreported case). However, after 25 years, there was no evidence of multiple sclerosis. A friend of hers came to our office and her long-term history of migraines was corrected. Thus, this woman came to our office to try this therapy. It completely corrected her problem. She subsequently kept her appointment with the neurologist to tell him of her good news. He strongly advised her to stop the medication because “there are controlled studies showing it does not work” (I am not aware of any such study) and “this drug could cause heart problems” (this is not true). She stopped the amphetamine on his advice and the headaches returned. Three months later at the urging of her husband she resumed the amphetamine and again her headaches dissipated.

Another woman with more than 20 years of severe retro-orbital stabbing pain attributed to keratoconus failed to respond to bilateral corneal implants but did respond very well to dextroamphetamine sulfate [59]. A 42-year-old woman sought infertility treatment. She stated that she would have to postpone investigation for a couple months because in two weeks she was going to have an experimental procedure to break her jaw to try to ameliorate severe headaches that were present for 22 years and not only occurred every day but were present for 70% of each day. The headaches were attributed to temporal mandibular joint syndrome. The procedure was to cost her $10,000 out of pocket and she was advised it would have about a 25% chance of providing some amelioration. She was advised to try dextroamphetamine sulfate first. She had more than 90% relief in ten days so surgery was cancelled. She remains headache free on 25 mg amphetamine salts extended release capsules.

These cases show that dextroamphetamine sulfate can help some women with chronic refractory headaches but one may wonder what percentage of women fail with this therapy. We presented a series at the 2014 American Association for Clinical Endocrinologists meeting in which we evaluated this therapy in 22 consecutive women with an average duration of headaches for 13.8 years who failed to gain improvement from beta blockers, topiramate, and ergotamine preparations. Only one woman reported no improvement and stopped therapy. There were 17 of 22 (77.3%) who reported almost complete or complete relief of headaches at six months and one year of therapy. Four women reported moderate relief (data not submitted for publication yet).

Chronic fatigue syndrome

Chronic fatigue syndrome seems to be more prevalent in females than males. This condition also seems to respond very well to amphetamine therapy. Recognizing this entity can prevent a multitude of needless expensive tests and po-
Fibromyalgia and inability to lose weight related to edema
Several conditions that are more prevalent in women that are refractory to other therapies but respond to dextroamphetamine sulfate include fibromyalgia [62], and inability to lose weight despite dieting (which is the only randomized controlled trial that we performed using dextroamphetamine sulfate) [63].

Other medical conditions not necessarily more prevalent in women
Other conditions refractory to standard therapy but responding extremely well to dextroamphetamine sulfate which have been described in published case reports include urticaria [64-66], long-standing eczema and keratosis pilaris [67], and pseudopheochromocytoma [68].

Other unique cases where the only therapy that corrected the condition was dextroamphetamine sulfate included restless leg syndrome, red meat allergy, post herpetic neuralgia, autoimmune hepatitis [69], hearing deficit, frozen shoulder syndrome, diplodia following orgasm [70], and a strange case of a woman who had multiple episodes per day of macroglossia which would cause her markedly enlarged tongue to protrude from her mouth and effect her speech and breathing which responded very well to the highest dosage of dextroamphetamine sulfate to date that I have prescribed – 130 mg per day (all unreported as yet).

Final thoughts
Over 20 years ago, the leading gynecologic textbook was Kistner’s Textbook of Gynecology. Robert Kistner was a professor of Obstetrics and Gynecology at Harvard University and was considered for many years as one of the leading authorities in endometriosis. I remember attending a plenary lecture at the American Society for Reproductive Medicine meeting some time over 20 years ago which was given by Dr. Kistner. He stated “If a teenager has dysmenorrhea, it is malpractice if the gynecologist does not perform a laparoscopy in order to prevent the condition from getting worse and causing pelvic adhesions”.

With the new information mentioned in this manuscript, I would like to make a new provocative statement: “The performance of a laparoscopy for dysmenorrhea in a teenager before trying dextroamphetamine sulfate first can be considered malpractice, especially if later the young lady is found to have diminished oocyte reserve, possibly as a consequence of laparoscopic removal of endometriosis and direct damage to ovarian tissue or indirectly by damaging ovarian blood supply”.

What role does genetics play in these various permeability disorders? Indeed there may be a genetic predisposition to have certain tissues develop diminished capacity to filter out harmful chemicals, toxins and bacteria. This would explain why Crohn’s disease, endometriosis, and other autoimmune diseases seem more prevalent in family members. The permeability defect can be so severe that symptoms begin in childhood. However, the defect may not manifest until later in adulthood until some acquired condition occurs, e.g., infection, trauma, or something that compromises sympathetic tone. Actually, there appears to be a genetic basis for sympathetic nervous system hypofunction. Hormonal changes can also influence permeability. This could be why the pain associated with the pelvic pain syndrome that has been previously, and in the author’s opinion, erroneously, attributed to endometriosis, exacerbates at certain times of the menstrual cycle.

One can exemplify this inter-relationship between genetics, acquired illnesses, sympathetic nervous system hypofunction, and the interaction of hormones. A pre-teen girl developed abdominal pain and failure to thrive. She was diagnosed with Crohn’s disease by colonoscopy and seemingly responded to mesalamine. She remained on mesalamine but developed similar symptoms in her mid 20’s. However, the colonoscopy was completely negative, as were all of her annual colonoscopies since childhood. She lost 14 kg down to 33 kg. Eventually she was diagnosed as having a pseudointestinal obstruction and she responded extremely well to dextroamphetamine sulfate and fully corrected the pseudo-intestinal obstruction [54]. This syndrome, which appeared suddenly in her mid 20’s, seemed to occur following a viral gastroenteritis that developed while on a cruise. This viral syndrome may be the acquired defect that exacerbated the genetic predisposition toward increased permeability of the small bowel and thus precipitated this problem of pseudointestinal obstruction.

Any evidence of genetic predisposition? Her mother has been treated for edema, pains in her legs with running, and unexplained loss of bladder control presumed to be related to multiple sclerosis which has been fully corrected by taking dextroamphetamine sulfate for 25 years. She had been fine from her symptoms of the sympathetic neural hyperalgies edema syndrome but developed breast cancer and was placed on letrozole tablets post-surgery. One year later, she...
developed severe shoulder pain and was misdiagnosed with the frozen shoulder syndrome by orthopedic specialists. She failed to respond to anti-inflammatory medications, analgesics, and physical therapy. We diagnosed her with the aromatase induced arthralgia syndrome, which completely disappeared after raising her dosage of dextroamphetamine sulfate from 30 to 45 mg extended release capsules (presented at the 2015 meeting of the American Association for Clinical Endocrinology [71]. Thus, in this case, the inheritance is for the defect in sympathetic tone as opposed to a specific tissue which has an intrinsic permeability defect.

The young lady with pseudointestinal obstruction stayed on the dextroamphetamine sulfate for five years and remained in complete remission. It was discovered during her investigation of the pseudointestinal obstruction that she had hypothalamic pituitary hypothyroidism. She had already responded to the amphetamine but she was placed on thyroid hormone. After five years of remission, it was decided to try to stop the amphetamines and see if any gastrointestinal symptoms remained abated. They did for five years while she remained on thyroid hormone. We believe that the weight loss caused the low thyroid problem by reducing thyroid stimulating hormone from the pituitary to try to conserve fuel which exacerbated the defect in sympathetic tone since thyroid hormone is a sympathomimetic amine [72]. Hypothyroidism can present with many of the symptoms of the sympathetic neural hyperalgesia syndrome and this syndrome can be mistakenly diagnosed with the thyroid hormone resistance syndrome because of presentation with similar symptoms of hypothyroidism despite normal thyroid values [73].

Though her mother manifested with edema which responded to sympathomimetic amine therapy, the young lady with pseudointestinal obstruction never manifested with edema. She conceived and did not suffer from any edema until her seventh month when it gradually became extremely severe so that she gained 29 kg (she started at 45 kg). It was suggested to re-institute the dextroamphetamine sulfate but her husband, a physician, was not in favor of this therapy because of potential unknown toxic effects to the fetus coupled with the fact that she remained normotensive and without proteinuria. However, at 38 weeks her serum creatinine rose precipitously and she was heading into renal failure (1.7 mg/dL). An emergency C-section was performed and her renal function returned to normal. Thus she developed the edema part of the sympathetic neural hyperalgesia syndrome which is related to increased capillary permeability leading to increased leakage of fluid from intravascular to extra-vascular space and the rennin-angiotensin aldosterone back-up system was not able to compensate for the loss of intravascular fluid leading to insufficient renal perfusion. Alternatively, chemicals related to increased cellular permeability may have caused decreased kidney function similar to her two previous bouts of decreased small bowel function. This case exemplifies that high levels of estrogen and/or progesterone can increase capillary permeability supporting the association of hormones and the sympathetic neural hyperalgesia edema syndrome.

**Future perspectives**

It is very likely that anyone reading this perspective who decides to treat the various mentioned disorders with dextroamphetamine sulfate will be very pleased with the outcome. Nevertheless, it is highly unlikely that this therapy will be widely used in the next five to ten years. The reason for this statement is, in general, widespread use of a drug generally requires a wide-spread advertisement campaign by pharmaceutical companies hoping to make a profit from a new drug. There are no pharmaceutical companies hoping to make a profit from dextroamphetamine sulfate for off-label use and there are no pharmaceutical companies promulgating this therapy.

It is unlikely that despite its efficacy and safety, any pharmaceutical company, including the present day manufacturers, will promote it because the drug is already available as a generic. Thus, there does not appear to be a potential profit in exchange for expenditure. The previous publications over 30 years would make it difficult to acquire a patent.

More widespread use, and thus help for a greater proportion of women (and men, too) who are needlessly suffering and not responding to “conventional” therapy can only increase in a limited manner by word of mouth. One way is to present the data at research meetings which usually frown on case reports but sometimes accept a series of cases. Another method is through publications and that is the motive behind writing this perspective: to provide one concise summary of other publications and to provide new anecdotes and a better perspective of this disorder.

Some physicians will only prescribe a new drug or use a new treatment that has been proven by a randomized controlled trial (RCT). Ethically and socioeconomically, I cannot perform one, though, I would do so if a company was willing to fund such a study not to gain more personal income but with the thought that demonstration of efficacy in an RCT will convince more physicians to adopt this treatment. Possibly, this article will stimulate some of the readership to conduct such a trial, and with their likely publication of the superiority of sympathomimetic amine therapy, more physicians will be convinced to try this therapy on patients resistant to standard therapy for pelvic pain, other pain symptoms, chronic fatigue, gastrointestinal motility disorders, and various skin conditions especially urticaria. I believe that those of you willing to try this treatment for the conditions described will be greatly rewarded. It may completely revolutionize your method of practice. If you share the same enthusiasm (after trying sympathomimetic amine therapy) (or if you do not), please publish your findings. Credibility increases if all the publications do not seem to come from just one center.
Potential new uses

It seems logical that this disorder of cellular permeability by possibly allowing unwanted chemicals to infuse into the endometrium with subsequent inflammation may be associated with an inflammatory endometrium leading to implantation failure (infertility or miscarriage). Our practice is geared toward evaluating a retrospective series, matched controlled study or even a randomized controlled study evaluating efficacy of sympathomimetic amine therapy for infertility and recurrent miscarriage.

Anecdotally, we have seen marked improvement in pregnant women with edema following treatment with dextroamphetamine sulfate. With all of the advancement in modern medicine, there is still not a good therapy for the prevention or treatment of pre-eclampsia. We only treat women through the first trimester. It is unlikely that we will have the opportunity to initiate a study for the prevention of pre-eclampsia with dextroamphetamine sulfate. Hopefully, this manuscript will interest some obstetrics and gynecology specialists to try this therapy for this condition and either publish case reports or randomized controlled studies.

Along the same lines, it seems reasonable that the abrupt rise in estrogen and/or progesterone with the ovarian hyperstimulation syndrome (OHSS) can cause marked edema and even anasarca in certain women, who already may have slightly compromised sympathetic tone, but sufficient sympathetic activity to preclude any clinical symptoms until there is further compromise by the new change in hormonal status. It serves to reason, therefore, that dextroamphetamine sulfate could be used to prevent or ameliorate OHSS. We have used this drug in circumstances of women following controlled ovarian hyperstimulation with more than 25 stimulated follicles and/or serum estradiol levels > 5,000 pg/mL but have never published any anecdotes because not all women who reach these criteria develop OHSS. Thus, we cannot state with certainty that failure to develop OHSS was because of ingesting the dextroamphetamine sulfate. Similar to the woman with the aromatase induced arthralgia syndrome, we have had many cases who seem to run a very benign course over the years of multiple sclerosis following sympathomimetic amine therapy, despite a more rocky early course pre-treatment. However, because of the nature of the disorder with long remissions, we can not state for sure the benign course was related to treatment with amphetamine.

Recently, the drug onansetron has been questioned as to its safety in treating nausea and hyper-emesis of pregnancy. Related to this, we will be offering treatment with amphetamine for severe nausea and vomiting during the first trimester (we generally follow the women for their first trimester). On what basis rests our optimism that amphetamine will relieve this type of suffering? One of the female medical students on an office rotation kept excusing herself to go to the bathroom. I inquired if she was alright and she said yes, she has emesis multiple times each day related to untreatable gastroparesis. She had suffered for ten years and was used to the symptoms. All her vomiting disappeared within two days of taking the dextroamphetamine sulfate.

There is a possibility that the rise in various hormones during pregnancy increases cellular permeability of the stomach in some people and if combined with some degree of sympathetic nervous system hypofunction those women may develop a type of gastroparesis or at least diminished stomach motility with resulting nausea and vomiting. The theory is that improving sympathetic tones will diminish the increase permeability of the mitochondria of gastrointestinal smooth muscle which was responsible for diminished gastric motility leading to nausea and vomiting.

References

Sympathomimetic amines are a safe, highly effective therapy for several female chronic disorders that do not respond well etc.


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Detection of a microgonadotropinoma by magnetic resonance imaging performed because of excellent response to controlled ovarian hyperstimulation despite elevated day 3 FSH

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Summary

Purpose: To determine if a better response than anticipated to controlled ovarian stimulation in a woman assumed to have diminished oocyte reserve based on an increased day 3 serum follicle stimulating hormone (FSH) level, could be related to a gonadotropinoma.

Materials and Methods: Magnetic resonance imaging (MRI) with and without gadolinium contrast was used in a woman who made 21 mature oocytes despite a history of day 3 serum FSH as high as 20 mIU/mL. Results: A pituitary microgonadotropinoma was detected. Conclusions: The presence of a better response than anticipated to controlled ovarian hyperstimulation (COH) with exogenous gonadotropins despite an increase in day 3 serum FSH should prompt a search for a possible gonadotropinoma.

Key words: Gonadotropinoma; Controlled ovarian hyperstimulation; Follicle stimulating hormone; Early follicular phase.

Introduction

A pituitary tumor secreting follicle stimulating hormone (FSH) and luteinizing hormone (LH) (referred to as a gonadotropinoma) is uncommon but probably underdetected because they frequently will not cause any abnormal symptoms or signs in contrast to pituitary tumors secreting prolactin or ACTH. Some have been detected because they have caused ovarian hyperstimulation in younger women [1].

Recently, a case was described where the gonadotropin cells were replaced by the tumor cells but the excess FSH and LH secreted was not biologically active leading to a false diagnosis of premature ovarian failure [2]. Interestingly, that tumor converted to a very large macroadenoma which replaced the gonadotropin cells and following a hypophysectomy, she was able to induce ovulation and achieve a live baby following treatment with exogenous gonadotropins at the age of 40 [2].

One way to consider the diagnosis of a gonadotropinoma in a pre-menopausal woman would by responding better than expected to exogenous gonadotropins despite an elevated day 3 serum FSH when controlled ovarian hyperstimulation (COH) is used for purposes of in vitro fertilization- embryo transfer (IVF-ET). A case exemplifying this caveat is presented.

Case Report

A 27-year-old woman who was evaluated for infertility at another infertility center was told she had diminished oocyte reserve and possibly poor oocyte quality based on an elevated day 3 serum FSH level. They advised her to proceed with IVF-ET immediately because she could soon be depleted of oocytes.

She failed to conceive after her first fresh and two frozen embryo transfer cycles where two embryos were transferred each time with 11 oocytes (eight metaphase II) retrieved. The second IVF-ET cycle using a micro-flare protocol provided 13 oocytes (11 metaphase II) and she conceived and delivered a live baby girl following the transfer of four embryos.

She sought an opinion with our group because of our experience with treating women with diminished oocyte reserve and because she was hoping to find a non-IVF solution for financial reasons. Her previous highest serum FSH was 20 mIU/mL. The couple had been told that the semen analysis of the male partner was perfectly normal but they had not measured antisperm antibodies. In fact, by direct immunobead testing we found that 70% of the sperm had anti-sperm antibodies attached.
A natural cycle investigation revealed that she made a mature follicle without follicle maturing drugs but had an abnormal post-coital test at that time. Thus, the conclusions were that the presence of the anti-sperm antibodies were immobilizing antibodies and intrauterine insemination (IUI) should be performed despite otherwise normal semen parameters [3]. Because these antibodies could also inhibit the attachment of the sperm to the zona pellucida, the sperm was first pre-treated with chymotrypsin-galactose to render the antibodies less biologically potent prior to IUI [4].

Serial sonographic studies found that she released the oocytes and based on her history of high serum FSH levels, despite forming mature follicles naturally, she was treated with vaginal progesterone in the luteal phase [5-7]. She failed to conceive after three IUI cycles with chymotrypsin-galactose treated sperm.

She wanted to try IVF-ET again. In her controlled ovarian hyperstimulation (COH) IVF cycle her day 2 serum FSH was slightly increased at 12 mIU/mL with a serum estradiol (E2) of 25 pg/mL. Normally, with diminished oocyte reserve we recommend mild ovarian hyperstimulation using no more than 150 IU instead of the 300 IU that we use for women with normal oocyte reserve [8, 9]. However, since her antral follicle count seemed quite adequate, and she previously conceived with a regimen of 600 IU of FSH, it was decided to use a conventional protocol of 225 highly purified FSH and 75 LH and FSH combined. Because of a very good response, the dosage was decreased to 225 IU total FSH, then 112.5, and finally 75 IU of FSH.

The peak serum E2 reached 2,668 pg/mL when human chorionic gonadotropin (hCG) 10,000 units were given. There were 22 oocytes retrieved of which 21 were metaphase II. Sixteen of the 21 mature oocytes fertilized following intracytoplasmic sperm injection (ICSI) and two mildly fragmented embryos of six- and eight-cells were transferred on day 3.

Thirteen embryos were cryopreserved – ten at the 2 pronuclear stage and three multi-cells (four, four, and six blastomeres). Six of the two pronuclear embryos were thawed and she transferred ten, eight-, and six-cell embryos but still failed to conceive. Eight more embryos were thawed and she transferred on day 3 an 8 and two seven-cell embryos but failed to conceive again.

She took a six-month break from the infertility before returning for another infertility consult. She was presented the option to consider returning to IUI with chymotrypsin-galactose treated sperm with luteal phase progesterone support or IVF-ET with some extra procedures, e.g., endometrial biopsy during menses, injection of luteal phase leuprolide acetate to stimulate gonadotropin releasing hormone receptors in the endometrium, or infusing the uterus with 500 units of hCG seven minutes before embryo transfer and finally using lower dosage (150 IU) FSH. However, based on her unexpected good response this time to half the dosage of FSH used in her previous infertility center, she was advised to have magnetic resonance imaging (MRI) of her pituitary to see if she may have an FSH secreting microadenoma.

Magnetic resonance of the brain with high-resolution images through the pituitary showed an isolated oval mass within the pituitary gland which showed no enhancement post-contrast. The measurements were 5.95 by 5.4-mm longitudinal by 10.8-mm transverse. As a result it displaced the pituitary infundibulum dorsally. Thus the MRI of the brain with and without gadolinium detected a pituitary microadenoma. Since her serum cortisol and prolactin levels were normal, the conclusion was that this was an FSH secreting gonadotropinoma.

Discussion

In contrast to the previously mentioned case report of the gonadotropinoma that presented as premature menopause, this case presented as a woman with regular menses but she was falsely considered to have diminished oocyte reserve related to increased early follicular phase serum FSH which was related to direct FSH secretion by a gonadotropinoma rather than lack of negative feedback from inhibin B [2].

One explanation to explain the aforementioned case of pseudo-ovarian failure was that either the entire lot of normal gonadotroph cells were replaced by tumor cells secreting immunoreactive FSH and LH, but biologically inactive, or perhaps significant hyperprolactinoma existed (because the tumor may have been a mixed prolactinoma and gonadotropinoma) and the high serum prolactin suppressed endogenous gonadotropins but not from the tumor [2]. Eventually, the gonadotropinoma cells were replaced by the macroprolactinoma and thus the tumor now only made prolactin and not gonadotropins when she reached her late 30’s [2].

The present case shows that a woman with FSH secreting gonadotropinoma can still spontaneously ovulate. A woman who responds far better to COH than expected for someone hypothesized to have diminished oocyte reserve based on day 2-3 serum FSH should be considered to possibly have a gonadotropinoma [2].

This condition of gonadotropinoma is probably underdetected in contrast to ACTH and prolactin secreting tumors which produce symptoms. Diagnosis is based on finding increased serum cortisol or prolactin level or inability to suppress cortisol levels with dexamethasone. Occasionally, a minority of gonadotropinomas will present with ovarian hyperstimulation in the absence of exogenous gonadotropin. Recently, Carteto-Branco et al. presented one such case and summarized data from previous cases of ovarian hyperstimulation with FSH and LH secreting tumors [1].

Recently, a case was described of a mixed microprolactinoma and gonadotropinoma that presented with high prolactin but normal FSH and LH. However, following the lowering of the prolactin level by cabergoline, this allowed the LH and FSH, which were suppressed by the hyperprolactinoma to now increase and cause mild ovarian hyperstimulation [10].

Whereas most macroprolactinomas do not start out as microprolactinomas, it is not clear what is the likelihood of a microgonadotropinoma growing to a macrogonadotropinoma. Thus, one could recommend transphenoidal microadenectomy or careful vigilance and benign neglect, or careful follow-up with MRI examination. The options were presented to this woman and she chose observation with a repeat MRI in six months.

This case exemplifies the importance of considering a gonadotropinoma in any woman with increased day 3 serum FSH who seems to respond much better than anticipated when stimulated with exogenous gonadotropins.
Detection of a microgonadotropinoma by magnetic resonance imaging performed because of excellent response to controlled ovarian etc.

References


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Introduction

In in vitro fertilization-embryo transfer (IVF-ET) cycles in women with normal egg reserve who receive conventional controlled ovarian hyperstimulation (COH) generally the serum estradiol (E2) will increase the day after the injection of human chorionic gonadotropin (hCG) related to the multiple follicles. In natural ovulatory cycles serum E2 levels generally drop after the luteinizing hormone (LH) surge.

Women with diminished egg reserve have been relatively successful with IVF-ET especially with minimal stimulation protocols [1]. A significant proportion of women with diminished egg reserve with minimal stimulation protocols may only develop one dominant follicle. Thus it would seem likely that serum E2 would be more likely to decrease in these women than women with normal egg reserve given conventional COH.

Some women included under “minimal stimulation” have fairly close to natural cycles with just a small boost of 75 IU FSH for one to three days or none at all [1, 2]. Possibly women diagnosed with diminished egg reserve whose serum E2 increases after the injection of human chorionic gonadotropin (hCG) have more follicles than those women whose serum E2 drops the day after hCG injection. The possibility exists however that the follicles with the poorest quality are more likely to demonstrate a decrease in serum E2 post-hCG injection. The possibility also exists that those women with a drop in serum E2 may be the ones more likely to release an egg before retrieval because the drop in serum E2 may be more likely to occur closer to ovulation and could suggest advancement of meiosis has already been initiated by a spontaneous LH surge initiating release of proteolytic enzymes and prostaglandins that will lead to detachment of the oocyte and thinning of the follicular wall.

A previous study of women aged ≤ 39 found no difference in live delivered pregnancy rates in women with diminished egg reserve when serum E2 dropped the day after hCG injection (19.0%) vs. those where the serum E2 increased (23.6%) or implantation rates (23.9% vs. 23.4%) [3]. However 34% in the group with the drop in E2 released the oocyte before retrieval vs. only 4.6% of those whose E2 increased [3]. The present study evaluated these parameters according to drop in serum E2 or not in women aged 40-42.

Materials and Methods

An observational study over a five-year time period was performed in women with diminished egg reserve receiving minimal FSH stimulation. IVF cycles included were those completely natural without any exogenous gonadotropin stimulation or those women not using more than 150 IU of FSH daily (except possibly increasing to 225 IU if in the late follicular phase a GnRH antagonist was initiated) [1, 2].

Only those women aged 40-42 with serum FSH on day 2 or 3 of ≥ 12 mIU/mL and ≤ five antral follicles were included. In some women with markedly diminished egg reserve, ethinyl E2 was used to lower the elevated serum FSH level to the-
A study performed in the early days of IVF found that a drop in the serum E2 the day after the hCG injection in women undergoing normal controlled ovarian hyperstimulation was associated with such poor pregnancy results that they suggested canceling the cycle [6]. However a subsequent study failed to corroborate these conclusions [7].

The present study is the first to evaluate the effect of a drop in serum E2 the day after the hCG injection during IVF-ET in women age 40-42 with diminished egg reserve. This study reached the same conclusions as the Meyer et al. study of women with normal ovarian reserve, i.e., the egg quality is comparable to those women who show a rise in serum E2 as evidenced by similar pregnancy rates.

Though a decrease in serum E2 the day after hCG injection was associated with a higher percentage of women releasing their eggs before oocyte retrieval, this did not seem to be related to a lower production of eggs since the number of embryos available for transfer in those where eggs were retrieved was the same. Since pregnancy and fertilization rates were also similar comparing both groups, it would appear that one of the main reasons for egg release before retrieval is associated with an earlier spontaneous rise in endogenous LH. Thus the egg quality in those women with a drop in E2 is similar to those with a rise in serum E2 but the chance of spontaneous ovulation before retrieval is more likely.

From a practical standpoint, if a drop in serum E2 was associated with a lower pregnancy rate, there would be enough time to cancel the retrieval which would be scheduled for the next day. Our policy is to perform an ultrasound on the day of oocyte retrieval shortly before starting anesthesia. Thus with comparable pregnancy rates to group 2, it would seem prudent to advise group 1 women to still keep their appointment for oocyte retrieval. Patients living at a distance requiring air travel could still have enough time to cancel depending on the distance if they think the odds of egg release are too high to warrant coming in for egg retrieval.

There were 17.1% (38/222) of the IVF-ET cycles reported by Meyers et al. in women with adequate egg reserve who showed a decrease in serum E2 the day after hCG injection compared to 22.1% (100/451) ET cycles in women with diminished egg reserve [7].

Thus a decrease in the serum estradiol level, the day after the injection of hCG in women aged 40-42 with diminished egg reserve using a minimal FSH stimulation protocol is not associated with a greater risk of failed fertilization or lower pregnancy rate following oocyte retrieval and embryo transfer, compared to similar women whose serum E2 rises the day after hCG. However, there is a greater chance of the egg releasing before the oocyte retrieval. Therefore the conclusion reached in this study is similar to the one reached in the sister study of women ≤ age 39 [3]. The pregnancy rates were lower but this would be expected because of their ages [8, 9].
The effect of a rise or fall of serum estradiol the day before oocyte retrieval in women aged 40-42 with diminished egg reserve


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Depressive symptoms and hormonal profile in climacteric women

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Summary

Objectives: The aims of the study were: evaluation of depressive symptoms in climacteric women, comparison of depressive symptoms between peri- and post-menopausal women, and assessment of a possible relationship between the presence of depressive symptoms and the hormonal profile of the studied women. Materials and Methods: The study included 45 peri-menopausal and 95 post-menopausal women admitted to the Department of Gynecological Endocrinology, Poznań University of Medical Sciences, because of climacteric symptoms. The following parameters were evaluated in all studied women: intensity of climacteric symptoms (Kupperman Index), intensity of depressive symptoms (Hamilton depression scale), serum concentrations of hypothalamic-pituitary-gonadal axis hormones (FSH, LH, 17β-estradiol), prolactin (PRL) and androgens [total testosterone, dehydroepiandrosterone sulfate (DHEAS)]. FSH, LH, 17β-estradiol, PRL, and total testosterone were evaluated by the immunoenzymatic methods and DHEAS was measured by the radioimmunological method. Results: Psychic and somatic manifestations of anxiety and fear, shallow sleep, and general somatic symptoms were the most frequent depressive symptoms in both studied groups. Both investigated groups differed in relation to the incidence and intensity of symptoms from the genital system (observed more often in post-menopausal women) and hypochondria (noted more frequently in peri-menopausal women). Numerous relationships between the incidence and intensity of certain symptoms and serum concentrations of the investigated hormones were found in both groups. The correlations were different in peri- and post-menopausal subjects.

Key words: Menopause; Depression; FSH; LH; PRL; 17β-estradiol; Total testosterone; DHEAS.

Introduction

Psychic, as well as vasomotor symptoms, including irritability, agitation, attention deficit, distrust even of family members and partners, sleep disorders, lack of libido, hyperactivity and quarrelsomeness, are a common clinical problem in menopausal women [1]. Their presence during menopause was the basis for the notion of climacteric depression or involutional melancholia, which was regarded as one of the forms of endogenous depression. The concept was developed by Kraepelin [2], who emphasized the diagnostic importance of uninhibited thought and will, presence of anxiety and fear, often accompanied by intensified suicidal tendencies, and who treated climacteric depression as a separate form of involutional melancholia.

According to Ballinger, depression may be diagnosed in about 50% of women seeking medical advice due to climacteric symptoms [3]. Freeman et al., [4] and Bromberger et al., [5] estimate that menopausal women are at a threefold higher risk for depressive symptoms. Martens et al., [6] report the relative risk for depression at that time of life to be 1.8. In most cases menopause-related depression runs a mild course [3]. The incidence of severe depression at the time of pre-, peri-, and post-menopause has been estimated at 5.8 - 11% [7, 8], 4 - 9.1%, and 1 - 9.8% [4, 7], respectively.

History of depression and severe premenstrual syndrome, as well as disturbed sleep and hot flashes that are supposed to be connected with incidence of depressive symptoms in the so-called ‘domino-effect’, are among the predictive factors of climacteric depression [4]. The typical for the menopause drop in the 17β-estradiol levels [9], changes in the cerebral blood flow [10], as well as genetic predisposition [11], are among the etiological factors for climacteric depression.

The aims of the study were: evaluation of depressive symptoms in climacteric women, comparison of depressive symptoms between peri- and post-menopausal women, and assessment of a possible relationship between the presence of depressive symptoms and the hormonal profile of the studied women.
Table 1. — Characteristics of the study groups (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peri-menopausal group</th>
<th>Post-menopausal group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.8±3.3**</td>
<td>56.1±4.4**</td>
</tr>
<tr>
<td>Time since last menstruation (months)</td>
<td>4.0±3.3**</td>
<td>67.6±5.1**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2±3.7</td>
<td>27.3±4.8</td>
</tr>
<tr>
<td>Kupperman Index</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>Hamilton scale</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>57.8±43.2**</td>
<td>77.6±29.7**</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>34.4±23.5</td>
<td>37.1±14.5</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>71.1±103.3**</td>
<td>20.5±22.5**</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>12.9±5.3*</td>
<td>10.6±4.3*</td>
</tr>
<tr>
<td>Total testosterone (ng/ml)</td>
<td>0.3±0.16</td>
<td>0.26±0.16</td>
</tr>
<tr>
<td>DHEAS (mg/dl)</td>
<td>1.5±0.8</td>
<td>1.29±0.65</td>
</tr>
</tbody>
</table>

Mann-Whitney test, *p < 0.05; **p < 0.001

Materials and Methods

The study included 45 peri-menopausal (still menstruating but with climacteric symptoms) and 95 post-menopausal (at least one year since the last menses) women, who were admitted to the Department of Gynecological Endocrinology, Poznań University of Medical Sciences, because of climacteric symptoms. Conditions unrelated to menopause that usually influence the occurrence of depressive symptoms, i.e. chronic diseases such as hypertension, neurological and mental diseases, digestive tract and endocrine system diseases, ischemic heart disease, history of ischemic episodes connected with the central nervous system, vascular changes within the peripheral vessels, and excessive alcohol consumption, constituted the exclusion criteria. Use of anti-depressants and hormone replacement therapy in the six weeks prior to the study also excluded the candidates from the study.

The intensity of climacteric and depressive symptoms was evaluated with the Kupperman Index [12] and the Hamilton depression scale [13], respectively, for all study participants. The BMI index was calculated with the use of the BMI = body mass/height² formula.

Serum FSH, LH, 17β-estradiol, prolactin (PRL), total testosterone, and dehydroepiandrosterone sulfate (DHEAS) levels were evaluated in all studied women. FSH, LH, 17β-estradiol, PRL, and total testosterone concentrations were tested by immunoenzymatic methods. Intra- and interassay coefficient of variation (CV) ranges were 1.2 - 3.3% and 2.0 - 5.6%, respectively. DHEAS level was evaluated with the radioimmunological method: intraassay CV and interassay CV ranges were 5.1% and 11%, respectively.

The following methods were used for the statistical analysis:
- Spearman’s test for the correlation between variables
- Mann-Whitney test for assessing the relationship between the existence of depressive symptoms and serum hormone concentrations
- Fisher’s test for comparing the incidence of depressive symptoms between the groups.

The study was approved by the Ethics Committee, Poznań University of Medical Sciences, and financed by the State Committee for Scientific Research (project no: 50305-01109136-12261-08039).

Table 2. — Incidence of depressive symptoms in the study groups.

<table>
<thead>
<tr>
<th>Depressive symptoms</th>
<th>Peri-menopausal group</th>
<th>Post-menopausal group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressive mood</td>
<td>18 (40%)</td>
<td>39 (41.1%)</td>
</tr>
<tr>
<td>Feeling of guilt</td>
<td>15 (32.2%)</td>
<td>38 (40%)</td>
</tr>
<tr>
<td>Suicidal thoughts and tendencies</td>
<td>9 (20%)</td>
<td>25 (26.3%)</td>
</tr>
<tr>
<td>Sleeping disorders</td>
<td>26 (57.8%)</td>
<td>55 (57.9%)</td>
</tr>
<tr>
<td>Shallow sleep</td>
<td>31 (68.9%)</td>
<td>67 (70.5%)</td>
</tr>
<tr>
<td>Waking up early</td>
<td>28 (62.2%)</td>
<td>57 (60%)</td>
</tr>
<tr>
<td>Loss of interest in activities</td>
<td>28 (62.2%)</td>
<td>58 (62.2%)</td>
</tr>
<tr>
<td>Slowness of movement</td>
<td>10 (22.2%)</td>
<td>22 (23.2%)</td>
</tr>
<tr>
<td>Sensorimotor anxiety</td>
<td>12 (26.7%)</td>
<td>18 (19%)</td>
</tr>
<tr>
<td>Psychic symptoms of anxiety and fear</td>
<td>34 (75.6%)</td>
<td>65 (68.4%)</td>
</tr>
</tbody>
</table>

Somatic symptoms of anxiety and fear

| Symptoms from the digestive tract    | 6 (16.3%)             | 16 (16.8%)            |
| General somatic symptoms            | 31 (68.9%)            | 68 (71.6%)            |
| Symptoms from the genital system    | 24 (53.3%)*           | 57 (60%)*             |
| Hypochondria                         | 12 (26.7%)*           | 9 (9.5%)*             |
| Weight loss                          | 1 (2.2%)              | 4 (4.2%)              |
| Self-criticism                      | 2 (4.4%)              | 6 (6.3%)              |

* Fisher’s test (p < 0.05)

Results

The studied groups differed significantly as far as age and time since last menstruation were concerned, that was the result of the criteria used for dividing the subjects. No differences in the BMI, mean Kupperman Index, and Hamilton scale scores were found between the groups. In the post-menopausal group, mean FSH concentration was significantly higher, whereas mean 17β-estradiol and PRL concentrations were significantly lower than in the peri-menopausal group. No differences in LH, total testosterone, and DHEAS levels were observed between the groups. Characteristics of the studied groups of peri- and post-menopausal women and the results with regard to the level of intensity of climacteric and depressive symptoms, as well as serum concentrations of the investigated hormones, are presented in Table 1.

The same depressive symptoms: psychic and somatic manifestations of anxiety and fear, shallow sleep and general somatic symptoms, were the most frequently observed in both studied groups. Both investigated groups differed in relation to the incidence and intensity of symptoms from the genital system (observed more often in post-menopausal women) and hypochondria (noted more frequently in peri-menopausal women). The incidence of depressive symptoms in both studied groups is presented in Table 2.

Numerous relationships between the incidence and intensity of certain symptoms and serum concentrations of
Depressive symptoms and hormonal profile in climacteric women

The correlations were different in peri- and post-menopausal subjects.

In the peri-menopausal group, the incidence and intensity of depressive mood, loss of interest in activities, and hypochondria were connected with elevated levels of FSH. The presence of psychic symptoms of anxiety and fear was correlated with higher levels of LH. Incidence and intensity of sleeping disorders, shallow sleep, loss of interest in activities and general somatic symptoms were connected with decreased levels of 17β-estradiol. Incidence and intensity of suicidal thoughts and tendencies, slowness of movement, somatic symptoms of anxiety and fear, and symptoms from the genital system were related to higher levels of total testosterone (suicidal thoughts and tendencies also to higher DHEAS). Increased intensity of slowness of motion was connected with lower PRL.

In the post-menopausal group, the incidence of shallow sleep was related to higher levels of FSH. Loss of weight and the intensity of that symptom was connected with higher LH concentrations. Incidence and intensity of suicidal thoughts and tendencies, as well as psychic symptoms of anxiety and fear, were correlated with higher 17β-estradiol (the latter was also related with higher levels of total testosterone). Higher intensity of waking up early was connected with lower PRL concentrations.

Levels of the investigated hormones, depending on the presence of selected depressive symptoms, as well as the relationship between symptom intensity and serum hormone concentrations are presented in Tables 3 (peri-menopausal women) and 4 (post-menopausal women).
Discussion

The present results show that intensity of depressive symptoms was similar in peri- and post-menopausal women. Ballinger [14] and Bungay et al., [15] suggest that symptoms of climacteric depression are more intense in the period of time proceeding the last menstruation and persist for about one year. The same authors point to the relationship between the intensity of depressive and climacteric symptoms. In the present study, the level of climacteric symptom intensity was similar both in peri- and post-menopausal women, what was probably the reason behind the similarities in the intensity of depressive symptoms. In both studied groups, the mean result of the Hamilton Scale was the basis for the diagnosis of moderate-intensity depression. Most authors underline that depression during menopause usually runs a mild course [7, 8]. The discrepancy between reports in the literature and the present results might be caused by that fact that the present study participants were referred to the department due to greater intensity of symptoms.

In both groups of women, similar depressive symptoms: psychic symptoms of anxiety and fear, somatic symptoms of anxiety and fear, shallow sleep, general somatic symptoms, were found in the majority of cases. It is in accordance with observations of other authors, who at the same time point to high incidence of suicidal thoughts and tendencies around the time of menopause [2]. Both investigated groups differed as far as incidence and intensity of symptoms from the genital system and hypochondria were concerned. Disorders of the genital system (dyspareunia,
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lack of libido) were observed more often and with greater intensity in post-menopausal women, whereas hypochondria was noted more frequently and with higher intensity in peri-menopausal women.

Other authors demonstrated similar results concerning symptoms from the genital system. Avis et al., [16] and Denuerstein et al., [17] reported decreased libido during menopause. Lack of sex drive depends on the intensity of climacteric as well as depressive symptoms. Llaneza et al., [18] found a reversed correlation between the level of intensity of the climacteric symptoms (Menopause Rating Scale) and symptoms from the genital system (Changes in Sexual Functioning Questionnaire). Mezones-Holguín et al., [19] demonstrated that sexuality (Female Sexual Function Index) was reversely correlated with depression and depended on the intensity of atrophic changes within the urogenital system. In the present study, disorders of the genital system included dyspareunia and lack of libido. The SWAN study analyzed these problems separately and concluded that lowered sex drive is noted more often in late peri-menopause and post-menopause, while dyspareunia is more prevalent in early peri-menopause and post-menopause [20].

The same investigation found that suicidal thoughts and tendencies were more frequently observed during post-menopause, while hyperactivity, early waking up, and appetite loss were typical of peri-menopause [20].

As far as hypochondria is concerned, Bungay [15] found it to be more intensified during post-menopause, similarly to insomnia, whereas anxiety and fear peaked during perimenopause. Similar results were reported by Ballinger et al., [3], who also demonstrated that the most frequent fear in women during menopause was the fear of breast cancer.

Estrogen deficiency is believed to be the main reason of psychic symptoms during menopause [21]. It is consistent with the present findings of a relationship between lowered levels of 17β-estradiol and higher, as well as more intensified, incidence of sleeping disorders, shallow sleep, loss of interest in activities and general somatic symptoms. Interestingly, these connections were observed only in peri-menopausal women. Moreover, other correlations, that are not present during peri-menopause, between higher levels of 17β-estradiol and higher incidence and intensity of suicidal thoughts and tendencies, as well as psychic symptoms of anxiety and fear, appear at the time of post-menopause. These investigations conclude that decreased 17β-estradiol is a significant risk factor for depression at the time of peri-menopause.

The present findings concerning the correlations between higher FSH levels and higher incidence and intensity of the depressive mood, loss of interest in activities, hypochondria and shallow sleep, as well as between a higher concentration of LH and the loss of weight, and psychic symptoms of anxiety and fear, constitute an indirect proof that 17β-estradiol plays a role in the etiology of depressive symptoms.

Freeman et al., [22] reported similar results and concluded that during pre-menopause the level of intensity of depressive symptoms correlates with higher concentrations of FSH, and in peri-menopause with lower levels of LH and inhibin B, as well as increased variability of estradiol, FSH and LH concentrations. Ryan et al., [23] found a relationship between the incidence of depression in post-menopause and the drop in the 17β-estradiol levels and increase in the FSH concentration in the course of two years. Sherwin et al., [24] demonstrated a correlation between the extent of estrogen decrease and lowered mood in women after surgical menopause.

The dependency between 17β-estradiol and intensity of depression was confirmed by studies on the effects of hormone replacement therapy in menopausal women. Saletu et al., [25] demonstrated that administration of conjugated estrogens at the dose of 1.25 mg/day significantly improved the clinical condition of post-menopausal women with mild depression. Ditkoff et al., [26] reported that conjugated estrogens used both, at the dose of 0.625 and of 1.25 mg/day for three months, relieved depressive symptoms in women after menopause. Klaiber [27] found that very large doses of conjugated estrogens (from five to 25 mg/day) administered for three months, significantly improved the mood in women with severe depression, non-responsive to conventional methods (anti-depressants, psychotherapy, electro-convulsive stimulation). Also, estrogen therapy was proven to prevent depression in the elderly [28].

Studies on the relationship between depression and serum testosterone levels brought divergent results. Bromberger et al., [5] found a dependency between the intensity of depressive symptoms in pre-menopause and increased serum testosterone concentration. Also, higher testosterone levels were observed in women with moderate depression during pre-menopause [29], as well as lack of age-related decrease in testosterone concentrations in postmenopausal women with moderate depression [30]. The present findings are similar to these results, as the authors confirmed a dependency between higher levels of total testosterone and incidence and intensity of suicidal thoughts and tendencies, slowness of movement, somatic symptoms of anxiety and fear, symptoms from the genital system, and psychic symptoms of anxiety and fear. Contradictory results are reported by Santoro et al., [31], who found a relationship between the intensity of depressive symptoms and decreased levels of total and free testosterone, as well as Turna et al., [32], who demonstrated a correlation between low levels of total testosterone and lack of libido in pre- and post-menopausal women. Studies on the use of testosterone in hormone replacement therapy seem to confirm its beneficial effect. That kind of therapy resulted in improved confidence and mood in most women, causing hyperactivity and irritability only in some subjects [33]. Zweifel and O’Brien [34], demonstrated that improved mood after high-dose androgen treatment was more significant than after androgens in...
combination with estrogens, only estrogens, estrogens in combination with gestagens, and gestagens alone.

Studies on the relationship between the incidence of depressive symptoms and DHEAS and DHEAS concentrations give divergent results. Apart from reports on lower intensity of depression in women with higher serum DHEAS concentrations [35, 36], there are findings that suggest a contradictory correlation [31, 37], or none at all [38]. The present authors found a higher intensity of suicidal thought and tendencies in peri-menopausal women with higher DHEAS levels. Similar results were demonstrated by Morrison et al., [8], who observed a higher intensity of depressive symptoms in younger women with higher DHEAS and older women with lower DHEAS. Yet another finding was reported by Schmidt et al., [35], who noted a dependency between the first episode of depression during peri-menopause and lowered levels of DHEAS.

The present study found a correlation between higher intensity of both, slowness of movement, and early waking up and lower levels of PRL. It seems to be the result of decreased 17β-estradiol concentration during menopause. PRL concentration at that time of life drops and the changes correlate with 17β-concentration levels [39]. As no changes in the PRL secretion are observed in depression, its increased concentration may be the result of antidepressant therapy [40].

Conclusions

Intensity of depressive symptoms during peri-menopause and post-menopause is comparable. The profiles of depressive symptoms of pre-menopause and post-menopause are similar. Hypochondria is most common during peri-menopause and lowered levels of DHEAS. The intensity of depressive symptoms in young women with higher DHEAS levels. Similar results were demonstrated by Morrison et al., [8], who observed a higher intensity of depressive symptoms in younger women with higher DHEAS and older women with lower DHEAS. Yet another finding was reported by Schmidt et al., [35], who noted a dependency between the first episode of depression during peri-menopause and lowered levels of DHEAS.

The present study found a correlation between higher intensity of both, slowness of movement, and early waking up and lower levels of PRL. It seems to be the result of decreased 17β-estradiol concentration during menopause. PRL concentration at that time of life drops and the changes correlate with 17β-concentration levels [39]. As no changes in the PRL secretion are observed in depression, its increased concentration may be the result of antidepressant therapy [40].

References

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Scar endometriosis is a gynecological complication that general surgeons have to deal with

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Summary
Background: Scar endometriosis is the presence of functional endometrium tissue in surgical incisions. It is a complication that develops after obstetrical or gynecological surgical procedures. As it presents with a mass adjacent to surgical incisions, general surgeons usually deal with it. The authors’ aim was to review and discuss the differential diagnosis, treatment methods, recurrence rate, and follow up of scar endometriosis. Materials and Methods: Data of patients diagnosed with incisional scar endometriosis between 2005 and 2012 were recorded retrospectively. Their initial symptoms, previous surgery histories, onset of symptoms after surgery, duration of symptoms, diagnostic modalities, treatment methods, pathological evaluations, and rate of recurrences were documented and analyzed. Results: Seventeen patients were diagnosed to have scar endometriosis. Former surgical histories were one hysterectomy, one vaginal birth with episiotomy, and 15 cesarean sections. Sixteen of the scar endometrioses were demonstrated on pfannenstiel incision and one on episiotomy scar. Only one recurrence was seen during follow up. Conclusion: Scar endometriosis should be taken into account in the surgical practice of incisional site masses of the abdominal wall. They should be excised totally for a proper treatment. Patients must be warned about malignancy risk.

Key words: Endometrioma; Cesarean section; Abdominal wall mass.

Introduction
Endometriosis is defined as the presence of endometriotic tissue outside the uterine cavity that is affected by hormonal changes [1]. It is usually seen in pelvic sites as ovaries, fallopian tubes, cul-de-sac, and rectovaginal septum. Among extrapelvic sites, abdominal wall is the most common localization. Usually after obstetrical or gynecological surgical procedures, due to implantation of endometrial tissue around the incision, extrapelvic endometrioses are mostly (1%) seen in the incisions of abdominal wall and rarely (0.06%) in episiotomy scars [2, 3]. Diagnosis is often difficult due to lack of specific symptoms and unexpected localizations. Scar endometriosis generally appears months and years after primary surgery [4]. Wherefore the localization on the abdominal wall, patients usually consult to general surgeons. On account of endometrioses being beyond the interest of general surgeons and their atypical localizations, cases are usually misdiagnosed or exact diagnoses are achieved after pathological examinations. This study is a brief review of scar endometriosis cases (n = 17) of patients that attended the present general surgery clinic. Their diagnosis, treatment, and follow-up were discussed according to general surgeons’ perspective.

Materials and Methods
Between the years 2005 and 2012, 17 patients were diagnosed to have scar endometriosis histopathologically at Baskent University Adana Hospital General Surgery Clinic. Their medical reports were analyzed and reviewed retrospectively.

Results
Seventeen patients were diagnosed histopathologically to have scar endometriosis. Ages of the patients were between 21 and 46 years (mean 32 ± 6). Of the patients 41.1% (n = 7) were nulliparous, others 58.9% (n = 10) were multiparous women. Among the patients that all had surgery (n = 17), majority (n = 15) had cesarean section, one had hysterectomy, and one had vaginal birth with episiotomy. All incisions used on the abdomen were pfannenstiel incisions. Endometriosis nodules were seen in 47% (n = 8) on the right side, 23.5% (n = 4) on the left side, and 17.6% (n = 3) at the middle of the pfannenstiel incisions. One of the patients had nodules situated in pfannenstiel incision bilaterally. One nodule was found in the episiotomy incision. Nodules were between 10-55 mm in diameter (mean 27.7 ± 12.3). Symptoms of 47.1% (n = 8) of patients was a palpable mass only and 52.9% (n = 9) had both nodule and cyclic pain. No tests were done for diagnosis of 23.5% (n = 4) of the patient prior to surgery.

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Serum CA 125 levels were studied for 35.3% (n = 6) of the patients. Ultrasonographic examination in 52.9% (n=9) and computed tomography in 11.8% (n = 2) of the patients were performed for diagnosis before surgery (Figure 1). All pathologic examinations confirmed scar endometriosis. Time elapsed from the last obstetric or gynecological surgery to the onset of the symptoms was two to 12 years (mean 6.4 ± 3.3). Duration of symptoms was six months to 11 years (mean 3 ± 3.2 years). Only one recurrence was seen after excision of the mass during follow up. The demographic characteristics and results of study parameters are summarized in Table 1.

### Discussion

Abdominal wall endometriosis is an example of extrapelvic endometriosis that is seen after cesarean section and hysterotomy, episiotomy, and after endometriosis operations, so called scar endometriosis. It is not unusual unfortunately with the increasing rate of cesarean sections. There can be three explanations for the pathogenesis of scar endometriosis. First one is metaplasia of primitive mesenchymal tissue as an endometriotic tissue [5]. This theory cannot explain why the nodule is mostly seen on the incisional scar. Second theory is hematogenic or lymphogenic transport of endometrial cells that have invasive potential [6]. Third theory is seeding of endometrial cells into subcutaneous tissue on the incision during hysterectomy in cesarean section and during episiotomy [7, 8]. In the present series scar endometrioses were seen one after hysterectomy, one after episiotomy, and the remaining were after cesarean section cases. In the literature most cases were also seen after cesarean sections, therefore seeding of endometrial cell theory can be more reasonable [2]. In cesarean section, while suturing the uterine incision, uterus is taken out of pelvis in terms of hemostasis. After placenta is removed, endometrial cavity is cleared by gauze to prevent residual placenta. These maneuvers facilitate the spillage of endometrial cells around the incision and abdominal wall. The endometriotic nodule is found not within the whole incision but usually at one side of it. On the other hand scar endometrioses are also seen after laparoscopic procedures or appendectomies and hernia repairs [9-11]. Seeding theory does not seem to be adequate to explain the pathogenesis in latter cases, although it is not known if pelvic endometriosis accompany in such cases.

Most patients had a symptom of a mass on the abdominal wall, but not all of them had cyclic or non-cyclic pain. Although cyclic pain is a pathognomonic finding for endometriosis, scar endometriosis may present with or without it.

Diagnosis of scar endometriosis is usually delayed to post-surgery. Exact diagnosis is usually done by pathologic examination. As patients usually attend to general surgeons who are not familiar with endometriosis, preoperative diagnosis is mostly thought to be tumor, hernia, abscess, fibroid or scar fibrosis. The mean time interval between primary surgery and the appearance of symptoms is approximately six years. This delay may also impair early diagnosis.

The diagnostic tools such as ultrasonography (USG), magnetic resonance imaging (MRI), and computed tomography (CT) scans may help the diagnosis of scar endometriosis. Zawin et al. suggested that MRI is the best method to diagnose scar endometriosis [12]. Beside MRI or CT scans, USG may be an easier and cheaper diagnostic tool and also may help to detect the localization and spreading of the nodule. Serum CA 125 level is not a good predictor for scar endometriosis, as long as the patient might already have endometriosis elsewhere, so positive result does not support and negative result does not exclude the diagnosis of scar endometriosis. Attentive questioning of patient history and careful physical examination with the support of USG can lead to the conclusion before surgery.

Among extrapelvic endometrioses malignancy risk of scar endometriosis is nearly 1% [13]. Histologic transfor-
mation of extrapelvic endometriosis to malignancy is seen mostly as endometrioid carcinomas, sarcomas, and clear cell carcinomas. Majority of malignancies arising from scar endometriosis detected in histologic examinations are mostly clear cell carcinomas (65%) and endometrioid carcinomas (22%) [13-15]. Clear cell carcinoma is a very aggressive tumor with a poor survival between six to 48 months and death rate of 33% [16]. Surgery must be recommended to avoid aggressive behavior of clear cell tumor. Excision achieved with a margin of one cm around the lesion is enough [17]. Although recurrence rate in the present series is 0.05%, it can be as high as 9.1% in literature [8]. Despite the previous publications highlighting medical treatment as an opportunity [11], the treatment of endometriosis today is undisputedly believed to be surgical excision of the lesion [14, 16]. Progesterone, danazol or GnRH agonists may limitedly help for control of pain due to endometriosis. These agents cannot ensure a lasting effect after ending medical treatment and only act temporarily. For prevention after operations, washing subcutaneous tissue following closure of the abdominal fascia or washing the pelvis may be an option. Nevertheless, there is no proof that such a mechanical step prevents from scar endometriosis.

**Conclusion**

In case of cyclic localized pain and palpation of nodules with or without pain on previous incisions on the abdominal wall of a woman, endometriosis should be kept in mind. Additionally if a nodule grows or becomes necrotic, malignancy should be considered. Ultrasonography can help in the diagnosis of endometriosis. In case of scar endometriosis, surgical excision is the way of treatment. Medical treatment is only a temporary solution for scar endometriosis. The patients do not desire surgical intervention, should be warned about cancer risk.

**References**


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Effects of estrogen intervention on the biomechanical characteristics of serum SOD, MDA, and middle cerebral artery in aged female rats

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Summary

Objective: This study aims to study the biological and biomechanical characteristics of the middle cerebral artery (MCA) in aged and estrogen-intervened aged rats, thereby providing biomechanical basis for clinics. Materials and Methods: Thirty 18-month-old Sprague Dawley (SD) rats, 30 18-month-old estrogen-intervened SD rats, and 30 four-month-old SD rats were studied. The estrogen-intervened rats were given estradiol benzoate on the fifth feeding day. Thirty-four days after the feeding, the serum of each rat was obtained. The radioimmunoassay was performed for the content determination of serum E2, ER, malondialdehyde (MDA), and superoxide dismutase (SOD). The tensile test was performed to evaluate the MCA of each rat. Results: Through the estrogen intervention, the serum contents of E2, ER, SOD, and MDA in old rats were restored to normal levels. The maximum stress, maximum strain, and elastic limit of the MCA in the aged estrogen-intervened rat group were greater than those of the non-intervened aged rat group, with a significant difference (p < 0.05). The elastic modulus in the aged estrogen-intervened rat group was less than that of the non-intervened aged rat group, with a significant difference (p < 0.05). Conclusion: E2 intervention can improve the flexibility, toughness, and compliance of MCA in aged rats.

Key words: Aged female rat; Estrogen; Middle cerebral artery; Mechanical properties.
crease of pressure in the normal female WKY. By contrast, MCA significantly expanded with the increase of pressure in the SH RSP group. In the section-stress elastic modulus figure, the normal female WKY group was stronger than the male group but was significantly weaker than the male group in the SH RSP group.

In past research, estrogen intervention was mostly focused on the bioresearch in aged women and animals. Rare domestic and international reports exist regarding estrogen intervention on the biomechanical properties of MCA. In this article, the authors report the quantitative clarification of the different results of estrogen intervention on the biomechanical characteristics and serum superoxide dismutase (SOD) and malondialdehyde (MDA) contents in the MCA of aged female rat and non-intervened aged female rats. Biomechanical and biological indicators were used to determine the effects of estrogen intervention, providing biological and biomechanical basis for the prevention of cerebrovascular disease in aged females.

Materials and Methods

Animals and grouping

Studies have shown that the weight difference of rats less than nine grams could effectively prevent errors caused by weight [18]. The experimental animals used were 30 18-month-old clean, female Sprague Dawley (SD) rats (body weight 290 to 330 grams) and 30 four-month-old SD rats (body weight 290 to 325 grams), which were provided by the Changchun High-tech Medical Experimental Animal Center (license number SCXK (Ji) 2003-0004). The animal laboratory safety level was ABSL-2, and the level of pathogen-carrying animal was clean level. Breeding was conducted in a room temperature of 20 ± 2°C, with good ventilation, relative humidity of 55% to 70%, and natural light. The animals were caged with free food and water. The diet was purchased from the experimental animal diet factory in Shenyang, with product standard code of DB-21741-93. Sixty 18-month-old female SD rats were randomly divided into estrogen-intervened group (30 rats) and non-intervened aged group (30 rats). Thirty four-month-old female SD rats were set as the young control group. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Jilin University.

Estradiol benzoate intervention

After the fifth feeding day, the intervention group rats were given estrogen estradiol benzoate (0.2 mg/kg, Batch No: 061101) through intramuscular injection every other day. The young control group and aged non-intervened group rats were not subjected to any treatment, and all rats were fed for 34 days.

Specimen preparation

On the 34th feeding day, the eye blood from the orbit was obtained from 15 rats of each group. The blood stood for a while, six ml fresh blood were centrifuged, and then left in open air for another 15 minutes, and centrifuged at low speed (2,000 r/min) for 20 minutes. The supernatant was stored at -40°C for future use. Craniotomy was then performed under the ASX-1 surgical microscope. The MCA was located and cut down with S-5 sterile plastic-handle scalpel. Fifteen specimens of each group were wrapped with saline-soaked cloth, placed in a saline bath, and stored at 4°C environment for future use.

Measurement of the specimen geometry dimension

The CG reading microscope was used to measure the MCA sample’s geometry as follows: length ten mm, diameter 1.0 mm to 1.02 mm for the young control group, length ten mm, diameter 1.0 mm to 1.02 mm for the estrogen-intervened aged female group, and length ten mm, diameter 1.0 mm to 1.02 mm for the non-intervened aged group.

The estradiol and estradiol receptor ELISA kit were used for the concentration determination of serum E2, ER, MDA, and SOD in each group. Microplate reader was used strictly in accordance with the estradiol and estradiol receptor ELISA kit instructions for the determination of serum E2 and ER concentrations in each group. The serum E2 and ER levels were measured using the radioimmunoassay (RIA) method. The MDA kit was used according to instructions, with thiobarbituric acid colorimetric method, in determining the serum MDA levels, which is expressed as MDA (nmol/ml)=(absorbance of measurement tube - absorbance of blank tube)/(absorbance of standard tube - absorbance of standard blank tube)×standard concentration (ten nmol/ml)×dilution times. The ultra SOD kit was used according to SOD kit instructions, with U3410 spectrophotometer and yellow purine oxidation enzyme (XO) method, for the SOD determination.

MDA determination

According to the MDA kit instructions, the thiobarbituric acid colorimetric determination method was used to measure the serum MDA content. Serum content (nmol/ml) = (absorbance of the sample tube - absorbance of the blank tube)/(absorbance of the standard pipe - absorbance of the standard blank)×concentration of the standard (ten nmol/ml)×dilution factor.

SOD determination

According to the SOD kit instructions, U3410 spectrophotometer and yellow purine XO method were used for the SOD determination.

Serum E2 and ER determination

The microplate reader and ELISA method were strictly used according to the estradiol and estradiol receptor ELISA kit instructions for the determination of serum E2 and ER concentrations. The serum E2 and ER levels were measured through RIA.

Longitudinal tensile tests of MCA

The CSS4500 automatic-control electronic universal testing machine was employed. Ten times of loading and unloading pre-tune treatments were performed for each sample according to reference [19]. The experimental ambient temperature was 36.5 ± 1.0°C. Each specimen was clamped in the test machine cartridge. The tensile test was conducted under a speed of two mm/min. The maximum stress, maximum strain, elastic limit, and stress–strain curve were determined. To maintain the humidity of the sample, leaching saline was performed to the specimen during the experiment. After the experiment, the computer automatically imported the experimental data and curve.

Statistical analysis

The SPSS16.0 software was used for the data analysis. Results were expressed as X ± s. The experimental data were statistically analyzed using the one-way ANOVA method. The Scheffe’s method was used to compare the groups. A p < 0.05 was defined as statistically significant.
Results

**MDA and SOD**

The measurement results of the serum MDA and SOD contents are shown in Table 1. The serum MDA content of the non-intervened group was higher than those of the estrogen-intervened and young control groups, with a significant difference ($p < 0.05$). By contrast, the serum SOD content of the non-intervened group was lower than those of the estrogen-intervened and young control groups, with a significant difference ($p < 0.05$). The serum SOD content of the estrogen-intervened group was higher than those of the young control and non-intervened groups, with a significant difference ($p < 0.05$).

**Serum E2 and ER contents**

The serum E2 content of the estrogen-intervened group was higher than those of the non-intervened and young control groups, with a significant difference ($p < 0.05$). However, the serum ER content of the estrogen-intervened group had no significant difference with those of the young control group ($p > 0.05$, Table 2).

**Brain artery tension and stress–strain function**

The experimental results of the brain artery tension in each rat group are shown in Table 3, and the stress-strain curves of MCA in each group are shown in Figure 1.

The maximum stress, maximum strain, and elastic limit strain values of MCA in the aged estrogen-intervened group were greater than those of the non-intervened group, with a significant difference ($p < 0.05$). The values of the modulus of elasticity at 13.3, 16.0 and 22.5 kPa of MCA in the aged estrogen-intervened group were less than those of the aged non-intervened group, with a significant difference ($p < 0.05$).

The dependence relationships among variables were obtained from the measured data and the total deviation. The stress–strain function of each group is expressed as follows:

- Young control group: $\sigma(e) = -0.0094e^4 + 0.8612e^3 + 2.5144e^2$
- Aged non-intervened group: $\sigma(e) = -0.0001e^5 + 0.0008e^4 + 0.7319e^3 + 0.2024e^2$
- Aged estrogen-intervened group: $\sigma(e) = -0.0001e^5 + 0.0011e^4 + 0.7795e^3 + 0.6919e^2$

![Figure 1. Longitudinal tensile stress-strain curves of different groups’ MCA.](image-url)
In Figure 1, the ordinate expresses the stress and the abscissa the strain. The strain of the brain artery walls in each group varied regularly under the stress. When the stress is 0 kPa to 16.0 kPa, the stress–strain curve exhibits an exponential relationship. The maximum stress, maximum strain, and elastic limit strain in the young control group were greater than those of the aged non-intervened and estrogen-intervened groups. When the stress exceeded 16.0 kPa, the strain of each group increased rapidly. The strain curve slope increased with stress, showing a non-linear relationship. A continuous force stretched occurred and extended at the wall fiber until reached the final fracture.

Discussion

The experimental results showed that the maximum stress, maximum strain, and elastic limit strain value of the cerebral artery in the aged estrogen-intervened group were greater than those of the aged non-intervention group, with a significant difference (p < 0.05). The estrogen intervention did change the mechanical properties of the cerebral artery in the aged female rats, which is in accordance with the expected results. The elastomeric components of the cerebral arterial wall include elastic, collagen, and smooth muscle fibers [20]. The thickness, hardness distribution, and spatial configuration type of the intracranial arteries are important factors for determining the vascular function and biomechanical properties [21]. Meng et al. [22] indicated that the structure of the cerebrovascular elastic tissue plays an important role in maintaining the tension and elasticity of the cerebral blood walls. Hayashi [23] comparatively analyzed the relationships of the elasticity and stiffness of the intracranial and the outer arteries and found that the modulus of the elasticity and stiffness of the arteries increased with the blood pressure and with the distance to the heart. Such increase is related to the difference of the ratios of wall stretch and collagen fibers, the number of smooth muscles, and the different arrangements of fibrous tissue structure. The reduction of arterial elasticity is considered as the early change of atherosclerosis, which is also a risk factor for plaque formation [24, 25]. As the female rats aged, arteriosclerosis appeared, leading to the content and arrangement direction changes of the arterial collagen and elastic fibers. Consequently, the displacement and strain of the MCA reduce under stress, which also reduces its elasticity and tenacity and increases its elastic modulus. The modulus of elasticity represents the arterial stiffness and hardness. The greater the modulus of elasticity, the smaller the expansion degree of the arterial wall and the worse the elasticity. The elasticity reduction in the aged female rats’ brain artery, indicates its poor compliance. In this study, the aged female rats were subjected to estrogen intervention, which improved the elasticity and toughness of the aged rats’ brain artery, reduced the elastic modulus of MCA in the aged female rats, and restored the MCA compliance of the cerebral artery in the aged female rats to some extent. The longitudinal tensile mechanical properties of the MCA in the aged estrogen-intervention group presented some recovery through the intervention, and the compliance also obtained some recovery. Therefore, estrogen intervention can increase the elasticity and compliance of the MCA in the aged female rats.

The elastic moduli of the cerebral artery in the aged female rats at the elastic stresses of 13.3, 16.0, and 22.5 kPa are greater than those of the aged estrogen-intervention group. The elastic modulus of the MCA increased with stress in each group. The elastic modulus represents the stiffness and hardness of an artery. The higher the elastic modulus, the greater the hardness of the arterial wall and the less the flexibility. In this experiment, the longitudinal stretching was applied to the cerebral arteries of the young, aged, and aged estrogen intervention rats [17]. Their mechanical properties were tested by applying an internal pressure to their arteries. Although different methods were used, the elastic modulus of the present experiment increased with stress similar to the result of reference [17]. Therefore, longitudinal stretching is feasible to be applied to the research of the mechanical properties of the arterial wall.

The SOD system is one of the important physiological mechanisms, effectively removing the excess free radicals. With the increase of age, this balance will gradually be destroyed, resulting in excess free radicals. Free radicals could cause DNA damage, leading to the mutation and tumor formation. Its superior ability to respond would oxidize a variety of cell substances, damaging biofilm and causing macromolecules, such as proteins and nucleic acids, to crosslink, which affect their normal functions. As an important antioxidant enzyme in vivo, SOD enables superoxide anion radicals to transform into the peroxide of hydrogen and oxygen ions, thereby reducing the lipid peroxidation and protecting the body tissues and cells from damage. Estrogen changes dramatically in females. The periodic secretion stops after 45 years to 50 years. Compared with the sexual maturity, estrogen decreases rapidly. The decline of the estrogen levels lead to the slow-down of the cholesterol metabolic rate, causing the acceleration of atherosclerosis, which in the long run would induce cerebral infarction. E2 can reduce the cerebral artery elasticin, plasma cholesterol, and atherosclerosis [1]. Estrogen has similar roles of neuroprotective agents, which could have a direct impact on the growth and function of the nerve cells [26]. In this study, the serum MDA content in the estrogen-intervened group significantly decreased. By contrast, the SOD activity of this group significantly increased compared with the non-intervened group. Hence, estrogen can increase the antioxidant capacity, inhibit the formation of lipid peroxidation products, and reduce oxygen-free radicals and lipid peroxidation product pair injury, thereby protecting the rat brain tissue.

Blood vessels are affected not only by the blood pressure but also significantly by the longitudinal tension. Therefore, the vascular tissue reconstruction under longitudinal tension has gradually been taken seriously in recent years. Previous studies on the E2 estrogen intervention on people or animals
have focused on the biological views [14-16]. In this experiment, the maximum stress, maximum strain, elastic strain limit, and the stress-strain curve of the MCA longitudinal stretching in the young, aged, and E2 estrogen-intervened rats were obtained, different from the previous studies. The elastic modulus corresponding to 13.3, 16.0, and 22.5 MPa were also obtained. Moreover, the arterial stress-strain relationship expression was established through the regression analysis, which can quantitatively clarify and comparatively analyze the MCA mechanical properties of each group. Biomechanics indicators, serum SOD, and MDA were utilized to judge the effect of E2 intervention on the aged female rats, providing a biomechanical basis for the prevention of cerebrovascular disease in aged women. The design, experimental methods, experimental data, and processing methods presented in this paper are innovative.

The sampling and preservation methods used in each group during the experiment are the same. The preset processing method used is also the same to reduce the experimental data errors by controlling the experimental ambient temperature and speed. Considering the limited experimental sample and the individual differences among the animals, the experimental data have certain discreteness. However, these data can still provide a certain reference for the mechanism research of the clinical estrogen interventions for preventing the cerebrovascular disease in aged women.

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References


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Female genital mutilation/cutting: an update

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Summary
Female genital mutilation/cutting (FGM/C) is a cultural practice involving several types of external female genitalia cutting. FGM/C is known to occur in all parts of the world but is most prevalent in 28 countries in Africa and the Middle East and among immigrant communities in Europe, Australia, New Zealand, Canada, and the United States. Studies of FGM/C suffer from many methodological problems including inadequate analysis and an unclear reporting of results. The evidence to link FGM/C to infertility is weak. The management of epidermal clitoral inclusion cysts includes expensive investigations like comprehensive endocrinology tests and MRI resulting in unnecessary anxiety due to delay in surgical treatment. Similarly, unnecessary cesarean sections or rupture of the infibulation scar continue to occur because of the inadequate use of intrapartum defibulation. A significant amount of efforts is required to improve and correct the inadequate care of FGM/C women and girls.

Key words: Female; Genital Mutilation; Cutting.

Introduction
In 1997, the World Health Organization (WHO), United Nations Children’s Fund (UNICEF), and United Nations Population Fund (UNPFA) jointly defined female genital mutilation (FGM) as “all procedures involving partial or total removal of the external female genitalia or other injury to the female genital organs for cultural or any other non-therapeutic reasons” [1]. In 2008, as a result of the involvement of more United Nations agencies and human rights organizations, a new statement was issued [2]. The terms FGC and female genital mutilation/cutting (FGM/C) were used instead of FGM to “reflect the importance of using non-judgmental terminology with practicing communities”. The United States Agency for International Development, American College of Obstetricians and Gynecologists (ACOG), and the American Academy of Pediatrics (AAP) use FGC instead of FGM in order to “reflect cultural sensitivity” [3-5]. FGM/C is a cultural practice involving several types of external female genitalia cutting. This review aims to assist health-care providers in recognizing and addressing the vast medical needs of the women and girls with a history of FGM/C.

FGM/C Types and Terminology
WHO classified female genital cutting into four types according to the extent of the cutting (Table 1) [2].

• Type I: the cutting, whether partial or total, of the clitoris together with the prepuce.
• Type II: the cutting, whether partial or total, of the clitoris together with the labia minora, while keeping or removing the labia majora.
• Type III: With or without the clitoris, either both labia minora and labia majora or just the latter is removed.
• Type IV: Includes piercing, pricking, and cauterization.

The WHO classification made the distinction between removal of the prepuce only (Type Ia) and removal of the prepuce with excision of part or all of the clitoris (Type Ib) [2]. In Type III (also known as infibulation) following the cutting, the two sides of the vulva are usually sutured together leaving a small tunnel for passage of urine and menstrual blood. The skin heals by forming a scar tissue bridging the vagina. Without suturing, the tissue will seal together with increased scarring. The latter is known as pseudo-infibulation [6]. Other terminologies are defibulation and refibulation. Defibulation implies incision of the scar tissue, usually prior to baby delivery. Refibulation is the re-joining the scar tissue after child birth to retighten the vaginal opening.

Prevalence
It is estimated that up to 140 million females worldwide have been subjected to FGM/C and that currently three million girls, most of them under 15 years of age, undergo the practice every year [2]. In addition, over 91 million African women are presently experiencing consequences of FGM/C. FGM/C is practiced mostly in Africa. However, it is also performed in Asia and the Middle East, and among immigrants in Europe, United States, Canada, Australia,
and New Zealand. In the United Kingdom alone, an estimated 86,000 women and girls have experienced FGM/C in their countries of origin, 10,000 are at risk, and another 3,000-4,000 experience FGM/C each year. In France, 20,000 women and 12,500 girls either have undergone or will undergo FGM/C [7]. Data from Switzerland suggest approximately 6,000 women and girls had experienced FGM/C [8]. Based on current trends, the majority of European countries will need to address growing numbers of women and girls who were victims of FGM/C [9]. In the United States, the 1990 census data showed an estimated 168,000 women have undergone or may undergo FGM/C. Based upon data from the 2000 census, approximately 228,000 women and girls have either experienced or are at risk for FGM/C (35% increase) [10].

Health consequences of FGC/M and its management

FGM/C has no proven health benefits; however, the existing literature documenting the complications associated with FGM/C has many shortcomings. Surveys from countries and areas in which FGM/C is practiced are lacking or nonexistent. Furthermore, many of the reported complications come from expert opinion rather than from large population-based surveys or studies with control groups. Therefore, uncertainty has persisted regarding the magnitude of the medical complications and consequences of FGM/C [11]. Despite all recent publications, the same concerns exist and even more damaging arguments about the motives for abolishing FGM/C have been raised [12]. FGM/C has been associated with short and long-term complications. Immediate complications include hemorrhage, pain, infection, fever, and death. Infections may be the result of use of non-sterile equipment and may include localized infection, abscess formation, HIV, hepatitis, septicemia, gangrene, or tetanus.

Long-term complications include dysmenorrhea, dyspareunia, recurrent vaginal and urinary tract infections, cysts, abscesses, keloid formation, consequences sexual dysfunction, infertility, and obstetrical complications can also occur. It appears that the more aggressive the type of cutting the more severe the complications. In Somalia, 39% of women reported complications after FGM/C [13]. The most common complications were hemorrhage (18.3%), infection (15%), urinary retention (4%), and/or septicemia (4%). Urinary retention in the first three days after the procedure is usually due to patient-avoidance of urination. Contact of urine with the recently operated raw parts of the external genitalia causes pain. Blood clot formation may also contribute to the urinary retention. Late complications were reported in 44% of the study population.

The most frequent delayed complication of FGM/C is epidermal clitoral inclusion cysts (ECICs) formation [14]. It develops due to filling of pockets of epithelium by fat, hair or fluid. The lack of anesthesia, poor hygiene, primitive instrumentation, and imprecision associated with FGM/C directly contribute to the formation of ECICs, which have been documented even after Type 1 [15]. A definitive history of previous FGM/C can be found in half of the ECIC cases. Spontaneous ECICs are exceedingly rare with only five documented cases in the literature [16]. ECICs can initially appear during the adolescent years or even later in life long after the initial procedure [17]. For clinicians unfamiliar with ECICs following FGM/C, the appearance of ECICs may generate unnecessary anxiety and work-up, including comprehensive endocrinology tests, chromosome analysis, ultrasonography, intravenous pyelography, and magnetic resonance imaging (MRI) [18-21]. The cyst may enlarge during pregnancy due to increased vascularity. Excision of the cyst during pregnancy may result in severe bleeding.

Surgical options regarding cyst excision include cystectomy with total clitoridectomy to prevent possible recurrence [22-25], cystectomy with clitoroplasty to preserve the clitoris for sexual fulfillment [26-27], and a surgical approach recommending only preserving the ventral clitoral skin for sexual satisfaction [28-29]. Osifo reported the presentation of a total of 37 females with post-genital mutilation clitoral epidermoid inclusion cyst, presented at two centers in Benin City, Nigeria, between January 2005 and December 2009 [30]. Fifteen (40.5%) were post-pubertal girls at an average age of 17 who could no longer cope with giant cysts measuring more than 3.5 x 6.5 cm in size. Ignorance, financial limitations, and the apprehension from anti-FGM/C agencies were reasons for late presentation. Subsequently, medical consultation was approached for the following reasons: rapid increase in size of cysts (100%) producing discomfort in the vulva (93.3%) patient, social stigmatization (80%), sexual difficulty (66.7%), and irritating swelling in the perineum (66.7%). Outpatient cystectomies including total clitoridectomy were performed with local anesthesia. The author recommended lifting the cyst and placing the incision distally and continue dissecting proximally to relieve the clitoris, thereby, preserving the ventral clitoral skin to attain orgasm and sexual satisfaction. No incidences of recurrence were recorded up to four years postoperatively.

<table>
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<th>Excision of</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
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<tr>
<td>Clitoral hood</td>
<td>a</td>
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<td>Prepuce</td>
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<td>Labia majora</td>
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<td>Labia minora</td>
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<td>Partial or Total</td>
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</table>

Table 1. — WHO classification of female genital mutilation.
Urinary problems are common complication of FGM/C. Progressive scar shrinkage may result in urethral strictures, urine retention, and slow urine streaming. Urinary calculi may develop under the infibulated scar resulting in sharp intermittent pain. Dirie and Lindmark reported dysuria (19.6%) and poor urinary flow (5%) [13]. Recently, Peterman and Johnson reported their findings regarding urinary and/or fecal incontinence using the most recent Demographic and Health Surveys in sub-Saharan Africa [31]. No evidence was found to suggest that FGM/C contributes to incontinence. Obstructed vaginal opening may cause candida and bacterial vaginitis. The infibulated scar makes the use of suppositories and topical medication ineffective. Oral medications are preferable. Dysmenorrhea and menorrhagia are typical complications of the infibulated scar which may be relieved by deinfibulation.

The psychological and sexual ramifications of FGM/C include psychological trauma, anxiety, depression, painful intercourse, and anorgasmia [32]. Studies on sexuality in women with FGM/C suffer from inadequate analysis and an unclear reporting of results. Obermeyer reviewed the available studies on sexuality in women with a history of FGM/C and found no evidence that it prevented sexual activity or the enjoyment of sexual relations [33]. However, a recent study using the female sexual function index questionnaire (a brief, multidimensional, validated tool for the assessment of sexual function) documented that women with FGM/C (even type I and II) experienced a statistically significant decrease in arousal, lubrication, orgasm, satisfaction, and overall scores, but not pain and desire scores, compared to women with no history of FGM/C [34].

The link between infertility and FGM/C is based on weak scientific evidence [35]. FGM/C may be indirectly responsible for infertility if sexual intercourse cannot occur, but this is very rare. However, a small case-controlled study was published indicating a positive association between the extent of FGM/C and primary infertility [36]. Despite all the problems and shortcomings of the study (study design, small number of patients, and lack of written consent as acknowledged by the authors), this study has been widely used as a “solid evidence” to link FGM/C with infertility [37]. Similarly, the transmission of sexual diseases, including HIV, and FGM/C has not been definitively proven [38].

The obstetric complications associated with FGM/C are well-documented. The risks of caesarean section, postpartum hemorrhage, episiotomy, and extended maternal hospital stay, resuscitation of the infant, and inpatient perinatal death were significantly increased among African women who had undergone FGM/C [39]. The obstetrical risks were further increased in infibulated women. Deinfibulation is necessary for a safe vaginal delivery. WHO recommends this procedure be performed by all health care providers, including nurses and midwives [40]. In contrast, in countries unfamiliar with FGM/C, deinfibulation is done in specialized clinics by “a senior person with extensive experience in dealing with reversal of the mutilation” [41].

Conclusion

FGM/C is performed for many reasons (e.g., cleanliness, aesthetics, prevention of still births in primigravida, promotion of social and political cohesion, prevention of promiscuity, improvement of male sexual performance and pleasure, increased matrimonial opportunities, and enhancement of fertility). Efforts have been made to abolish the practice; however, recent evidence suggests FGM/C continues among Muslims [42-44]. Actually, FGM/C predates Islam and is practiced by Christians, Jews, and followers of indigenous African religions [45]. The holy Quran, which is the first source of Sharia law, does not mention FGM/C. The second source is Hadith, and controversy exists about FGM/C and Hadith. Some scholars believe that there is reliable evidence in Hadith that cutting of the prepuce only is Sunna while others disagree. The traditional religious approach to abandon FGM/C adopted by the West has been propagated as a “unanimous agreement” among Muslim scholars, and states not only is there no religious evidence for FGM/C but also significant medical consequences associated with the procedure. Overall, this blanket approach has failed and has created resistance against abandoning the practice. The argument against FGM/C based on the medical consequences led to an increasing “list” of complications without appropriate scientific documentation, as well as the medicalization of the procedure [46]. In this updated review of the literature of the complications of FGM/C concerns still exist regarding epidemiological flaws of the studies. With respect to the management of women and girls, fear of criminalization and prosecution may lead to an unnecessary delay in reporting the complications of the procedure [30]. Similarly, unfamiliarity with the practice may lead to unnecessary, extensive, and expensive work-ups and treatment. Proper education of all health providers is needed to manage current victims of this practice.

References

Introduction

Skin-to-skin care, also called the ‘kangaroo care,’ is practiced on newborns, usually preterm infants, wherein the infant is held close to the mother’s chest. The mother and the infant are bonded skin-to-skin and chest-to-chest, which provides warmth and security for the infant [1]. Given that infants cannot verbally express their pain and that their underdeveloped neural system makes them insensitive to pain stimulation-induced ache and stress, their feelings of pain have not been discussed much in previous studies [2-4]. Pain does not only cause neonatal hyperalgesia, high oxygen consumption, high metabolism, acid electrolyte imbalance and other complications among infants, but can also affect their nervous system’s structure and function which results in a series of long-term changes in their behaviour, such as inattentiveness, hyperactivity, and other disorders. Pain can also cause short-term negative reactions among infants. Pain stimulations, such as heel lancing [5], nasogastric tube placement, and mechanical ventilation cause significant physiological reactions like accelerated heart rate [6], decreased oxygen saturation [7], increased blood pressure (BP) [8], and intracranial pressure (ICP) [9], which changes the cerebral blood flow that results to periodic hypoxemia, fluctuation in BP levels, reperfusion injuries, and venous congestion. The infant responds to these stimulations via behavioural reactions such as crying, changing of facial expressions (wrinkling of brows, squeezing of eyes, and etching deep lines around their noses), moaning, and changing of body posture and movement. The most acknowledged among these responses are the infant’s crying and changing of facial expressions [10]. An infant who experiences pain at an early stage undergoes long-term behavioural changes afterwards. For example, the male infant who is circumcised without any anaesthesia becomes more sensitive to pain stimulation, such as when he receives a vaccine shot after four to six months [11]. Staying in the Neonatal Intensive Care Unit (NICU) is also found to significantly increase the infant’s sensitivity to pain. Twenty-eight-week preterm infants who have stayed in the NICU for four weeks have developed a stronger sensitivity to pain as compared to 32-week preterm infants. Such sensitivity to pain is directly related to the frequency of invasive medical procedures. Compared to the full-term infants, infants with very low birth weight who have undergone prolonged hospitalization or repetitive invasive medical procedures develop somatic symptoms in 4.5 years, along with attention deficit hyperactivity disorder (ADHD), learning difficulties, cognitive and behavioural dysfunctions, and poor adaptability [12]. Pain control can be categorized into two groups, namely, drug and non-drug. Non-drug pain relief methods, such as skin contact, touching, feeding of syrup and other non-nutritive sucking, are commonly used by mothers in fear of the drugs’ side effects to their children. Chen and Liu [13] found that infants can perceive pain and respond via significant physiological reactions. The field of healthcare does not think highly of pain...
among infants, which lead to ineffective pain relief measures for infants who undergo diagnostic and therapeutic operations. Such topic has been largely ignored as doctors cannot completely determine safe medicine dosages for infants and certain non-drug pain relief methods lack systematic research. Therefore, the infants’ pain must be recognized, prevented, and quantified at an early stage to effectively intervene with their health and to maintain their physiological and psychological stabilities. Non-drug methods can sufficiently reduce pain among infants. This study aimed to investigate the effects of skin-to-skin care in the heart rate, transcutaneous oxygen saturation, facial expressions, physical activity, and sound performance of infants, to evaluate the feasibility of this pain relief method, and to provide a reasonable theory for the mitigation and prevention of neonatal pain.

Materials and Methods

Subjects

Between April 2010 and December 2010, the authors selected 40 infants, 15 male and 25 female, from the present hospital’s obstetrics ward as their study subjects. Every subject underwent heel lancing 72 hours after their birth. The infants were selected based on the following criteria: (1) weighed greater than 2.5 kg, (2) obtained a ten-point Apgar Score five minutes after their birth, (3) gestational age greater than 37 weeks, and (4) the mother must sign Informed Consent Agreements and voluntarily participate in this research. Neonates were excluded from the study based on the following criteria: (1) if they cried 15 seconds before the heel lancing, (2) if they had received sedatives and analgesics within 24 hours, (3) if they had received surgery; (4) if they had congenital anomalies, (5) if they had infections that required antibiotics, (6) if they had experienced abortive heel lancing beforehand, and (7) if their mothers had a cold, fever, or gastroenteral illness. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Wuxi Maternity and Child Health Hospital Affiliated to Nanjing Medical University. Written informed consent was obtained from all participants.

Grouping method

Random number table method was used to divide ten infants into groups at a single time. The selected infants were assigned a number ranging from 0 to 9 based on their birth order. Those assigned with numbers 0 to 4 were placed in the intervention group, whereas those assigned with 5 to 9 were placed in the control group. This selection method was repeated until both groups had 20 infants.

Intervention method

Control group: After the infants were routinely bathed, they were wrapped up in clothes to reduce body temperature loss. They remained wrapped up during and one minute after the heel lancing. Intervention group: 20 minutes before the heel lancing, the authors approached the mothers on their bedsides and helped them prepare for the kangaroo care. The room temperature was set between 26°C and 28°C, the doors and windows were closed, the mothers were lifted head-side of bed, and the infants were handed to them still wrapped up in clothes or covers. Afterwards, certain pieces of clothing were removed to reveal large portions of the infants’ skins. The infants were then held close to their mothers’ naked chests. The authors assisted the mothers to place their infants in a vertical or frog position skin-to-skin to their chests. They also instructed the mothers on how to hold their infants’ buttocks with one hand and the other hand over their backs to avoid the infants from slipping and to enhance the skin-to-skin contact. The nurse administered heel lancing after 15 minutes. The mother and the infant would remain in their positions during and one minute after the heel lancing.

Physiological measures

The research process was divided into three phases, namely, ten seconds before the heel lancing, during the heel lancing (from puncturing the heel until a sufficient amount of blood had been drawn), and the recovery phase (ten seconds after the heel lancing). A multifunction monitor was used to record the infant’s heart rate and oxygen saturation at three different phases. The researcher would record the infant’s heart rate and oxygen saturation every ten seconds before the heel lancing to the end of the recovery phase.

Pain score

Douleur Aiguë Nouveau-né (DAN) Scale [14] was used to quantify the infant’s pain. The infant’s facial expression was scored between 0 to 4 points, the body movements between 0 to 3 points, and crying between 0 to 3 points, which totalled to 10 points. The score reflected the intensity of the pain among the selected infants. The researcher also recorded the duration of the infant’s facial expressions and crying.

Statistical analysis

SPSS v15.0 software was used to process the data and to express all information in mean ± SD (standard deviation). The measurement data, such as birth weight, were expressed in mean and SD. The numerical data, such as gender or type of delivery, were expressed in frequency and percentage and were analyzed via T test. The duration of the facial expression and crying were analyzed by the Wilcoxon rank-sum test (Mann-Whitney U test). The heart rate and oxygen saturation rate were analyzed via Repeated Measure ANOVA.

Results

General condition

The intervention and control groups had no statistically significant difference in their birth weight, gestational age, sex, and type of delivery (p > 0.05) (Table 1).

Table 1. — Comparison of general information between control group and study group (X±S).

<table>
<thead>
<tr>
<th>General Information</th>
<th>Study Group (N=20)</th>
<th>Control Group (N=20)</th>
<th>Intervention Group (N=20)</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3,740±3,337</td>
<td>3,337±4,091</td>
<td>-1.170</td>
<td>0.249</td>
<td></td>
</tr>
<tr>
<td>Gestational age (week)</td>
<td>39.3±0.63</td>
<td>39.3±0.94</td>
<td>-0.216</td>
<td>0.830</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td>2.333</td>
<td>0.062</td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean</td>
<td>33</td>
<td>16</td>
<td>17</td>
<td>0.406</td>
<td>0.419</td>
</tr>
</tbody>
</table>

Between April 2010 and December 2010, the authors selected 40 infants, 15 male and 25 female, from the present hospital’s obstetrics ward as their study subjects. Every subject underwent heel lancing 72 hours after their birth. The infants were selected based on the following criteria: (1) weighed greater than 2.5 kg, (2) obtained a ten-point Apgar Score five minutes after their birth, (3) gestational age greater than 37 weeks, and (4) the mother must sign Informed Consent Agreements and voluntarily participate in this research. Neonates were excluded from the study based on the following criteria: (1) if they cried 15 seconds before the heel lancing, (2) if they had received sedatives and analgesics within 24 hours, (3) if they had received surgery; (4) if they had congenital anomalies, (5) if they had infections that required antibiotics, (6) if they had experienced abortive heel lancing beforehand, and (7) if their mothers had a cold, fever, or gastroenteral illness. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Wuxi Maternity and Child Health Hospital Affiliated to Nanjing Medical University. Written informed consent was obtained from all participants.
Table 2. — Comparison of infantile heart rate at different time spot between groups (bpm).

<table>
<thead>
<tr>
<th>Item</th>
<th>Before HL</th>
<th>10 s</th>
<th>20 s</th>
<th>30 s</th>
<th>40 s</th>
<th>50 s</th>
<th>60 s</th>
<th>After HL</th>
<th>Time</th>
<th>Interaction</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention group</td>
<td>118.05±</td>
<td>6.09</td>
<td>124.95±</td>
<td>3.62</td>
<td>132.30±</td>
<td>4.93</td>
<td>138.30±</td>
<td>3.39</td>
<td>145.40±</td>
<td>4.76</td>
<td>151.60±</td>
</tr>
<tr>
<td>Control group</td>
<td>120.60±</td>
<td>5.62</td>
<td>136.70±</td>
<td>5.17</td>
<td>144.80±</td>
<td>5.00</td>
<td>154.65±</td>
<td>5.58</td>
<td>162.65±</td>
<td>4.37</td>
<td>170.35±</td>
</tr>
</tbody>
</table>

*p < 0.01

Table 3. — Comparison of infantile oxygen saturation rate at different time spot between groups (%).

<table>
<thead>
<tr>
<th>Item</th>
<th>Before HL</th>
<th>10 s</th>
<th>20 s</th>
<th>30 s</th>
<th>40 s</th>
<th>50 s</th>
<th>60 s</th>
<th>After HL</th>
<th>Time</th>
<th>Interaction</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention group</td>
<td>98.35±</td>
<td>0.75</td>
<td>88.00±</td>
<td>4.08</td>
<td>84.75±</td>
<td>3.39</td>
<td>86.15±</td>
<td>6.22</td>
<td>87.70±</td>
<td>7.89</td>
<td>82.15±</td>
</tr>
<tr>
<td>Control group</td>
<td>97.40±</td>
<td>1.39</td>
<td>84.95±</td>
<td>9.88</td>
<td>81.50±</td>
<td>9.61</td>
<td>78.95±</td>
<td>7.13</td>
<td>78.50±</td>
<td>8.61</td>
<td>76.15±</td>
</tr>
</tbody>
</table>

*p < 0.05

Table 4. — Comparison of pain index between groups.

<table>
<thead>
<tr>
<th>Items</th>
<th>Average rank-sum of control group</th>
<th>Average rank-sum of intervention group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain facial expression</td>
<td>21.75</td>
<td>9.25</td>
<td>0.041</td>
</tr>
<tr>
<td>time (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crying time (s)</td>
<td>22.28</td>
<td>10.72</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Infantile heart rate

Table 2 compares the two groups’ neonatal heart rates at different time periods. The neonatal heart rates were stable before the blood collection. The intervention group’s change in heart rate was less than that of the control group before the blood collection and the recovery stage. Repeated measurement of variance (F = 369.695, \( p = 0.000 \)) indicated a statistically significant difference in the two groups’ heart rates at different time points. The time * grouping (F = 5.390, \( p = 0.000 \)) showed a statistically significant difference in the interaction time and packet.

Infantile oxygen saturation rate

Table 3 compares the oxygen saturation of the infants at different time points. During the blood collection, the neonatal oxygen saturation decreased in varying degrees among the two groups, in which the intervention group’s blood oxygen saturation remained at approximately 80% before recovering to 98.10% after ten seconds from the blood collection. Moreover, the intervention group experienced less reductions in oxygen saturation compared to the control group at each time point.

Pain index

Table 4 shows a statistically significant difference (\( p < 0.01 \)) between the two groups’ pain indicators.

Discussion

Kangaroo care is a non-drug therapy, which has been identified by few Chinese studies to have an intervention effect on pain [15]. By examining the infant’s heart rate, transcutaneous oxygen saturation, and acute pain during a randomized, controlled trial, the present authors observed that the skin contact between the mother and child had an intervention effect on neonatal pain. Heel lancing is the most common medical procedure for neonatal pain [16]. Pain stimulation leads to a series of physiological responses, which cause both short- and long-term effects. A proper and effective assessment of infantile pain, along with a positive and effective intervention, can eradicate its harmful effects [17]. By holding them close to their mothers’ chests, the infants have 82% less chances to cry and 65% less chances to make facial expressions during the heel lancing [18]. The present experiment shows that the infants in the intervention group had cried (41.90 ± 8.93 s) and had made facial expressions (71.15 ± 16.45 s) for a shorter time than those in the control group. The intervention group also has a DAN score (5.85 ± 0.98) that is lower than that of the control group. A statistically significant difference between the two groups was observed (\( p < 0.01 \)). Kangaroo care reduces the infants’ responses to pain by providing them a safe and cozy environment. The mothers’ comfort and encouragement has a major role in the infants’ resistance to pain stimulation [19]. Table 2 shows that before heel lancing, the control and intervention groups had no statistically significant difference on heart rate (\( p > 0.05 \)), which indicates the absence of pain stimulation among infants before the heel lancing. However, the heart rate changes among the two groups during the seven ten-second intervals ten and 60 seconds after the heel lancing. Based on time, interaction, and grouping factors, there is a statistical significance between the two groups (\( p < 0.01 \)). Given that time equals to F = 369.695, \( p = 0.000, \)
Table 2 shows the statistical significance of the two groups’ heart rates at different time periods. During the heel lancing, both groups’ heart rates accelerates (Time * Grouping: F = 5.390, p = 0.000) which indicates the statistical significance of time and grouping interaction. The heart beat acceleration rate of the intervention group is much lower than that of the control group. Skin-to-skin contact between the mother and infant reduces the changes in the infant’s heart rate during the pain stimulation. Other studies also found that while administering kangaroo care, the mothers’ respiration, heart beat, and blood flow all mimicked their condition when the infants are still in their uterus, which enhances the security of the infants [20]. Infants become more secure when their mothers hold them close to their chests, touch them, and let them feel their heartbeat [21]. The present data shows that after the heel lancing, the heart rate of the intervention group became lower than 160 bpm. Therefore, the kangaroo care is proven to sufficiently support the infantile ventricular relaxation and to reduce the negative effects to the infant’s cardiac output and heart rate acceleration.

During kangaroo care, the mother’s temperature is quickly transferred to the newborns. After an hour of kangaroo care, the newborns’ body temperature reaches the normal level and stays within that range [22]. Body temperature is the major factor to the infant’s blood oxygen saturation rate. There is no statistical significance between the control and intervention groups’ blood oxygen saturation rate before the heel lancing. However, a statistically significant difference (p < 0.05) was observed between the two groups’ blood oxygen saturation rate during the seven ten-second intervals ten and 60 seconds after the heel lancing. During the heel lancing, the blood oxygen saturation rate of both groups dropped significantly, which indicates that acute pain lowers the infant’s blood oxygen saturation rate. Table 3 shows Time: F = 71.377, p = 0.000. The two groups’ blood oxygen saturation rates had a statistical significance at different time spots. During the four ten-second intervals 30 to 60 seconds after the heel lancing, the blood oxygen saturation rate of the control group became lower than 80%. However, 60 seconds after the heel lancing, the blood oxygen saturation rate of the intervention group became lower than 80%. Given that Time * Grouping: F = 2.358, p = 0.024, the time and grouping interaction shows a statistical significance. Although both groups’ blood oxygen saturation rates dropped during the heel lancing, the blood oxygen saturation rate of the intervention group remained around 80%. During the last ten seconds of the heel lancing, the infantile blood oxygen saturation rate recovered to 98.10%. During most time spots, the infantile blood oxygen saturation rate of the intervention group was lower than that of the control group. All of these findings indicate that skin contact between the mother and infant significantly reduces the duration of pain stimulation. Therefore, this pain relief method prevents and ultimately reduces the infantile hypoxemia.

When the pain stimulation is unavoidable during the infantile period, kangaroo care can be administered to reduce the hypoxemia that is caused by pain and improve the development of the infants’ systems [23]. The present study proved that the skin contact between the mother and infant reduces the effects of pain stimulation for full-term infants during heel lancing. Unfortunately, this research was conducted at a small-sample scale. In actual practice, the inaccuracy of many factors, such as environmental factors, crying of infants, and probe position of life monitor, had different effects on experimental data collection, which ultimately reduced the sample size. The present authors hope to continue this research at a much broader population. After improving their surveillance method, they hope to find an effective way to reduce the effects of pain stimulation to infants, to help develop their personalities and intelligence at an early stage, and to improve their social adaptability.

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References
Effect of skin contact between mother and child in pain relief of full-term newborns during heel blood collection


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Do combined psychological stress examinations predict pregnancy outcome in an assisted reproductive technology program?

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Summary

Purpose of Investigation: To investigate prospectively if the pregnancy outcome in infertile women undergoing assisted reproductive technology (ART) is predictable by a combination of psychological stress examinations on the day of embryo/blastocyst transfer. Materials and Methods: From April 2012 to May 2012, 114 women aged 42 years old or less underwent transfer of morphologically-good embryo/blastocyst(s) in the present in vitro fertilization (IVF) center. Immediately before the transfer, salivary secretion was obtained and frozen. α-amylase and cortisol concentrations were quantified using biochemical assays. In addition, patients were asked to answer General Health Questionnaire 28 (GHQ28) and Zung’s Self Rating Depression Scale (SDS) following transfer. The results were compared between the pregnant group and non-pregnant group. Results: There were no significant differences in the age of the infertile couples between the pregnant group and non-pregnant group as well as body mass index of the infertile women. The GHQ28 and SDS scores were similar between the two groups, as were the salivary α-amylase and cortisol concentrations. Conclusion: This prospective study failed to demonstrate the predictivity of the pregnancy outcome by psychological stress examinations in infertile women in an ART program, even though these tests were used in combination.

Key words: Assisted reproductive technology; Pregnancy outcome; Psychological tests; Salivary stress markers.

Introduction

The idea that psychological stress triggers major health problems, including cardiovascular and endocrinological diseases, has been widely accepted [1]. Studies also suggest the relationship between psychological stress and infertility including in vitro fertilization (IVF) failure and recurrent miscarriages [2, 3]. Psychological stress has been measured using questionnaires and endocrine markers such as α-amylase and cortisol. Recent advance in laboratory examinations demonstrated that the concentration of these molecules is accurately measurable in the saliva secretion as well as in the plasma [4, 5]. In the research of reproductive biology and pathology, these endocrine markers have been evaluated in the plasma samples, whereas a few studies utilized the secretion of the salivary glands.

Given the burden in infertility screening and treatment, repetition of venipuncture may further increase the psychological stress of the patients. Using less invasive salivary cortisol and α-amylase measurement and written questionnaires, we aimed to clarify the association between psychological stress and pregnancy outcome prospectively in an assisted reproductive technology (ART) program.

Materials and Methods

One-hundred and thirteen women who underwent embryo/blastocyst transfer in the present IVF center from April 2012 to May 2012 were enrolled in the study under informed consent [6]. The patients aged 43 years or more and/or with morphologically poor embryos/blastocysts were excluded from this study. This study was approved by our Institutional Review Board.

Immediately before the transfer, one ml of salivary secretion was obtained by their passive drooling. The sample was collected directly in a tube and stored at -20°C until measurement. α-amylase concentration was determined using a commercially available kinetic reaction assay [4], whereas cortisol concentration was quantified using a highly sensitive enzyme immunoassay [5]. The quantification was done in duplicate and the mean value was evaluated for comparison.

During 30-minute bed rest following the transfer, patients were asked to answer two written psychological tests: General Health Questionnaire 28 (GHQ28) [7] and Zung’s Self Rating Depression Scale (SDS) [8]. Serum HCG concentration was measured using an automated enzyme immunoassay on the 11th day following day-3 early cleavage embryo transfer or on the ninth day following day-5 blastocyst transfer. According to the manufacturer instruction, the values with two IU/L or more were regarded as a positive pregnancy test.

Statistical analysis was performed between the pregnant group and non-pregnant group. The scores and values were compared using Student’s t test. A p value less than 0.05 was considered significantly different.

Results

There were no significant differences (p > 0.13) in the age of the infertile couples between the pregnant group (female partner 36.6 ± 3.4 years, and male partner 38.0 ± 5.0 years, mean ± SD) and non-pregnant group (female partner 37.4 ±
Table 1. — Characterization of the pregnant group and nonpregnant program in an ART program.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant group (n = 36)</th>
<th>Non-pregnant group (n = 77)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.6 ± 3.4</td>
<td>37.4 ± 4.3</td>
<td>0.32</td>
</tr>
<tr>
<td>Age, male partner (years)</td>
<td>38.0 ± 5.0</td>
<td>38.6 ± 4.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.0 ± 3.0</td>
<td>21.0 ± 3.0</td>
<td>0.13</td>
</tr>
<tr>
<td>GHQ28 score</td>
<td>5.0 ± 3.7</td>
<td>5.1 ± 4.9</td>
<td>0.90</td>
</tr>
<tr>
<td>SDS score</td>
<td>37.2 ± 6.3</td>
<td>36.7 ± 6.8</td>
<td>0.74</td>
</tr>
<tr>
<td>Salivary α-amylase concentration (µg/dl)</td>
<td>196.0 ± 144.6</td>
<td>202.0 ± 133.2</td>
<td>0.83</td>
</tr>
<tr>
<td>Salivary cortisol concentration (IU/ml)</td>
<td>0.16 ± 0.10</td>
<td>0.15 ± 0.02</td>
<td>0.46</td>
</tr>
</tbody>
</table>

All data are shown as mean ± standard deviation.

4.3 years, and male partner 38.6 ± 4.7 years), as well as body mass index of the infertile women (22.0 ± 3.0 kg/m² in the pregnant group vs 21.0 ± 3.0 kg/m² in the non-pregnant group (Table 1). The GHQ28 and SDS scores in the pregnant group (5.0 ± 3.7 and 37.2 ± 6.3, respectively) were comparable to that in the non-pregnant group (5.1 ± 4.9 and 36.7 ± 6.8, respectively) (p > 0.74). Finally, the salivary concentration of α-amylase (196.0 ± 144.6 mg/dl in the pregnant group vs. 202.0 ± 133.2 mg/dl in the non-pregnant group) and cortisol (5.1 ± 4.9 IU/ml in the pregnant group vs. 36.7 ± 6.8 IU/ml in the nonpregnant group, respectively) was also similar between the two groups (p > 0.46).

Discussion

The effect of psychological stress on reproduction remains controversial. While some studies support the negative impact of psychological stress on pregnancy, others deny it [9-15]. The discrepancy among the studies largely comes from the methodological variances and confounding factors. Researchers often use the time-to-pregnancy as a main outcome measure to assess the relationship between psychological factors and infertility [2, 9], but this parameter is frequently biased by diverse infertility etiologies such as ovarian reserve, tubal patency, intercourse frequency, and sperm count and motility. To reduce these biases, the authors limited the subjects to the infertile couples undergoing ART programs.

In this study, the authors did not find any significant differences in the molecular stress markers (salivary cortisol and/or α-amylase concentrations) and questionnaires (GHQ28 and SDS scores) between the pregnant and nonpregnant group following IVF-embryo transfer cycle. The strength of the present data is that prospective multiple measurements were adopted for evaluation. These results suggest that assessment of psychological stress in human reproduction is not easy and simplistic, although further investigations are required to reduce the intervention of the confounding factors. Some investigators reported that the level of these stress markers in infertile patients is higher than in fertile women [12, 15], implying that having infertility itself is a stressful condition.

Psychological stress measurement with four independent examinations including salivary secretory markers and written questionnaires failed to predict the pregnancy outcome in an ART program. These findings indicate that the results of IVF depend on various factors and larger studies are still required to detect the impact of psychological stress on pregnancy outcome.

References

Introduction

Unexplained infertility is a common cause of infertility [1]. Although there is no consensus about which tests are essential for diagnosing unexplained infertility, it has been recommended that all basic tests for infertility should be normal including laboratory assessment of ovulation, tests of anatomic structure of the uterine cavity and fallopian tubes, and semen analysis [2-4]. Therefore, unexplained infertility is a diagnosis of exclusion.

Tests for fallopian tube patency are important in the evaluation of infertility but are sensitive only for gross defects. It may be difficult to detect subtle anatomic or functional problems of the fallopian tubes that may have negative effects on fertility [5-8].

The tubal lumen caliber and course of the fallopian tube may contribute to infertility. The intramural tubal lumen caliber may be narrow (0.1 mm) [9]. The fallopian tubes are tortuous in most patients, but the intramural segment of the fallopian tube may be straight in most patients [10, 11]. In addition, hormonal changes associated with the menstrual cycle have effects on physiologic activities of the fallopian tubes. During the estrogen dominant phase of the menstrual cycle, transisthmic flow may decrease and contractions of tubular musculature may increase [12, 13]. During the proliferative phase of the menstrual cycle, the cilia beat less frequently than they do after ovulation and the proportion of ciliated cells decreases along the tube [14,15].

Patients who have multiple tubal tortuosities and who have laparoscopy may have a higher incidence of conception, possibly because tubal insufflation may alleviate tubal obstruction. A higher incidence of conception may occur after laparoscopy because of removal of minor obstruction such as fine intraluminal adhesions or thickened mucous plugs [16]. Amorphous material may form a cast in the tubal lumen in 33% women who have segmental tubal resection because of proximal tubal obstruction [17].

There is a need for effective techniques to address the subtle functional and anatomic tubal problems that may prevent pregnancy. Hydrotubation is a technique in which a liquid mixture or saline solution (aqueous or oil-soluble medium) is flushed through the cervix into the uterine cavity and fallopian tubes. Although it is unknown whether hydrotubation increases fertility, hydrotubation may improve the frequency of pregnancy because it may mechanically dislodge dense material in the tubes [18]. Previous studies have used different volumes and contents of saline solution, but the duration of flushing (not time-limited) has not been described quantitatively [19-23].

The present authors hypothesized that treatment with time-limited hydrotubation may be a useful empirical method to improve the frequency of pregnancy for women with unexplained infertility. The beneficial effects of hydrotubation in previous studies (that had no time limitation during the procedure) may have been associated with the

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Summary

**Purpose:** To evaluate time-limited hydrotubation combined with clomiphene citrate as treatment for unexplained infertility. **Materials and Methods:** In this unblinded, randomized controlled trial of patients who had unexplained infertility, 40 patients were treated with time-limited hydrotubation (saline, 20 ml; flushed within 20 to 30 seconds) and clomiphene citrate (total, 70 cycles) and 40 patients were treated with clomiphene citrate alone (total, 74 cycles). All women underwent an ovulation induction protocol with clomiphene citrate (100 mg/d orally for five days, from day 3 to day 7 of the cycle). Hydrotubation was performed after detection of the dominant follicle. **Results:** There were 15 pregnancies in the 80 patients (19%) (total, 144 stimulated cycles; 10% pregnancies per cycle). The frequency of clinical pregnancy per cycle was significantly greater in patients who were treated with hydrotubation and clomiphene citrate (nine pregnancies per cycle [13%]) than those treated with clomiphene citrate alone (two pregnancies per cycle [3%]; odds ratio, 5.3; 95% confidence interval, 1.1 to 25.5; *p* ≤ 0.05). The frequency of pregnancy per patient (total, clinical, or chemical) was similar for the two treatment groups. The frequency of live birth or abortion per cycle or patient was similar between the two treatment groups. **Conclusion:** Time-limited hydrotubation and clomiphene citrate may increase the frequency of clinical pregnancy per cycle in women who have unexplained infertility.

**Key words:** Obstetrics; Fallopian tubes; Ultrasonography; Conception; Pregnancy.
mechanical force of flushing and pharmacologic effects of different agents added to the hydrotubation solution. In the present study, the authors evaluated the mechanical effect of hydrotubation with saline alone in a time-limited fashion. When the ejection velocity may reach a maximum value, hydrotubation may be more effective because of higher mechanical force.

Materials and Methods

Subjects

This was an unblinded, randomized controlled trial comparing the efficacy of time-limited hydrotubation and clomiphene citrate or clomiphene citrate alone on the frequency of pregnancy in women who were diagnosed with unexplained infertility. The study was performed in accordance with the Code of Ethics of the Declaration of Helsinki [24]. The study protocol was approved by the Ataturk University Medical Faculty Ethical Committee (registered approval number 2009/3-06).

Participants were recruited from 116 consecutive couples undergoing infertility counseling at the Infertility and Assisted Reproduction Unit, Department of Obstetrics and Gynaecology, Ataturk University from May 2009 to April 2010. A complete infertility investigation had been performed before referral to the Assisted Reproduction Unit. Women were included in the study when they had unexplained infertility and (1) ≥ two years (two to five years) unprotected sexual intercourse, (2) ≥ two semen analyses from their partner that were normal, (3) age 18 to 35 years, (4) confirmation of bilateral tubal patency by hysterosalpinogram or diagnostic laparoscopy, (5) confirmation of normal ovaries from their partner that were normal, (6) serum follicle-stimulating hormone level < 75 pg/ml at day 3 (menstrual cycle), and (7) regular menstrual cycles during the previous six months.

Exclusion criteria were (1) a history of ovarian and/or adnexal surgery, (2) presence of major medical or pelvic organic diseases or endocrine conditions such as thyroid disease or hyperprolactinemia, (3) use of hormonal medication within the previous six months before the first study visit, (4) women with body mass index ≥ 30 kg/m², (5) women with ovarian or tubal abnormalities, and (6) subjects with male factor infertility.

There were 20 couples that did not satisfy the inclusion criteria. In the 96 eligible couples, 80 couples accepted inclusion into the study and gave informed consent for ovulation induction with or without hydrotubation. Subjects were randomized for treatment using sequentially numbered opaque sealed envelopes that were given to a nurse who was not involved in the study and who assigned women to two study groups: (1) hydrotubation and clomiphene citrate (40 patients) and clomiphene citrate alone (40 patients).

Treatment and evaluation

All patients had ovulation induction with clomiphene citrate 100 mg/d orally for five days, from day 3 to day 7 of the menstrual cycle. After spontaneous bleeding, endometrial thickness and follicle measurements were performed on day 3 and 12 of the cycle with a transvaginal ultrasonography unit that was equipped with a five to seven MHz endovaginal probe. Ultrasonographic examinations were repeated on days 13 and 14 when the leading follicle diameter was < 18 mm on day 12. After one or two follicles with diameter ≥ 18 mm were detected on ultrasonography, the couples were advised to have sexual intercourse on alternate days for one week. If ≥ three mature follicles (≥ 18 mm) developed, the intervention was cancelled and the couples were advised to avoid sexual intercourse for the subsequent two weeks. Ovulation was monitored by midluteal phase serum progesterone or by ultrasonography. Endometrial thickness was measured on the day that the dominant follicle was detected.

In the patients who were randomized for treatment with hydrotubation and clomiphene citrate, time-limited hydrotubation was performed once per treatment cycle after detection of the dominant follicle (≥ 18 mm). A sterile vaginal speculum was inserted into the vagina, and the vagina and cervix were cleansed with sterile saline (ten ml). A pediatric Foley catheter (8 French) was introduced into the cervical canal and pushed beyond the cervical os into the uterine cavity (2.5 cm). The balloon was insufflated with saline (three ml) to obstruct the cervical canal. Saline solution (20 ml) was flushed into the uterine cavity and tubes, and the time of flushing was limited to 20 to 30 seconds (mean, 26). In patients who reported abdominal pain or discomfort during flushing, the ejection velocity of saline was decreased.

Six patients in the study group and four patients in the control group, who had no pregnancy after the first cycle, refused treatment in the second menstrual cycle. All women were followed until it was evident that pregnancy was unsuccessful or pregnancy was confirmed successful by serum β-human chorionic gonadotropin level (>20 IU/L). Patients with positive β-human chorionic gonadotropin level were further followed for presence of an intrauterine gestational sac, yolk sac, and fetal pole on ultrasonography by a radiologist who was blinded to the study. Chemical pregnancy was diagnosed if serum β-human chorionic gonadotropin level was >20 IU/L, and it did not rise to the level of >1,500 IU/L and no intrauterine gestational sac with double line signal was detected in transvaginal ultrasonography in control examinations. Clinical pregnancy was diagnosed if fetal pole was detected within the gestational sac. The authors used the term abortion for clinical pregnancies. Chemical pregnancies were not included in abortion rate. A maximum of two treatment cycles per patient were planned. The primary outcome measure was frequency of pregnancy per cycle.

Data analysis

Data analysis was performed with statistical software (Statistical Package for Social Sciences, version 11.5). Normality was evaluated with Shapiro-Wilk test. Results were reported as median (range, minimum to maximum) or number (percent). Comparisons between groups were made with Mann-Whitney test for continuous data and Fisher exact test or Yates or Pearson χ² test (chi-square test) for categorical data. Odds ratios with 95% confidence intervals were calculated for frequency of pregnancy. Statistical significance was defined by p ≤ 0.05.

Results

The study groups were similar in age, body mass index, duration of infertility, frequency of previous unsuccessful assisted reproduction treatment, number of cycles, and number of follicles ≥ 18 mm (Table 1). Median endometrial thickness was significantly greater in the patients who were treated with hydrotubation and clomiphene than those treated with clomiphene citrate alone (Table 1).

There were 15 pregnancies that occurred in the 80 patients (19%) (total, 144 stimulated cycles; 10% pregnancies per cycle) (Table 2). In four patients who had hydrotubation and clomiphene citrate and two patients who
had clomiphene citrate alone, pregnancy occurred in the first cycle. The frequency of clinical pregnancy per cycle was significantly greater in patients who were treated with hydrotubation and clomiphene citrate than those treated with clomiphene citrate alone (Table 2). The frequency of total or chemical pregnancy per cycle was similar for the two treatment groups (Table 2).

The frequency of pregnancy per patient (total, clinical, or chemical) was similar for the two treatment groups (Table 3). The frequency of live birth or abortion per cycle or per patient was similar between the two groups (Tables 2 and 3). No multiple pregnancies occurred in either treatment group. In the patients who were treated with hydrotubation and clomiphene citrate, four patients (10%) experienced discomfort that began during hydrotubation and persisted approximately for 20 minutes. Vital signs were normal during and after hydrotubation, and there were no other complications. There was one patient in each group who developed mild ovarian hyperstimulation syndrome that resolved spontaneously without hospitalization. There was no ectopic pregnancy in either group.

### Discussion

The present study showed a significantly greater frequency of clinical pregnancy per cycle in patients who were treated with time-limited hydrotubation and clomiphene citrate than those treated with clomiphene citrate alone. Hydrotubation may increase the frequency of pregnancy by improving subtle problems of the fallopian tubes that may cause unexplained infertility [18]. The frequency of total pregnancy, chemical pregnancy, or live birth (per cycle or per patient) was not significantly altered by hydrotubation.

In a previous study, the frequency of pregnancy was not improved by hydrotubation with a large volume of saline (50 ml) performed one day before intrauterine insemination [19]. However, hydrotubation with a solution that included an antibiotic, hyaluronidase, and a steroid (10 to 30 ml), performed during the proliferative phase of the menstrual cycle for three consecutive days, was associated with a high frequency of subsequent pregnancy (62.5% within three months) in patients who had unexplained infertility and normal hysterosalpingogram [20]. Possible explanations for this finding include a mechanical effect of hydrotubation, plug weakening by hyaluronidase, treatment of undiagnosed infection with antibiotics, and anti-inflammatory effects of steroids [20]. In addition, pertubation with dilute lidocaine in a balanced salt solution (10 to 20 ml), one day before intrauterine insemination, resulted in significant improvement in frequency of pregnancy (14.9% vs 3.2%) in women diagnosed with unexplained infertility, possibly because of the effect of lidocaine to reduce sperm phagocytosis [21, 25]. However, another study of hydrotubation with low dose lidocaine in saline (20 ml) or saline alone, one day before intrauterine insemination, showed similar clinical frequency of pregnancy with or without lidocaine (17.4% vs 11.2%) in patients with unexplained infertility [22]. A review of 11 randomized trials showed that

### Table 1. — Clinical characteristics of patients who were treated for unexplained infertility.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hydrotubation and clomiphene citrate</th>
<th>Clomiphene citrate alone</th>
<th>p ≤  †</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>40</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>No. cycles</td>
<td>70</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>22.5 (19 to 32)</td>
<td>24 (18 to 32)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.8 (22.4 to 27.3)</td>
<td>24 (22.4 to 26.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (y)</td>
<td>3.3 (2 to 5.8)</td>
<td>3.5 (2.1 to 5.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Patients who had previous unsuccessful assisted reproduction treatment</td>
<td>16 (40)</td>
<td>17 (43)</td>
<td>NS</td>
</tr>
<tr>
<td>No. cycles</td>
<td>2 (1 to 2)</td>
<td>2 (1 to 2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. follicles per cycle ≥ 18 mm</td>
<td>1 (1 to 2)</td>
<td>1 (1 to 2)</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>8 (6 to 11)</td>
<td>7 (5 to 10)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Data reported as number, median (range, minimum to maximum), or number (%); † NS, not significant (p > 0.05)

### Table 2. — Frequency of pregnancy per cycle in patients who were treated for unexplained infertility.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hydrocytubation and clomiphene citrate</th>
<th>Clomiphene citrate alone</th>
<th>95% Confidence Interval</th>
<th>p ≤  †</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cycles</td>
<td>70</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy per cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11 (16)</td>
<td>4 (5)</td>
<td>3.3 (1.0 to 10.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical</td>
<td>9 (13)</td>
<td>2 (3)</td>
<td>5.3 (1.1 to 25.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Chemical</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td>1.1 (0.2 to 7.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth</td>
<td>8 (11)</td>
<td>2 (3)</td>
<td>4.7 (1.0 to 22.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Abortion</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Data reported as number (%); † NS, not significant (p > 0.05)

### Table 3. — Frequency of pregnancy per patient in patients who were treated for unexplained infertility.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hydrocytubation and clomiphene citrate</th>
<th>Clomiphene citrate alone</th>
<th>95% Confidence Interval</th>
<th>p ≤  †</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>40</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy per patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11 (27.5)</td>
<td>4 (10)</td>
<td>3.4 (1.0 to 11.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical</td>
<td>9 (22.4)</td>
<td>2 (5)</td>
<td>5.5 (1.1 to 27.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Chemical</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>1.0 (0.1 to 7.5)</td>
<td>NS</td>
</tr>
<tr>
<td>First cycle</td>
<td>4 (10)</td>
<td>2 (5)</td>
<td>2.1 (0.4 to 12.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth</td>
<td>8 (20)</td>
<td>2 (5)</td>
<td>4.8 (0.9 to 24.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Abortion</td>
<td>1 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Data reported as number (%); † NS, not significant (p > 0.05)
tubal flushing with oil soluble medium versus no intervention was associated with a significant increase in the frequency of pregnancy [23]. The beneficial effects of hydrotubation in women with unexplained infertility may be attributed to the mechanical force of flushing or pharmacologic effects of the various agents added to the hydrotubation solution.

The duration of flushing may be another important factor that may differ between the studies about hydrotubation. In previous studies, the flushing technique had not been standardized and was described qualitatively (slowly or steadily). The present study investigated time-limited hydrotubation (20 to 30 seconds) on frequency of pregnancy in women with unexplained infertility. Adequate mechanical force may be required to dislodge debris in the fallopian tubes, and this may necessitate sufficient flow velocity of saline in the tubes within a limited time. However, high flow velocity may cause intolerable adverse effects such as abdominal pain. Future study may define the maximum flow velocity that may be tolerated during hydrotubation.

Conclusion

Time-limited hydrotubation and clomiphene citrate may increase the frequency of clinical pregnancy per cycle in women who have unexplained infertility. Hydrotubation was well tolerated and there were no complications. Limitations of the present study include the small sample size, which may have limited the potential to demonstrate other significant differences in results between the two treatment groups. The present study provides justification for future larger randomized trials to further investigate time-limited hydrotubation in women with unexplained infertility and to define the optimal volume and ejection velocity of solution during the procedure.

References


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Polycystic ovarian syndrome (PCOS) is the most common gynecologic endocrine disorders among adolescents and women of childbearing age; its incidence rate accounted for 5%~10% of premenopausal women [1]. PCOS’ basic characteristics are long-term anovulation or rare ovulation, polycystic ovaries’ alteration, high androgen hormone in blood, and clinical manifestations of heterogeneity. Recent studies have found that PCOS not only affects the reproductive system, but also other systems, leading to a complex multisystem syndrome; it also results in related metabolic disorders including insulin resistance, high androgen hormone in blood, abnormal glucose metabolism, and lipid metabolism with increased cardiovascular risk [2]. Given the high incidence of PCOS and its great harm to patients, researchers are focused on how to diagnose and treat patients with PCOS, and prevent patients from long-term metabolic complications. Patients with PCOS often have anovulatory infertility; even if patients achieve pregnancy, they also encounter a higher probability of early abortion compared to normal women. Therefore, the present authors retrospectively analyzed endocrine and metabolic alterations and induction of ovulation of 579 patients with PCOS, who visited their reproductive centers. When treated, they were given oral Diane-35 and metformin hydrochloride. The authors then detected and evaluated the indexes, and assessed treatment efficacy evaluation criteria during the treatment of PCOS; this information can provide a basis for the clinical medication.

Materials and Methods

Study design and populations

The authors collected 579 patients with PCOS in Reproductive Center of Zhongshan City Bo’ai Hospital from October 2006 to October 2012, aged from 20 to 39 years; the average age was 27.93 years, the standard deviation of age was 3.70 years. They diagnosed PCOS according to Rotterdam’s criteria formulated in 2003 [3] they ruled out those patients as having late-onset congenital adrenal hyperplasia, or Cushing’s syndrome, or hyperprolactinemia, or thyroid disease and hormone secretion tumor. The authors divided 579 PCOS patients into two groups, namely obese group and non-obese group, according to the principle when patients’ body mass index (BMI) was greater than or equal to 25 kg/m², they considered a patient as obese, otherwise they considered a patient as non-obese. Therefore, the present authors retrospectively analyzed endocrine and metabolic alterations and induction of ovulation of 579 patients
the total 579 patients. They also assessed degree of insulin resistance (IR) to target organ using homeostasis model assessment of insulin status (HOMA-IR) [4], HOMA-IR is equal to fasting insulin (FINS) value multiplied fasting blood glucose (FBG) value, then divided 22.5. HOMA-IR ≥ 1.95 is diagnosed as insulin resistance in the present center. According to this, they further divided 579 patients with PCOS into altogether four groups, namely group A, group B, group C, and group D. The non-obese without insulin resistance of group A consisted of 171 patients. The non-obese with insulin resistance of group B consisted of 263 patients. The obese without insulin resistance of group C consisted of 34 patients. The obese with insulin resistance of group D consisted of 111 patients.

Ultrasound diagnosis of PCOS
All subjects were in the follicular phase, when patients had amenorrhea; if the authors did not see the sizes of follicles that were greater than ten mm and corpus luteum in two ovaries using B ultrasound on pelvic cavity, they considered these patients to be in the follicular phase, then their blood was drawn. There were special messenger recording the sizes of uterine and ovarian, sizes and numbers of antral follicle in each side of ovary by transvaginal ultrasonography color Doppler ultrasonic diagnostic apparatus, which was used for the ultrasound diagnosis of PCOS.

Determination of reproductive endocrine hormones
The authors collected ten ml fasting elbow vein blood of each subject from the third to fifth day of the menstrual cycle, however in the amenorrhea patients, the date was not restricted. Then centrifugally collected serum, was preserved at -20°C, and then used to test LH, FSH, estradiol (E2), T, prolactin (PRL), sex hormone-binding globulin (SHBG), fasting glucose (FG), fasting insulin (FINS), and the authors calculated FAI and HOMA-IR. Also these hormones were determined by chemiluminescence method with automatic electrochemistry luminescence immunity analyzer and reagents; the difference between the same batch number of reagent’s datas was less than 2.8%, and the difference between the different ones was 4.3%.

Definitions of diseases
The diagnosis criteria for PCOS referred to one revised by European Society of Human Reproduction and Embryology, and Rotterdam working group of American Society for Reproductive Medicine (ESHRE/ASRM) [3], when a patient has two of the following three articles, she can be diagnosed as having PCOS, namely: 1) anovulation or rare ovulation; 2) clinical manifestations of polycystic ovary under ultrasound, which was used for the ultrasound diagnosis of PCOS.

Therapy methods
The authors divided the 579 patients with PCOS into two groups, namely insulin resistance group and no insulin resistance group. Patients in insulin resistance group began to take orally Diane-35, which included two mg cyproterone acetate and 0.035 mg ethinyl estradiol for each tablet, at the fifth day of menstruation, one tablet daily, and continued to take orally Diane-35 at the fifth day of next menstruation. At the same time patients in insulin resistance group took orally metformin hydrochloride enteric-coated tablets, twice a day, each time 500 mg, and continuously. After three months of taking the tablets, indexes were detected and observed. Patients in no insulin resistance group began to take orally Diane-35 at the fifth day of menstruation, one tablet daily, and continued to take orally Diane-35 at the fifth day of next menstruation, one tablet daily, continuously. After three months of taking the tablets, indexes were detected and observed. At four months after the treatment, the authors began to induce patients ovulation with CC for five days, once daily, once 100 mg, at the fifth day of menstruation. When it was seventh day of menstrual cycle, they injected 75 U HMG in the patients and adjusted the dosage of HMG according to follicular development degree. They then began to monitor the sizes of follicle one time the other day, using B ultrasound from ninth to 11th day. When follicles’ diameter was greater than equal to 15 mm, they changed to daily continuous observation until ovulation. When follicles’ diameter was up to 18 mm, they intramuscularly injected 10,000U human chorionic gonadotropin (HCG) in the patients, and after injection the patients engaged in sexual activity twice in 24-48 hours. The authors then rechecked ovulation after 36 hours from the HCG injection with B ultrasound. B ultrasound showed signs of dominant follicle ovulation; collapse, reduced volume, contour clear follicle echo area disappeared, detecting a small amount of fluid in the pelvic cavity. After 72nd hour from the HCG injection, if there was no dominant follicle ovulation, then they were diagnosed as having luteinized unruptured follicle syndrome (LUFS). After two weeks of the ovulation, the authors detected HCG in patients’ urine with urine pregnancy test; if negative, the patients received the above ovulation method for two months, and if positive, their blood HCG levels were assessed. The authors examined and determined the number of gestational sacs and fetal heart sounds with B ultrasound after five weeks of ovulation, and recorded patients’ delivery.

Statistical analysis
A SPSS 13.0 statistic analysis software was used to analyze the data. The values of LH, FSH, E2, T, and PRL represented normal distribution, shown in ±s and the differences of the indexes’ values between groups were used by the independent t samples T test for comparison. The values of FAI, FINS, and HOMA-IR and other indicators showed non-normal distribution and numerical values were represented with median and inter-quartile range (Md ± IQR); the differences of the indexes’ values between groups were used by the non-parametric Wilcoxon test and logistic regression analysis for statistically assessment. The differences of sensitivity and specificity between groups were assessed by chi-square test for comparison. The AUC-ROC was used by Medcalc statistical software for analysis. The differences of ovulation and pregnancy delivery between groups were used by t-test to compare. When p was less than 0.05, the difference between groups was significant and had statistical value.

Results
Analysis of biochemical changes before and after treatment in 579 PCOS patients between groups
After treatment, the values of FAI in four groups were lower than those before therapy and there were significant differences. Compared with those before treatment, SHBG levels in four groups were elevated and there were significant differences. After treatment, LH levels and LH/FSH ratios in group A, or group B, or group C were lower than those before therapy and there were significant differences. However, compared with those before treatment, HOMA-IR and FINS in group A were elevated, while HOMA-IR...
Table 1. — Comparison of various biochemical indexes between before and after treatment of different methods in non-obese group (x̄ ± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>LH (IU/l)</th>
<th>LH/FSH</th>
<th>T (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cure</td>
<td>After cure</td>
<td>Before cure</td>
<td>After cure</td>
</tr>
<tr>
<td>A</td>
<td>171</td>
<td>11.79 ± 6.22</td>
<td>7.13 ± 3.369</td>
<td>1.81 ± 0.93</td>
</tr>
<tr>
<td>B</td>
<td>263</td>
<td>10.12 ± 5.62</td>
<td>6.74 ± 2.79#</td>
<td>1.65 ± 0.91</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>SHBG (nmol/l)</th>
<th>FAI</th>
<th>FBG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cure</td>
<td>After cure</td>
<td>Before cure</td>
<td>After cure</td>
</tr>
<tr>
<td>A</td>
<td>171</td>
<td>52.82 ± 26.22</td>
<td>116.93 ± 58.83#</td>
<td>4.55 ± 2.89</td>
</tr>
<tr>
<td>B</td>
<td>263</td>
<td>46.15 ± 26.33</td>
<td>109.42 ± 54.83#</td>
<td>5.53 ± 3.30</td>
</tr>
</tbody>
</table>

Table 2. — Comparison of various biochemical indexes between before and after treatment of different methods in obese group (x̄ ± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>LH (IU/l)</th>
<th>LH/FSH</th>
<th>T (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cure</td>
<td>After cure</td>
<td>Before cure</td>
<td>After cure</td>
</tr>
<tr>
<td>C</td>
<td>34</td>
<td>8.47 ± 3.96</td>
<td>6.07 ± 2.34#</td>
<td>1.41 ± 0.67</td>
</tr>
<tr>
<td>D</td>
<td>111</td>
<td>7.68 ± 4.50</td>
<td>6.93 ± 3.82</td>
<td>1.24 ± 0.70</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>SHBG (nmol/l)</th>
<th>FAI</th>
<th>FBG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cure</td>
<td>After cure</td>
<td>Before cure</td>
<td>After cure</td>
</tr>
<tr>
<td>C</td>
<td>34</td>
<td>57.16 ± 40.28</td>
<td>117.60 ± 62.20#</td>
<td>4.55 ± 2.88</td>
</tr>
<tr>
<td>D</td>
<td>111</td>
<td>35.61 ± 23.88</td>
<td>107.63 ± 52.17#</td>
<td>7.15 ± 4.55</td>
</tr>
</tbody>
</table>

*Compared between before and after treatment of two groups, #p < 0.01, *p < 0.05.

and FINS in group B and group C were decreased, and there were all significant differences (as shown in Tables 1 and 2).

**Analysis of ovulation status after treatment of 579 patients with PCOS**

As shown in Table 3, after treatment there were 373 patients with ovulation and 61 patients with no ovulation in non-obese group; there were 101 patients with ovulation and 44 patients with no ovulation in obese group. Ovulation rate in non-obese group was 85.94%, while ovulation rate in obese group it was 69.66%; the difference of ovulation rates between two groups was significant (p < 0.01). After treatment of ovulation, SHBG levels of patients with ovulation in both groups were higher than those of patients with anovulation in both groups, while FAI and FINS of patients with ovulation in both groups were lower than those of patients with anovulation in both groups, and there were all significant differences. LH level and LH/FSH ratio of patients with ovulation in non-obese group were lower than those of patients with anovulation, while HOMA-IR of patients with ovulation in both groups were higher than those of patients with anovulation, and there were all significant differences.

**Comparison of indicators after treatment of patients in obese group and in non-obese group between delivery of live birth patients and abortion patients**

After treatment, there were 147 patients that had delivery and 42 patients that had miscarriage in non-obese
group; there were 40 patients that had delivery and 22 patients that had miscarriage in obese group. After the differences were compared between biochemical indicators when patients in obese group or in non-obese group had delivery of live births or in pregnancies whose gestational age was less than 12 weeks, and in ones with spontaneous abortions after treatment. It was shown that T, FAI, FINS, HOMA-IR, and other indicators when patients in obese group or in non-obese group had delivery of live births or pregnancy were all lower than those that had spontaneous abortions after treatment and there were significant differences. While SHBG level of patients that had delivery was higher than those that had spontaneous abortions, and there were significant differences (Table 4).

Discussion

Patients with PCOS complicated with infertility is mainly caused by ovulation obstacle, and their infertility is related to insulin resistance, high androgen hormone levels in blood, and high level of LH. Women with oligomenorrhea or amenorrhea have about a 90% chance of being diagnosed with PCOS [1]. Therefore it is extremely important to adopt earlier treatment before ovulation induction. Diane-35 is commonly used drug for treating patients with PCOS, which contains cyproterone acetate, has strong progesterone activity, so it can reduce ovarian androgen secretion through the inhibition of LH secretion and bind to androgen receptors of target cells, thereby blocking androgen action of peripheral target organs. It can also inhibit 5 alpha reductase activity in skin. It has been reported that

### Table 3. Comparison of various biochemical indexes between before and after treatment of patients in non-obese group and in obese group (x̄± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>LH (IU/l)</th>
<th>LH/FSH</th>
<th>FBG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non obesity</td>
<td>434</td>
<td>6.74 ± 2.82</td>
<td>7.92 ± 3.94#</td>
<td>1.07 ± 0.48</td>
</tr>
<tr>
<td>Obesity</td>
<td>145</td>
<td>6.43 ± 2.74</td>
<td>7.42 ± 4.88</td>
<td>1.03 ± 0.41</td>
</tr>
</tbody>
</table>

### Table 4. Comparison of various indexes after treatment of patients in non-obese group and in obese group between delivery of live births patients and abortion patients (x̄± s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N (cases)</th>
<th>LH (IU/l)</th>
<th>LH/FSH</th>
<th>T (nmol/l)</th>
<th>SHBG (nmol/l)</th>
<th>FAI</th>
<th>FINS (mU/l)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non obesity</td>
<td>189</td>
<td>6.88 ± 2.97</td>
<td>7.43 ± 3.04</td>
<td>1.11 ± 0.52</td>
<td>1.16 ± 0.45</td>
<td>1.50 ± 0.59</td>
<td>1.76 ± 0.59*</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>62</td>
<td>7.42 ± 3.40</td>
<td>6.05 ± 1.91</td>
<td>1.10 ± 0.45</td>
<td>1.07 ± 0.34</td>
<td>1.49 ± 0.57</td>
<td>1.92 ± 0.51*</td>
<td></td>
</tr>
</tbody>
</table>

*Compared between ovulation and anovulation patients of two groups, #p < 0.01, *p < 0.05.

*Compared between miscarriage and delivery patients of two groups, #p < 0.01, *p < 0.05.
cyproterone acetate increased ability of insulin growth factor-1 (IGF-1) binding protein, decreased the level of free insulin growth factor, thereby reducing efficacy of IGF-1 in androgen synthesis process [5]. Diane-35 contains estrogen that can increase SHBG levels, and reduce free androgen levels. However, the present study observed that Diane-35 lowered the level of androgen, at the same time, it did not improve insulin sensitivity, can even cause abnormal glucose tolerance in non-obese patients with PCOS; this result was similar to ones reported in the literature [6-8]. Considering that cyproterone acetate in Diane-35 has glucocorticoid activity and promotes gluconeogenesis, and is against insulin action, therefore non-obese patients with PCOS should be considered to use oral contraceptives having no glucocorticoid activity, while PCOS patients with insulin resistance should use insulin sensitizer metformin, thereby lowering insulin levels, reducing insulin resistance, improving ovarian function, and glucose metabolism function of PCOS patients, treating high androgen hormone levels in blood, and restoring menstruation and ovulation function.

The present research showed that SHBG levels in patients with ovulation after ovulation induction was much more elevated than in patients that were anovulatory, while the levels of FAI and FINS in patients with ovulation after ovulation induction were lower than in patients that were anovulatory, and there were significant differences between two groups. Abroad there have been similar reports [9]. This study suggests that SHBG and FAI levels can all be considered as effective treatment indexes to evaluate whether treatment measures both in either obese or non-obese patients with PCOS are effective or not.

This research showed that the levels of T and FAI and FINS and HOMA-IR in abortion group were higher than that in delivery group after treatment, while the SHBG level was lower than that in delivery group. The reason why patients with PCOS had abortion may be related to high androgen hormone levels in blood and insulin resistance. SHBG level decreasing in patients with PCOS elevates the level of free androgens in serum, which further promotes expression of the androgen receptor (AR) in the local endometrial. AR has similar effect to progesterone receptor (PR), also reduces the expression of integrin α V β3 in PCOS patients, which may be associated with implant failure and high abortion rates of patients with PCOS. Homeobox gene HOXA10 is one of the molecular markers on endometrial receptivity, and is regulated by steroid hormone. Some research has found that testosterone of ovarian origin in vitro down-regulated HOXA10 expression in endometrial cells of Ishikawa, and found that there was HOXA10 mRNA expression decrease in endometrial biopsy specimens of PCOS patients, which might also be one cause of endometrial receptivity found in patients with PCOS [10, 11]. Recent studies suggest that insulin resistance elevating HOMA-IR may lead to recurrent spontaneous abortion [12]. Obesity is one of the risk factors of spontaneous abortion [13], and obese patients with PCOS have varying degrees of insulin resistance [14].

Insulin resistance resulted in PCOS patients’ endometrial hyperplasia abnormal and function defect. Early pregnancy immune inhibitory glycoprotein glycodelin may inhibit the immune response of endometrial to embryos the insulin-like growth factor-binding protein-1 (IGFBP-1) is advantageous to the embryo in the adhesion process of maternal-fetal interface [15], but hyperinsulinemia has a negative impact on the pre-implantation environment by reducing the expression of glycodelin and IGFBP-1 [11]. Jennifer et al. [16] observed that IGFBP-1 and glycodelin in PCOS patients’ serum during their early pregnancy were significantly lower than that in the control group. Thus speculated that the two reduced proteins may be related to occurrence of spontaneous abortion of PCOS patients. Hyperinsulinemia also can upregulate the level of plasminogen activator inhibitor-1 (PAI-1), so that induced thrombosis affects the placental blood supply, making the trophoblast dysplasia lead to miscarriage [17]. Yilmaz et al. [18] found that PCOS patients have hyperhomocysteinemia which was positively associated with insulin resistance; hyperinsulinemia leads to hyperhomocysteinemia. Hyperhomocysteinemia may increase vascular endothelial oxidative stress response, activation of platelets, promote thrombosis, stimulate vascular smooth muscle cell proliferation, promote endothelial apoptosis, interfere with maternal-fetal interface endometrial blood flow, and vascular integrity, rendering the endometrial environment conducive to embryo growth, or more likely lead to early abortion [19-21]. In addition, there is subtle relationship between hyperinsulinemia and high androgen hormone levels in blood; hyperinsulinemia inhibits hepatic SHBG synthesis, causing free androgen increased in the body. The reports suggested that through research on intima of women with PCOS, SHBG expression decreased in endometrial stromal of PCOS patients with insulin resistance may lead to abnormal steroid environment, and also to change of regulation mechanism, thus leading to abortion. Insulin resistance and hyperinsulinemia might become a central link of abortion in overweight patients with PCOS [22-24]. Combining the present research with practical clinical significance, the authors believe that SHBG and FAI can be used as an effective therapeutic evaluation index during ovulation treatment and artificial pregnancy.

In conclusion, this study suggests that whether there is insulin resistance or not, SHBG and FAI can be used as an effective treatment evaluation index, both in either obese or non-obese patients with PCOS, which can cue information of ovulation treatment and pregnancy outcome. Monitoring the levels of SHBG and FAI can guide clinical medication during the treatment of PCOS patients and can thus reduce the abortion rate, which has an important significance in guiding aristogenesis and good fostering.
References


Possible role of perineal ultrasound in the diagnosis of cystocele

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² Institute of Radiology, Second University of Naples, Naples
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Summary
Objective: Perineal ultrasound has not yet been adequately evaluated in relation to the diagnosis of anatomical descensus of pelvic organs. Therefore, the aim of the present study was to assess whether it is possible to carry out a topographical comparison between bladders in normal seat and prolapsed ones and to quantify the extent of descensus. Materials and Methods: The authors selected 140 women, divided into three groups (two control groups and one case group). All patients underwent urogynaecological examination, according to the Pelvic Organ Prolapse Quantification (POP-Q), and perineal ultrasound to evaluate pubo-bladder distance. Results: Considering the data recorded in the two control groups, the authors established the physiological pubo-bladder distance between 27-33 mm at rest and 25-30 mm under stress. In the group with cystocele, the pubo-bladder distance was significantly lower: 20 mm at rest and three mm under stress (mean value). The authors also performed a classification of ultrasound cystocele in four stages, in accordance with clinical staging. Conclusions: In conclusion, the present data show the excellent potential role of perineal ultrasound in the diagnosis of cystocele, but it is necessary to perform randomized studies to standardize the method.

Key words: Perineal ultrasound; Cystocele; Pubo-bladder distance.

Introduction
Perineal ultrasound is being considered a more and more reliable diagnostic technique in the assessment of the defects of pelvic static and urinary continence. It allows to evaluate these aspects both from an anatomic and a functional point of view through the study of the movements of pelvic organs from a position at rest (static) to a developing situation induced by the increase in abdominal pressure (dynamic) [1]. For this purpose, ultrasound can be carried out with different types of ultrasonic transducers (convex, linear, endocardial, and transrectal probes), and through multiple approaches (perineal/vulval, endovaginal, transanal, and endoanal) [2].

It is well known that with passing of time pelvic organs - especially bladder and urethra in the urinary system, uterus and vagina in the genital one- undergo topographical changes as a response to previous traumatic stimuli such as pregnancies, deliveries or previous pelvic surgery, or as a response to molecular stimuli due to physiological variations such as estrogen deficiency typical of menopausal period [3]. These topographic changes would be of little significance if they did not correspond to connected pathological alterations that inevitably lead to functional and/or anatomical disorders (urinary incontinence and/or prolapse of pelvic organs) with effects on women’s quality of life [4, 5]. With regards to this matter, in the last decades there have been many therapeutic advancements in urogynaecology, but still nowadays attention is mainly drawn to the improvement of diagnostic sensitivity in order to implement appropriate prevention measures or carry out early diagnoses of pelvic floor disorders. Perineal ultrasound is part of this debate as it is nowadays regarded in literature as an effective diagnostic support in the assessment of urethral mobility and of bladder neck typical of stress urinary incontinence [6-10]. On the contrary, such a method has not yet been properly judged in relation to the quantitative diagnosis of anatomic descensus of pelvic organs [11, 12]. Concerning this, the employment of magnetic resonance for imaging recently gained a more and more significant role in the assessment of urethral mobility and of bladder neck typical of stress urinary incontinence [13-16]. The negative factors of this technique are the economical aspect and its limited accessibility. The latter only refers to the gynaecology diagnosis as, in radiology, magnetic resonance is definitely considered the preferred exam thanks to its precision and the reduction of operator’s subjectivity. Owing to these limits, to prove that perineal ultrasound is highly reliable in the diagnostic assessment of genital prolapse, a non-invasive and low cost technique should be available. Such tech-
nique, associated with clinical examination, would considerably improve the diagnostic sensitivity of Pelvic Organ Prolapse-Quantification (POP-Q) [17].

In 2012, Chantarasorn and Dietz [18] carried out a study with the clinical and radiological comparative assessment of cystocele type II and type III based on the retrovesical angle, between urethra and trigonum, according to the classification suggested by Green [19]. This classification identifies two types of cystoceles: Green type II with retrovesical angle ≥ 140°, called “open”; and Green type III with retrovesical angle ≤ 140°, called “intact”. The variation of this angle allows to establish the type of defect: central in cystoceles with intact retrovesical angle ≤ 140° and lateral in cystoceles with open retrovesical angle ≥ 140°.

In spite of the attempts to standardize precise ultrasound indexes for cystocele diagnosis, to this day we still do not have objective and certified data to be taken as a reference when employing this method.

On the strength of these premises, the aim of the present study was to consider whether it is possible to employ ultrasound to carry out a topographical comparison between bladders in normal seat (control group) and prolapsed bladders (patients with clinical diagnosis of cystocele) and whether it is possible to quantify the descensus.

Materials and Methods

This observational prospective study was approved by the ethics committee of the present department and the authors obtained the informed consent of all the patients selected. They singled out 140 women and divided them into three groups which referred to the gynaecology departments (general and menopausal medical practices) or to the Centro del Pavimento Pelvico (the Centre for the Pelvic Floor).

The first objective of this study was to employ ultrasound to compare the position of the bladder in a group of young, nulliparous women (average age: 30 years) with the one in a group of nulliparous women who went through menopause since at least five years (average age: 53 years). Such comparison was needed to establish whether, in the absence of risk factors, the position of the bladder remains unchanged in the two groups, and to correctly define the control group. The second objective was to employ ultrasound to diagnose cystocele in patients with such a clinical diagnosis and cystocele and whether it is possible to quantify the descensus.

The third group (study group C) involved 60 peri-post-menopausal women with an average age of 54 years (SD ± 0.9 - 0.6) and a clinical diagnosis of cystocele, regardless of its stage. Cystocele clinical staging was carried out through urogynaecology assessment after voiding the bladder and in lithotomy position using the POP-Q standardization system. After the identification of pointers on the anterior and posterior walls of the vagina and of the cervix, the authors carried out the assessment of their descensus in relation to the hymen, both at rest and under stress.

All the patients were subject to an accurate anamnesis in order to exclude women with hereditary collagen diseases, obesity (BMI ≥ 30 kg/m²) or previous pelvic surgery. In addition, every patient was submitted to transvaginal ultrasound assessment of the vagina in order to evaluate the degree of bladder fullness and the presence of possible pathologies that may have discouraged the perineal approach, which allowed differential diagnoses for possible urethral diverticular pathologies in presence of defects of the anterior wall.

After the enrolment phase, all the patients were submitted to perineal ultrasound both static (at rest) and dynamic (during the Valsalva manoeuvre). This procedure was carried out through translabial ultrasound using a convex probe 3.5 MHz positioned lengthwise the vulval opening and slightly inclined upwards, while the patient was in dorsal lithotomy position. Positioning the probe in this way allows to obtain the best assessment of all the anatomic structures of the pelvic floor and also the mid-sagittal view of the anterior perineum.

The pubic bone appears like an oval image surrounded by a regular filament (curved ligament) on the left of the screen. The anechoic longitudinal structure in the centre of the screen is the urethra, on top of that can be seen the bladder surmounted by the uterus. Finally, the vaginal canal is displayed on the right of the screen, next to the urethra. As the pubic bone is the only fixed structure among those observed, it is the reference point for the scanning. The ultrasound reference point taken into account in the assessment of the cystocele was the distance between the bladder base and a straight line passing by the lower margin of the pubis symphysis (P-line) (Figure 1).

In the control groups (A and B), the distance from the P-line was measured at one cm from the bladder neck; while in the study group (C) it was measured from the lower part of the bladder base. Since the authors supposed that in physiological conditions the regular urethral-bladder angle is at a distance of about one cm from the posterior bladder wall, they chose to measure the P-line in the control group at one cm from the bladder neck. In addition, when assessing the P-line in both control groups, they detected that the angle between the P-line and the pubic axis was always about 30° in all the patients, with a non statistically significant standard deviation. Therefore, taking into account this random observation, they decided to measure the P-line as to make the angle always correspond to 30° also in the study group in order to create a reproducible method (Figure 2).

The examination was carried out with half-full bladder (250 cc) to avoid an excessive bladder fullness that may have affected the cooperation of the patient in performing the Valsalva manoeuvre and therefore altered the values assessed. The value was correctly evaluated through bladder scan. For every patient, the measurement of these parameters was both static and dynamic. In the dynamic phase, the increase in intra-abdominal pressure was obtained asking the patient to perform a progressive top-down push without breathing. Thanks to the cine-loop method (the ability to memorize and reproduce images), the authors could observe a stop-motion of the peak of the abdominal push and detect the point of maximum descensus of the bladder base.
Possible role of perineal ultrasound in the diagnosis of cystocele

In respect to the P-line. When the bladder base went beyond the P-line, the values obtained were marked as negative. The ultrasound values recorded were observed by two different operators and the measurements were carried out looking at the stop-motion corresponding to the peak of the abdominal push.

Statistical analysis

The data are recorded as median, range, and standard deviation. The average of the pubo-bladder distance in the three groups was analysed through the Student t test. The concordance evaluation between the clinical and the ultrasound analysis, as well as the concordance evaluation between the operators, were evaluated according to the Cohen’s kappa coefficient.

Results

The first objective was to establish some physiological ultrasound values in the two control groups. In nulliparous, healthy and young patients of control group A, the authors observed that the bladder base was at least at 28 mm from the P-line at rest (range 28/33 mm) and was at least at 25 mm during the Valsalva manoeuvre, at the peak of the abdominal push (range 25/30 mm).

In the nulliparous, healthy, and menopausal patients of control group B, we observed nearly identical values, as the difference detected was non statistically significant. More specifically, we observed that the bladder base is at least at 26.7 mm from the P-line at rest (range 26/32 mm) and at least at 24.8 mm during the Valsalva manoeuvre, at the peak of the abdominal push (range 24/30 mm).

On the basis of these data, the authors suggest that the physiological range of the pubo-bladder distance at rest is between 27 and 33, while the physiological range of the bladder descensus under stress is between 25 mm and 30 mm.

In the study group affected by cystocele, the pubo-bladder distance detected by ultrasound was markedly lower at rest—median value: 20 mm (range 0/30)—with a median value of three mm under stress (20/-21 mm). The results are shown in Table 1.

During the ultrasound assessment, the authors observed that all the patients with the same cystocele grade in the POP-Q had nearly the identical median pubo-bladder distance. Therefore, they divided the patients in four ultrasound stages. The ultrasound staging in four stages (from I to IV) was carried out consistently with the clinical staging by POP-Q system and resulted in: stage I = 16 patients; stage II = 21 patients; stage III = 17 patients; stage IV = six patients (Figures 3-6).

More specifically, patients with a median value of the pubo-bladder distance during Valsalva of 20 mm (range 10/20 mm) were appointed to the sub-group with grade I.

Table 1. — Clinical characteristics and perineal ultrasound measurements (median values and range).

<table>
<thead>
<tr>
<th></th>
<th>Control group A (healthy women)</th>
<th>Control group B (healthy women)</th>
<th>Study group (women with cystocele)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=40</td>
<td>n=40</td>
<td>n=60</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.0 (22 – 35)</td>
<td>53.0 (48 – 61)</td>
<td>54.0 (45 – 60)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 (20 – 28)</td>
<td>24.0 (20 – 28)</td>
<td>25.0 (21 – 29)</td>
<td>0.061</td>
</tr>
<tr>
<td>Distance bladder base – P-line at rest (mm)</td>
<td>31.0 (28 – 33)</td>
<td>31.0 (28 – 33)</td>
<td>20.0 (0 – 30)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Distance bladder base – P-line under stress (mm)</td>
<td>27.0 (25 – 30)</td>
<td>27.0 (25 – 30)</td>
<td>3.0 (0 – 21)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Retrovesical angle</td>
<td>≥ 150°</td>
<td>≥ 150°</td>
<td>≥ 140° (38 patients)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤ 140° (22 patients)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. — Pubo-vesical distance (D2), between bladder base and P-line (D1).

Figure 2. — Angle between P-line (D1) and pubis longitudinal axis (D2).
cystocele (n = 18); patients with a median value of the pubo-bladder distance during Valsalva of 4 mm (range 0/9 mm) were appointed to the sub-group with grade II cystocele (n = 20); patients with a median value of the pubo-bladder distance during Valsalva of -4 mm (range -1/-10 mm) were appointed to the sub-group with grade III cystocele (n = 15); and patients with a median value of the pubo-bladder distance during Valsalva of -14 mm were appointed to the sub-group with grade IV cystocele (n = 7). The values of the pubo-bladder distance are reported in Table 2. The results are statistically significant for one p < 0.01. The concordance between the clinical and the ultrasound diagnosis proved to be excellent (Cohen’s kappa = 0.92, p < 0.0001). The concordance between the two operators proved to be good (Cohen’s kappa = 0.794, p < 0.0001).

Discussion

The risk factors responsible for anatomical and functional disorders of the pelvic static are numerous; among them pregnancies, deliveries, bronco-pneumopathic diseases, pelvic surgery, menopause, etc. As a matter of fact, their multifactorial etiopathogenesis causes these pathologies to be remarkably frequent to such an extent that pelvic floor dysfunctions are nowadays considered a real social problem both due to their high incidence and the impact they have on the quality of life [20-22]. This justifies the considerable at-
tention the research gave to the diagnostic-therapeutic approach to these pathologies in the last years [23-25]. Although the objective urogynaecological examination is currently considered the main diagnostic tool for pelvic floor pathologies, the evidence of the diagnostic reliability of some imaging techniques -proved by many studies reported in literature- suggests that in the future such methods could contribute to reach the gold standard in urogynaecological evaluation.

To this day, the technique that proved a reliable accuracy in the assessment of the prolapse of the pelvic organs is the dynamic magnetic resonance, but it is difficult to employ in clinical practice [26-29]. Since perineal ultrasound would be easy to employ in clinical practice, as well as non-invasive and inexpensive, many attempts have been made to standardize ultrasound models that may be used as diagnostic support in the assessment of urogynaecological dysfunctions. In the last decades, perineal ultrasound was employed to assess urethral hypermobility in relation to stress urinary incontinence [7], or to evaluate the therapeutic success of incontinence correction techniques through sling. Nonetheless, the data proving its possible role in the assessment of pelvic organs prolapse are still insufficient. Concerning this, the aim of the present study was to assess whether it is possible to establish the regular topographic position of the bladder with relation to the pubic bone, and hence to diagnose and stage a possible cystocele. Therefore, the authors established a reference line which passes by the lower margin of the pubis symphysis (P-line) using the pubis -the only fixed structure-as the main reference point to measure the distance between the mentioned line and the bladder base [30-33]. To define the physiological ranges, the authors observed two control groups of nulliparous women, one with an average age of 30, and the other with an average age of 53 in order to exclude possible biases due to menopause. They noticed that the difference between the pubo-bladder distance in the two groups was not statistically significant and therefore it is possible to use the values obtained as possible ranges for the regular physiological position of the bladder in the pelvis. On the strength of the present results, the authors considered as physiological a pubo-bladder distance under stress with values higher than 25 mm, with a range from 25 to 30 mm. Although many cut-offs were suggested [34], they must underline that no reference value is universally acknowledge in literature. In addition to this, they noticed that the angle between the P-line and the pubic axis in all these patients was always about 30°, with a non-statistically significant standard deviation. This value can be considered a random observation, but they used it as possible reproducibility index of the technique employed and therefore they established the P-line for women in the study group always at 30° on the pubic axis. Once they had established the physiological values, they assessed the patients median values according to the cystocele grade detected in the POP-Q and noticed that the pubo-bladder distance was nearly identical in patients with the same grade of cystocele.

The present results suggest that perineal ultrasound has a good diagnostic effectiveness. Nonetheless two main issues may limit its standardization in the assessment of pelvic floor dysfunctions: The absence of fixed reference points (the pubic bone is the only fixed point); and the quantification of the Valsalva stress for the dynamic measurements.

As far as the specific standardization of the Valsalva stress and the increase in intra-abdominal pressure are concerned, the attempts to standardize the manoeuvre were not widely spread. Nevertheless, this problem seems to be unimportant for the purpose of the dynamic evaluation. As a matter of fact, Dietz et al. [35] proved that nearly every woman can produce pressures that may generate a maximal descensus ≥ 80% and therefore the standardization of the pressures provoked by the Valsalva manoeuvre is to be considered useless.

Despite the actual reliability of the perineal ultrasound, this method must be regarded only as a diagnostic support, since it cannot absolutely replace the clinical assessment of cystocele, which is nowadays the only standardized approach. Although many studies confirm a good diagnostic concordance between the clinical examination and the ultrasound evaluation, it is possible to detect some differences, most of all due to the fact that the reference point in clinical examinations is the hymen, while it is the pubic bone in the ultrasound assessment.

Conclusions

In conclusion, the present data show the excellent potential role of perineal ultrasound in the diagnosis and classification of cystocele, but it is also evident that further data are needed. Moreover, this method could be useful to carry out effective preventive interventions, for example when a pubo-bladder distance equivalent to a grade I cystocele could be considered a predictive factor of the descensus and could therefore suggest the employ of preventive protocol for pelvic-perineal rehabilitation. These data surely represent an hypothesis and further validation is needed through randomized trials on a considerably wider population to establish a possible standardization.

References


Fetal abdominal wall defects: six years experience at a tertiary center

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²Department of Radiology, Faculty of Medicine, Dokuz Eylül University, Izmir (Turkey)

Summary
The authors’ aim was to detect the associated anomalies and their effect on the management of the fetuses with omphalocele and gastroschisis. Between the period of 2007-2013, the data of fetuses with abdominal wall defects were analyzed. Chromosomal abnormalities and associated morphologic anomalies diagnosed by ultrasonography and autopsy were evaluated. Of the 61 fetuses, ten (20.4%) omphalocele cases and nine (17.3%) gastroschisis cases were isolated. Chromosomal abnormalities were found in seven fetuses with omphalocele cases. All fetuses with abnormal karyotypes had multiple additional anomalies. Termination rate was 65.3% for omphalocele group versus none in the gastroschisis group. To give better counseling about the prognosis and outcome of the fetuses with abdominal wall defects, detection of additional anomalies as well as type of the defect are essential tools even if the karyotype is normal.

Key words: Abdominal wall defects; Chromosomal abnormality; Fetal anomaly; Gastroschisis; Omphalocele.

Introduction
Congenital anterior abdominal wall defects comprise a wide range of anomalies, with omphalocele and gastroschisis being the most common types. Although these defects are categorized under the same heading, their incidences, clinical properties, evaluation, and management differ from each other. The diagnosis of abdominal wall defects can be made sonographically as early as 12 weeks. The early detection of an abdominal wall defect however, is alone not enough to make a proper decision about the prognosis of the fetus.

In the absence of legal termination of pregnancy, antenatal diagnosis seems to have no significant impact on the outcome of neonates with anterior abdominal wall defects [1]. On the other hand, in countries where the termination of pregnancy is allowed, counseling the parents is possible through a detailed morphologic and genetic evaluation of the fetuses. If the karyotype is normal, management of pregnancy mostly depends on the presence of additional structural anomalies [2].

The aim of this study was to determine the role of associated chromosomal and structural anomalies to the management in fetuses with abdominal wall defects.

Materials and Methods
The present study was performed retrospectively from the recordings of the fetuses with abdominal wall defects from 2007 to 2013 in Izmir Tepecik Training and Research Hospital, which is the most widely circulating tertiary center in Aegean region of Turkey. Institutional Review Board approval was taken from the center. The fetuses diagnosed with abdominal wall defects were reevaluated again in the authors’ perinatology department to identify the type of the defect and additional anomalies.

Women whose fetuses had abdominal wall defects were counseled with a genetician for chromosomal analysis. After obtaining informed consents of the parents, chorionic villus sampling, amniocentesis or cordocentesis were performed according to the gestational ages. Termination of pregnancy and autopsy were offered to the women when additional anomalies with bad prognostic outcomes or abnormal karyotypes were detected. All of the ultrasonographic findings of the terminated fetuses were confirmed with postmortem examination. Findings of autopsy and prenatal ultrasonography were evaluated together to identify the all associated anomalies.

The fetuses with the defects apart from omphalocele and gastroschisis were excluded from the study. The authors collected information regarding the maternal ages, gestational ages at diagnosis, fetal genders, fetal karyotypes, prenatal ultrasound, and postmortem findings. Fetuses were grouped as isolated, which had no additional anomalies other than omphalocele and gastroschisis. The anomalies secondary to the primary defects such as pulmonary hypoplasia and intestinal malformations were not considered as additional anomalies. Ectopia cordis and cloacal extrophy malformations were thought as parts of the Pentalogy of Cantrell (supraumbilical omphalocele - congenital heart anomalies - lower sternal, anterior diaphragmatic and pericardial defects) and omphalocele - cloacal extrophy - imperforate anus - spinal defects (OEIS) syndromes, respectively. The women who did not terminate their pregnancies were examined with ultrasonography until end of their gestations. Additional anomalies detected at later gestational weeks during follow up were also recorded.

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Results

During the six-year period, 63 cases of abdominal wall defects were identified. Among these defects 49 were omphalocele, 12 were gastroschisis, and two were body stalk anomaly. Clinical properties of fetuses with omphalocele and gastroschisis are shown in Table 1. Two omphalocele cases were from dichorionic twin pregnancies. Mean maternal age of the study population was 26.9 ± 6.3 years. For omphalocele and gastroschisis groups, mean maternal age was 27.2 ± 6.3 and 24.6 ± 6.7 years, respectively. The mean gestational age at diagnosis was 16.1 ± 3.5 weeks for omphalocele and 17.4 ± 3.4 weeks for gastroschisis group. Female to male ratio of the fetuses was 2.1 in omphalocele group and 1.4 in gastroschisis group.

Table 1. — Clinical properties of fetuses with omphalocele and gastroschisis.

<table>
<thead>
<tr>
<th></th>
<th>Omphalocele</th>
<th>Gastroschisis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>49 (80.3%)</td>
<td>12 (19.7%)</td>
<td>61 (100%)</td>
</tr>
<tr>
<td>Maternal age*</td>
<td>27.2 ± 6.3</td>
<td>24.6 ± 6.7</td>
<td>26.9 ± 6.3</td>
</tr>
<tr>
<td>Gestational age at diagnosis*</td>
<td>16.1 ± 3.5</td>
<td>17.4 ± 3.4</td>
<td>16.3 ± 3.5</td>
</tr>
<tr>
<td>Female-male ratio</td>
<td>33/16 : 2.1</td>
<td>7/5 : 1.4</td>
<td>40/21 : 1.9</td>
</tr>
<tr>
<td>Isolated</td>
<td>10 (20.4%)</td>
<td>9 (75%)</td>
<td>19 (31.2%)</td>
</tr>
<tr>
<td>Non chromosomal syndromes</td>
<td>3 (6.1%)</td>
<td>-</td>
<td>3 (4.9%)</td>
</tr>
<tr>
<td>Chromosomal syndromes</td>
<td>7 (14.3%)</td>
<td>-</td>
<td>7 (11.5%)</td>
</tr>
<tr>
<td>Associated anomalies</td>
<td>39 (79.6%)</td>
<td>3 (25%)</td>
<td>42 (68.8%)</td>
</tr>
<tr>
<td>Termination</td>
<td>32 (65.3%)</td>
<td>-</td>
<td>32 (52.5%)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD;

Table 2. — Distribution of associated anomalies in fetuses with omphalocele and gastroschisis.

<table>
<thead>
<tr>
<th>Anomalies</th>
<th>Omphalocele</th>
<th>Gastroschisis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>20</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>Neural tube defect</td>
<td>15</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Choroid plexus cyst</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Corpus callosus agenesis</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Dandy walker malformation</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Skeletal system</td>
<td>20</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Vertebral deformity</td>
<td>7</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Club foot</td>
<td>6</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Limb*</td>
<td>5</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Sacral agenesis</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Cardiac</td>
<td>12</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Hypoplastic left heart</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>DORV</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Face and neck</td>
<td>7</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Urinary</td>
<td>6</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Multicystic kidney</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Duplex ureter</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>PUV</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Hydrops</td>
<td>6</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>5</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Hyperechogen bowel</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Imperforate anus</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Genital</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Ambiguous genitalia</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Bifid uterus</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Cloacal extrophy</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Ectopia cordis</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>12</td>
<td>1</td>
<td>13</td>
</tr>
</tbody>
</table>

*Excluding club foot; DORV: double outlet right ventricle; PUV: posterior urethral valve.

Table 3. — Associated anomalies of fetuses with chromosomal abnormalities.

<table>
<thead>
<tr>
<th>Case</th>
<th>Maternal age</th>
<th>Gestational age at diagnosis</th>
<th>Ultrasound findings</th>
<th>Autopsy findings</th>
<th>Karyotype</th>
<th>Karyotype method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>21</td>
<td>DORV, meningomyelocle, single umbilical artery</td>
<td>Bilateral radius and thumb agenesis, horseshoe kidney</td>
<td>47,XX+18 AC</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>17</td>
<td>Diaphragmatic hernia, single umbilical artery, bilateral club foot, micrognathia, clinodactyly, absent nasal bone</td>
<td>Hyoplastic left heart, duplex kidney</td>
<td>47,XX+18 AC</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>19</td>
<td>Bilateral choroid plexus cysts, meningomyelocle, ascites, hyoperechogen bowel, bilateral club foot, bilateral clenched hands, AVSD</td>
<td>Ambiguous genitalia, polydactyly in right foot, left heterotaxy</td>
<td>47,XY+18 AC</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>16</td>
<td>Increased nuchal fold, bilateral choroid plexus cysts, AVSD</td>
<td>Micrognathia, bilateral clenched hands</td>
<td>47,XX+18 AC</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>18</td>
<td>PUV, hypoplastic left heart</td>
<td>Corpus callosus agenesis, microphthalmia</td>
<td>47,XY+18 AC</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>16</td>
<td>Increased nuchal fold, bilateral choroid plexus cysts, micrognathia</td>
<td>Bilateral club foot, umbilical cord cyst</td>
<td>47,XX+18 AC</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>16</td>
<td>Cystic hygroma, hydrops, VSD</td>
<td>Horseshoe kidney</td>
<td>45,X AC</td>
<td></td>
</tr>
</tbody>
</table>

AC: amniocentesis; AVSD: atrioventricular septal defect; CCA: corpus callosus agenesis; DORV: double outlet right ventricle; PUV: posterior urethral valve; VSD: ventricular septal defect.
Of all the abdominal wall defects, 19 fetuses (ten omphalocele and nine gastroschisis cases) were found to have no additional anomalies. The most common associated anomalies were central nervous system, cardiac and skeletal defects in omphalocele group, whereas minor anomalies (single umbilical artery, choroid plexus cyst) were found in gastroschisis cases. There were three syndromes other than aneuploidies associated with omphalocele, two of them were OEIS cases, and one of them was a Pentalogy of Cantrell case. Distribution of the associated anomalies according to their sites are shown in Table 2.

Seven chromosomal abnormalities, which included six Trisomy 18 and one Turner syndromes were found. All of the chromosomal abnormalities were detected from the fetuses with omphaloceles and multiple additional anomalies (Table 3). In the present study, 52.5% of the pregnancies with abdominal wall defects and 65.3% of pregnancies with omphaloceles were terminated. No fetus was terminated in gastroschisis group.

**Discussion**

In this study, of the 61 fetuses with abdominal wall defects, 49 were associated with omphalocele, 12 were with gastroschisis. The high ratio of omphalocele to gastroschisis cases (4:1) in the present study compared to other studies was related to the characteristic of the study population [3]. It is thought that gastroschisis is caused by environmental factors rather than genetic. Vasoconstrictive agents, smoking, alcohol, and young maternal age were the most blamed causative factors [4, 5].

Termination of pregnancy is considered as an option in counseling women about their fetuses with abdominal wall defects. Determining type of the defect is helpful in some extent to give information about outcomes of the fetuses but more prognostic factors are needed. Various factors such as size of the defect, presence of liver herniation, and degree of pulmonary hypoplasia were reported [6-8]. However, among these factors associated structural and chromosomal abnormalities were the most essential factors affecting outcomes of the fetuses.

In the present study, chromosomal abnormalities were found in 14.3% of omphalocele cases. Trisomy 18 was the most common chromosomal abnormality seen in fetuses with omphaloceles as stated in previous studies but other trisomies, monosomies, deletions, and triploidies were also reported [9, 10]. The rate of structural anomalies of fetuses with omphalocele was 76.7%, similar to most studies, which varied from 27% to 91% [2, 6, 11-17]. Central nervous system defects were the most common associated anomalies followed by skeletal and cardiac defects. Neural tube defects were detected in more than half of the central nervous system anomalies. There was no chromosomal abnormality in fetuses with isolated omphaloceles in the present study. In the previous studies, the chromosomal abnormalities ranged between 10.3% and 31.9% for omphalocele and were all associated with structural anomalies [3, 6, 13-16]. This finding does not mean that fetal karyotyping is not needed in isolated omphaloceles. The high rate of aneuploidies among omphalocele cases and the possibility of missing additional anomalies by ultrasonography mandate fetal karyotyping in fetuses with omphaloceles.

The necessity of prenatal karyotyping in gastroschisis cases is subject to some debate. The present authors did not detect any chromosomal abnormality in fetuses with gastroschisis as reported in some studies [6, 13-17]. On the contrary, there are studies reporting low rates of aneuploidies including trisomy 13, 18, 21, and sex chromosome abnormalities [2, 13, 18-21]. Among these studies except that performed by Barisic et al. [21], abnormal karyotypes were found in multiple additional anomalies. Although the present number of cases of gastroschisis were not sufficient to interpret, it seems reasonable not to offer prenatal karyotyping in isolated cases. Haddock et al. [22] reported that gastroschisis is not associated with major anomalies. Similarly, the anomalies of gastroschisis cases in the present study were minor (choroid plexus cyst, single umbilical artery). However, recent many studies reported the association of major anomalies with gastroschisis with a range of 5.3% - 53.2% [2, 6, 13-16, 18, 20]. The present authors believe that actual frequencies should be lower than reported due to the inclusion of minor anomalies or the ones that were consequences of gastroschisis such as intestinal atresia and malrotation.

Some congenital syndromes other than aneuploidies such as OEIS, Pentalogy of Cantrell, and Beckwith Wiedemann syndrome are more common in fetuses with omphaloceles. In the present study, of the 49 omphalocele cases, two fetuses with OEIS and one fetus with Pentalogy of Cantrell syndromes were diagnosed via autopsy findings. It is difficult to detect some syndromes in utero and certain characteristic findings such as macroGLOSSIA in Beckwith Wiedemann syndrome appear later in gestational weeks. The wide variation in expression may be the reason that Beckwith Wiedemann syndrome was not diagnosed among terminated fetuses. Gastroschisis is associated rarely with syndromes compared to omphalocele [2, 13]. Likewise, the present authors did not detect any finding suggesting part of a syndrome.

Of the fetuses with omphaloceles, 65.3% were terminated. When the present authors excluded the fetuses with abnormal karyotypes, termination rate for pregnancies with omphaloceles were still high (59.5%) due to the lethal or serious anomalies. Further, even if no prenatal karyotype had been performed, termination would have still offered to these aneuploid fetuses. The present authors found that associated structural malformations played more important role than aneuploidies in determining the pregnancy termination. For this reason, prenatal diagnosis of abdominal wall
defects should lead to a comprehensive search for associated structural anomalies. No fetuses with gastroschisis were terminated owing to the abnormal karyotype or additional anomalies. Since the prognosis was better in gastroschisis than any other abdominal wall defects, the present authors do not offer karyotyping in isolated gastroschisis. However, correct prenatal ultrasonographic detection of isolated gastroschisis cases should be more accurate than reported [13, 18].

Conclusion
When a fetus with abdominal wall defect is encountered, distinguishing the type should be the first step of the evaluation. It is also important to follow these fetuses at certain intervals in order to detect all associated anomalies. In conditions where the defect is suspicious or additional anomalies are associated, prenatal karyotype analysis should be done.

References

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Ultrasonographic wall thickness measurement of the upper and lower uterine segments in the prediction of the progress of preterm labour

Department of Obstetrics and Gynecology, Suez Canal University, Ismailia (Egypt)

Summary
Objective: To assess the role of ultrasonographic measurement of the upper and lower uterine segments wall thickness in predicting the progress of preterm labour in patients presenting with preterm labour pains. Study design: Fifty pregnant women presenting at Obstetrics Department – Suez Canal University, Egypt with regular lower abdominal pains and diagnosed as having preterm labour were enrolled in the study. Materials and Methods: Measurements of the upper and lower uterine segments wall thickness by transabdominal ultrasonography in-between contractions and with full bladder were taken. The upper/lower uterine wall thickness ratio was calculated and correlated to the progress of the preterm labour and to the response to tocolytics. Results: The ultrasonographic upper/lower uterine wall thickness ratio was directly related to the progress of preterm delivery (PTD). The change in this ratio is correlated inversely with the response to tocolysis. Using the ROC curve, when the upper/lower uterine wall thickness ratio was ≤ 1.26 the sensitivity was 94.74 and the specificity was 100.00, and when the ratio was ≤ 1.52 the sensitivity was 100.00 and the specificity was 83.33. Conclusions: These data may serve as a baseline ultrasonographic reference values for further studies in prediction the progress of preterm labour in patients presenting with preterm labour pains.

Key words: Ultrasound; Upper uterine segment thickness; Lower uterine segment thickness; Prediction; Preterm Labour.

Introduction
The diagnosis of preterm labour is generally based upon clinical criteria of regular painful uterine contractions (four every 20 minutes or eight every 60 minutes) accompanied by cervical dilatation (≥ one cm) and/or effacement (≥ 80%) [1].

Traditional methods for predicting women destined to deliver preterm based on obstetric history, demographic factors, and symptoms are neither sensitive nor specific [2-3]. A number of biologic markers in serum, amniotic fluid, and cervical secretions have been evaluated for their potential to predict preterm delivery (PTD). The most commonly used biochemical approach for differentiating women who are at high risk for impending PTD from those who are not at high risk is measurement of fetal fibronectin (fFN) in the cervicovaginal secretions. The test is performed alone or in conjunction with sonographic assessment of cervical length [4-5].

By the end of pregnancy the body of the uterus is divided into two segments which are anatomically distinct. The upper uterine segment is mainly concerned with contraction and is thick and muscular while the lower segment is prepared for distention and dilatation and is thinner. The lower segment has developed from the isthmus and is about eight to ten cm in length. When labour begins, the retracted longitudinal fibers in the upper segment pull on the lower segment causing it to stretch; this is aided by the force applied by the descending head or breech [6].

As the upper and lower uterine wall thickness were found to change in opposite directions throughout normal labour, the upper becomes thicker and lower becomes thinner, the authors thought of determining the ratio between the wall thickness of the upper and lower uterine segments, and to use this ratio as an indicator in predicting the progress of PTDs in patients represented by preterm labour pains.

Materials and Methods
The study was carried out as prospective cohort study aiming to assess the role of ultrasonographic measurement of upper and lower uterine segments wall thickness in predicting the progress of PTDs in patients presenting with preterm labour pains. This study was carried out in the Department of Obstetrics and Gynecology at Suez Canal University Teaching Hospital, Ismailia – Egypt and included 50 pregnant women with preterm labour pains admitted at Obstetrics and Gynecology Departments and enrolled in the study after fulfilling the study criteria and after obtaining the ethical approval from the faculty ethics committee.

The studied population included pregnant women presenting between 24 and 36 weeks gestation with early established preterm labour pains (regular uterine contractions with cervical dilatation one cm and less than three cm and/or cervical effacement of 80%). Other inclusion criteria involved single intrauterine pregnancy, intact membranes, primigravida or multigravida, and previous his-
tory of preterm labour. Pregnant women with late established preterm labour pain (cervix ≥ three cm dilatation), twin pregnancy, preterm premature rupture of membranes, uterine anomalies, uterine fibroids, history of previous cesarean section, and low lying or anterior wall placental insertion were all excluded.

Detailed account of the different steps of the protocol was explained to the patient and informed consent for participation in the study was obtained. The data collected from the patients included their medical history, clinical and ultrasonographic examinations, investigations and treatments, and then all the patients were followed up till the time of delivery.

Transabdominal ultrasonographic examination was done using an ultrasonographic machine with a curvilinear probe with a 3.5-MHz frequency to measure the uterine wall thickness using the following technique: patients were placed in supine position and slightly tilted to the left. Then the uterus was centralized in the midline. The transabdominal sonographic examination was carried out with the bladder full to allow good visualization of the lower uterine segment. The lower uterine segment wall thickness was measured at two cm above the internal os (Figure 1). The upper uterine segment was measured at a point in the anterior uterine wall midway between the cervix at the level of the internal os and the fundus at the point of maximum convexity (Figure 2). Placental tissue is not included in the measurement. The myometrium is defined as a layer of homogenous echogenicity from the serosal surface to the decidua [9]. The image field was magnified before taking the measurement which was obtained between uterine contractions and by the same observer. At least three measurements were obtained from the uterine wall and the average value was calculated.

The patients’ treatment included antibiotics, tocolytics, and corticosteroids. Antibiotics are given in the presence of infection such as urinary tract infection or a positive high vaginal swab. Calcium channel blocker (nifedipine) oral capsules ten mg orally every 15 minutes for one hour was the tocolytic regimen of choice. Corticosteroids for fetal lung maturity were given as betamethasone 12 mg I.M. injection two doses 24 hours apart.

The outcome recorded included time of delivery in weeks, mode of delivery, maternal and fetal complications of delivery,
Table 1. — Demographic and laboratory data for the entire study population.

<table>
<thead>
<tr>
<th></th>
<th>Responders (N = 38)</th>
<th>Non-responders (N = 12)</th>
<th>Student (t) test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>23.79 ± 2.22</td>
<td>22.83 ± 1.70</td>
<td>1.4</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>GA</strong></td>
<td>31.92 ± 2.63</td>
<td>31.33 ± 3.31</td>
<td>0.64</td>
<td>0.53</td>
</tr>
<tr>
<td><strong>US GA</strong></td>
<td>31.86 ± 2.81</td>
<td>30.67 ± 4.44</td>
<td>1.1</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Hb%</strong></td>
<td>9.86 ± 0.89</td>
<td>8.83 ± 0.45</td>
<td>2</td>
<td>0.05*</td>
</tr>
<tr>
<td><strong>Pus cells</strong></td>
<td>17.63 ± 15.44</td>
<td>25.83 ± 13.7</td>
<td>1.2</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>TLC</strong></td>
<td>15.95 ± 4.77</td>
<td>20.9 ± 0.57</td>
<td>3.6</td>
<td>0.0008**</td>
</tr>
</tbody>
</table>

*Statistically significant
GA = gestational age; US GA = ultrasound gestational age
Hb% = hemoglobin concentration, TLC = total leucocytic count

Results

The study population were divided into two groups: group 1 were those patients whose preterm labour pains stopped with tocolytics (38 patients) and group 2 were those patients who did not respond to tocolytics and went into delivery prematurely (12 patients).

For the whole study population; the maternal age ranged between 20 and 28 years with a mean of 23.56 ± 2.13. The parity ranged between para 0 (nullipara) and para 2. There was no significant difference in maternal age, parity or previous miscarriage between the two groups. The gestational age (by date and ultrasound) at presentation ranged between 25 to 34 weeks with an average of 31 weeks. No difference was noted between the two groups (Table 1).

Of note, the hemoglobin concentration and the total leucocytic count (TLC) were significantly different between the two groups (Table 1). Also all women included in the study had pus cells in urine that ranged between ten and 100 /HPF with a mean of 19.60 ± 21.51. No difference was noted between the groups.

Table 2 shows the descriptive statistics of uterine wall thickness of all patients included in the study. The maximum thickness noted in the upper uterine segment was 14.5 mm while it was 13 mm in the lower segment. In some cases the lower uterine segment was thicker than the upper giving rise to an upper/lower segment ratio of as low as 0.39. Seventy-two percent of patients (N = 36) had an upper/lower segment ratio of < 1.5 while 28% (N = 14) of patients had a ratio of ≥ 1.5.

In responders to tocolysis, the ultrasonographic measurement of the upper uterine segments wall thicknesses ranged between 3.5 and 14.5 mm with a mean of 6.27 ± 2 mm which was significantly thinner compared to the upper uterine wall thickness in non-responders, which ranged between 7.2 and 11.1 mm with a mean of 9.61 ±1.4 mm (Table 2). However, there was no significant difference between the lower uterine segment wall thicknesses between the study groups. In responders, the thickness ranged between two and 19 mm with a mean of 6.68 ± 3.39 mm, while in non-responders the thickness ranged between three and seven mm with a mean of 4.87 ±1.43 mm (Table 2).

In responders, the upper/lower uterine segments ratio ranged between 0.39 and 1.52 with a mean of 1.01 ± 0.23,
Table 3. — Criterion values and coordinates of the ROC curve.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+LR</th>
<th>-LR</th>
<th>+PV</th>
<th>-PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1.26</td>
<td>94.74</td>
<td>100.00</td>
<td>0.053</td>
<td>100.0</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>≤1.5</td>
<td>97.37</td>
<td>83.33</td>
<td>5.84</td>
<td>0.032</td>
<td>94.9</td>
<td>90.9</td>
</tr>
<tr>
<td>≤1.52</td>
<td>100.00</td>
<td>83.33</td>
<td>6.00</td>
<td>0.00</td>
<td>95.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

+LR: positive likelihood ratio; -LR: negative likelihood ratio; +PV: positive predictive value; -PV: negative predictive value.

Table 4. — Descriptive statistics for neonatal outcome.

<table>
<thead>
<tr>
<th></th>
<th>Responders (N = 38)</th>
<th>Non-responders (N = 12)</th>
<th>Student t test (t)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>37.13 ± 1.19</td>
<td>30.67 ± 4.44</td>
<td>9.5</td>
<td>&lt;0.000*</td>
</tr>
<tr>
<td>Neonatal weight (kg)</td>
<td>3.12 ± 0.38</td>
<td>1.56 ± 0.46</td>
<td>15.4</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>One minute Apgar score</td>
<td>9.03 ± 0.94</td>
<td>6.42 ± 1.08</td>
<td>8.1</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

*Statistically significant

while in non-responders this ratio ranged between 1.5 and 2.6 with a mean of 2.06 ± 0.4 and this difference was statistically significant (Table 2).

Figure 3 shows the ROC curve sensitivity and specificity of the ultrasonographic measurement of the ratio between the upper and the lower uterine segments wall thickness.

Table 4 shows the descriptive statistics of neonatal outcome of the study with a mean gestational age at delivery of 37.13 months in responder that was statistically significant compared to that of non-responders (30.67 weeks). Six neonates (15.8%) in the responders group required neonatal ICU admission compared to all neonates (100%) in the non-responder group.

Neonatal weight ranged between 2,200 and 4,200 grams with a mean of 3.12 ± 0.382 responders and ranged between 900 and 2,250 grams with a mean of 1.56 ± 0.46 in non-responders, with a significant p-value. One minute Apgar score ranged between 7 and 10 with a mean of 9.03 ± 0.94 in responders and ranged between 5 and 8 with a mean of 6.42 ± 1.08 in non-responders with significant p-value (Table 4).

Discussion

Aside from the digital vaginal examination and cervical assessment by ultrasonography, obstetricians have limited ability to predict the progress of a pregnant woman who is having contractions prematurely. Predicting the progress of such women is of utmost importance as obstetric and neonatal interventions and procedures would differ if the possibility of labour continuing is very high.

There are some predictive biochemical markers that can be used to predict the progress the preterm labour in symptomatic pregnant women such as fetal fibronectin, phosphorylated insulin-like growth factor binding protein, and estriol. Other few potential future biomarkers are still under trial-like cytokines, matrix metalloproteinases, relaxin, stress-related biomarkers, endocannabinoids, and pregnancy associated plasma protein A.

As measuring of the myometrial thickness by transabdominal ultrasound is a non-invasive method, the present authors proposed measuring the upper and the lower uterine segments in patients presenting with preterm labour pains and use the absolute value and/or the ratio between the upper and lower uterine segments as predictors of the progress of labour in women who present with preterm labour pains.

To the best of the authors’ knowledge, there is paucity of previous studies in the literature studying the uterine wall thickness during preterm labour. Only few studies used ultrasonography to measure uterine wall thickness, and the investigators concentrated on the lower uterine segment before and during labour in a full term pregnancy. It has been used in different studies to correlate the lower uterine segment thickness to the success of trial of vaginal delivery after cesarean section and the risk of uterine rupture in these cases [7, 8].

Tanik et al. compared the ultrasonographic measurement of the lower uterine segment wall thickness with intrapartum findings in 50 pregnant women with previous history of caesarean sections, and they found sensitivity 100%, specificity 82%, positive predictive value 87%, and negative predictive value 100% confirming the reliability and safety of ultrasound in evaluating the uterine wall thickness [7]. Also, Rosenberg et al. found that the risk of uterine rupture or dehiscence is directly related to the degree of lower uterine segment thinning measured at or around 37 weeks of gestation, and in particular this risk increases significantly when the thickness is 3.5 mm or less. [8]

In 2005, Buhimschi et al., showed that the sonographic evaluation of myometrial thickness may represent an alternative clinical tool for the prediction of a short latency interval in women with prelabour premature rupture of membranes (PPROM) in 45 pregnant women with singleton pregnancy. They concluded that significant thickening of the anterior and fundal walls of the uterus follows PPROM and that a thick myometrium in non-labouring patients with PPROM is associated with longer latency interval [9].

In the present study, the authors have excluded any factor that may affect the uterine wall thickness such as history of previous cesarean section or PPROM. Also, anterior wall and/or low lying placenta were excluded as Degani and Leibvitz found that the myometrial thickness was significantly increased behind the placental insertion site, as compared to other portions of the uterine wall, and attributed this to increased vascular tissue elements in the myometrium at placentaion area [10].

The present authors have chosen the point midway between the cervix and the fundus to represent the thickness of the upper uterine wall while the lower uterine wall thickness was measured two cm above the internal os with a full bladder to clearly identify the myometrium-bladder interface.
Both measurements were taken while the ultrasound probe was oriented in the midline sagittal plane. The authors have found that these points are easily reproducible.

Durnwald and Mercer, in their study of the myometrial thickness in pregnancy, used the same points as representing both uterine wall thicknesses, and they also found no significant difference in myometrial thickness between the second and third trimesters. At both trimesters, the myometrial thicknesses were less compared to the first trimester [11].

Determining the ratio between the wall thickness of the upper and lower uterine segments and using this ratio in predicting the progress of PTDs is novel. Durnwald and Mercer found that myometrial thickness of the different parts of the uterus was greater in multipara compared to primiparous women. Also, women with a high body mass index (BMI) are more likely to have an anterior wall measurement greater than those with women with a BMI < 30 kg/m² [11]. For this reason, an upper/lower uterine segment ratio would be more accurate in representing the changes of the uterine walls compared to the absolute values as these can be influenced by parity and BMI.

The study population was divided into two groups based on their responses to tocolytic therapy: group 1 (responders) and group 2 (non-responders). It was found that the response to tocolysis correlated inversely with changes in the ratio between the ultrasonographic measurement of the upper and the lower uterine segments wall thickness. Using a cutoff ratio, the authors found that when the ratio was ≤ 1.26, the sensitivity was 94.74, and the specificity was 100.00, and when the ratio was ≤ 1.52, the sensitivity was 100.00 and the specificity was 83.33. This means that the higher the ratio, the more likely that the woman will continue to labour and the less likely that the uterine contractions would respond to tocolytics. Changes of the ratio can be explained by the fact that during the course of labour – term or preterm - the lower uterine segments wall thickness may add to the clinical examination findings in women at risk of preterm delivery. Am. J. Obstet. Gynecol., 1990, 162, 748.


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References
Clinical and radiographic characteristics in pulmonary endometriosis: based on five cases

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2Department of Respiratory Medicine, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou
3Department of Reproductive Endocrinology, Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou (China)

Summary

Objective: This study aims to improve the diagnostic and therapeutic efficacy through analysis of clinical and radiographic characteristics in pulmonary endometriosis. Materials and Methods: This retrospective study was conducted from January 1998 to December 2008. The clinical and radiographic characteristics of five patients diagnosed as pulmonary endometriosis were evaluated. Results: Among the five female patients of reproductive age, one case presented with recurrent pneumothorax, four cases presented with recurrent hemoptysis. Episode of pneumothorax and hemoptysis had the close association with the menstrual cycle. Except for pneumothorax case, the computed tomography (CT) scans during menstruation showed patchy opacification or infiltration (n=4). Histopathologic examination of the resected specimen conformed typical endometrial tissue in the lungs. Misdiagnosis occurred involving spontaneous pneumothorax (n=1), pulmonary tuberculosis (TB) (n=3), and bronchiectasis (n=1). Conclusion: Pulmonary endometriosis is prone to misdiagnosis. The combination of medical history and CT scans in association with menstrual cycle was useful to make the differential diagnosis after effective diagnostic treatment of hormone therapy.

Key words: Computed tomography; Lung; Endometriosis; Hormone therapy.

Introduction

Endometriosis is classically defined as an extratubal growth of endometrial tissue (glands and stroma) and it affects about 10% to 15% of women in reproductive age [1]. Even though endometriosis is usually located in the pelvis, it can occur in non-gynecologic sites involving thoracic, peritoneum, brain, etc. The different manifestations of non-gynecologic endometriosis vary completely depending on the location of the lesion [2]. Pulmonary endometriosis is diagnosed when endometrial tissue can be found within the respiratory system. It is rare but well-documented [3]. The clinical presentation of pulmonary endometriosis is hemoptysis or pneumothorax/hemothorax and the most common visiting branch of the hospital is emergency room or General Physician without specificity in radiographic findings. This retrospective study was conducted to evaluate the clinical and radiographic characteristics in pulmonary endometriosis.

Materials and Methods

Ethics

This retrospective study was conducted in Department of Respiratory Medicine, the 2nd Affiliated Hospital, Zhejiang Chinese Medical University, Hangzhou, China from January 1998 to December 2008. This study was implemented in accordance with the declaration of Helsinki and received approval from the Ethics Committee of Zhejiang Chinese Medical University. Written informed consent was obtained from all participants.

Materials

The mean age of five patients diagnosed as pulmonary endometriosis was 33 years (age range: 25-43) and the duration of the medical history was three months to six years. The final diagnosis was based on medical history alone or diagnostic hormone therapy or thoracoscopy.

Clinical evaluation

The clinical and radiographic characteristics of five patients were evaluated in details containing inpatient assessment and chest computed tomography (CT) scans, Laboratory tests including erythrocyte sedimentation rate (ESR), tumor markers, tuberculosis-SPOT (TB-SPOT), fiberoptic bronchoscopy, bronchoalveolar lavage, and sputum cytology were also investigated. The CT scans was performed during and two weeks after menstruation.

The reason of performed surgery using thoracoscopy was recurrently severe hemoptysis in one patient (the amount of hemoptysis was 100 ml per day).

Results

Clinical characteristics

Among the five female patients of reproductive age, one case presented with recurrent pneumothorax. Other four cases presented with recurrent hemoptysis, occurring usually during menstruation, sometimes before and after the menstruation. The amount of hemoptysis ranged from ten to 100 ml per day. One of the four cases had the pulmonary endometriosis combined with pelvic endometriosis, the other three cases presented with single lesion in lungs.
Underlying condition

Other than a history of drug-induced abortion (n=4) or menstrual colic (n=2), none of them had remarkable underlying diseases that could cause hemoptysis or pneumothorax (Table 1).

Clinical manifestation

All of the five patients had no history of fever, productive cough or chest pain. The physical examination and laboratory investigations were normal. Radiology evaluation: radiographic findings showed that the compression volume of left lung was 55% in the pneumothorax patient. The CT scans of the remaining four cases indicated that patchy opacification or infiltration (n=4) with uneven density and well-defined margin (Figures 1, 2). The location of the lesion was confined to the segments of the lungs, including the right upper lobe in one case, the right middle lobe in one case, the right lower lobe in one case, and the left lingular lobe in one case.

Pathology findings

Histopathologic examination of the resected specimen confirmed typical endometrial tissue in the lungs (n=1). The presence of both the stroma with hemosiderin-laden macrophages and endometrial-type epithelium met the diagnosis criteria of endometriosis. In addition, both the stroma and glands had the positive immunoreaction for estrogen and progesterone receptors.

Misdiagnosis

Misdiagnosis occurred involving spontaneous pneumothorax (n=1), pulmonary TB (n=3), and bronchiectasis (n=1). The recurrent pneumothorax case was admitted to hospital with misdiagnosis and endured thoracentesis or chest tube thoracoscopy.

Table 1. — Clinical characteristics and CT findings of pulmonary endometriosis during menstruation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical presentation</td>
<td>Chest pain</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Past medical history</td>
<td>Abortion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Menstrual colic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CT findings</td>
<td>Pneumothorax</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Patchy opacification</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Infiltration</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. — Misdiagnosis and treatment of pulmonary endometriosis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misdiagnosis</td>
<td>Spontaneous pneumothorax</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Bronchiectasis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Treatment</td>
<td>Thoracentesis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hormone therapy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thoracoscopy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
include ill- or well-defined opacities, nodular lesions, thin-walled cavities, cystic changes, and bullous formation [7]. All of these lesions may vary in size during the menstrual cycle and may disappear after the menses [8]. If similar parenchyma changes during twice menstrual cycle present in same pulmonary segment, and no abnormality or hemoptyysis cease during the inter-menstrual period, the pulmonary endometriosis should be suspicious.

The choice of treatment in pulmonary endometriosis should be individualized to application. Hormone therapy adjusting menstrual period could treat women of relatively elder or dispense with reproduction. Pulmonary endometriosis could be relieved naturally if women of reproductive age become pregnant. Lesions could be resected by surgery to cure recurrent life-threatening hemoptyysis. As a benign lesion, pulmonary endometriosis is prone to be misdiagnosed as acute and chronic pneumonia, active TB/ tuberculoma, fungal infection or tumor, sarcoidosis, abscess, etc [9].

The combination of medical history and CT scans in association with menstrual cycle was useful to make the differential diagnosis after effective diagnostic treatment of hormone therapy. In the women of reproductive age, hemoptyosis or pneumothorax without obvious inducing factors should be considered with a diagnosis of pulmonary endometriosis.

References


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Recurrence rate of ovarian endometriosis in patients treated with laparoscopic surgery and postoperative suppressive therapy

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University of Belgrade, Medical faculty of Belgrade Clinic of Gynecology and Obstetric “Narodni Front”, Belgrade (Serbia)

Summary

Introduction: The testing represented a prospective study that was performed at the Gynaecology and Obstetrics Clinic “Narodni Front” in Belgrade during a two-year period. The study encompassed female patients with ovarian endometrioma operated with laparoscopic surgery. The research objective was to determine the percentage of occurrence of relapses in patients operated for endometriosis of the ovary in relation to the stage of the disease and the type of performed operation, and which were receiving suppressive therapy with gonadotropin-releasing hormone (GnRH) analogues after the surgery compared to those who were not receiving suppressive therapy after the operation. Materials and Methods: The recurrence of endometriosis on the ovary of the test and control groups was monitored during the first year after surgery. In all patients ultrasound checks were done every month during the first six months after surgery, and then every three months for the next six months. In all patients in whom the recurrence, i.e. endometrioma on the ovary larger than three cm was revealed postoperatively by ultrasound, the laparoscopic removal of the endometrioma was performed again as well as the histopathological examination of the material. Results and Conclusion: There was no statistically significant difference in the distribution of recurrence of endometriosis between the groups formed according to the type of surgical technique (cystectomy or cystotomy). The recurrence of endometriosis occurred later in the group of patients in which the treatment GnRH analogues was applied after the surgical treatment. The recurrence of endometriosis in more severe stages (Stage III and IV) occurs later in the group of patients in which the treatment GnRH analogues is applied after the surgical treatment. Key words: GnRH analogues; Ovarian endometrioma; Cystectomy; Cystotomy; Laparoscopy.

Introduction

Endometriosis is one of the common diseases in the reproductive age of women. It affects 5-10% of the total female population and is revealed in 40% of infertile women and 60% of women with chronic pelvic pain [1, 2]. The treatment of endometriosis can be surgical and with medication. In the last ten years gonadotropin-releasing hormone (GnRH) analogues have been used in therapy [3, 4]. The use of GnRH analogues substantially reduces the symptoms of endometriosis [5]. Despite great progress in surgical techniques and due to the nature of the disease, endometriosis often relapses. The research objective was to determine the percentage of occurrence of relapses in patients operated on for endometriosis of the ovary in relation to the stage of the disease and the type of performed operation, and which were receiving suppressive therapy with GnRH analogues after the surgery, compared to those who were not receiving suppressive therapy after the operation.

Materials and Methods

The testing represented a prospective study that was performed at the Gynaecology and Obstetrics Clinic “Narodni Front” in Belgrade during a two-year period. The study encompassed 200 female patients with ovarian endometrioma operated on with laparoscopic surgery. The patients were from 20-45 years of age. The indications for the surgery were set based on the anamnesis, the pelvic ultrasound examination, and the serum concentrations of cancer antigen 125 (CA 125). The operated patients were divided into two groups, the test and control groups. The test group consists of 100 patients operated with laparoscopy due to endometrial cysts of ovary and in which suppressive therapy with GnRH analogues was applied after surgery. Out of the GnRH analogues, triptorelin was administered in the form of acetate, i.e. dipherelin 3.75 mg intramuscularly once a month, or goserelin in the form of acetate, i.e. zoladex 3.6 mg subcutaneously into the anterior abdominal wall, once per month. GnRH analogues were administered over the course of four to six months depending on the severity of the endometriosis. Depending on the type of surgery, all tested patients were divided into two subgroups. The first subgroup consists of patients who had undergone laparoscopic cystectomy, while the other subgroup consists of patients who had undergone laparoscopic cystotomy and coagulation of the cyst capsule. The decision regarding which type of surgery was performed in patients was made by the surgeon, based on the surgical report. The degree of severity of endometriosis was assessed according to the classification of the American Society for Fertility and Sterility (AFS classification). In relation to the degree of progress of endometriosis, all patients in the test group were divided into three subgroups as follows: patients with II, III and IV stage of endometriosis.
Results

The study encompassed 200 patients. Laparoscopic cystectomy was performed in 112 (56%) patients and laparoscopic cystotomy in 88 (44%) patients. Out of the total of 200 patients, 100 of them received GnRH analogues postoperatively and the other 100 patients received no suppressive therapy with GnRH analogues postoperatively. Out of the total of 200 patients, GnRH analogues were administered in 44 (22%) patients after a laparoscopic cystectomy and in 56 (28%) patients after a laparoscopic cystotomy. Sixty-eight (34%) patients after laparoscopic cystectomy and 32 (16%) patients after laparoscopic cystotomy did not receive GnRH analogues.

Table 1 shows the distribution of patients by stage of endometriosis according to the type of operation and application of suppressive therapy. The differences were not statistically significant in terms of the type of operation and stage of endometriosis between these two groups of patients (p < 0.05).

The stage of endometriosis shows a statistically significant correlation with the type of applied laparoscopic operation. In stage II of endometriosis, significantly more frequently laparoscopic cystectomy was applied and in the III and IV stage laparoscopic cystotomy (p < 0.01).

Table 2 shows the distribution of patients according to the recurrence of endometriosis in relation to the type of operation and application of suppressive therapy. The obtained data were statistically analyzed by using descriptive and analytical statistical methods. As to descriptive statistical methods, measures of central tendency and measures of variability were used, and from the analytical methods the chi-square test, Kruskal-Wallis, and the t-test.

Table 3 shows the distribution of patients according to the recurrence of endometriosis usually occurred four
months after the first therapy session. The statistical analysis showed no significant difference in the distribution of recurrence of endometriosis among groups of patients in stage III and IV of endometriosis (p > 0.05). In the entire group of patients with more severe stages of endometriosis (stages III and IV), the distribution of the period in which endometriosis occurred, does not show a statistically significant difference (p > 0.05).

### Discussion

The study encompassed 200 patients that were operated for endometriosis of the ovary with a laparoscopic technique. Forty-four (22%) patients were treated with GnRH analogues after laparoscopic cystectomy, whereas 68 (34%) patients were not given GnRH analogues after it; 56 (28%) patients received GnRH analogues after laparoscopic cystotomy, whereas 32 (16%) patients did not receive GnRH analogues after it. In 66.7% of the patients that were receiving GnRH analogues after a laparoscopic cystectomy, recurrence was diagnosed seven to 12 months after the first therapeutic treatment, while the percentage amounted to 60% in patients in which treatment with GnRH analogues was applied after laparoscopic cystotomy. In the group of patients in which therapy with GnRH analogues was not applied after a laparoscopic cystectomy, 48.5% had a recurrence of endometriosis four to six months after the first therapeutic treatment. The study in Korea that examined the effect of GnRH analogues after laparoscopic surgery of ovarian endometriosis showed that this therapy has a positive effect on the prevention of recurrence after six months of treatment [6, 7].

Patients that did not receive the suppressive therapy with GnRH analogues after a laparoscopic cystotomy had a recurrence of endometriosis in the period of one to three months after the first therapy in 45.5% of the cases. The postoperative use of GnRH analogues after a laparoscopic cystectomy of the endometrial cyst in the ovary reduces the relapse rate and this rate for 18 months after surgery was 6% [8] and 9.6% [9], while in patients who did not use this therapy, it amounted to 16.6% [8]. Other authors suggest that the excision of ovarian endometrioma may reduce the

<table>
<thead>
<tr>
<th>Stage of endometriosis</th>
<th>Recurrence (months)</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>1-3</td>
<td>4-6</td>
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<tr>
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</tr>
<tr>
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</table>
after the first therapeutic treatment. In the group of patients that were not receiving GnRH analogues after the surgical procedure, in 39% there was a recurrence of endometriosis one to three months after the first therapeutic treatment, and in 46% four to six months after the first therapeutic treatment. In the group of patients where no therapy with GnRH analogues was applied after the laparoscopic cystectomy, 38.2% of patients had a recurrence of endometriosis one to three months after the initial therapeutic treatment. In 40.6% of the patients who did not receive suppressive therapy after the laparoscopic cystotomy, had a recurrence of endometriosis one to three months after the first therapeutic treatment. Through the statistical analysis of the data no significant differences in the distribution of recurrence of endometriosis between the groups formed according to the type of surgical technique (cystectomy or cystotomy) were found.

There was a statistically significant difference in the recurrence of endometriosis between groups of patients based on the applied therapy. The application of GnRH analogues shows a statistically significant correlation with the recurrence of endometriosis. This suggests that the recurrence of endometriosis emerged later in the group of patients in which the treatment with GnRH analogues was applied after surgical treatment. The postoperative use of GnRH analogues after the laparoscopic cystectomy of the endometrial ovarian cyst reduces the relapse rate and that rate for 18 months after surgery was 6% (30) or 9.6% (31), and in patients who did not use this therapy it was 16.6% (30). Other authors suggest that the rate of recurrence of ovarian endometrioma 12 months after laparoscopic surgery is 16.5% and that the postoperative use of GnRH analogues significantly reduces the rate of recurrence of the disease. They state that if GnRH analogues are used during the first three months postoperatively, the rate of recurrence 12 months after the surgery is 17.9%; 24 months after the surgery is 12.5% and 36 months after the surgery is 25%. If GnRH analogues are administered during the first six months after the surgery the recurrence rate is significantly lower; 12 months after surgery is 4.3%, 24 months after surgery is 5.3%, and 36 months after surgery is 5.3%. [15]. A study in Italy followed the recurrence in patients in which laparoscopic treatment of endometriosis was performed. One group was treated with GnRH analogues and the other group received a placebo therapy. Comparing the results, they have come to the conclusion that there was no significant effect of suppressive therapy and that recurrence depended on the type of surgical techniques [16].

When we look at all patients with stage III and IV of endometriosis, regardless of the applied therapeutic modalities, the highest percentage of relapse occurred four months after the application of the first therapeutic treatment. The study in Korea analyzed the effect of GnRH treatment on recurrence in patients with stage III and IV of endometriosis after laparoscopic surgery. The results showed a lower incidence of relapse in patients who were treated with GnRH analogues for six months as compared to those who were treated for only three months. The difference was not statistically significant. The therapy with GnRH analogues in the duration of six months after the laparoscopic surgery had a favourable impact on the recurrence of endometriosis [17].

Conclusion

There was no statistically significant difference in the distribution of recurrence of endometriosis between the groups formed according to the type of surgical technique (cystectomy or cystotomy). The application of the therapy with GnRH analogues shows a statistically significant correlation with the recurrence of endometriosis. This suggests that the recurrence of endometriosis occurred later in the group of patients in which the treatment GnRH analogues was applied after the surgical treatment. The application of therapy with GnRH analogues in the group of patients with more severe stages of endometriosis shows a significant correlation with the recurrence of endometriosis. This suggests that the recurrence of endometriosis in more severe stages (stages III and IV) occurs later in the group of patients in which the treatment GnRH analogues is applied after the surgical treatment.

References

Recurrence rate of ovarian endometriosis in patients treated with laparoscopic surgery and postoperative suppressive therapy


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Non-invasive prenatal diagnosis of fetal RhD by using free fetal DNA

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1Department of Obstetrics and Gynecology, Beykoz State Hospital, Istanbul
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Summary

Objective: Anti-D immunoglobulin is applied to all pregnant women having RhD incompatibility to prevent hemolytic disease of the newborn. The aim of this study is to determine fetal RhD status in the Rh incompatible pregnancies with an non-invasive technique; free fetal DNA isolation from maternal circulation. In the case of Rh incompatibility especially with a history of previous fetal anemia, it can be beneficial to know Rh status antenatally in terms of monitoring fetuses with Rh positive (RhD(+)) status consciously.

Materials and Methods: Total free DNA was isolated in 50 Rh negative (RhD(−)) pregnant women, who had RhD alloimmunisation with their husbands. The gene in isolated DNA was investigated with TagMan prob and real time PCR by using primers belonging to exon 7 of the RhD gene.

Results: The authors analyzed 50 RhD(−) women by using quantitative real time PCR technique. Five of them were RhD(−) and the rest of them were found to be RhD(+). After birth one of the infants who were analyzed as RhD(+) were found to be RhD(−).

Conclusion: The detection of fetal RhD status by using a non-invasive method from maternal circulation was found to be possible. Assessing fetal RhD status non-invasively by using free fetal DNA in maternal blood will be cost-efficient, avoiding unnecessary indirect Coombs test and unnecessary Rhogam applications that is used in RH incompatible pregnancies. This study will throw a fresh light on prenatal diagnosis.

Key words: Prenatal diagnosis; Fetal DNA; RhD gene.

Introduction

Hemolytic disease of the newborn which causes fetal anemia, neonatal icterus, and even death is caused by rhessus (Rh) group incompatibility between maternal and paternal blood groups [1]. Anti-D alloimmunisation has been reduced with the implementation of prophylactic anti D treatment in all pregnancies with Rh incompatibility [2] It is known that in a predominantly white population, however about 38% of these women would be carrying an RhD negative [RhD(−)] fetus and would receive treatment unnecessarily [3].

In 1997 a new era in prenatal diagnosis was introduced to science by Lo et al., non-invasive fetal RhD genotyping from maternal plasma was considered a valuable tool in the identification of pregnancies at risk of hemolytic disease of the fetus and newborn [4].

The Rh blood system is a very polymorphic system, RhD and RhCE are located in the region of p36.12-p34.3 on chromosome 1, and they are 97% homologous to each other. Each of these genes consist of ten exons and they contain 69 kb of DNA. The regions of exon 7 and exon 10 within the RhD gene are the areas of focus [5]. The present study is based on detection of exon 7 region to determine the presence of RhD gene.

Materials and Methods

Peripheral five ml blood sample was taken from 51 Rh(−) pregnant women at 5-40 weeks of gestation, having Rh incompatibility with their husbands who admitted for pregnancy follow up at the Istanbul Bilim University, Medical Faculty Obstetrics Department. Blood was centrifuged 15' at 4,100 g, and upper part, plasma was stored at -80°C until the DNA isolation day. The total free DNA isolation was performed according to free DNA isolation procedure of High Pure PCR template preparation kit. The samples that were saved at -80°C were first centrifuged at 13,000 rpm for 10' and upper fluids were taken, 200 µl “binding buffer” and 40 µl “proteinase K,” were placed on each sample, and they were placed at 70°C in a water bath for 10’. Isopropanol in the amount of 100 µl was also added to them and the mixtures were taken to strainer eppendorf and centrifuged until all of the liquid passed to the bottom; 500 µl “inhibitor buffer” was added to eppendorfs and centrifuged. Two centrifuged were then also formed by adding 500 µl “wash buffer” each time. Next 50 µl “elution buffer” was added to strainer eppendorfs and centrifuged, the sub-tube which had free DNA after centrifuge was taken to -20°C until the day of Real time PCR. Real time PCR was then performed under conditions as shown in Tables 1 and 2. Specific primers (5′-CTC CAT CAT GGG CTA CAA-3′, 5′-CCG GCT CCG ACG GTA TC-3′) for region exon 7 which belongs to RhD and TaqMan prob (5′-FAM AGC AGC ACA ATG TAG ATG ATC TCT CCA TAMRA-3′); FAM [6 carboxyfluorescein] and TAMRA [6 carboxytetramethyllrhodamine] were the fluorescent reporter dyes and quencher dye was used. The tubes which contained RhD genes were used for...
positive control, and the tubes that were known not to contain RhD gene were used for negative control. The tubes containing the NTC (PCR mixture without DNA) reaction mixture was used for the determination of contamination.

Results

One patient was excluded because of first trimester abortion. The total DNA of 50 women who were known to have Rh incompatibility was analyzed with positive control, negative control by TagMan prob method. Five samples were found to be RhD(−) and the rest were Rh positive [RhD(+)]. After birth, RhD status of the fetus were corrected; all of the patients who were found to be RhD(−), were RhD(−) and 44 patients who were found to be RhD(+) were RhD(+). Only one patient who was found to be RhD(+) was RhD(−) in fact. Sensitivity was 100%, specificity was 83.3%, positive predictive value was 97.7%, and negative predictive value was 11.3%.

Discussion

Hemolytic disease of the fetus and newborn (HDFN) has been a prevalent pathology of pregnancy and a major obstetric problem, with an important impact on fetal and neonatal morbidity and mortality. The introduction of postpartum and antenatal anti-D immunoglobulin prophylaxis for phenotypic RhD(−) pregnant women, has dramatically improved the risk of the affected fetuses and the incidence of D sensitisation has decreased [6] The current strategy for monitoring RhD(−) pregnant women at high risk for HDFN relies on serial assessment of maternal antibody levels, paternal screening, administration of anti-D prophylaxis for RhD(−) pregnant women, and when necessary, fetal monitoring using ultrasound and Doppler and intrauterine fetal blood sampling [7]. It is known that a kind of human blood product, anti-D immunoglobulin carries risk for blood-borne infections and the supplies are limited worldwide. Availability of non-invasive diagnostic assay of fetal RhD status makes it possible to restrict use of antenatal prophylactic anti-D immunoglobulin. Identification of fetal Rh status will prevent approximately 40% of women having RhD(−) fetus from being vaccinated [8]. The population which were included in the present study had 12% of RhD(−) infants. The difference in ratios of RhD(−) infants between the present study population and general population may be attributed to narrow number of individuals.

Fetal RhD genotype can be determined with a high level of accuracy by analysis of fetal DNA circulating in maternal plasma and serum [9]. If the fetus is known to be RhD(+), especially with a history of anemic or hydropic fetus, close monitoring may be rendered. Further investigations may result as a change in algorithms in management of pregnancies with RhD incompatibility like Rhogam application doses and timing of vaccine.

Several studies examined the prenatal accuracy of fetal RhD genotyping from analysis of circulating cell-free fetal DNA (ccff DNA) in maternal plasma which ranged from 32% to 100% [10]. Non-invasive determination of fetal RhD genotype usually relies on DNA amplification by PCR, and detection of chromosome 1 specific sequences in maternal plasma [4]. The present authors used exon 7 on chromosome 1 to determine Rh status of the infant.

One previous study with a population of 2,000 patients revealed 0.8% rate for the infants being RhD(−) but found to be RhD(+) [11]. The present study had one patient (2%) who was actually RhD(−) but found to be RhD(+). Incidence seems to be high in the present study, and the authors attribute this result to small amount of their study population.

Müller et al. highlighted the importance of transport time on sample quality. At the room temperature, after six days even the concentration of fetal DNA remains same, total DNA amount increases. Thus, maternal DNA contamination is higher than average [2]. Rouillac – Le Scellour et al. do not recommend to study with samples older than 48 hours [12]. The present authors did centrifugation and stabilization in hours and they do not relate their false positive result with transport time.

In the Caucasian population deletion of homozygous RhD gene is the main cause of negative phenotype in contrast with black Africans who do not have homozygous deletion but carry one or two variant genes, the RhD pseudogene or RHD-CE-Ds hybrid gene. [13,14] Rouillac-Le et al. found 31 patients to have pseudogene or hybrid gene in 893 patients, Chinen et al. found two in 102 patients, Gunel et al. found two in 40 patients. [12, 15, 16] Multiplex PCR for more than one region should be performed to detect this hybrid or pseudogenes [16]. The present authors performed only exon 7 in this study, not exon 10 therefore one patient with a false positive result may have pseudogene.
It has been previously shown that fetal DNA passage in maternal circulation increases in pathological pregnancies associated with placental abnormalities such as preeclampsia, preterm labor, and pregnancies with karyotypically abnormal fetuses [4]. None of the patients in the present study had abnormality during pregnancy follow up. Only one patient had early first trimester abortion and she was excluded.

Conclusion

Fetal RhD status could be determined non-invasively from maternal circulation. This will obstruct anti immunoglobulin use in pregnant women carrying RhD(−) fetus. Determining RhD(+) of the fetus gives opportunity for timely close monitoring due to hydrops. Fetal DNA isolation from maternal blood will soon provide diagnosis of aneuploidies and single gene disorders non-invasively.

References


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Novasure impedance control system versus microwave endometrial ablation for the treatment of dysfunctional uterine bleeding: a double-blind, randomized controlled trial

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Summary
Purpose of investigation: To compare the efficacy and safety of two different second-generation ablation devices, Novasure impedance control system and microwave endometrial ablation (MEA), in cases of abnormal uterine bleeding (AUB). Materials and Methods: This is a randomized controlled trial that took place in a single Gynecological Department of a University Hospital. Sixty-six women with dysfunctional uterine bleeding (DUB), unresponsive to medical treatment, were included in the trial. The ratio of women allocated to bipolar radio-frequency ablation or MEA was 1:1. Follow-up assessments were carried out at three and 12 months post-ablation. The present main outcome measure was amenorrhea rates 12-months post-treatment. Results: The rate of amenorrhea at 12-months post-ablation was significantly higher in women treated by Novasure (25/33; 75.8%) as compared to those treated by MEA (8/33; 24.2%) (rate difference: +51.5%, 95% CI: +27.8 to +67.7). Conclusion: In women with DUB, endometrial ablation with Novasure bipolar radiofrequency impedance-controlled system is associated with increased rates of amenorrhea at 12-months post-treatment as compared to the MEA method.

Key words: Abnormal uterine bleeding; Novasure; Microwave endometrial ablation.

Introduction
Abnormal uterine bleeding (AUB) is one of the most common problems in women of reproductive age, with a prevalence of more than 5% [1]. It is estimated that menorrhagia affects 10-30% of menstruating women at any time [2] and about 20% of referrals of women to their gynecologists are due to menstrual disorders [3].

AUB may be due to anatomical, endocrine, hematological and iatrogenic factors, although several cases of AUB occur without any obvious pathology. The latter was usually referred under the term of dysfunctional uterine bleeding (DUB), which accounts for about half the cases of excessive menstrual blood loss [4]. Since 2011 with the FIGO classification system (PALM-COEIN terminology) [5] in cases of AUB the term DUB was discouraged, although it is still commonly used throughout the gynecological community. Nowadays nonstructural causes of AUB, as well as non-identified disorders of hemostasis (AUB-C), ovulatory disorders (AUB-O), endometrial disorders (AUB-E), and non-classified causes (AUB-N) compose the large group of what we used to categorize as DUB.

Several treatment options have been proposed in cases of AUB. Medical treatment is considered as a first-line treatment and includes tranexamic acid, non-steroidal anti-inflammatory drugs, combined oral contraception pill, progestogen, danazol, gonadotropin releasing hormone analogues (GnRH-a), and levonorgestrel releasing intra-uterine system. In cases of AUB resistant to medical treatment, physicians should offer women an alternative surgical treatment, choosing between hysteroscopic and non-hysteroscopic endometrial ablation techniques and hysterectomy [6].

The effectiveness of the reported treatment options for AUB has been evaluated and reviewed in many publications. Surprisingly there is lack of randomized controlled trials (RCTs) comparing most of the ablation devices. Due to this fact, recently, a systematic review had to employ methods of network meta-analysis in order to provide indirect evidence regarding the comparative efficacy of many second-generation ablation devices [7].

The aim of this randomized controlled trial is to compare the efficacy and safety of two different second-generation ablation devices, Novasure impedance control system and microwave endometrial ablation (MEA), in cases of DUB.
Materials and Methods

A randomized controlled trial was performed in the 1st Department of Obstetrics and Gynecology, “Papageorgiou” University Hospital of Thessaloniki, Greece from January 2008 until December 2010. Both ethics committees of the hospital and the Aristotle University of Thessaloniki approved the study, while at the same time the protocol of this RCT was registered in ClinicalTrials.gov (ID: NCT01173965).

Population

Women with DUB, as indicated on the pictorial blood assessment chart (PBAC) described by Higham et al. [8] with a score of more than 150 points, were eligible for the trial. All women included in the trial suffered from AUB for more than a year, unresponsive to medical therapy, and had already completed their family planning. All patients were younger than 50 years old, had to have a normal cervical cytology test, a negative pregnancy test, and a follicular stimulating hormone (FSH) level of less than 20 mIU/ml. Women with coagulopathies or thyroid gland dysfunction were excluded from the trial. In order to exclude uterine pathology, all women were subjected to transvaginal ultrasound scan and endometrial biopsy (either by dilatation and curettage or hysteroscopy).

Interventions and outcomes

After signed informed consent was obtained, every patient was scheduled for surgical intervention. Demographic data of each patient were registered, as well as data regarding the history and the clinical condition of their AUB. The presence of dysmenorrhea was also recorded and its intensity was determined by means of a visual analogue scale (VAS). Pre-treatment of the endometrium with three monthly doses of 3.75 mg leuproide acetate was performed in all patients before randomization, which was undertaken with the use of a computer-generated table of random numbers. Although usage of GnRH-a is not recommended in cases of Novasure ablation, according to guidelines, in order to insure patient blinding to the allocated treatment, the authors prescribed GnRH-a to all patients. To ensure allocation concealment, this table of random numbers was not disclosed to the recruiting physicians. The ratio of women allocated to bipolar radio-frequency ablation or microwave endometrial ablation was 1:1.

The ablation treatment in both groups was performed by an experienced senior gynecologist according to the instructions of the manufacturers. The procedures were carried out as day-cases, under general intravenous anesthesia. Analgesia was pursued with the administration of 40 mg parecoxib sodium intramuscularly during treatment. The duration of each treatment was recorded by each device as net ablation time. The patients were blind to the allocated ablation technique.

Follow-up assessments were carried out at three and 12 months post-ablation. At three months after the procedure, women were contacted by telephone and interviewed. They were asked to report whether: a) they were amenorrhoic or not, b) there was need for analgesics immediate post-ablation, c) they were experiencing dysmenorrhea or not, d) they would consider that their clinical condition improved or not and, e) whether they were satisfied with the method or not. At 12-months post-ablation, patients were asked to visit the out-patient clinic of the University Department. During this visit, a physician who was unaware of the allocated treatment modality examined the women. Duration and clinical characteristics of menstruation were registered, as well as information concerning patient satisfaction and potential improvement (or not) in everyday life. At the same time, a Higham’s pictorial chart was also completed [8]. The presence of amenorrhea at 12-months post-ablation was the primary outcome measure. Furthermore, it was noted whether another additional intervention or hysterectomy had been performed during this time due to method failure.

Power analysis

Since at the time of protocol set-up there was no published data on the efficacy of MEA, explicitly on women with DUB, an analysis of ten such cases treated with MEA in the present department, provided the best estimate for the proportion of patients with amenorrhea at 12 months post-ablation (20%). Regarding the efficacy of Novasure, data from a published RCT were used [9]. Based on these assumptions, it was estimated that 33 patients in each group would be sufficient to detect a difference in the efficacy of the two methods (achievement of amenorrhea at 12-months after treatment) from 0.20 (MEA) to 0.56 (Novasure) [9] using a two-tailed Fisher’s Exact test with a=0.05 and b=0.20.

Statistical analysis

The normality of distribution of continuous variables was tested with the use of the Shapiro-Wilk test. In case of normal distribution of values, the results between the two groups were compared with the use of Student’s t-test, whereas in case of non-normality, the Mann-Whitney U test was used. Categorical variables were compared between groups with the use of the Fisher’s Exact test. All analyses were performed according to the intention-to-treat principle. Statistical significance was set at a level of 0.05. The Statistical Program for Social Sciences was used for all statistical analyses.

Results

According to the recruitment plan, 66 patients were enrolled in this study (MEA: n=33 – Novasure: n=33). Every patient was treated according to the group that was allocated to by the randomization table. The minimum follow-up of 12 months after treatment was completed by all randomized patients.

The baseline characteristics of the population analyzed in this study are depicted in Table 1. Overall, no statistically significant differences were observed between the two groups compared in terms of age, weight and body mass index (BMI), as well as, in number of previous pregnancies, menstruation patterns, and previous history of AUB. Similarly, the mean concentration of hemoglobin (as assessed during the initial work-up) was comparable between the two arms of this study.

Regarding treatment characteristics (Table 2), the length of the procedure was significantly increased in the MEA group. Endometrial ablation using either Novasure or MEA was successfully performed in all cases, while no major or minor complications were noted in the patients included in this RCT. The use of analgesics immediately post-ablation was required more often in patients treated with MEA as compared to those in whom Novasure was used (Table 2).

Follow-up at three months

All 66 patients had a follow-up telephone interview at three months after the endometrial ablation procedure (Table 3). Less women reported dysmenorrhea in the No-
Novasure impedance control system versus microwave endometrial ablation for the treatment of dysfunctional uterine bleeding etc.

The Novasure group (n=2) as compared to the MEA group (n=4), although this difference was not statistically significant (rate difference: -6.1%, 95% CI: -21.9 to +9.3). Most of patients in the Novasure group (n=22) reported amenorrhea, while only few patients in the MEA group (n=9) respectively (p = 0.003) (Table 3). In terms of clinical improvement and patient satisfaction both methods had similar rates (MEA: 97% vs. Novasure: 100%).

Follow-up at 12 months

Twelve months after the endometrial ablation, all patients (n=66) returned for a follow-up visit. Dysmenorrhea was reported more often from patients who were treated with MEA, yet this difference was not statistically significant (rate difference: +18.2%, 95% CI: -0.5 to +35.5) (Table 4). Similarly, the intensity of the pain, as measured by the VAS was not significantly different.

The rate of amenorrhea at 12-months post-ablation was significantly higher in women treated by Novasure (25/33; 75.8%) as compared to those treated by MEA (8/33; 24.2%) (rate difference: +51.5%, 95% CI: +27.8 to +67.7). Overall, the profile of blood loss seemed to be more favorable in women treated by Novasure (Table 4). In line with this finding, was the fact that the PBAC score was significantly decreased in the Novasure group when compared with the MEA group and that the mean difference in this score for each group from recruitment to 12-months post-ablation was significantly higher in the Novasure group. The mean level of hemoglobin was not significantly different in the two groups (Table 4).

Finally, more patients in the Novasure group (33/33; 100%) reported that they were satisfied with the results of the procedure before their 12-month follow-up visit (Table 4). One presented cryptomenorrhea five months after the endometrial ablation, yet this difference was not statistically significant (rate difference: +18.2%, 95% CI: -0.5 to +35.5) (Table 4). Similarly, the intensity of the pain, as measured by the VAS was not significantly different.

The rate of amenorrhea at 12-months post-ablation was significantly higher in women treated by Novasure (25/33; 75.8%) as compared to those treated by MEA (8/33; 24.2%) (rate difference: +51.5%, 95% CI: +27.8 to +67.7). Overall, the profile of blood loss seemed to be more favorable in women treated by Novasure (Table 4). In line with this finding, was the fact that the PBAC score was significantly decreased in the Novasure group when compared with the MEA group and that the mean difference in this score for each group from recruitment to 12-months post-ablation was significantly higher in the Novasure group. The mean level of hemoglobin was not significantly different in the two groups (Table 4).

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### Table 1. — Baseline characteristics.

<table>
<thead>
<tr>
<th></th>
<th>MEA (n=33)</th>
<th>Novasure (n=33)</th>
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<tr>
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<td>71.6 (8.0)</td>
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<td>26.1 (4.6)</td>
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<tr>
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<tr>
<td>3: 4</td>
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<td>Menstruation (days)d</td>
<td>7 (1)</td>
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<tr>
<td>PBACe</td>
<td>554 (119.1)</td>
<td>622 (218.6)</td>
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<tr>
<td>No: 16</td>
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<tr>
<td>VASg</td>
<td>4 (7)</td>
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<td>Hgb (g/dL)h</td>
<td>11.3 (0.7)</td>
<td>11.2 (2.2)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

1: Results are presented as median (interquartile range) and compared with Mann Whitney U test; b: Results are presented as mean (standard deviation) and compared with the Student’s t-test; c: Results are presented as counts and compared with the Fisher’s Exact test.

### Table 2. — Treatment parameters.

<table>
<thead>
<tr>
<th></th>
<th>MEA (n=33)</th>
<th>Novasure (n=33)</th>
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<tr>
<td>Duration of treatmenta (sec)</td>
<td>76.8 (9)</td>
<td>67.0 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minor complicationsb</td>
<td>Yes: 33</td>
<td>Yes: 33</td>
<td>n/a</td>
</tr>
<tr>
<td>Major complicationsb</td>
<td>Yes: 33</td>
<td>Yes: 33</td>
<td>n/a</td>
</tr>
<tr>
<td>Use of analgesicspost-ablationc</td>
<td>Yes: 9</td>
<td>Yes: 0</td>
<td>0.002</td>
</tr>
<tr>
<td>Hgb (g/dL)d</td>
<td>12.9 (0.99)</td>
<td>12.9 (1.03)</td>
<td>0.90</td>
</tr>
</tbody>
</table>

1: Results are presented as median (interquartile range) and compared with Mann Whitney U test; b: Results are presented as counts; c: Results are presented as counts and compared with the Fisher’s Exact test. n/a: not applicable.

### Table 3. — Follow-up at three months.

<table>
<thead>
<tr>
<th></th>
<th>MEA (n=33)</th>
<th>Novasure (n=33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmenorrheaf</td>
<td>Yes: 4</td>
<td>Yes: 2</td>
<td>0.67</td>
</tr>
<tr>
<td>No: 31</td>
<td>No: 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjective blood loss</td>
<td>Amenorrhea: 9</td>
<td>Amenorrhea: 22</td>
<td>0.003</td>
</tr>
<tr>
<td>Clinical status improvementa</td>
<td>Yes: 32</td>
<td>Yes: 33</td>
<td>1.0</td>
</tr>
<tr>
<td>Method satisfactiona</td>
<td>Yes: 32</td>
<td>Yes: 33</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a: Results are presented as counts and compared with the Fisher’s Exact test.

### Table 4. — Follow-up at 12 months.

<table>
<thead>
<tr>
<th></th>
<th>MEA (n=33)</th>
<th>Novasure (n=33)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmenorrheaf</td>
<td>Yes: 8</td>
<td>Yes: 2</td>
<td>0.08</td>
</tr>
<tr>
<td>No: 25</td>
<td>No: 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjective blood loss</td>
<td>Amenorrhea: 8</td>
<td>Amenorrhea: 25</td>
<td></td>
</tr>
<tr>
<td>Clinical status improvementa</td>
<td>Yes: 28</td>
<td>Yes: 33</td>
<td>0.053</td>
</tr>
<tr>
<td>Method satisfactiona</td>
<td>Yes: 30</td>
<td>Yes: 33</td>
<td>0.24</td>
</tr>
<tr>
<td>Improvement in everyday lifea</td>
<td>No: 3</td>
<td>No: 0</td>
<td></td>
</tr>
<tr>
<td>Need for further treatmenta</td>
<td>Yes: 2</td>
<td>Yes: 0</td>
<td>0.49</td>
</tr>
</tbody>
</table>

a: Results are presented as counts and compared with the Fisher’s Exact test; b: Results are presented as median (interquartile range) and compared with Mann Whitney U test; c: Results are presented as mean (standard deviation) and compared with the Student’s t-test.
post-ablation and was treated by hysterectomy, while the second woman presented continuous menorrhagia and requested permanent treatment by hysterectomy ten months after her primary treatment. No patient had been submitted to an additional intervention in the Novasure group. The only patient in this group that was still experiencing menorrhagia based on her PBAC score, reported a significant improvement as compared to her clinical status before entering the study (PBAC difference: -603.0) and was unwilling to undergo further treatment.

Discussion

This RCT comes to the conclusion that, in women with DUB, endometrial ablation with the Novasure bipolar radiofrequency impedance-controlled system is associated with increased rates of amenorrhea at 12-months post-treatment as compared to the MEA method.

The superiority of Novasure in terms of amenorrhea achievement was evident from the first follow-up interview, already at three-months post-treatment. Furthermore, it seems that most of the therapeutic potential of either Novasure or MEA is expressed as early as three-months post-treatment and this finding appears to be in line with the available evidence [9-11].

It should be noted though, that at three-months post-treatment, most of the patients in the MEA group that did not achieve amenorrhea, reported hypomenorrhea. This might be the reason that led most of the patients in both groups reporting a substantial improvement in their clinical condition and expressing their satisfaction with the method of endometrial ablation.

Both methods appeared to be equally safe, since no major or minor complications were noted in any of the two groups. The Novasure method was completed in less time than what was required for MEA. However, this difference in time is small, and it is not likely to be of clinical importance. The use of analgesics postoperatively was more frequent in the MEA group as compared to the Novasure group (RD: +27.3%, 95% CI: +11.2 to +44.2).

To the best of the authors’ knowledge the present is the first RCT comparing these two second-generation techniques of endometrial ablation (i.e. Novasure vs. MEA). Most RCTs that have been published so far have compared second with first generation devices of endometrial ablation [12-18]. Only five RCTs evaluating second-generation devices are available in the literature [9-11, 19, 20]. These include head-to-head comparisons between: a) Novasure bipolar radiofrequency impedance-controlled endometrial ablation and thermal balloon [9, 10, 20] b) Novasure bipolar radiofrequency impedance-controlled endometrial ablation and hydrothermoablation [19] and, c) MEA and thermal balloon [11].

These studies have been reviewed and meta-analyzed in a recent publication [7]. Based on the results of this meta-analysis, Novasure bipolar radiofrequency impedance-controlled endometrial ablation was associated with increased amenorrhea rates at 12-months post-treatment as compared to thermal balloon (OR: 4.56, 95% CI: 2.24-9.26). At the same time, direct evidence did not indicate a significant difference between MEA and the thermal balloon (OR: 1.13, 95% CI: 0.70-1.82), although a network meta-analysis performed suggested that potentially the MEA is superior to the thermal balloon in terms of amenorrhea at 12-months post-ablation (OR: 1.66, 95% CI: 1.01-2.71).

Regarding the comparison of Novasure with MEA, indirect evidence produced through a network meta-analysis suggested that MEA might be associated with decreased rates of amenorrhea at 12-months as compared to Novasure, although this result was not statistically significant (OR: 0.66, 95% CI: 0.36-1.21) [7]. The data from the present study seem to confirm the direction of the effect that was previously suggested through that indirect evidence.

One of the strong points of this study is the fact that the population analyzed was exclusively women with DUB. Most of the studies that have been published so far have not specifically targeted this population and have, in general, included women with abnormal menstrual bleeding due to various pathologies. Furthermore, according to the pre-specified inclusion/exclusion criteria, all women that were included in this RCT should have had a basal FSH less than 20 IU/L. In this way, the possibility of peri-menopausal amenorrhea during the follow-up period was reduced and thus, the actual efficacy of the two methods was evaluated. On the other hand, the present study is also characterized by some limitations that need to be commented. Although, the sample size of this study had been decided a priori based on a proper power analysis, it is not large, and thus, relatively wide confidence intervals have been produced. Evidently, the accumulation of high quality evidence in the future will produce a much more accurate estimate of the underlying effect size.

Another issue that the present authors had to take under consideration was whether to pre-treat endometrium with GnRH-a to all patients, or just women in MEA group, since thinning of the endometrium prior to the application of Novasure is not recommended according to guidelines. They decided to prescribe GnRH-a to all patients in order to insure patient blinding to the allocated treatment. In the present authors’ opinion, pretreatment might play a small but crucial role in the final results by improving amenorrhea rates in Novasure group, but more evidence is needed to prove their hypothesis.

Conclusion

This study is the first to provide evidence on the comparative efficacy of the Novasure bipolar radiofrequency impedance-controlled system and MEA. Based on the re-
sults of this RCT, Novasure seems to be associated with increased rates of amenorrhea at 12-months after the procedure as compared to the MEA.

References


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Optimizing the modified laparoscopic Vecchietti procedure

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Summary

Objective: To enhance the modified laparoscopic Vecchietti procedure. Materials and Methods: A case series of five women with Mayer-Rokitansky-Küster-Hauser syndrome at the Department of Obstetrics and Gynecology, King Abdulaziz University Hospital, Jeddah, Saudi Arabia underwent the modified laparoscopic Vecchietti procedure with intraperitoneal placement of sutures. This involved perforation of the vaginal dimple by a straight thread guide with two threads attached to the olive followed by pulling the two threads intra-peritoneally and through the abdominal wall to the traction device by grasping instruments under laparoscopic control. Results: Intraperitoneal placement of the sutures was easily done without complications in all five women. The operative time was 50 ± 10 (mean ± SD) minutes. After five postoperative days, the average vaginal length was seven to 7.5 cm. Two women were able to have vaginal intercourse without problems. After six months to one year of follow up, the vaginal length was at least ten cm and no postoperative complications occurred. Conclusions: Intraperitoneal placement of sutures makes the modified laparoscopic Vecchietti procedure easy and appealing. Furthermore, it avoids potential damage to the vital structures at the pelvic side walls.

Key words: Vaginal agenesis; Vecchietti procedure; Intraperitoneal suture approach.

Introduction

Vaginal agenesis occurs between one in 4,000 to one in 10,000 live female births [1]. Its differential diagnosis includes Mayer-Rokitansky-Küster-Hauser syndrome, androgen insensitivity syndrome, and intersex conditions [2]. Mayer-Rokitansky-Küster-Hauser is the most common form of vaginal agenesis and the second most frequent cause of primary amenorrhea. It is associated with normal ovarian function, normal female karyotype, and the presence of secondary sexual characteristics. It is due to congenital aplasia of the Müllerian ducts. Congenital anomalies of the upper urinary tract may occur in 30-40% of cases. Structural anomalies of the vagina and urogenital system are often challenging to diagnose and treat [3]. Treatment of vaginal agenesis is considered controversial. The modified laparoscopic Vecchietti procedure is gaining popularity especially in Europe [4]. Brucker et al., in 2008, reported improvements in the technique to make it safer, shorter, more effective, and less traumatic [5]. This involves using a new traction device and a modified laparoscopic procedure. The most time-consuming step of the procedure is the threading of the sutures through the eye of the suture carrier [6]. Furthermore, the bilateral retroperitoneal tunneling of the sutures over the lateral pelvic side walls requires experience and is potentially dangerous. The objective of this study was to report an enhancement of the modified laparoscopic Vecchietti procedure by avoiding the threading of the sutures through the eye of the suture carrier and the bilateral retroperitoneal tunneling with the intraperitoneal placement of sutures.

Materials and Methods

Approval by the Institutional Review Board (IRB) was obtained. Between November 2011 and February 2012, five women with Mayer-Rokitansky-Küster-Hauser syndrome at King Abdulaziz University Hospital, Jeddah, Saudi Arabia gave informed consent after proper counseling for the use of intraperitoneal approach in the placement of sutures during the modified laparoscopic Vecchietti procedure. The vaginal dimple (Figure 1) was perforated from outside by a straight thread guide with the two threads (Terylene 3+4) attached to the olive as reported by Brucker et al. [5]. The two threads were laparoscopically detached from the straight thread guide (Figure 2) and the thread guide was removed. The integrity of the urinary bladder and rectum were checked by cystoscopy and rectal examination. The threads were pulled intraperitoneally and through the abdominal wall (Figure 3) to the traction device by laparoscopic grasping instruments. This step avoids the bilateral retroperitoneal passages of the curved thread guide, the threading into the guide, and the subperitoneal pulling of the threads as reported before [5]. The traction device was placed at the level of the umbilicus and not suprapubically to allow normal axis and potential more length of the neo-vagina. Similarly, urethral catheterization and not suprapubical was done until removal of the traction device. This is because the olive only (without the segmented dummy) was used. Pain relief postoperatively was achieved by epidural anesthesia. This allowed daily tightening of the traction threads. After discharge from the hospital, vaginal dilators were used by the patients and they were followed regularly in the outpatient clinics.

Results

The age of the women was 26 ± 3 years (mean ± SD). Four women had a diagnosis of Mayer-Rokitansky-Küster-Hauser syndrome and one woman had the diagnosis of androgen insensitivity syndrome.
sensitivity syndrome. Four women presented with sexual problems. For two of these women, these problems led to divorce. One unmarried woman was not in a sexual relationship and presented with primary amenorrhea. For the woman with testicular feminization syndrome, the testes had been removed in a prior procedure. Intraperitoneal placement of sutures was easily done without complications in all five women. The operative time was 40 ± ten minutes. After five postoperative days, the average vaginal length was determined to be six to seven cm. Two married women were able to have vaginal intercourse without problems and three unmarried women did not yet have sexual relationship after the procedure.

Discussion

There is a lack of consensus regarding the best treatment for vaginal agenesis. In Mayer-Rokitansky-Küster-Hauser syndrome, it is thought that dilatation should be the first step, followed by surgery if necessary. Vaginal dilatation was first reported by Frank in 1938 [7]. It involves the use of graduated vaginal dilators placed at the introital dimple for 20 to 30 minutes three times daily for several months. For many reasons, including the lack of compliance, fatigue of the patients, and awkwardness of the various positions used [8], and in the present society due to refusal based on cultural attitudes, the surgical approach is favored. There are many reported surgical procedures which indicate that there is no agreement on what is the best option and that there is no single technique that is ideal for all deviations of vaginal anatomy [9]. The most frequently used technique by gynecologists in the United States is the McIndoe procedure [10]. In contrast, in Europe the modified laparoscopic Vecchietti procedure has been more popular [4]. The original Vecchietti procedure reported in 1965 involved laparotomy and dissection of the space between the urinary bladder and the rectum to pass a needle with two threads from the inside to the outside to attach them to the olive [11]. The threads were then passed extraperitoneally lateral to the rectus muscles to the traction device. Fedele et al., in 1994, published the laparoscopic version of the Vecchietti procedure [12]. Another larger study was published in 2008 [4]. Here, the space between the bladder and the rectum was dissected laparoscopically and the Vecchietti’s thread-bearing needle was passed from inside to the outside by piercing the pseudohymen to bring the threads (which were attached to the olive) to the peritoneal cavity and then outward by passing them extraperitoneally through the abdominal wall. Brucker et al., in 2008, reported a modification in the technique [5]. The procedure was done without dissection of the space between the bladder and rectum “vesicorectal tunneling.” The vaginal dimple was pierced from the outside to the inside with a straight thread guide with the two threads attached to the olive. The threads were laparoscopically detached from the thread guide and the thread guide was retracted. Then the curved thread guide was advanced retroperitoneally, down to the upper pole of the vagina. Each thread was threaded into the guide and drawn back subperitoneally through the abdominal wall. This most time-consuming step of the procedure requires experience and is potentially dangerous. The present study avoids the threading into the eye of the suture carrier and the retroperitoneal passage of the threads over the important structures in the pelvic side walls. Intraperitoneal placement of the sutures makes the modified laparoscopic Vecchietti procedure simple, rapid, and appealing.
References


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Outcomes and management strategies in pregnancies with early onset oligohydramnios

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Obstetrics and Gynecology, Celal Bayar University School of Medicine Obstetrics and Gynecology Department, Manisa (Turkey)

Summary

Objective: To evaluate the outcomes and management options in pregnancies with early onset oligohydramnios. Materials and Methods: The file datas of all pregnancies diagnosed as oligohydramnios or anhydramnios before 27 gestational weeks between January 2006 and September 2013 were evaluated retrospectively. The underlying pathology and associated anomalies, karyotype analysis, the outcome of the pregnancy (either termination or labour), and gestational week at the time of diagnosis were analyzed. Results: A total of 54 pregnancies were evaluated; mean gestational week at the time of the diagnosis was 19.8 ± 4.6. Mean maternal age was 27.28 ± 6.03. Thirty-seven pregnancies were anhydramniotic, 13 fetuses had associated anomalies, five of them had multicystic dysplastic kidney, five had bilateral renal agenesis, one had hypoplastic right heart syndrome, one had clubfoot, and one had ventricular septal defect and cleft palate. Karyotyping was normal regarding the fetuses with structural anomalies. Nineteen patients had premature preterm rupture of membranes and 39 patients had termination of pregnancy. Conclusion: The prognosis of early onset oligohydramnios is poor. Main determinant is gestational week at the time of the diagnosis.

Key words: Antenatal ultrasound; Oligohydramnios; Congenital anomalies of kidney; Preterm premature rupture of membranes.

Introduction

Amniotic fluid is vital for the normal development of the fetus. The regulation of the amniotic fluid depends on the maternal and fetal amniotic structures: in early pregnancy, amniotic fluid mainly consists of the maternal transudate. The chorioamniotic membrane serves as a selective barrier for the molecules for the free passage of water and electrolytes. The contribution of the embryo is minimal during this period. However, hypotonic urine excretion by the fetal kidneys occurs during the 12 gestational weeks. After 24-26 gestational weeks, fetal kidneys and lungs become primary regulators for the amniotic fluid. At term, 800-1000 ml amniotic fluid is produced daily by fetal urine excretion. Approximately 340 ml amniotic fluid is produced by fetal lungs, 170 ml of that contributes directly to the amniotic fluid, the other 170 ml is reabsorbed by the fetal lung. 500 to 1000 ml fluid is swallowed by the fetus daily at term, and 200-500 ml fluid flow occurs intramembranously every day [1].

Researchers and clinicians used different thresholds in order to define the abnormalities in amniotic fluid volume (AFV). In general, oligohydramnios is decreased AFV according to the gestational week [2]. Oligohydramnios is defined differently by different researchers: single vertical pocket (SVP) < 0.5 cm by Mercer et al. [3], SVP < two cm by Manning et al. [4], SVP < three cm by Halperin et al. [5]. Magan et al. proposed the two diameter pocket (vertical x horizontal) < 15 cm [6]. Amniotic fluid index (AFI) < 5th percentile according the gestational age is defined by Moore et al. [7]. AFI < five cm by Pheylan et al. [8], < seven cm by Dizon-Townson et al. [9], and AFI < eight cm by Jeng et al. [10].

The present authors aimed to evaluate the main causes of early onset oligohydramnios and discuss the management strategies in case of preterm premature rupture of the amniotic membranes rupture (PPROM) and in case of oligohydramnios with renal origin.

Materials and Methods

The file records of all pregnancies diagnosed as oligohydramnios or anhydramnios before 26 gestational weeks between January 2006 and September 2013 were evaluated retrospectively in our perinatology outpatient clinic. A total of 54 cases were included in the study. The underlying pathology and associated anomalies, karyotype analysis, the outcome of the pregnancy (either termination or labour), and gestational week at the time of diagnosis were analyzed. Oligohydramnios definition is regarded as the SVP < two or AFI < five cm. The underlying etiology was evaluated by ultrasonography. Pelvic examination with sterile speculum was made to exclude PPROM. PPROM cases were administered antibiotic therapy with their first admission, usually via intravenous route. Tocolysis was not applied. The study was approved by the Institutional Ethics Committee.
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Discussion

Normal amniotic fluid shows mainly the intact fetal gastrointestinal and urinary system. It is vital for the maintenance of normal fetal development. Reduced AFV in the first and early second trimester raises questions about renal functions: the clinician should evaluate the kidneys by ultrasonography: do fetal kidneys present? If yes, do they appear normal in shape and size? If the answer is yes, then oligohydramnios should be due to renal origin. In the present study the most common renal anomaly associated with oligohydramnios was renal agenesis and multicystic dysplastic kidneys. The prenatal recognition rate of the associated renal anomalies was 10/56 (17.8%). This is much lower than the true incidence of the renal origin oligohydramnios cases. Approximately 50% of oligohydramnios cases may have associated renal and urinary tract anomaly. However, in case of severe oligohydramnios, it is very difficult to screen the fetus appropriately. Furthermore, fetal prognosis is poor. Klaassen et al. evaluated 23 fetuses with renal-origin oligohydramnios, 16 of which had congenital kidney anomalies, four autosomal recessive polycystic kidney disease, and three renal tubular agenesis; 30% of them died mostly due to pulmonary hypoplasia and renal failure [11].

If renal origin could be ruled out by sonographic examination, then PPROM is to be suspected [12]. PPROM before 24 gestational weeks is less than 1% of all pregnancies [13]. The management of PPROM before viability is controversial. If anhydramnios has occurred or if any evidence of chorioamnionitis is found, termination of pregnancy is inevitable. However, cases with small leakage of amniotic fluid without any infection sign can be managed expectantly. Dinsmoor et al. evaluated 46 cases with PPROM before viability threshold, 43 of which elected expectant management with a mean latency period 13 days (range: 0-96). Overall survival rate was 47%. Only 37% of the survivors had severe sequelae [14]. In the present study, 18 cases had PPROM before 24 gestational weeks, eight of them had termination of the pregnancy, ten cases managed were expectantly. Similarly to the study of Dinsmoor et al., mean latency period was 20 days in the present study group. The main limitation of the present study was the lack of the data regarding the newborn sequela. Regarding PPROM, use of corticosteroids, use of tocolytic agents, and the type and mode of antibiotics are controversial. Recent studies highlighted that steroids should not be used before 24 weeks. The repetitive doses do not add any benefit [15]. Regarding the tocolytic use, Cochrane review comprising of eight studies with 408 pregnancies between 23-27 weeks concluded that tocolytics may increase the risk of intra-amniotic infection and increase the long term sequela such as cerebral palsy [16]. The different antibiotic regimens are not superior to each other; however co-amoxiclav should be not used as it increases the risk of necrotizing enterocolitis [17]. Another problem is where to follow-up these pregnancies: at home or at hospital? The recent studies suggest that follow-up at home may be a good option. In a review with 116 cases showed that patients followed up at home had approximately ten days less hospital stay and they had lower cesarean rates [17]. In the present department, the authors follow-up the PPROM pregnancies at home only if there is no amniotic fluid leakage since three days, if there is no anhydramnios, and if there is no clinical and laboratory sign of infection.

Table 1. — Descriptive data of the study population.

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean maternal age (mean ± SD)</td>
<td>27.28 ± 6.03</td>
</tr>
<tr>
<td>Gravida (mean ± SD)</td>
<td>1.98 ± 1.13</td>
</tr>
<tr>
<td>Parity (mean ± SD)</td>
<td>0.63 ± 0.75</td>
</tr>
<tr>
<td>Abortus (mean ± SD)</td>
<td>0.35 ± 0.69</td>
</tr>
<tr>
<td>Gestational week (mean ± SD)</td>
<td>19.8 ± 4.6</td>
</tr>
</tbody>
</table>

Table 2. — Associated anomalies with oligohydramniotic cases.

<table>
<thead>
<tr>
<th>Associated anomaly</th>
<th>N</th>
<th>%</th>
<th>Karyotyping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clubfoot</td>
<td>1</td>
<td>1.9</td>
<td>Normal</td>
</tr>
<tr>
<td>Hypoplastic right heart</td>
<td>1</td>
<td>1.9</td>
<td>Normal</td>
</tr>
<tr>
<td>Multicystic dysplastic kidneys</td>
<td>5</td>
<td>9.4</td>
<td>Normal (n=3)</td>
</tr>
<tr>
<td>Renal agenesis</td>
<td>5</td>
<td>9.4</td>
<td>Normal (n=2)</td>
</tr>
<tr>
<td>VSD + cleft palate</td>
<td>1</td>
<td>1.9</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table 3. — Features of early PPROM cases.

<table>
<thead>
<tr>
<th>Description</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early PPROM</td>
<td>18</td>
<td>30.0</td>
</tr>
<tr>
<td>Latency (days) (mean ± SD)</td>
<td>20.0 ± 14.57</td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Cesarean</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Results

A total of 54 pregnancies were evaluated; mean gestational week at the time of the diagnosis was 19.8 ± 4.6. Mean maternal age was 27.28 ± 6.03 years. The descriptive data is shown in Table 1. Thirty-seven pregnancies were anhydramniotic, 13 fetuses had associated anomalies, five of them had multicystic dysplastic kidney, five had bilateral renal agenesis, one had hypoplastic right heart syndrome, one had clubfoot, one had ventriculoperitoneal defect (VSD) and cleft palate (Table 2). Karyotyping was offered for the cases with associated anomalies, but performed only in eight cases. Karyotype analysis was normal regarding the fetuses with structural anomalies. Eighteen patients had PPROM. Mean latency period was 20.0 ± 14.57 days for labour, five of which had cesarean and five of which had vaginal labour (Table 3). Mean birth weight of newborns was 782 ± 35 grams (512-1,072 grams). Thirty-nine patients had termination of pregnancy.
The prognosis mainly depends on the gestational week, duration of the oligohydramnios, and the severity of oligohydramnios. Even when the fetus had no genetic or structural anomalies, early onset oligohydramnios may cause pulmonary hypoplasia, fetal contractures, and lethal infection dangerous for both the mother and the fetus. The main mechanism for the associated of pulmonary hypoplasia is thoracic compression and the improper breathing movement due to anhydramnios or severe oligohydramnios.

The present study has some limitations. Firstly, the authors could not provide the neonatal outcomes of the newborns as the record system failed. Secondly, they do not know the true incidence of associated renal anomalies in this study, because the parents did not want fetal postmortem biopsy after pregnancy termination. However, the present study is important as the clinicians face commonly with oligohydramniotic pregnancies before viability threshold. From that point of view, this study shows some practical clues for the clinicians.

**Conclusion**

Pregnancies with anhydramnios detected before 24 gestational weeks should be offered termination. However, beyond 26 gestational week, in case of PPROM, expectant management may be offered. Antibiotherapy should be initiated. The pregnant woman must be followed up every two days by leucocyte count and C-reactive protein levels. If chorioamnionitis is suspected, delivery should be performed.

**References**


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Surgical management of intrauterine devices migrated towards intra-abdominal structures: 20-year experience of a tertiary center

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Summary

Aims: To share surgical management experiences of intra-abdominal intrauterine devices (IUDs) in tertiary center. Material and Methods: A total of 27 patients were retrospectively analyzed. This retrospective study was conducted between September 1992 and April 2013 at Department of Obstetrics and Gynecology Tepecik Research and Training Hospital, Izmir, Turkey. Demographic findings, diagnostic methods, and operative notes of patients were obtained from the patient file. Findings: Of the 27 IUDs, nine (33.3%) were in omentum, four (15%) were in Douglas pouch, one in left sacrouterine ligament, one in uterovesical space and one in fundus posterior, six (22%) in left adnexial region, one in abdominal wall, one was subdiaphragmatic, one in ligamentum latum, and one in jejunum. Almost all of the patients had TCu-380 A IUDs. Seventeen patients (63%) were managed by laparoscopy, whereas laparotomy was required in ten (37%). Adhesions were found in 23 of 27 (85%) patients with varying degrees. In four cases the incision was extended due to adhesions. Conclusion: A missing string was the first finding of an intra-abdominal IUD. Pelvic ultrasonography, X-ray, and hysteroscopy methods should be performed in order to detect the localization of IUD in case of a missing string. Surgical approach should be the first treatment option for intra-abdominal IUDs.

Key words: Intrauterine device (IUD); Migration; Intra-abdominal structures.
Of the 27 IUDs, nine (33.3%) were in omentum, four (15%) were in Douglas pouch, one in left sacrouterine ligament, one in uterovesical space and one in fundus posterior, six (22%) in left adnexal region, one in abdominal wall, one was subdiaphragmatic, one in ligamentum latum, and one in jejunum. Two patients were planned hysteroscopic approaches, however IUD was removed laparoscopically as almost whole of IUD was out of the uterus. Almost all of the patients had TCu-380 A IUDs.

Seventeen patients (63%) underwent laparoscopy and the operation was switched to laparotomy due to intensive adhesions in four (23.5%). Operation was performed through a mini-laparotomy with a three to four cm incision in remaining ten cases (37%). Incision necessitated to extend due to adhesions only in seven cases. Varying degrees of adhesions were detected in 23 out of 27 patients (85%). No adhesions were detected in four patients (15%). One patient who was detected not to have adhesions had an IUD inserted 12 months ago and another had one inserted three months ago. Major complications (vascular or intestinal injury) occurred in no patients. IUD was removed from jejunum with a one-cm incision as it was in jejunum in only one case. Jejunum was repaired with primary sutures. Pomeroy method of tubal ligation was performed in the course of IUD extirpation in nine cases as they desired tubal ligation. A statistically significant difference could not be found between mini laparotomy and laparoscopic surgery in terms of operative time and post-operative duration of hospital stay. Mean duration of hospital stay was two days. Patients were uneventful in postoperative follow up.

Discussion

IUDs have been widely used since 1960. While the first used types were biologically inactive polyethylene lippes-loop IUDs, second generation copper and hormone-releasing IUDs which were developed in 1970s are used today. In Turkey, 62.6% of reproductive age women are married and 15.7% of them use IUDs as contraceptive method [4]. This ratio is 33% in China and 5.3% in developed countries [8].

IUDs are used widely as they are reversible, safe, inexpensive, effective, and easily applicable. However they also have side effects like increased menstrual bleeding, pain, infection, and perforation. These side effects are seen more particularly within the first months following IUD insertion.

Risk of uterine perforation is in the ratio of 0.87/1,000 [4]. IUD type (copper-containing IUDs are more risky), application time (following delivery or curettage etc), uterine size, position, and experience of the operator are the factors affecting perforation rates. IUD may place at any site in the abdomen if perforation occurs [10].

Following the procedures during insertion of IUD would minimize perforation risk. Immediate laparoscopy may be applied if perforation is recognized during the procedure. Most of the perforations are not recognized during insertion as in our study and women may stay asymptomatic for months. Therefore women who are inserted IUDs must be called for gynecologic examination 6-12 weeks after insertion and informed about coming for controls once in two years thereafter [11].

Transvaginal ultrasonography and direct graphies should be performed in case of a missing string and IUD should be considered to have fallen without noticing only after these examinations.

Current treatment of intra-abdominal IUDs is surgical removal however opposite opinions also exist. Adoni et al. detected no intra-abdominal adhesions in 11 patients who were detected to have intra-abdominal IUDs. Of these IUDs, four were normal and seven were copper IUDs. They reported that intra-abdominal IUDs were not needed to be removed [12]. Similarly, Markovitch et al. reported no adhesions in three cases and recommended not to remove IUDs in asymptomatic patients.

On the contrary to these opinions, World Health Organization recommends immediate surgical intervention due to risk of intensive adhesions, chronic pain, and even infertility when an intra-abdominal IUD is detected [13]. In the present study, varying degrees of adhesions were detected in 23 out of 27 patients (85%). Laparotomy was needed due to intensive adhesions in four cases. However according to another opinion, adhesions develop in the early period and these prevent migration of IUD, therefore operations would not be useful as they would further increase adhesions [5]. Beside adhesions, neighbouring organ injury due to mi-

<table>
<thead>
<tr>
<th>Table 1. — Distribution of age, parity and duration of IUD use.</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.49</td>
<td>7.8</td>
<td>20.0-50.0</td>
</tr>
<tr>
<td>Parity</td>
<td>2.75</td>
<td>2.06</td>
<td>1.0-9.0</td>
</tr>
<tr>
<td>Duration of IUD use (month)</td>
<td>20.62</td>
<td>43.21</td>
<td>0.5-144.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. — Distribution of delivery type, IUD localization and surgical method.</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of previous delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23</td>
<td>85</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>IUD localization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omentum</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>Douglas</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Adnexial region</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Surgical method</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparotomy</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>17</td>
<td>63</td>
</tr>
</tbody>
</table>

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grated IUD is dangerous and it may migrate to distant regions from which IUDs are more difficult to remove. IUDs which migrated to iliac vein, right iliac bifurcation, and retroperitoneal region are reported in literature. Therefore retroperitoneal region and abdominal wall should certainly be examined if IUD cannot be found during the operation.

Intra-abdominal foreign bodies precipitate also infection besides adhesion. None of the present 27 patients developed abscess formation however abscess formation is reported in the ratio of 15-20% in literature [12].

Finally, the patient’s knowing that she carries a foreign body in her abdomen may lead to psychological and medicolegal problems. In the present patients, all intra-abdominal IUDs were surgically removed as they could lead to intra-abdominal organ perforations, infection, and chronic pelvic pain.

In conclusion, patients should be controlled with certain intervals after IUD insertion even if they have no symptoms and it should be kept in mind that a missing string may indicate an intra-abdominal IUD. Localization of IUD should be determined with ultrasonography and direct graphs. According to the results of the present study, diagnosed intra-abdominal IUDs should be removed surgically even if they are asymptomatic. However publications which do not support this result also exist. Further studies are needed in order to create a definite opinion about this issue.

References


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The impact of LH, E2, and P level of HCG administration day on outcomes of in vitro fertilization in controlled ovarian hyperstimulation

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**Summary**

**Objectives:** The objective of this study was to evaluate the impact of luteinizing hormone (LH), estradiol (E₂) and progesterone (P) levels on the day of human chorionic gonadotropin (HCG) administration on outcomes of in vitro fertilization (IVF) in controlled ovarian hyperstimulation (COH). **Study Design:** In this retrospective study, 129 infertile women undergoing IVF / intracytoplasmic sperm injection (ICSI) treatments were included; these cycles were stratified according to LH levels of ≥ 1.12 IU/L or < 1.12 U/L and according to E₂ levels of ≥ 1,005.89 pmol/L or < 1,005.89 pmol/L. The main outcome measure was the clinical pregnancy rate. **Results:** The clinical pregnancy rate was significantly higher in the group with LH ≥ 1.12 IU/L than in the group with LH < 1.12 IU/L (43.28% vs. 30.65%, p < 0.05). The clinical pregnancy rate was also higher in the group with E₂ ≥ 1,005.89 pmol/L than in the group with average E₂ < 1,005.89 pmol/L (42.86% vs. 30.51%, p < 0.05). Among the LH, E₂, and P levels on the day of HCG administration, LH level was the most important predictor of outcomes of IVF in COH. The present data showed an adverse effect of low serum LH level (LH < 1.12 IU/L) on the day of HCG administration on clinical pregnancy rate. E₂ level can also predict the outcomes of IVF in COH. **Conclusions:** Low serum LH level (LH < 1.12 IU/L) and low serum E₂ level (average E₂ < 1,005.89 pmol/L) on the day of HCG administration led to low clinical pregnancy rates, while the P level on the day of HCG administration may have had little effect on clinical pregnancy.

**Key words:** In vitro fertilization-embryo transfer (IVF-ET); Controlled ovarian hyperstimulation (COH); Luteinizing hormone (LH); Estradiol (E₂); Progesterone (P).

**Introduction**

Improving the clinical pregnancy rate of in vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) is currently the focus of many researchers.

Luteinizing hormone (LH) is the glycoprotein hormone secreted by the gonadotropin cells of the anterior pituitary. LH is known to be important in oocyte growth and maturation [1], it can promote the proliferation and differentiation of theca cells to produce androgen, which synergistically increases estrogen production. In the late follicular phase, LH helps produce small quantities of progesterone (P), thereby contributing to the promotion of positive estrogen feedback. The estradiol (E₂) level reflects follicle secretion and it is necessary for follicular development and maturation. Synthesis of E₂ is related to the development of dominant follicles, and serum E₂ concentration is the most meaningful indicator to assess follicular maturation. E₂ level relates to the number and size of follicles within the two sides of the ovaries. The average E₂ level of each follicle has greater predictive significance to determine the day of human chorionic gonadotropin (HCG) injection than the total E₂ level. It has been reported that the E₂ level could predict the outcome of IVF-ET treatment [2-4]. Serum P is mainly secreted by granul lutein cells and theca luteal cells. P can inhibit uterine contraction, reduce the sensitivity of the uterus to oxytocin, work against estrogen’s function of endometrial proliferation to improve glandular secretion, and is conducive to embryo implantation and development. There are several reports regarding the impact of HCG administration day levels of LH, E₂, and P on the outcomes of IVF/ICSI-ET in controlled ovarian hyperstimulation (COH), but the results vary [2, 3, 5-11]. This study discussed the predictive value of serum LH, average E₂ level of follicles ≥ 14 mm in diameter and P level measured on the day of HCG administration on outcomes of IVF/ICSI-ET in COH, and determine the optimal time for HCG administration.

**Materials and Methods**

A total of 129 infertile women underwent IVF/ICSI-ET treatments at the Reproductive Medicine Center of NJPH from January 2010 to May 2011. Inclusion criteria were as follows: (1) younger than 40 years, (2) first cycle, (3) absence of moderate...
Table 1. — Demographic information of groups of higher (A1) and lower (A2) LH levels on the day of HCG administration.

<table>
<thead>
<tr>
<th></th>
<th>Group A1 (LH ≥ 1.12 IU/L)</th>
<th>Group A2 (LH &lt; 1.12 IU/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>67</td>
<td>62</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>30.10 ± 4.40</td>
<td>29.66 ± 4.11</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility period, years</td>
<td>4.34 ± 3.12</td>
<td>4.19 ± 2.68</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>22.01 ± 2.53</td>
<td>22.16 ± 3.06</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FSH, miu/ml</td>
<td>8.18 ± 2.53</td>
<td>7.87 ± 2.68</td>
<td>NS</td>
</tr>
<tr>
<td>Basal LH, miu/ml</td>
<td>4.54 ± 2.08</td>
<td>4.73 ± 2.84</td>
<td>NS</td>
</tr>
<tr>
<td>Basal E2, pmol/L</td>
<td>185.61 ± 114.83</td>
<td>160.33 ± 81.28</td>
<td>NS</td>
</tr>
<tr>
<td>Basal PRL, ng/ml</td>
<td>17.06 ± 8.58</td>
<td>15.88 ± 8.44</td>
<td>NS</td>
</tr>
<tr>
<td>Basal T, ng/ml</td>
<td>1.79 ± 2.56</td>
<td>4.19 ± 19.35</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>11.65 ± 3.81</td>
<td>12.77 ± 5.24</td>
<td>NS</td>
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</tbody>
</table>

Note: Continuous data are presented as the means ± SD.

Table 2. — Demographic information of groups with higher (B1) and lower (B2) E2 levels on the day of HCG administration.

<table>
<thead>
<tr>
<th></th>
<th>Group B1 (average E2 ≥ 1005.89 pmol/L)</th>
<th>Group B2 (average E2 &lt; 1005.89 pmol/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>70</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>Age, years</td>
<td>30.01 ± 4.73</td>
<td>29.75 ± 3.64</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility period, years</td>
<td>4.30 ± 2.27</td>
<td>4.23 ± 2.43</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>21.12 ± 2.36</td>
<td>21.24 ± 2.83</td>
<td>NS</td>
</tr>
<tr>
<td>Basal FSH, miu/ml</td>
<td>8.44 ± 2.57</td>
<td>7.54 ± 2.56</td>
<td>NS</td>
</tr>
<tr>
<td>Basal LH, miu/ml</td>
<td>5.03 ± 2.78</td>
<td>4.16 ± 1.95</td>
<td>NS</td>
</tr>
<tr>
<td>Basal E2, pmol/L</td>
<td>184.77 ± 108.85</td>
<td>160.05 ± 88.75</td>
<td>NS</td>
</tr>
<tr>
<td>Basal PRL, ng/ml</td>
<td>16.72 ± 7.52</td>
<td>16.22 ± 9.59</td>
<td>NS</td>
</tr>
<tr>
<td>Basal T, ng/ml</td>
<td>3.96 ± 18.21</td>
<td>1.73 ± 2.75</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>12.11 ± 3.52</td>
<td>12.40 ± 5.51</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Continuous data are presented as the means ± SD.

Figure 1. — ROC curve of LH levels on the day of HCG administration.

Figure 2. — ROC curve of average E2 levels on the day of HCG administration.
The impact of LH, E2, and P level of HCG administration day on outcomes of in vitro fertilization in controlled ovarian hyperstimulation

or severe endometriosis, and severe hydrosalpinges, (4) absence of cancelled cases of implantation to prevent severe ovarian hyperstimulation syndrome (OHSS) or cases of OHSS, (5) Basal day 3 FSH level is under 10 mIU/ml, and (6) the antral follicle count (AFC) > 6. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of NJPH.

Prior to treatment, all patients received 21 contraceptive pills followed by gonadotropin-releasing hormone agonist (GnRH-a) for down-regulation from day 17 to day 21 of the oral contraceptive regimen. FSH and/or human menopausal gonadotropin (HMG) were started on day 3 to day 6. HCG 10,000 IU was given on the night when four or more follicles reached ≥ 16 mm in diameter, three or more follicles reached ≥ 17 mm in diameter or two or more follicles reached ≥ 18 mm in diameter. Oocyte retrieval was performed 34-36 hours later, and ET was performed three days after oocyte retrieval. All patients received intramuscular P as luteal support from the date of oocyte retrieval. Blood HCG was measured 14 days after ET and ultrasound was conducted 28 days after ET, and clinical pregnancy was defined as the presence of an intrauterine gestational sac and a fetal heart beat.

On the day of HCG administration, all patients had five ml of venous blood drawn at the Clinical Testing Center of NJPH between 7:45 and 8:00 am. The samples were centrifuged at 2,000 rpm for 15 minutes. Sera samples were tested using an immunoassay system automatic chemiluminescence analyzer and the immune serum chemiluminescence detection kit to determine the levels of serum LH, E2, and P. These hormones on the HCG day were all within the normal range.

All data management and storage were completed with Reproductive Medicine Clinical Management System Software Version 9.9 of NJPH. SPSS 17.0 was used to conduct analyses, including ROC curve statistics. Student’s t-test and x² test, and p values < 0.05 were considered to be statistically significant. Data are presented as the mean ± standard error of the mean.

Results

Of 129 fresh ET cycles, the causes of infertility included the following: tubal or pelvic factors (76 cycles, 58.91%), male factor (19 cycles, 14.73%), mild endometriosis (three cycles, 2.33%), unknown causes (six cycles, 4.65%), and combined male and female factors (25 cycles, 19.38%). Age, years of infertility, BMI, basic endocrine level, and AFC were not significantly different between groups (all p > 0.05; Tables 1 and 2).

In this study, there were 48 clinical pregnancies and 81 cases of pregnancy failure. According to the statistical ROC curves, the area under the curve for serum LH, average E2 of follicles ≥ 14 mm in diameter and P levels on the day of HCG administration were 0.617, 0.552, and 0.443, respectively. Patients were divided into groups based on serum LH levels on the day of HCG administration as follows: one Group A1 with ≥ 1.12 IU/L and Group A2 with < 1.12 IU/L. The sensitivity of this measure to predict pregnancy was 0.60, and the specificity was 0.531. Similarly, patients were divided into groups based on the average E2 levels of follicles ≥ 14 mm in diameter as follows: Group B1 with ≥ 1,005.89 pmol/L and Group B2 with < 1,005.89 pmol/L. The sensitivity of this measure to predict pregnancy was 0.625, and the specificity was 0.506. (ROC curves are shown in Figures 1, 2, and 3).

As shown in Table 3, groups with high and low LH levels show a statistically significant (p < 0.05) difference between the number of follicles ≥ 14 mm in diameter on the day of HCG administration and clinical pregnancy rates. Table 4 shows that groups with high and low E2 levels for follicles ≥ 14 mm in diameter differ by LH and P levels, the number of follicles ≥ 14 mm and ≥ 16 mm in diameter on the day of HCG administration, and clinical pregnancy rate (p < 0.05). (Tables 3 and 4).
Discussion

Only when serum LH levels are above the threshold of LH in the body can LH maintain normal follicle development and hormone secretion [12]. Some researchers have proposed that appropriately complementary LH helps to improve the pregnancy outcome of IVF in some patients [13], while other researchers have argued that normal women with significantly lower LH levels after down-regulation of COH can still obtain a good pregnancy outcome [14].

Fleming et al. found that when LH < 1 IU / L in the mid-follicular phase, E2 levels decreased, thus leading to adverse pregnancy outcomes [14]. Lu et al.’s study showed that the clinical pregnancy rate (58.33%) when serum LH was ≥ 1.58 IU / L on the day of HCG administration was significantly higher than when LH was < 1.58 IU / L (34.48%) [15]. A recent meta-analysis showed that the addition of recombinant LH to IVF cycles may improve implantation and clinical pregnancy in patients of advanced reproductive age [16].

In the present study, the authors found that group A2 had more follicles ≥ 14 mm and ≥ 16 mm in diameter than group A1 (< 0.05), the clinical pregnancy rate was also lower than group A1 (< 0.05). These results suggest that there is an specifically response in group A1 in the process of ovulation as a moderate amount of LH can prevent estrogen decline and reduce FSH dosage. A lack of LH may affect follicular development, resulting in the specifically poor response to FSH. Fleming’s study suggested that low serum levels of LH were not conducive to follicular growth but could reduce the differences in follicular development; the FSH dose increases, while the number of retrieved oocytes increases with increased administration time of FSH [17]. A moderate LH level is important for the maturation of oocytes and maintenance of luteal functions. This
research offered some basis for the evaluation of moderate serum LH level of <1.12 IU/L on the day of HCG administration in COH and found that the clinical pregnancy rate in this group decreased significantly as compared to the other groups studied.

In COH, injecting HCG when the average E2 level of each mature follicle reached 732-1,098 pmol/L achieved a maximum rate of embryo implantation and clinical pregnancy, while the lowest clinical pregnancy rate was reported with E2 levels below 732 pmol/L [2]. It has been reported that E2 > 2,936 pmol/L leads to a higher pregnancy rate, but an overly high level of E2 is not conducive to embryo implantation. Others have suggested that the E2 level on the day of HCG administration or E2 pre-treatment has no correlation with pregnancy rate [6, 18-21]. While another paper showed that serum E2 levels had a concentration-dependent effect on the pregnancy outcome, suggesting an optimal range of E2 level for achieving a successful pregnancy: 3,000-4,000 pg/ml for women < 38 years and 2,000-3,000 pg/ml for women ≥ 38 years [22].

In the present study, the authors found that group B1 had higher LH and P levels on the day of HCG administration day compared to group B2 (p < 0.05). Between groups B1 and B2, the difference in the number of follicles ≥ 14 mm or ≥ 16 mm in diameter on the day of HCG administration was statistically significant (p < 0.01). As the number of follicles significantly increased in group B2, it may be inferred that an excessive number of follicles resulted in a decline in the average E2 level of each follicle, which may affect the quality of follicles. Therefore, individualized treatments are needed in the process of COH to avoid the growth of too many follicles, which not only increases the risk of ovarian hyperstimulation but also has an adverse impact on pregnancy outcome. There was a significant difference in the number of follicles ≥ 14 mm on the day of HCG administration between groups B1 and B2 (p < 0.05). The significant reduction in the number of follicles in the group with a higher clinical pregnancy rate suggests a relationship between the number of follicles and clinical pregnancy. The clinical pregnancy rate was lower in group B2 than in group B1 (p < 0.05). Synchronizing the process of IVF/ICSI-ET is the key to improving the clinical pregnancy rate. A low E2 level may not adequately increase the number of spiral vessels, glands, and glandular secretion after ovulation, thus reducing endometrial perfusion with inadequate activation of cytokines and causing follicular and endometrial development to be asynchronous. The present authors found that in COH, the average E2 level of follicles ≥14 mm in diameter on the day of HCG administration was related to pregnancy outcome and that the clinical pregnancy rate was significantly lower in the group with an average E2 level < 1,005.89 pmol/L.

Endometrial receptivity is an important factor affecting IVF-ET pregnancy outcomes. Studies suggest that serum P level on the day of HCG administration in IVF-ET did not affect the follicular quality but affected endometrial receptivity, resulting in lower clinical pregnancy rates and implantation rates [10, 21].

According to the ROC curves generated in the present study, the area under the curve of serum P level on the day of HCG administration was 0.443. This suggests that the serum P level on the day of HCG administration may have no effect on pregnancy outcomes of IVF/ICSI-ET. However, according to Ochtenkühn et al., live birth rate in cycles with GnRH-a was significantly lower in women with P levels ≥ 2.0 ng/ml (17.4%) on the day of HCG administration as compared with women with P levels < 1.5 ng/ml (24.6%) and 1.5-1.99 ng/ml (26.7%) [23]. One new study reported that P/E2 ratio on day of HCG administration improves the prediction of IVF outcome when compared to serum P levels alone [24]. So further studies are needed to elucidate this issue.

In summary, this retrospective study arrived at the preliminary conclusion that among the three serum markers, LH level, average E2 level of follicles ≥14 mm in diameter, and P level on the day of HCG administration, serum LH level had the strongest predictive value for pregnancy outcome. When serum LH level was < 1.12 IU/L on the day of HCG administration, the clinical pregnancy rate following IVF/ICSI-ET was significantly lower. At the same time, using ROC curves, the present authors concluded that when serum LH levels were greater than 2.14 IU/L, 87.7% of IVF/ICSI-ET patients had negative pregnancy outcomes. Therefore, serum LH level should be maintained within a certain range during the process of COH; when LH < 1.12 IU/L, adding supplemental LH may help to further increase the pregnancy rate. Monitoring the LH level may also help to determine the optimal time to inject HCG, with the goal to further enhance the pregnancy rate among patients undergoing IVF/ICSI-ET. In COH cycles, the average E2 level of follicles ≥ 14 mm in diameter on the day of HCG administration was related to pregnancy outcome; when average E2 level < 1,005.89 pmol/L, clinical pregnancy rate was significantly lower. This study indicated that serum P level on day of HCG administration may have had little effect on the pregnancy outcomes of IVF/ICSI-ET.

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References


Follicular phase serum and follicular fluid glycodelin measurements in gonadotropin-releasing hormone (GnRH)-antagonist assisted reproduction cycles: A prospective cohort study

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Summary

**Purpose:** To establish the serum pattern for glycodelin and to investigate the possible correlations of serum and follicular fluid (FF) glycodelin with clinical pregnancy in gonadotropin-releasing hormone (GnRH)-antagonist controlled cycles. **Materials and Methods:** A prospective observational study conducted with 80 infertile couples who received a GnRH-antagonist controlled cycle. Glycodelin levels were measured in FF, day 2-3, and ovarian pick-up (OPU)-day serum samples. **Results:** There were no significant differences in serum glycodelin concentrations in either the early follicular phase or the preovulatory phase, and in FF glycodelin concentrations between clinically pregnant and non-pregnant patients. OPU-day serum glycodelin was found to be significantly higher than early follicular serum glycodelin level in all patients whether pregnancy occurred or not. **Conclusion:** Although day 2-3 and OPU-day measurements of serum glycodelin levels were not significant in predicting clinical pregnancy, the pattern of serum glycodelin seems different in GnRH-antagonist controlled cycles than natural and GnRH-agonist controlled cycles.

**Key words:** Assisted reproductive technology; Glycodelin; Gonadotropin releasing hormone antagonist.

Introduction

Approximately 80-85% of assisted reproductive technology (ART) cycles reach the embryo transfer phase, as reported in the 2009 ART report. It was also reported that the clinical pregnancy rate was approximately 36% per cycle, with more than one embryo transfer administered in approximately 86% of cycles [1]. In general, failure of implantation appears to be one of the most important contributors to decreased ART success [2].

Implantation consists of the apposition, adhesion, and invasion phases; all of these steps are regulated by molecules derived from embryonic and maternal sources. Several molecules, such as integrin, L-selectin ligand, and leukemia inhibiting factor, have been demonstrated to play a role in the initiation and continuation of implantation. Glycodelin is one of the most important of these molecules [3]. Glycodelin is secreted from endometrial glands and, to a lesser degree, the salpinx, hematopoietic cells and ovaries [4]. In normal menstrual cycles, glycodelin concentrations are low during ovulation, and the highest levels are observed at the end of the luteal phase. The peak glycodelin serum level occurs six to eight days after the progesterone peak [5], and in cycles that result in conception, plasma glycodelin rises rapidly after implantation [6].

It is believed that glycodelin has a particularly important impact on the implantation phase [7] and may play a role in endometrial receptivity [8]. However, in a study by Bentin-Ley et al., endometrial flushing fluid glycodelin concentrations measured on the first day after the luteinizing hormone (LH) peak in normal menstrual cycles, could not predict pregnancy in the subsequent in-vitro fertilization (IVF) cycle [9]. In contrast, patients with lower serum glycodelin levels in the normal menstrual cycle had higher pregnancy rates in the subsequent ovarian stimulation-IVF cycle [10]. Although pregnancy prediction was not examined, serum glycodelin levels in long gonadotropin-releasing hormone (GnRH)-agonist IVF cycles were significantly lower up to the seventh day of the spontaneous cycle. After the seventh day, the serum glycodelin in both stimulated and non-stimulated cycles reached equally low values [5]. However, circulating concentrations of glycodelin reach a nadir in the peri-ovulatory phase in both stimulated and non-stimulated cycles; therefore, the pattern of serum glycodelin changes during the follicular phase in natural and long GnRH-agonist
IVF cycles appears to be similar, although the concentration is lower in GnRH-agonist cycles.

Based on these data, it is likely that serum glycodelin plays a role in the development of pregnancy in natural and GnRH-agonist IVF cycles. In contrast, some evidence suggests that GnRH-antagonists may have detrimental effects on endometrial receptivity and embryonic implantation, which may contribute to the decreased pregnancy rate in GnRH-agonist cycles [11]. Controversies regarding the endometrial receptivity differences between GnRH-agonist protocols, GnRH-antagonist protocols, and natural cycles still remain [3]. However, there have been no studies investigating the glycodelin pattern in GnRH-agonist ART cycles; therefore, glycodelin pattern changes in GnRH-agonist cycles may be one of the causes of the differences between GnRH-agonist and antagonist cycles.

Glycodelin is also synthesized by the granulosa and theca cells, released in follicular fluid (FF) and taken up by cumulus cells [12]. In natural conception cycles, glycodelin regulates the functional competence of spermatozoa [13]. To date, the effect of follicular fluid glycodelin levels on the success of ART cycles has not been studied.

The aim of the present study was to establish the serum concentration pattern for glycodelin in GnRH-agonist ART-intracytoplasmic sperm injection (ICSI) cycles and to investigate the possible correlations between serum and follicular glycodelin and clinical pregnancy in these treatment cycles. To the authors’ knowledge, no studies have investigated the role of follicular phase serum glycodelin levels and follicular fluid-glycodelin levels in GnRH-agonist cycles.

Materials and Methods

This prospective cohort study was performed between June 2011 and May 2012 at the Eskisehir Osmangazi University, Medical Faculty-Center for Reproductive Health. The study was approved by the Ethical Review Board of the hospital. Informed consent was obtained from all patients prior to participation in the study. To ensure that only couples with male-factor infertility were included in the study, females were examined to rule out female-factor infertility. To identify pathologies that may have caused subfertility, patients were evaluated with a pelvic examination and a patient history. In addition, no additional endocrine disorders or endometriosis were detected. To exclude anovulation, all women were determined to have a progesterone level of > 3 ng/ml on day 21 of their menstrual cycle. Day 3 basal ultrasonography and hormone profiles of all women demonstrated the following: follicle stimulating hormone (FSH) < 10 mIU/ml, estradiol <40 pg/ml, and an antral follicle count (AFC) > 6. However, although we ruled out probable endometriosis with the absence of specific signs/symptoms for endometriosis and with the presence of a normal gynecological pelvic examination, the authors did not perform routine laparoscopy (the gold standard in the diagnosis of endometriosis) in the diagnostic work-up of the study patients. They also did not detect any findings on ultrasonography that may be associated with endometriosis or other gynecological pathologies. Furthermore, only women who never smoked were included because any amount of smoking could affect the results. Women who smoked or had findings compatible with endometriosis, anovulation, polycystic ovarian syndrome, chronic pelvic inflammation, or diminished ovarian reserve were excluded from the analysis.

A total of 80 couples admitted for ART-ICSI treatment who met the World Health Organization (WHO) criteria [14] for primary infertility due to severe oligoasthenoteratospermia [severe oligospermia (< 5 million sperm/ml), asthenospermia (< 5% progressive motility), or teratospermia (< 4% normal forms by strict criteria)] were selected for the study.

A total of 80 females received controlled ovarian hyperstimulation (COH) with a fixed-dose GnRH-agonist protocol. As soon as menstruation began, ovarian stimulation was initiated with 150-250 U/day of recombinant FSH (rec-FSH). On the sixth day of stimulation, 0.25 mg of cetrorelix was started and was continued until the administration of human chorionic gonadotropin (hCG). Recombinant hCG was administered when ≥ three follicles grew to ≥ 17 mm, and oocyte pick-up (OPU) was performed under sedation in the 36th hour after hCG was administered. On the oocyte-retrieval day, sperm were collected in a sterile plastic container from males who had refrained from ejaculation for three to five days prior to the procedure. Following sperm preparation using the density-gradient method, the ICSI procedure was performed with sperm that were selected by embryologists two to three hours after OPU. At 16-18 hours, the fertilization-two pronucleus check was conducted on embryos that were developed in cleavage stage medium, and embryos were observed until the third day after OPU. On the third day, embryologists performed controls and selected one Grade I embryo, classified according to the European Society for Human Reproduction and Embryology (ESHRE) consensus, for transfer [15]. All transfers were performed under ultrasonography using a soft transfer catheter.

Serum hCG levels were measured in all patients on the 14th day after embryo transfer. Patients were considered pregnancy-positive if their hCG level was >50 IU/L. In patients with a positive initial hCG measurement, a second assessment was performed at 48 hours to ensure a two-fold increase in hCG. Transvaginal ultrasonography was performed three weeks later in patients who exhibited an increase in hCG, and if fetal structures and fetal cardiac activity were observed, the patient was considered to be clinically pregnant.

On day 2-3, blood serum samples were obtained. In addition, FF and blood serum samples were simultaneously obtained from 80 women during the OPU procedure. FF samples from the leading follicles were collected in each woman and pooled. Only the visually blood-free FF samples were included in the analysis. FF and serum samples were immediately centrifuged at 1,000 g for 15 minutes, and the supernatants were collected and stored in tubes at -20 °C until they were needed for the assays. Glycodelin was measured with a glycodelin enzyme-linked immunosorbent assay (ELISA) kit. The intra-assay CV was <8%, the inter-assay CV was <10%, and the minimum detectable level of glycodelin was typically less than 7.8 pg/ml.

This study was statistically evaluated using SPSS IBM 20. For all variables, the Shapiro Wilk test was used for normality. For normally distributed variables, the paired sample t-test and independent sample t-tests were used; the mean ± the standard deviation values were reported. The Wilcoxon signed ranks test and the Mann Whitney test were used to evaluate non-normally distributed variables, and the median (25%-75%) percentiles were reported.
Results

The clinical characteristics of 80 couples with male-factor infertility are summarized in Table 1. No significant differences were noted in the women’s age, BMI, duration of infertility, day 3 FSH-LH-estradiol levels or total AFC between couples who had a clinical pregnancy and those who did not have a clinical pregnancy. The clinical pregnancy rate was 25%.

The median value of cycle day 2-3 serum glycodelin for all patients was 1.61 (1.13-2.13) ng/ml. The median value was 1.96 (1.40 - 2.66) ng/ml for the group in which pregnancy occurred and 1.53 (1.09 - 2.0) ng/ml for the group in which pregnancy did not occur. Although the glycodelin level was higher for the group in which pregnancy occurred, the difference was not significant ($p > 0.05$, Table 2).

The median value of the OPU-day serum glycodelin for all patients was 4.49 (2.47 - 10.02) ng/ml. The median value was 5.24 (2.92 - 11.95) ng/ml for the group in which

Table 1. — Characteristics of the pregnant and non-pregnant patients.

<table>
<thead>
<tr>
<th></th>
<th>Clinically pregnant (n=20)</th>
<th>Clinically non-pregnant (n=60)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.85±3.77</td>
<td>31.0±5.01</td>
<td>0.489</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.45±4.74</td>
<td>25.50±4.07</td>
<td>0.958</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.91±2.83</td>
<td>5.76±2.41</td>
<td>0.819</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>4.42±3.10</td>
<td>4.41±2.18</td>
<td>0.977</td>
</tr>
<tr>
<td>E₂ (pg/ml)</td>
<td>35.03±15.13</td>
<td>46.21±43.91</td>
<td>0.270</td>
</tr>
<tr>
<td>Duration of Infertility (years)</td>
<td>9.02±5.67</td>
<td>7.64±5.66</td>
<td>0.347</td>
</tr>
<tr>
<td>AFC total</td>
<td>12.6±6.95</td>
<td>12.7±6.86</td>
<td>0.955</td>
</tr>
<tr>
<td>Unexplained infertile couples (n)(%)</td>
<td>11-27.5</td>
<td>29-72.5</td>
<td></td>
</tr>
<tr>
<td>Male factor infertile couples (n)(%)</td>
<td>9-22.5</td>
<td>31-77.5</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation (SD)

Abbreviations: BMI: Body Mass Index; FSH: follicle stimulating hormone; LH: luteinizing hormone; AFC: antral follicle count.

$p$-value < 0.05 was considered statistically significant.

Table 2. — Cycle characteristics, ART outcomes, and glycodelin concentrations of patients.

<table>
<thead>
<tr>
<th></th>
<th>All cases (n=80)</th>
<th>Clinically pregnant (n=20)</th>
<th>Clinically non-pregnant (n=60)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation duration (days)</td>
<td>8.57 (7.0-10.0)</td>
<td>9.0 (7.25-10.0)</td>
<td>8.0 (7.0-9.0)</td>
<td>0.233</td>
</tr>
<tr>
<td>Gonadotropins used (IU)</td>
<td>2541.56 (1800-3131.25)</td>
<td>2362.50 (1781.25-3775.0)</td>
<td>2312.50 (1800-3018.75)</td>
<td>0.498</td>
</tr>
<tr>
<td>Total oocytes retrieved (n)</td>
<td>10.0 (5.0-15.75)</td>
<td>11.0 (6.25-17.0)</td>
<td>9.50 (5.0-15.0)</td>
<td>0.449</td>
</tr>
<tr>
<td>Serum glycodelin on day 2-3 (ng/ml)</td>
<td>1.61 (1.13-2.13)</td>
<td>1.96 (1.40-2.66)</td>
<td>1.53 (1.09-2.0)</td>
<td>0.060</td>
</tr>
<tr>
<td>Serum glycodelin on OPU-day (ng/ml)</td>
<td>4.49 (2.47-10.02)</td>
<td>5.24 (2.92-11.95)</td>
<td>4.28 (2.20-8.59)</td>
<td>0.250</td>
</tr>
<tr>
<td>Follicular fluid glycodelin (ng/ml)</td>
<td>6.40 (4.22-8.84)</td>
<td>6.47 (4.04-9.52)</td>
<td>6.40 (4.22-8.84)</td>
<td>0.996</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>%72</td>
<td>%73</td>
<td>%71</td>
<td>0.990</td>
</tr>
</tbody>
</table>

Data are expressed as median (25-75%). Abbreviations: OPU: oocyte pick-up. $p$-value < 0.05 was considered statistically significant.

Figure 1. — Median serum glycodelin levels during different phases of GnRH-antagonist ART cycle. In all patients ($p < 0.001$), clinically pregnant ($p < 0.001$), and non-pregnant ones ($p < 0.001$) serum glycodelin increased significantly during the follicular phase.
pregnancy occurred and 4.28 (2.20 - 8.59) ng/ml for the group in which pregnancy did not occur; however, this increase was not significantly different ($p > 0.05$, Table 2).

The day 2-3 and the OPU-day serum glycodeolin concentrations for all patients were 1.61 (1.13 - 2.13) and 4.49 (2.47 - 10.02), respectively ($p < 0.001$). The median values were 1.96 (1.40 - 2.66) and 5.24 (2.92 - 11.95) ($p < 0.001$) for patients who became pregnant, and 1.53 (1.09 - 2.0) and 4.28 (2.20-8.59) ($p < 0.001$) for patients in which pregnancy did not occur (Figure 1).

The median value of glycodeolin in the FF collected on the OPU-day for all patients was 6.40 (4.22 - 8.84) ng/ml. The median value was 6.47 (4.04 - 9.52) ng/ml for the group in which pregnancy occurred and 6.40 (4.22 - 8.84) ng/ml for the group in which pregnancy did not occur. The FF glycodeolin level was slightly higher in patients who became pregnant than in patients who did not; however, the difference was not significant ($p > 0.05$, Table 2).

**Discussion**

In all patients, whether pregnancy occurred or not, serum glycodeolin pattern in GnRH-antagonist controlled cycles was totally different from the natural cycles [10] and the GnRH-agonist controlled cycles [5]; OPU-day serum glycodeolin was found to be significantly higher than early follicular serum glycodeolin level (Figure 1). Additionally, there were no significant differences in serum glycodeolin concentrations in either the early follicular phase or the pre-ovulatory phase between clinically pregnant and non-pregnant patients. In addition, there were no differences in the follicular fluid glycodeolin concentrations between the two groups. Therefore, the serum or follicular fluid glycodeolin levels were not predictive of clinical pregnancy in GnRH-antagonist cycles.

The ability of normal menstrual cycle serum glycodeolin levels to predict pregnancy in IVF cycles was evaluated by Westergaard et al. in a study of 19 patients [10]. The authors found that for all of the measurements in various phases of the menstrual cycle, including the early follicular phase, patients with low glycodeolin levels exhibited highest pregnancy rates in the subsequent IVF cycle. As stated in the present results, at the beginning of the cycle (day 2-3), serum glycodeolin concentrations were higher in patients who became pregnant with the GnRH-antagonist treatment cycle; however, this difference was not statistically significant (1.96 ng/ml vs. 1.53 ng/ml) ($p = 0.060$). Although the present authors did not make serial measurements during the three normal menstrual cycles as did Westergaard et al. [10], they evaluated the results of 80 patients at the beginning of the GnRH-antagonist ICSI cycle during the unmedicated period (day 2-3). Therefore, the present findings contradict the widely held belief that low serum glycodeolin concentrations in the early follicular phase are correlated with a higher probability of pregnancy. Additionally Westergaard et al. reported that glycodeolin peaked in the early follicular and late luteal phases during the ovarian stimulation cycle, as well as in natural cycles, and that the levels were lowest in the late follicular and ovulation phases [10]. Again contrary to these findings in literature, median OPU-day glycodeolin levels were higher than the median day 2-3 glycodeolin levels in all patients (4.49 ng/ml vs. 1.61 ng/ml; $p < 0.001$), in clinically pregnant (5.24 ng/ml vs. 1.96 ng/ml; $p < 0.001$) and non-pregnant ones (4.28 vs. 1.53 ng/ml; $p < 0.001$) in the present study (Figure 1). Furthermore, glycodeolin does not decrease towards mid-cycle in GnRH-antagonist cycles as in natural cycles; on the contrary, it increases. So it is probable that serum glycodeolin pattern in GnRH-antagonist cycles may not be same as in natural cycles and/or in non-controlled (with GnRH-agonist or GnRH-antagonist) IVF cycles, and GnRH-antagonist may effect serum glycodeolin in a totally different way.

In another study with 51 patients who underwent long GnRH-agonist protocol ART-IVF treatments, Bersinger et al. demonstrated that, up to seven days prior to the LH peak, the glycodeolin was much lower than in patients in natural cycles. Furthermore, they found that the lowest glycodeolin level, measured on the OPU-day, was similar to the natural cycle LH peak phase [5]. To the present authors’ knowledge, there are no studies in the literature that investigate the glycodeolin pattern in GnRH-antagonist cycles or its ability to predict clinical pregnancy. Based on the present results, the OPU-day glycodeolin level was higher than the day 2-3 level in patients who underwent ART-ICSI cycles with a fixed-dose GnRH-antagonist protocol (4.49 ng/ml vs. 1.61 ng/ml, $p < 0.001$). These data may contradict other reports in the literature because of the differences in the protocols. Furthermore, glycodeolin does not decrease towards mid-cycle in GnRH-antagonist cycles as in GnRH-agonist cycles; instead, it increases. Additionally, OPU-day serum glycodeolin levels were slightly higher in patients who became clinically pregnancy after treatment; however, the difference was not statistically significant (5.24 ng/ml vs. 4.28 ng/ml, $p = 0.250$). According to the present results, it is probable that serum glycodeolin pattern in GnRH-antagonist COH cycles may be totally unlike from GnRH-agonist controlled COH cycles.

Currently, conducting an ART cycle with a GnRH-antagonist is a more patient-friendly method for initiating ovarian stimulation and provides physicians with a possible alternative to GnRH-agonist treatment. Additionally the emergence of GnRH-antagonists in ART cycles has enabled the development of milder ovarian stimulation protocols that may have a reduced impact on endometrial receptivity [16]. However, concerns remain regarding the possible detrimental effects of GnRH-antagonists on endometrial receptivity [17, 18]. Therefore, although the authors did not study endometrial receptivity, the different serum glycodeolin patterns in GnRH-antagonist cycles and the natural and GnRH-agonist cycles may be very important in this re-
Follicular phase serum and follicular fluid glycodelin measurements in gonadotropin-releasing hormone (GnRH)-antagonist assisted etc.

References


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Higher abnormal fertilization, higher cleavage rate, and higher arrested embryos rate were found in conventional IVF than in intracytoplasmic sperm injection

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Summary
Objective: The aim of this study was to investigate whether performing different fertilization technologies (intracytoplasmic sperm injection [ICSI] and in vitro fertilization [IVF]) may affect the result of fertilization in the normal fertilization cycles. Study design: The authors performed a retrospective analysis of 164 cycles using sibling oocytes in combined IVF/ICSI with achieved a normal fertilization (≥ 25%) both conventional IVF and ICSI in this infertility centre. Results: It was found that there were no differences in 2PN rate (70.25% vs 70.60%), but higher cleavage rate in ICSI than IVF insemination (98.99% vs 96.81%), higher arrested embryos rate in IVF than ICSI in 2PN group (20.00% vs 13.95%), and higher abnormal fertilization 1PN (3.87% vs 1.92%) and 3PN (3.63 vs 0.854%) in IVF than ICSI. Conclusion: there were some differences fertilization outcomes between ICSI and IVF, which may be related to different procedures between two techniques.

Key words: IVF; ICSI; Embryos cleavage; Arrested embryos; 1PN.

Introduction
In assisted human reproduction, there are two major technologies: conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [1]. Conventional IVF procedure, designed mainly to deal with female infertility, involves mixing individual eggs in a petri dish with spermatozoa in overnight incubator, in which sperm-egg fertilization will occur. ICSI is the procedure for injecting a selected sperm cell into the middle of an egg by micro-injection system. ICSI is being used mostly for two indications: severe male-factor infertility and failed or low fertilization in conventional IVF previously.

There are several differences between the two kind’s fertilization technologies. ICSI is more invasive than IVF, and oocytes degeneration is a common phenomenon after performing ICSI [2]. During IVF, the more immature oocytes underwent maturation, and have an opportunity to fertilization with spermatozoa in overnight incubator [3]. During IVF, sperm-eggs fertilization is more natural and random, compared with ICSI. Therefore, can the aforementioned different procedures between ICSI and IVF affect the fertilization outcome? To address the issue, excluding the impact of fertilization failure, the present authors performed a retrospective analysis of 164 cycles using sibling oocytes in combined IVF/ICSI with achieved a normal fertilization (≥ 25%) both conventional IVF and ICSI (January 2011 and June 2012) in the present infertility centre.

Materials and Methods
Patients
Between January 2011 and June 2012, 164 cycles using sibling oocytes in combined IVF/ICSI with a normal fertilization (≥ 25%), both conventional IVF and ICSI, were considered for the study in the infertility centre. Patients’ age ranged from 22 to 43 years, with a mean (±SD) of 32.30 ± 3.9 and main causes of infertility were tubal (n = 66), unexplained infertility (n = 11), endometriosis (n = 5), ovulatory dysfunction (n = 22), uterus (n = 4), and male factor (n = 56). All patients signed written informed consent. Only the first cycle’s therapies were included in the current study.

Ovarian stimulation
Patients were down-regulated with gonadotropin releasing hormone (GnRH) agonist using long or short protocols and stimulated with follicle stimulating hormone (FSH) and human menopausal gonadotropin (hMG). Ovarian activity was monitored by regular ultrasound scans and serum sex hormone assays. A dose of 10,000 U of human chorionic gonadotropin (hCG) was administered when the leading cohort follicles reached a diameter of 18 to 20 mm. Oocyte retrieval was performed through vaginal puncture under ultrasound guidance 36-38 hours after hCG.
Table 1. — Fertilization, cleavage, and embryos results after the 164 cycles using sibling oocytes in combined IVF/ICSI with fertilization rate ≥ 25% obtained both IVF and ICSI.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2PN</th>
<th>ICSI</th>
<th>0PN</th>
<th>ICSI</th>
<th>1PN</th>
<th>ICSI</th>
<th>(≥3PN)</th>
<th>ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocytes (%)</td>
<td>70.25</td>
<td>70.60</td>
<td>16.99</td>
<td>15.94</td>
<td>3.87 b</td>
<td>1.92</td>
<td>3.63 c</td>
<td>0.854</td>
</tr>
<tr>
<td>Cleavage (%)</td>
<td>96.81 a</td>
<td>98.99</td>
<td>29.54</td>
<td>26.34</td>
<td>73.44</td>
<td>81.48</td>
<td>98.33</td>
<td>91.66</td>
</tr>
<tr>
<td>Arrested embryos (%)</td>
<td>20.00 b</td>
<td>13.95</td>
<td>61.45</td>
<td>67.80</td>
<td>65.96</td>
<td>63.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Available embryos (%)</td>
<td>98.44</td>
<td>97.40</td>
<td>84.38</td>
<td>78.95</td>
<td>81.25</td>
<td>75.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good-quality embryos (%)</td>
<td>73.70</td>
<td>75.21</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
| Data were analysed using the chi-squared test. All analyses were performed with Statistical Package for Social Sciences version 17.0 (SPSS). Statistical significance was defined as \( p < 0.05 \).}
final fate for those “rescued” oocytes during IVF? The Palermo study [5] showed that oocytes with sudden breakage membrane was lower number of cleaved embryos compared to the other two membrane patterns (successful injection and difficult breakage) that resulted a similar cleavage ability. Therefore, it is may be that the oocytes tending to degenerate may have had a lower cleavage rate. In the current study, 2PN embryo cleavage rate was lower in IVF than ICSI, and it is probable due in part to the fact that the more oocytes, which would degenerate after ICSI, were “temporarily rescued” by performing IVF. Moreover, during IVF, the more immature oocytes underwent maturation, and had an opportunity to be fertilized with spermatozoa in overnight incubator; it is therefore difficult to study the developmental potential of in vitro matured oocytes during IVF. However, there were some studies addressing in vitro matured oocytes in ICSI cycles, which may give some explanation [6-8]. Balakier et al. found that the cleavage rate was lower in in-vitro matured oocytes compared with MII oocytes at denudation [6]. Shu et al. and Strassburger et al. found that in vitro matured oocytes had higher percentages of arrested embryos than in vivo matured oocytes [7, 8]. Based on the above results, it is possible that the number of in vitro matured oocytes obtain normal fertilization during IVF than ICSI, so it would result in a lower cleavage rate and in more arrested embryos during IVF than ICSI. The results of the present study agree with the above assumption, as it was demonstrated that there was a lower cleavage rate and more arrested embryos in IVF compared with ICSI. All in all, IVF have a lower cleavage rate and the more arrested embryos compared with ICSI, probably partly due to the mode of fertilization.

There are no differences in 0PN cleavage rate between IVF and ICSI (29.54% vs 26.34 %), which suggest that some oocytes fail in fertilization in the normal cycles due to intrinsic oocyte factors and not due to whether the sperm enters the egg.

In the current study, the notable finding was that the 1PN rate was significantly higher in IVF insemination than ICSI (3.87% vs 1.92%), which is however difficult to explain. There were some suggested mechanisms for the appearance of a 1PN zygote, including parthenogenetic activation, asynchronous development of pronuclei, failure of either male or female pronucleus formation, and “pronuclear fusion” [9, 10]. Nagy et al. found that a higher proportion of oocytes develop two pronuclei asynchronously after IVF than after ICSI [11]. They explained that there was a much greater time-span in IVF oocytes between the formation of the male pronucleus and the formation of the female pronucleus, compared with ICSI [11]. It was perhaps because, with ICSI, there were not only sperm factors present in the oocyte to initiate activation, but also a mechanical stimulus in itself. While in IVF oocytes this process was initiated only by the sperm factors, which might require a longer time to trigger the same effect. Hence, the higher 1PN rate in IVF insemination than ICSI was related to a higher proportion of oocytes developing two pronuclei asynchronously after IVF than after ICSI. Another possibility is the procedures of formation of “pronuclear fusion”. Male nuclear envelope and female nuclear envelope were formed just in the same area at the same time. So a common membranous envelope was formed to surround the male or female pronucleus [12]. Levron et al. suggested that it may occur when a spermatozoon enters or is deposited very close to the oocyte spindle [12]. However during ICSI, the sperm is injected away from the meiotic spindle and there is a less chance of “pronuclear fusion” phenomenon occurring, which partly resulted in the more 1PN occurring after IVF, as compared to after ICSI.

In the present study, 3PN rate was significantly higher in IVF than ICSI (3.63% vs 0.854%), which is easy to explain. Among the several mechanisms giving rise to triploid zygotes, dispermy is recognized to be the most common, which was overcome by performing ICSI. Jun et al. suggested performing ICSI in patients with a high incidence of triploidy in prior IVF cycles [13].

All in all, higher cleavage rate in ICSI than IVF insemination and higher arrested embryos rate in IVF than ICSI in 2PN group and higher abnormal fertilization (1PN or 3PN) in IVF than ICSI may be partly related to different procedures between ICSI and IVF.

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Case Reports

The confounding effect of the development of idiopathic orthostatic edema and thyrotoxicosis on weight fluctuation related to effects on free water clearance in a woman with long-standing surgically induced panhypopituitarism and diabetes insipidus

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Summary

Purpose: To evaluate the effect of idiopathic orthostatic edema and the effect of thyrotoxicosis on weight fluctuation and fluid retention in the presence of surgically induced panhypopituitarism and diabetes insipidus controlled with hormone replacement. Materials and Methods: Dextroamphetamine sulfate was used for weight gain when no other etiologic factor was found. Methimazole was used when weight loss occurred when serum T4 and free T4 indicated thyrotoxicosis. Results: Sympathomimetic amine therapy very effectively controlled the weight gain and methimazole controlled the weight loss. Conclusions: Hypopituitarism and diabetes insipidus controlled with hormone replacement do not protect against fluid retention from idiopathic edema.

Key words: Sympathetic hypofunction; Diabetes insipidus; Thyrotoxicosis; Panhypopituitarism; Weight loss.

Introduction

There are certain endocrine disorders that are associated with weight gain, related to an inability to adequately clear free water. Though the condition known in the past as idiopathic orthostatic edema is associated with secondary hyperaldosteronism, the main mechanism for edema and subsequent weight gain is the increased capillary permeability associated with the increase in hydrostatic pressure associated with standing [1]. The increase in capillary permeability seems to be related to hypofunction of the sympathetic nervous system, as evidenced by significant improvement in edema and weight loss following the treatment with the sympathomimetic amine dextroamphetamine sulfate [2]. Evidence that the secondary hyperaldosterone plays only a minor role was shown in the same study by finding only a mild decrease in weight with spironolactone over a six-month period and yet these patients had a much more profound effect during the second six months with dextroamphetamine sulfate [2].

Thyroid hormone is a sympathomimetic amine. Weight gain and fluid retention are also believed to be related to increased capillary permeability when hypothyroidism is present, and weight loss by the use of thyroid hormone to improve weight gain has a similar mechanism to dextroamphetamine sulfate for idiopathic orthostatic edema, i.e., correcting the capillary permeability defect. Because hypofunction of the sympathetic nervous system and the resulting increase cellular permeability leads to many other pathological states, especially pain syndromes and muscle fatigue and motility defects, the condition has been renamed the sympathetic neural hyperalgesia edema syndrome [3].

The case presented here describes weight and fluid changes in a woman with secondary hypothyroidism with appropriate thyroid hormone replacement and diabetes insipidus adequately controlled with DD arginine vasopresion (DDAVP) when she developed idiopathic orthostatic edema followed subsequently with thyrotoxicosis related to Grave’s disease.
Case Report

A 14-year-old girl who was slowly losing vision bilaterally was diagnosed with an optic glioma and underwent craniotomy and six weeks of radiation treatment. Her pituitary gland was injured, whether by the tumor or subsequent treatment. At that time, it was noted that her cortisol level was low to normal. She presented to us soon after for primary amenorrhea. She was diagnosed with hypogonadotropic hypogonadism and started on conjugated estrogen and medroxyprogesterone acetate, which brought on normal periods.

At age 27, she underwent a diagnostic laparoscopy which revealed a blockage in one fallopian tube. At this time, her weight was stable at 49 kg. The following year, she gained two kg over a few months despite dieting and exercising three times a week. She was started on hydrocortisone ten mg BID for persistently low cortisol and synthroid 0.05 mg daily. Over the next one to two years, she continued to gain weight, reaching 76 kg maximum. Now, at age 29, she was diagnosed with idiopathic edema and started on dextroamphetamine sulfate. She lost the first 3.6 kg over four months. She was also diagnosed with diabetes insipidus, which could usually be controlled with fluid restriction, although she used DDAVP when necessary.

Two years later, at age 32, she started IVF after completing 12 cycles of human menopausal gonadotropins, which had only resulted in one ectopic pregnancy in her only patent tube. After some failed cycles and two miscarriages, she delivered a child at age 35. She had stopped all medications during the pregnancy and expected significant weight gain, but only gained 15 kg before delivery. She was back down to her normal weight three weeks later. Within two weeks, however, she started to retain fluid again. She restarted DDAVP and dextroamphetamine sulfate ten mg BID. She continued with these medications plus prednisone, in lieu of hydrocortisone, L-thyroxin, and estrogen/progesterone replacement. She continued regular follow-ups and had small fluctuations in weight, with the most significant weight gain in her late 40s.

At the age of 55, she had an acute exacerbation of the idiopathic edema, suddenly gaining eight kg over one week, with extreme fluid retention in the legs and feet. Her weight at this time was close to 68 kg. Increasing dextroamphetamine sulfate from ten mg BID to 15 mg BID, she lost most of the weight over the next two months. The medication was subsequently decreased to 15 mg daily.

Less than a year later, she began losing a significant amount of weight. She went down to 46 kg. It was thought that she might be in a remission with the idiopathic edema. Decreasing the dextroamphetamine sulfate to ten mg daily had no effect, so additional thyroid studies were sought. Serum thyroxine (T4) and free T4 were both high, and thyroid stimulating immunoglobulins were also high. Thyroid imaging with uptake revealed a normal- sized thyroid with increased two- and 24-hour thyroid uptakes, consistent with Grave’s disease. Methimazole was started at this time.

Currently, her TSH and T4 are checked every three weeks and thyroid medications adjusted as needed. She continues prednisone 7.5 mg daily, DDAVP, and dextroamphetamine sulfate ten mg daily. She is at her normal weight with minimal fluid retention.

Discussion

In this woman’s case her diabetes insipidus was caused by the absence of the posterior pituitary hormone vasopressin. The symptoms are polyuria and polydipsia untreated but she was reasonably well-controlled with DDAVP with her being able to sleep most of the night without drinking. This study shows that correcting diabetes insipidus does not protect against the development of severe edema and weight gain from the sympathetic neural hyperalgesia edema (idiopathic orthostatic edema) syndrome. The marked weight loss in a short time with thyrotoxicosis shows that the weight loss is possibility also related to loss of fluid. It is not clear if the quick marked weight loss in a short time may have been related to the concomitant presence of diabetes insipidus.

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Late diagnosis of positive HIV serology in pregnancy incidentally discovered by the widespread appearance of Kaposi’s sarcoma

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Summary
The authors report a case of Kaposi’s sarcoma (KS) found in a pregnant woman. On discovery, the condition had spread throughout her body as is characteristic in some cases of individuals with HIV-positive serology. She was unaware of her HIV positive status. Her HIV infection had been diagnosed at the same time as KS at her last prenatal consultation. The newborn was delivered by an uncomplicated cesarean section. Appropriate treatment and multidisciplinary management after childbirth resulted in complete remission.

Key words: Kaposi’s sarcoma; Pregnancy; HIV.

Introduction
Initially described by the Viennese dermatologist Moritz Kaposi, Kaposi’s sarcoma (KS) is a tumorous disease, multifocal in nature, and characterized by excessive proliferation of endothelial and fibroblast cells [1-4]. KS can be primarily categorized into four types: the epidemic of AIDS-related type, described in young homosexual men [5, 6]; the immunocompromised, and the classic or sporadic variant, and the endemic form, called African KS especially observed in Equatorial Africa [1, 7]; This latter form causes a chronic infection and death typically occurs within ten to 15 years of initial infection [8]. It is characterized by skin lesions, mainly localized in the lower extremities. They can be more aggressive with mucocutaneous and visceral spread requiring systemic treatment [9]. Kaposi disease (KD) in pregnancy is often observed in the immunocompromised, and is uncommon because of its sensitivity to chorionic gonadotropin hormones (CGH) [10].

The authors describe a case, rarely depicted in the literature, of a more extensive presentation of KD, widespread throughout the body of an HIV-1-positive pregnant woman.

Case Report
The patient was 30-years-old and carrying a poorly monitored pregnancy estimated at eight months gestation. She was seen for a generalized skin rash associated with frequent episodes of diarrhea. Physical examination revealed a well appearing woman with widespread skin lesions characterized as macula in nature (Fig. 1) and accompanied by prurigo. The obstetrical examination and ultrasound were within normal limits. Other findings included a positive serology for HIV-1, a viral load of 500 cells / ml, CD4 count 100/mm3, and moderate anemia of nine g/dl. The histological analyses of her skin lesions were in favor of KS with foci of spindle cells, evidence of neovascularisation, and an inflammatory infiltrate consisting of predominantly of CD8+ cells. A planned cesarean delivery resulted in the birth of an apparently well-appearing newborn (Apgar score 8 and 10), weighing 3,100 grams. A postoperative course without acute incidents enabled her transfer to a dermatological center on the 5th postoperative day for better management of her skin condition. Cryotherapy and monochemo-therapy with vincristine was performed and complete remission (CR) was achieved after three months.

Discussion
KD is relatively uncommon, more often seen in immunocompromised men than women. The association with HIV was first described in homosexual men (> 30%) [10]. Very few publications describe KD in pregnant women. This case is the first reported in decades in this country. The risk of developing KD is higher in HIV-infected persons [11] and usually occurs in young subjects (sex ratio: 8/1). Specifically, there is a 20,000-fold increase in the risk of developing KS in people with AIDS compared to the general population, and a 300-fold increase in the same risk in the HIV-immunocompromised versus persons immunocompromised from other causes. Consequently, 15% of patients with AIDS develop KS.

The incidence of HIV infection remains a major concern in poor countries because it affects more the female
population. The vulnerability of women is linked to their low socio-economic conditions, cultural practices, and high-risk behaviors, such as polygamy, and poor hygiene, respectively.

KD is an AIDS-related opportunistic infection with pregnancy further compounding the disease, leading to a more advanced state immunosuppression, which may explain widespread skin lesions (Figures 1 to 4). The very extensive nature seen in this case is more due to the immunosuppressed state of the person than the aggressiveness of the sarcoma itself. KD was diagnosed by histological analysis of the skin lesions (macula). Mucosal damage is infrequent [11]. Late diagnosis of HIV infection is related to the fact that the patient had not had any medical consultation and had as recourse only traditional treatments. She declined HIV screening in the first month of pregnancy. Refusal is often motivated by fear of stigmatization, and rejection by the spouse and family.

In this case, support was specific and multi-disciplinary. Due to the absence of initial antiretroviral treatment and a high viral load, a planned cesarean section was performed to decrease the maternal to neonatal risk of HIV transmission. The newborn was in good health (Apgar score 8 and 10).

ARV treatment was started immediately after birth in both mother and child, in parallel with that of KD. In the present country, there is no radiation therapy. So the authors prescribed, as Monelle Sone et al. [8], monochemothry with DTIC because of better tolerance, relatively low cost, and availability. Monochemothry with vincristine, velbe, VP16 or DTIC was used by many authors [8-11].
CR with complete disappearance of skin lesions was observed four months later. Olweny had observed nearly 90% of CR with combined-chemotherapy based on vincristine, actinomycin Dm and DTIC [12]. Mouelle Sone et al. [8] used combined-chemotherapy in critical cases. Immune therapy with interferon does not appear to play a role in the therapeutic protocols [8]. CGH treatment is still experimental but has already been discussed [13,14].

Maternal prognosis of KD is relatively good especially with a multi-disciplinary team management approach. During pregnancy, there was no fetal risk of contamination by KD, but the fetus is very exposed to HIV due to premature birth or inadequacy of ARV treatment, and high viral load (>400 copies / ml). In the present case, the infant was sent in a neonatology department where the classical assessment was normal.

KD in pregnancy is very infrequent. In case of late diagnosis of HIV infection, a cesarean delivery improved fetal prognosis. Maternal prognosis is generally better when treated early and appropriately.

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Rupture of maternal splenic artery aneurysm and fetal demise

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Summary
Splenic artery aneurysm (SAA) is the third most common intra-abdominal aneurysm. This condition, which occurs predominantly in young women, is generally asymptomatic and frequently discovered during pregnancy upon rupture. Reported maternal and fetal mortality are respectively 75% and 72.5-95%. A 40-year-old woman gravida 4 para 3 was referred to the obstetrical emergencies at term for loss of consciousness, nausea, vomiting, and hypotension. At admission, she had developed upper abdominal pain. Fetal demise and hemoperitoneum were diagnosed. An abdominal computed tomography (CT) scan revealed SAA rupture. An emergency hemostatic splenectomy was performed followed by a cesarean section with a favorable subsequent outcome. SAA rupture should be considered in the differential diagnosis of acute abdominal pain during pregnancy. Prompt multidisciplinary management is essential for patient’s survival.

Key words: Aneurysm rupture; Splenic artery; Pregnancy; Fetal demise.

Introduction
Splenic artery aneurysm (SAA) is the third commonest intra-abdominal aneurysm and the commonest visceral artery aneurysm (60%) [1-4]. This condition is generally asymptomatic and frequently discovered in women of childbearing age (58%) [1, 3, 5].

Because of the paucity of symptoms, the true prevalence of SAA is unknown in general population, but autopsy reports mention a rate of 0.01% to 10.4% [2]. In 69% of cases, it is detected during the third trimester of pregnancy and in 95% of cases upon rupture. Maternal and fetal mortality rates are estimated, respectively, 75% and 72.5-95% [1-4, 6-8]. These high mortality rates are probably explained by the minimal prodromal symptoms, acute deterioration after SAA rupture, and signs that mimic other common obstetric emergencies, such as placental abruption, uterine rupture, amniotic fluid embolization, and in early pregnancies, rupture of an ectopic pregnancy [7, 8].

Case Report
A 40-year-old woman gravida 4 para 3 was referred to the obstetrical emergencies of the Geneva University Hospitals (HUG) at 37 weeks of gestation for loss of consciousness, nausea, vomiting, and hypotension. At admission, she had developed upper abdominal pain, and signs that mimicked other common obstetric emergencies, such as placental abruption, uterine rupture, amniotic fluid embolization, and in early pregnancies, rupture of an ectopic pregnancy [7, 8].

Constrictions or vaginal bleeding and the uterine cervix was closed. She had no signs of preeclampsia, except presence of proteinuria. Fetal heart sounds were absent and fetal demise was confirmed upon ultrasound examination. Abdominal ultrasound revealed a massive hemoperitoneum. Hemoglobin level dropped from 120 g/l to 98 g/l with no other alterations in blood tests. Hemodynamic shock was diagnosed and resuscitation was conducted during those investigations with macromolecules and vasoactive amines. Given the patient’s hemodynamic shock and the unknown origin of the hemoperitoneum, an abdominal computed tomography (CT) scan was organized and showed a SAA rupture (Figures 1 and 2). An emergency xypho-pubic laparotomy was performed. At peritoneal opening, a hemoperitoneum of three liters was confirmed. A hemostatic splenectomy was performed to stabilize the patient hemodynamically, followed by a cesarean section, with extraction of a stillborn fetus weighing 3,100 g. Pre- and per-operative resuscitation efforts involved transfusion of eight units of packed red blood cells, seven fresh frozen plasma, cyklokapron and fibrinogen. After splenectomy and weaning from pressors, the patient remained stable. She was transferred to the intensive care unit for 48 hours, with magnesium sulfate administered for the first 24 hours because of suspicion of preeclampsia. Blood pressure and biological markers remained within normal range. Fifteen days after surgery, an infection of a splenic lodge collection was diagnosed. Introducing of empirical antibiotic therapy subsequently adapted to bacterial sampling from cultures (methicillin-sensitive Staphylococcus aureus) allowed the fluid collection to dissipate and the clinical status to improve. The patient was discharged on the 28th postoperative day. She underwent the usual post-splenectomy vaccinations and psychological follow-up was initiated for post-traumatic stress disorder and fetal loss. Histology of the surgical sample confirmed a rupture of a SAA, which was less than two cm, at splenic hilum level.

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Over 100 cases of SAA rupture during pregnancy have been described. The first recorded case was by Beussier in 1776 and the first favorable maternal outcome after treatment was reported by Mac Leod in 1940 [1]. Pregnancy is a known risk factor for SAA rupture. The risk of rupture during pregnancy is estimated at about 20% [6]. Two-thirds of ruptures happen during the third trimester, 13% during the second trimester, 13% per-partum, and 6% in the post-partum period [1, 2, 6, 9]. Prevalence is higher in women suffering from portal hypertension (7%), which increases the risk of rupture earlier on in the pregnancy [6].

Discussion

During pregnancy, fluctuations in estrogen, and progesterone levels alter arterial wall structure (intimal hyperplasia, interruption of the elastic lamina, and fibrous dysplasia of the media) [1]. Furthermore, splenic artery elasticity is reduced by increased secretion of relaxin hormone in the third trimester [1]. These combined effects stress the vessels wall. Pregnancy is accompanied by hemodynamic changes which add a mechanical component to rupture: increase in cardiac output and blood volume, relative portal congestion, and compression of iliac vessels and the aorta by the gravid uterus, increasing blood pressure in the upper abdomen [1]. The combined effects of these factors are cumulative with each successive pregnancy, increasing not only the incidence of SAA but also the frequency of rupture that is believed to grow as parity rises [1, 2].
A retrospective study by Ha et al. examined maternal characteristics and clinical presentations for these SAA. The median age was 28 years, the average number of pregnancies was 2.3, and the mean gestational age at rupture was 34.5 weeks. The average size of aneurysms was 2.25 cm, with more than half of ruptures occurring at aneurysm sizes less than two cm [1]. The most frequent location was the splenic hilum, as in the presented case. Most of the SAA were discovered upon rupture, with symptoms such as upper abdominal pain (epigastric or left hypochondrium) with dorsal or left shoulder irradiation (Kehr’s sign), nausea and vomiting, and hemodynamic instability or shock.

In 20-25% of cases, rupture is preceded by a pre-rupture syndrome: malaise, hypotension, and abdominal pain resolving spontaneously and occurring within 48 hours of aneurysmal rupture [3, 9]. Before admission, the present patient suffered from inaugural malaise without precipitating factor, with loss of consciousness, and no report of abdominal pain at home, upper abdominal pain appearing on arrival at the hospital. This first episode seems to correspond to the aneurysmal pre-rupture syndrome: the aneurysm ruptures in the omental cavity, creating malaise through sudden hypotension and is at first stemmed by blood clots or the omentum itself in the lesser sac, blocking the foramen of Winslow, and stabilizing the patient. After a latency period (from minutes to 72 hours), an increase pressure in the lesser sac leads to an intraperitoneal hemorrhage in the large omentum cavity with clinical ascertainment of a hemorrhagic shock (Figures 3 and 4). This phenomenon provides additional time for diagnosis and thus increases the chance of both maternal and fetal survival.

SAA rupture during pregnancy is a surgical emergency requiring prompt multidisciplinary management. Ha et al. showed that factors statistically significantly associated with increased maternal mortality were multiple aneurysms, hemodynamic instability, and absence of a general surgeon during laparotomy [1, 3, 6]. Hemodynamic instability and rupture outside of labor were predictive factors of high neonatal mortality, as in the present case.

In the present case, an abdominal CT-scan was performed before surgery in order to determine the location of the bleeding source. Radiological investigations could be considered a waste of time in the emergency of the situation [10], but the patient was still hemodynamically stable. Moreover, the obstetrical team had no reason to suspect uterine rupture or another obstetrical complication, and localizing the true origin of the bleed enabled the authors to justify a multidisciplinary surgical team. This decision is turn allowed for faster control of blood loss, by performing a xypho-pubic laparotomy, and splenectomy before fetal extraction.

Present recommendations differ as to whether propose elective aneurysmal resection surgery or embolization in cases of fortuitous discovery of an SAA over 2-2.5 cm [5, 11] in diameter in women of childbearing age or even under this threshold [12]. If the aneurysm is discovered during pregnancy, the optimal time to operate is in the second trimester, after embryogenesis [1, 13]. Elective surgery can be performed by laparoscopy or laparotomy, and mortality is less than 0.5-1.3% [14].

Conclusion

SAA rupture should be considered as a differential diagnosis in any pregnant woman presenting severe upper abdominal pain or hypovolemic shock. Early recognition and prompt multidisciplinary management are essential for both mother and fetus survival.

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The use of fresh frozen plasma for reproduction in severe factor V deficiency

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Summary

Objective: Severe factor V (FV) deficiency is rare. There are case reports describing pregnancy outcomes in women with FV deficiency and one case report of successful pregnancy following the use of fresh frozen plasma (FFP) in several cycles of ovulation induction and intrauterine insemination and at delivery. The authors report another case to support the use of FFP for reproduction. Case: A 27-year-old woman with severe FV deficiency was given FFP at the time of ovulation induced with clomiphene citrate, human menopausal gonadotropin (hMG), and human chorionic gonadotropin. Intrauterine insemination (IUI) was done 35 hours later. She became pregnant with twins and delivered vaginally at 36 weeks of gestation with the prophylactic use of FFP. Conclusion: Fresh frozen plasma can be offered for reproduction to women with severe FV deficiency.

Key words: Fresh frozen plasma; Reproduction.

Introduction

Factor V (FV; proaccelerin or labile factor) is an essential cofactor for the activation of prothrombin to thrombin [1]. Inherited FV deficiency is rare, with a prevalence of approximately one in 1,000,000 [2]. Type I (quantitative) deficiency can be severe, which is characterized by very low or unmeasurable levels of FV antigen and coagulant activity, with a moderate to severe bleeding phenotype [2]. Mild or moderate type I deficiency is characterized by FV plasma levels ≥ 20%, and approximately half of these patients are asymptomatic for bleeding episodes [1, 2]. Due to the rarity of this disorder and the small number of cases reported in the literature, information on its clinical presentation and management are limited [3,4]. With respect to FV deficiency in reproductive-aged women, there are case reports describing pregnancy outcomes in approximately 30 women [5], and one case report of successful pregnancy following several cycles of ovulation induction and intrauterine insemination [6]. The authors report an additional case, where a woman with severe FV deficiency delivered twins after prophylactic fresh frozen plasma (FFP) administration, ovulation induction, and intrauterine insemination (IUI).

Case Report

A 27-year-old Arab woman, born of consanguineous parents, was diagnosed with severe FV deficiency at the age of 14 years when she presented with acute abdomen at the time of ovulation. At that time, her FV antigen level was 5% of normal. Thereafter, she took oral contraceptives for 13 years to prevent recurrence of ovulation-related hemoperitoneum. After her marriage at age 20, the couple was counseled against pregnancy because of the associated risks; however, after seven years they desired to have children. The oral contraceptive was stopped. She was given two units of FFB at the time of spontaneous ovulation for two cycles, and after ovulation induction with clomiphene citrate 100 mg from day 2 of the cycle for five days for another two cycles. Subsequently, she was given clomiphene citrate 100 mg from day 2 of the cycle for five days, and human menopausal gonadotropin (hMG) 150 units on day 9 of the cycle. When the mature follicle reached 18 mm, 5,000 units of human chorionic gonadotropin (hCG) intramuscularly was given with the FFP. IUI was done 35 hours later. She became pregnant with twins and delivered vaginally at 36 weeks of gestation with the prophylactic use of FFP. She was sent home in good general condition on the third postpartum day. Follow-up visits in the clinic were satisfactory.

Discussion

Pregnancy and childbirth in women with severe FV deficiency present a special clinical challenge. Advanced planning, in addition to a good understanding and awareness of the potential complications, are essential in ensuring an optimal outcome. Iwase et al., in 2011, reported the first successful pregnancy in a woman with severe FV deficiency after controlled ovarian stimulation with hMG and IUI under the prophylactic use of FFP [6]. Their patient underwent five treatment cycles before achieving a pregnancy that ended in spontaneous abortion. The successful
pregnancy was achieved on the third post-abortion cycle, with an emergency cesarean section performed at 31 weeks of gestation. In the present case, the authors escalated their interventions, beginning with FFP around the time of spontaneous ovulation for two cycles, followed by two cycles with FFP, ovulation induction, and timed intercourse. Finally, the present case underwent a single cycle with ovulation induction, prophylactic FFP, and IUI, which produced the successful pregnancy. With respect to ovulation-related hemoperitoneum in FV deficiency requiring treatment, only three cases have been reported in the literature [6].

Postpartum hemorrhage is increased in women with severe FV deficiency. Recommendations for the management of labor and postpartum period rely on substitution therapy with FFP [3]. The present case experienced post-partum hemorrhage after administration of FFP; however, it was successfully managed without complications using standard procedures.

Controlled ovulation with clomiphene and hMG, prophylactic FFP, and IUI were used to achieve pregnancy in a woman with severe FV deficiency. The present case further supports the feasibility of this approach, which can be offered to other women with severe FV deficiency who previously avoided pregnancy because of the associated risks. Appropriate monitoring and care must be provided.

Conclusion

Fresh frozen plasma can be offered for reproduction to women with severe FV deficiency.

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Pregnancy associated with melanoma and fetal anomalies: a case report and review of literature

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Summary
The estimated incidence of melanoma complicating pregnancy has ranged from 0.1 to 2.8 per 1,000 pregnancies. Here the Authors present a case of a 40-year-old pregnant woman, who was admitted to the Clinic of Obstetrics and Gynaecology in 26 weeks of gestation, with diagnosis of melanoma and suspected with fetal anomaly, as possible bowel obstruction, and polyhydraminos. The melanoma was asported with a wide local excision under local anesthesia. Histological evaluation revealed melanoma Stage Ib (Clark IV, Breslow thickness 1.2 mm, pT2a). Lymph node sonography of neck, axilla, inguinum, abdomen, and pelvis as well as chest radiography did not demonstrate any evidence of metastatic disease. After vaginal delivery at 37 weeks of gestation, the female preterm hypotrophic newborn was transferred to the Institute for Neonatology and underwent resection of duodenojejunal atresia with tapering duodenoplasty and duodenojejunal termino-terminal anastomoses due to intestinal obstruction. No evidence of the melanoma was found in the placenta. Two years later the child was healthy and the mother was disease-free.

Key words: Melanoma; Pregnancy; Congenital anomalies.

Introduction
The estimated incidence of cancer diagnosed in pregnant women is one in 1,000 pregnancies and is predicted to rise as childbearing is shifted towards later reproductive ages [1]. The most common cancers associated with pregnancy are cervical, breast cancer, melanoma, lymphomas, and leukemia. Melanoma represents a life threatening situation not only for the mother, but also for the fetus due to potential aggressive therapy and fetal metastasis. The estimated incidence of melanoma complicating pregnancy has ranged from 0.1 to 2.8 per 1,000 pregnancies [2]. Although melanoma occurring during pregnancy challenges the physician, only a few studies regarding this pathology have been published. Little is known regarding the exact mechanisms whereby maternal cancer may pose risk to a developing fetus.

Case Report
A 40-year-old woman, with three previous deliveries was admitted to the Clinic for Obstetrics and Gynaecology in 26 weeks of gestation, for pregnancy complications including threatened preterm delivery and gestational diabetes mellitus. Additional finding was a suspicious cutaneous lesion on the patients back, first noticed by dermatologist few days before hospitalization. After the diagnosis of melanoma, a wide local excision. Pathological evaluation revealed melanoma Stage Ib (Clark IV, Breslow thickness 1.2 mm, pT2a). Before the operation the physical examination of lymph nodes was carried out to check whether the melanoma had spread to them. Sonography of neck, axilla, inguinum, abdomen and pelvic lymph nodes, and chest radiography were negative for metastases. Sentinel node biopsy was not performed. The predictive values of the serum protein S100 as a tumor marker and lactate dehydrogenase in the post surgical follow up staging of patient were measured and evaluated.

Gestational diabetes was ruled out and with no further symptoms of preterm delivery, the patient was discharged from the hospital two weeks later. Genetic examination with amniocentesis performed at 17.2 weeks of gestation, for age risk factor diagnosed female fetus with normal chromosomes (46XX).

A suspicion of abnormality that caused bowel obstruction and polyhydraminos was revealed by ultrasound; moreover a dilated stomach (40 x 20 cm) and small intestine with stenosis or atresia, and intrauterine growth restriction (IUGR) were evidenced.

After spontaneous vaginal delivery at 37 weeks of gestation, female newborn child weighed 1,950 grams, length 48 cm, head circumference 33 cm, and Apgar score 3. Immediately after birth, the newborn was transferred to the Institute for Neonatology for surgery that consisted in resection of duodenojejunal atresia with tapering duodenoplasty and duodenojejunal termino-terminal anastomoses. The pathology report of specimen revealed cystic duplication of intraluminal duodenojejunal transition zone. No evidence of the melanoma was found in the placenta. The mother and child are well and alive two years later.

Discussion
Melanoma is one of the most commonly diagnosed cancer during the childbearing age and pregnancy. Melanoma is now a major cause of death due to cancer in women of childbearing age, and the incidence rates are dramatically increasing.
There have been concerns in the past that hormonal and immunological changes occurring during pregnancy could be important in the development of melanoma. The most recent controlled studies suggest that pregnancy has no effect on survival in woman diagnosed with melanoma during pregnancy.

Based on a limited data, pregnancies associated with the diagnosis of localized melanoma do not appear to worsen prognosis [3].

Since 1866 when the first case report appeared, only 87 patient cases of placental or fetal metastasis have been reported. Although melanoma is the most common malignancy to metastasize to placenta, accounting 27 of 87 (31%) patient cases, metastasis of melanoma to the fetus appears to be a rare event [1].

Although pregnant women with melanoma should be treated similarly to non-pregnant ones, pregnancy status of patient limits the treatment options [4, 5]. Surgery is a definitive treatment for early stage of disease (Stage I et II melanoma), and this does not differ between pregnant and non-pregnant women, whereas the treatment of pregnant women with Stage III and IV melanoma is less clear and more difficult. Generally, as a treatment option, surgery poses the least risk to the fetus and may be considered the safest cancer treatment option for some cancers, especially after the first trimester. Careful monitoring of the mother and baby is important in order to keep the risk to a minimum.

Most authors agree that anesthesia (general, local, regional) does not affect the development of embryo and fetus. The maternal death rate due to anesthesia and surgery is negligible during the first trimester and does not appear to increase the incidence of major birth defects. With local anesthesia local resection can be performed regardless of the trimester without increased risk [6].

Only a few data exist regarding birth outcome in women with breast cancer, relating melanoma and Hodgkin’s disease. A recent study of birth outcome in women who were diagnosed with melanoma and Hodgkin’s disease: a review” Clin. Epidemiol., 2010, 23, 7.


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Rupture of uterine serosal hematoma: delayed complication of uterine perforation

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Summary
Uterine perforation, a major complication of dilatation and curettage (D&C), is typically recognized at the time of the procedure. Large defects in the uterine wall or injury to other intraabdominal organs can result in an acute abdomen requiring immediate surgical treatment. On the other hand, small perforations usually resolve on their own without any long-term consequences. Here, the authors report a case of delayed hemoperitoneum, ten days after the D&C evacuation of an early pregnancy. Initially, intramural pregnancy was the suspected etiology. However, histopathology suggested that the inciting event was the rupture of a serosal uterine hematoma, which likely resulted from an incomplete uterine perforation during D&C. The patient did well after undergoing an uneventful laparoscopy.

Key words: D&C; Delayed complication; Uterine perforation; Laparoscopy.

Introduction
One of the most common complications of dilatation and curettage (D&C) of early pregnancy is uterine perforation. Usually, conservative therapy is sufficient for if the perforation is small, caused by a small instrument such as a uterine sound [1]. However, even a relatively small perforation can lead to a potentially lethal hemorrhage requiring an emergent operation as an immediate or short-term consequence [2]. Here, the authors report an unusual case of delayed acute haemoperitoneum secondary to rupture of a uterine serosal hematoma caused by an incomplete uterine perforation.

Case Report
A 29-year-old, gravida 2, para 1, woman arrived at the present emergency department with acute abdominal pain and vaginal bleeding developed earlier that day. On systemic examination, there was marked lower abdominal tenderness with rebound tenderness. She also showed mildly anemic conjunctiva. The patient was in a hypovolemic state with a blood pressure of 85/50 mmHg, heart rate of 90 bpm, and body temperature of 36.2°C. Urine pregnancy test was positive; however she had a history of D&C ten days previously. Therefore, the test was regarded as a false positive, detecting remnant serum beta-human chorionic gonadotropin (beta-hCG). Transvaginal ultrasound showed normal thin endometrium with no evidence of intrauterine pregnancy and a large amount of fluid collection in the pelvic cavity, suggestive of haemoperitoneum (Figure 1). Diagnosis of ruptured ectopic pregnancy previously missed, was made, and exploratory laparoscopy was performed. On laparoscopic examination, a 1.5 cm sized bleeding mass was identified at the surface of uterine fundus. Other pelvic organs were found to be grossly normal (Figure 2). Again, the lesion was believed to be an ectopic gestation at an unusual site such as an intramural pregnancy, and excision was performed uneventfully. However, histopathologic study of the specimen revealed only mesothelial cells covering hematoma without any trophoblastic tissue (Figure 3). The patient recovered well and her serum beta-hCG level at postoperative day 9 was 14.3 mIU/ml, down from initial levels of 650 mIU/ml.

Discussion
D&C during early pregnancy is a relatively safe procedure. Common complications directly associated with the procedures are infection, hemorrhage, uterine perforation, and incomplete abortion [3]. The actual incidence of uterine perforation during surgical evacuation of early pregnancy is uncertain because many cases can go undetected by the operator during the procedure [1]. The most commonly perforated sites are the uterine fundus, which is relatively avascular compared to the cervix and lateral body. If uterine perforation is suspected, simple observation in the outpatient setting is usually appropriate for detecting any significant internal bleeding. Transvaginal ultrasound is also often helpful in demonstrating free fluid in the pelvic cavity indicating haemoperitoneum. Severe haemoperitoneum is a rare acute consequence of uterine perforation requiring emergency surgical treatment. In this case, the authors encountered a delayed haemoperitoneum occurring ten days after the procedure. It appears that the cause was the rupture of a serosal hematoma at the uterine fundus, as there were no uterine defects or injured vessels. The authors suspect that the hematoma was caused by an incomplete uterine perforation, not penetrating the uterine serosa. The protruding mass was likely the accumulation of blood under the serosal surface, which eventually ruptured. The literature describes a similar case, that of a uter-
ine perforation during surgical evacuation occurring at an area thinned by the scar of a previous cesarean section. The defect was filled by the formation of a hematoma [4]. However, in the present case the rupture of the hematoma was an unusual delayed complication of uterine perforation resulting in acute life-threatening haemoperitoneum. This case illustrates the potential need of close follow-up of asymptomatic patients and careful instructions regarding symptoms suggestive of internal bleeding, when perforation is suspected. Also, low threshold for high suspicion for perforation during the procedure is fundamental to uterus and visceral organs.

References

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Pulmonary hypertension (PH) is a disorder defined by elevated mean pulmonary arterial pressure. Outcome of pregnancy in patients with PH have a high maternal mortality rate and early recognition of this condition with prompt evaluation and careful management is crucial.

Case Report

A 30-year-old woman presented at the present institute gravid, 23 weeks gestation, with dyspnea on mild exertion, which became progressively more severe during the course of the pregnancy. She was heterozygous for a bone morphogenetic protein receptor type 2 mutation. Her medical history included surgical repair of a congenital pulmonary valve stenosis at the age of three years, with only mild residual pulmonic stenosis. She was then asymptomatic with normal pulmonary pressure, according to echocardiography. An echocardiogram conducted at presentation showed no signs of tricuspid regurgitation (TR), but indirect variables, including dilated main pulmonary artery and flattening of the intra-ventricular septum, suggested PH. Right heart catheterization performed at 24 weeks gestation confirmed diagnosis of mild PH with mean pulmonary artery pressure of 32 mmHg, normal wedge pressure without pharmacological reversibility. During pregnancy, oxygen therapy was administered to maintain saturation above 90%, enoxaparin was administered as anticoagulation therapy. At 25 weeks gestation, intravenous (IV) treprostinil was started and titrated up to 25 ng/kg/min. At 33 weeks gestation, an elective cesarean section was performed using spinal anesthetic technique, and a healthy baby girl was born. The patient was treated with intensive diuretic therapy to maintain negative fluid balance and with oxygen due to severe hypoxemia. She was admitted to the intensive care unit for seven days, and then to the internal medicine department for another two weeks. She was discharged at 21 days postpartum with oxygen therapy. Her condition gradually improved, and at 12 weeks follow-up, she no longer required oxygen therapy. Treprostinil was gradually withdrawn and she is currently being treated only with ambrisentan, ten mg daily. Figure 1 shows the pulmonary artery pressure estimated by echocardiography and functional capacity (FC) levels from baseline before pregnancy to 14 weeks postpartum.

Discussion

The woman described insisted on continuing with the pregnancy, despite the current recommendation for early termination of pregnancy [1]. While early reports estimated 50% maternal mortality in PH patients, rates have decreased to 17% [2]. During pregnancy, blood volume, heart rate, cardiac output, and myocardial oxygen consumption increase considerably. In normal pregnancy increased lung plasma volume results in decrease in peripheral vascular resistance, which enables accommodation of the progressively increasing right ventricular load. In women with PH, peripheral vascular resistance does not decrease, and there is thus no restraint of the mounting pressure on the right ventricle. Treatment of PH requires a multi-disciplined approach, which is especially critical during pregnancy. The selection and combination of therapies depends on many factors, such as clinical severity, drug efficacy, and side effects. Among the advanced vasodilator therapies that have become available in the last decade for the treatment of PH, the prostacyclin analogues are the most potent medication [3]. As a pregnancy category B drug, treprostinil can potentially be used during pregnancy. Though the relatively long half-life of
treprostinil enables subcutaneous administration, [4] continuous IV administration is still considered the most potent means of administration [5]. The authors decided to initiate IV treprostinil therapy, and not oral therapy, in gestational week 25, despite only mild PH. They selected this potent treatment due to their anticipation of increasing pulmonary pressure consequent to the physiology of pregnancy. Repetitive measures of echocardiogram and functional capacity measured during pregnancy confirmed their expectations.

Documents of treprostinil treatment during pregnancy are few. Subcutaneous treprostinil was administered to one parturient woman in Jais et al.’s registry [6]. IV treprostinil was administered to four women with severe PH; all four delivered at term, without maternal or fetal mortality. [7] The present authors attribute their patient’s survival to their adherence to an intensive therapeutic protocol. Treatment of PH during pregnancy is a relevant medical issue, since some women become pregnant despite medical advice and the high risks involved, and others are diagnosed with the disease only during pregnancy. This case demonstrates positive maternal and fetal outcomes following therapy with intravenous treprostinil, commenced prior to hemodynamic and clinical deterioration.

References


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Pentalogy of Cantrell accompanied by scoliosis and pes equinovarus deformity at 12 weeks gestation

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Summary
Cantrell’s pentalogy (CP) is a rare syndrome characterized by defects in the lower sternum with ectopia cordis, anterior diaphragm defects, midline supraumbilical abdominal wall defects, defects in the diaphragmatic pericardium, and congenital heart disease. The authors report a 12-weeks gestation with multiple fetal anomalies suggesting the diagnosis of CP (a large thoraco-abdominal defect with herniating liver and bowel, heart deviated anteriorly with concomitant ventricular septal defect), and the ‘S’ shaped fetal spine due to increased lumbar lordosis and scoliosis with accompanying pes equinovarus deformity. Chorionic villus sampling was performed due to increased nuchal translucency (3.7 mm). The fetal karotype was found to be 47, XX, +21 (trisomy 21). In the literature, three scoliosis cases have been reported accompanying the CP along with multiple anomalies and one concomitant pes equinovarus deformity has been reported previously.

Key words: Pentalogy of Cantrell; Scoliosis; Pes equinovarus deformity; Prenatal diagnosis.

Introduction
Cantrell’s pentalogy (CP) was first described in 1958 [1]. Cantrell Haller and Ravitch reviewed the literature to identify similar conditions and proposed five core features for this syndrome. CP is a rare combination of anomalies, comprising: a defect of the lower sternum, midline suprambilical abdominal wall defect, deficiency of the anterior diaphragm, deficiency in the diaphragmatic portion of the pericardium with communication between the pericardial and peritoneal cavities, and congenital heart defect [1]. The full spectrum is estimated to have an incidence of 5.5 per one million live births [2]. There is male dominance with a male to female ratio of 2.7 : 1. With prenatal ultrasonography, the CP usually can be diagnosed in the first trimester of pregnancy [3]. Pentalogy is often incompletely present, with various combinations of two or three defects that are more common. The etiology and pathogenesis are still unknown and usually occurs as a sporadic event. Here, the authors presented an early prenatal diagnosis of incomplete CP with Trisomy 21 accompanying vertebral column anomalies and pes equinovarus deformity.

Case Report
A 23-year-old woman was referred at 12 weeks gestation with multiple fetal anomalies (a large thoraco-abdominal wall defect, heart deviated anteriorly). She had no significant obstetrical history. In the obstetric ultrasonography, single, living fetus with 60.5 mm crown-rump length and compatible with 12 weeks was detected. Ultrasonographic assessment identified a large exomphalos and inferior sternal defect, with the heart deviated anteriorly, which suggested the diagnosis of CP. The authors could not fully determine cardiac anomalies due to gestational age and deviated heart, but tricuspid regurgitation was detected and with transvaginal ultrasonography, a large ventricular septal defect was seen. The fetal spine could not be clearly evaluated due to increased lumbar lordosis and scoliosis. The vertebral column was ‘S’ shaped and was accompanied by pes equinovarus deformity. Nuchal translucency was also increased (3.7 mm).

Current findings and the prognosis of the disease were explained to the family. Chorionic villus sampling was performed. The genetic examination revealed a 47, XX, +21 (trisomy 21). The parents opted for termination. Pregnancy was terminated within 12 hours 600 microgram in total of vaginal misoprostol. In the macroscopic examination of female fetus, a wide median defect was seen, including abdominal wall and lower thorax. Intra-abdominal organs, liver and bowel, herniated by this defect, and anteriorly deviated heart were seen (Figure 1). The fetal spine was ‘S’ shaped due to increased lordosis and scoliosis. There was no concomitant neural tube defect however pes equinovarus deformity was seen in the right foot (Figure 2).

Discussion
CP is a rare syndrome characterized by defects in the lower sternum with ectopia cordis and various congenital heart diseases, anterior diaphragm defects, midline supraumbilical abdominal wall defects, and diaphragmatic pericardial defects.

In 1972, Toyoma, further classified the degree of expression of CP into three classes. Class I is a definite diagnosis with all five defects present. Class II is a probable diagno-
sis with four of the five defects expressed, and Class III is considered an incomplete expression with varying combinations of defects present [4]. CP is often incompletely present, as seen in this case. The present case was represented with ectopia cordis, ventricular septal defect, supraumbilical abdominal wall defect, with herniated organs including liver and bowel. However lower sternal defect, anterior diaphragm defect, and diaphragmatic pericardial defect could not be evaluated due to gestational age. If pericardial effusion can be seen, associated anterior diaphragmatic hernia and diaphragmatic pericardial defects may be suspected, but the present fetus did not have pericardial effusion. The etiology and pathogenesis of the CP are still unknown. The hypothesis underlying this condition probably originates between the 14 and 18 days of embryonic life, with an alteration in the migration of the primordial mesodermic structures of the medial line [5]. This results with failure of ventral wall closure and incomplete external primordial bands fusion. Incomplete closure and fusion would account for defects in the chest and abdominal wall, aplasia of the anterior diaphragm, and the associated pericardium. Organs may eviscerate through the resulting sternal and abdominal wall defects. Ectopia cordis is characterized by complete or partial displacement of the heart outside the body. Intracardiac anomalies are described in CP including the most common ventricular septal defect, but also tetralogy of Fallot, atrial septal defects, and ventricular diverticulum [1]. Several cases of the CP reported in the literature had central nervous system and craniofacial malformations, such as cleft lip and palate, craniorachischisis, encephalocele, and hydrocephalus [6-8]. Various other associated anomalies have been reported; limb defects such as clubfoot, absence of tibia or radius, hypodactyly and abdominal organ defects such as gallbladder agenesis and polysplenia [9-11]. This syndrome has also been reported occasionally in association with sirenomelia. In the present case the fetus also had increased lordosis and severe scoliosis concomitant with pes equinovarus deformity. In the literature, three scoliosis cases have been reported accompanying CP, along with multiple anomalies and one concomitant pes equinovarus deformity has been reported [12, 13].

Most cases are believed to occur sporadically and no recurrences have been reported. Although sporadic in most of the described infants, X-linked recessive inheritance was suggested for some families, and genes located on the X-chromosome (Xq25-q26.1) may be involved in some of the reported cases [5]. There are much suspicious conditions as chromosome imbalances, an early amniotic sac rupture, and amniotic bands [14, 15].

Prenatal diagnosis is important because the prognosis is poor and the disease is lethal. With prenatal high resolution ultrasound and fetal magnetic resonance imaging, CP can
usual diagnosed in the first trimester of pregnancy as in the present case [3]. However, diagnosis can be especially established during the second trimester in 60% of cases with incomplete defects [16].

Chromosomal abnormalities especially should be kept in mind and with increased nuchal translucency measurement prenatal diagnostic methods should be performed. In the present case, chromosome examination result was found as 47, XX,+21 but most frequently defined chromosomal anomaly associated with pentalogy is Trisomy 18 which can be found in 5-10% of cases [17].

Although survival of the CP depends on the size of the abdominal wall defect, extent of the cardiac defect, and presence of associated anomalies, because of the poor prognosis, termination of pregnancy should be offered in cases diagnosed at early weeks.

In conclusion, presence of fetal abdominal wall defects and increased nuchal translucency in ultrasonography should be carefully investigated for the diagnosis of CP and chromosomal anomalies. As in the present case, CP can be accompanied by chromosomal abnormality. Therefore early diagnosis is important for making appropriate counseling to the family and aid in decision-making regarding pregnancy.

References


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Conservative surgical management of multiple myometrial abscesses; an unusual case with review of the literature

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Summary
Myometrial abscess, especially with multiple foci, is quite rare and previous literature prevalently discusses unique locus of intramyometrial abscesses, usually treated with hysterectomy accompanied with or without bilateral salpingo-oophorectomy. The presented case, to the authors’ knowledge, is the first multiple myometrial abscess case treated with conservative surgical approach.

Key words: Intramyometrial; Abscess; Multiple; Conservative.

Introduction
Pelvic inflammatory disease (PID) is an infection of the female upper genital tract that involves any combination of the uterus, endometrium, ovaries, fallopian tubes, pelvic peritoneum, and adjacent tissues. PID is accepted mostly a result of ascending infection from the lower to upper genital tract and usually associated with more than one microorganism [1]. Although there are different guidelines regarding the diagnostic criteria, in case of suspicion of PID, treatment should be immediately commenced and the patient should be followed up, since a short delay in treatment may result in severe cases [2]. For PID, the positive predictive value of clinical diagnosis is 65-90% compared to laparoscopic diagnosis [2]. Although the first choice of treatment is combined regimen of antibiotics, in cases with suspicion of abscess, frequently surgical intervention or ultrasonography-guided drainage is required in the course of the management.

Few cases with myometrial abscess have been reported in literature and differential diagnosis is quite complicated most of the time. A 29-year-old patient with multiple intramyometrial abscesses treated with combined antibiotic and conservative surgery is mentioned in this case report with review of the literature.

Case Report
A 29-year-old gravidity 1 parity 1 patient was admitted to Gynecology outpatient clinic with pelvic pain and mucopurulent leucorrhrea. She claimed that she had delivered with cesarean section 16 months prior, and three months after the delivery, an intrauterine device (IUD) was applied in another gynecology clinic followed by removal of the IUD one month later due to pelvic pain. Two months after that, she had an operation due to tubo-ovarian abscess in an another medical center.

Gynecological examination revealed mixed type mucopurulent leucorrhrea accompanied by cervical tenderness. She did not have fever (36.5°C). Laboratory findings were as follows: Hb: 11.4 g/dL, WBC: 13x10⁹ / L (4-10 x 10⁹ / L), hsCRP: 0.2 mg/L (0-0.5 mg/L), sedimentation: 24, βhCG: negative, CA 125: 62 IU/ml (0-35), and liver function and renal function tests were within normal range. Ultrasonographically there were multiple hypoechogenic foci in myometrium with the largest diameter of two cm accompanied by normal appearing ovaries (Figure 1).

The patient was hospitalized with diagnosis of PID and intravenous combined antibiotherapy (ceftriaxone and metronidazole) was started and given for ten days. Urine, vaginal, and blood cultures did not reveal any microorganisms and the infection parameters did not rise, although she had subfebrile periods during the follow up. Ultrasonographic findings did not improve during the medical therapy and Doppler ultrasonography revealed an increased vascularity in a solid five-cm area which could not be distinguished from myometrium, containing non-serous cystic lesions, the largest with a two-cm diameter. Additionally magnetic resonance imaging was done for further evaluation and revealed multiple peripheric contrast positive cystic lesions with dense content, accompanied by minimal fluid in the pelvic area (Figure 2). Due to incomplete remission in the clinical presentation, surgery was planned. In laparotomy, the uterus was enlarged and there were multiple adhesions among uterus and surrounding intestinal loops. The uterus grossly had multiple cystic areas each full of mucopurulent discharge (Figure 3). All the grossly available cystic areas were drained and all the defects in myometrium were sutured,and a sample of pus was taken from the discharge for culture. Pathological examination of the myometrial pieces were reported as ‘dystrophic calcification and severe pus in myometrium’.

Escherichia coli and coagulase negative staphylococci were isolated from the pus culture. Postoperatively the patient was discharged after eight-day combined gentamycine + clindamycine therapy.

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Ten days later she was admitted for the second time due to pelvic tenderness and subfebrile fever. Microbiological examination of blood and vaginal and urine cultures did not reveal any microorganisms. Antibiotherapy was begun (ceftriaxone and metronidazole) and continued for 12 days, resulting with a favourable clinical outcome; the patient was discharged from the hospital with full recovery.

Discussion

PID among IUD users is most strongly related to the insertion process and to the background risk of sexually transmitted diseases. Conditions which represent an unacceptable health risk if an IUD is inserted are current PID, current purulent cervicitis, and chlamydial or gonorrhreal infection [3]. Studies regarding IUD and PID revealed that the rate of PID within 20 days after insertion was 9.6 per 1,000 women years [4]. Also PID risk was found to be similar with depot medroxyprogesterone (DMPA), hormone releasing IUD or combined oral contraceptive usage even eight years after IUD insertion [4]. Additionally Steenland et al. reported that even postabortion insertion of IUD, did not increase PID risk but only resulted in a higher expulsion rate [5]. The mentioned case had IUD inserted three months after the delivery and removed one month after insertion. It seems to be beneficial to routinely control the IUD applied one month after the intervention since the risk for a pelvic infection is highest in the first month and early management in case of any suspicion of infection may prevent further complications.

Removal of the preexisting IUD in PID, is also controversial in the literature. In this case the insertion time after delivery was three months and the device was removed about one month later because of pain possibly due to arising pelvic infection. The present authors, however, did not have adequate data regarding whether medication was given at that time or what the medication consisted of. Tepper et al. in a review reported that retaining IUD after the diagnosis of PID had similar or better outcomes than removal [6]. Also, the timing of the removal of IUD is an important issue. The possible contributing variable for Tepper et al.’s results may be the removal of IUDs on admission [7-9].

In differential diagnosis, tubo-ovarian abscess, adenomyosis, degenerated myoma or a pyomyoma were excluded in this case. The previous surgery for tubo-ovarian abscess and subfebrile fever in follow up favored mostly an infectious etiology, such as intramyometrial abscess or pyomyoma instead of other alternative gynecological diagnosis. Secondary infection of an uterine leiomyoma after an abortion or transvaginal instrumentation resulting with pyomyoma is quite rare and there are few case reports in literature [10]. Also a spontaneous conversion of a leiomyoma to pyomyoma in a virgin patient without expected risk factors has been reported. In cases with triad of bacteremia or sepsis, leiomyoma uteri, and no other apparent source of infection the diagnosis of pyomyoma should be included in differential diagnosis [11]. Absence of previous uterine leiomyoma
history excluded the diagnosis of pyomyoma in this case. After all, the suspicion of intramyometrial purulent collection and incomplete remission in clinical presentation of the patient made it mandatory to perform a diagnostic laparotomy.

Abscess formation in myometrium is not a frequent situation accompanying PID. Although it depends on the clinical situation and hemodynamics of the patient, in similar cases, the mostly preferred treatment is surgery consisting of hysterectomy with or without sапingo-oophorectomy. Parsons et al. reported a 65-year-old case with ruptured appendicitis which seemed to have perforated into the uterine fundus. The intrauterine lesion was reported by computed tomography scan as an altered density in myometrium [12]. The authors had to perform total abdominal hysterectomy and bilateral sапingo-oophorectomy. Kuah et al. in Singapore, also presented another 46-year-old case with an intramyometrial abscess which was treated by total abdominal hysterectomy and bilateral sапingo-oophorectomy [13]. In the presented case, since the patient had desire for preservation of fertility, the operation was conducted in a conservative manner which also resulted in full recovery with the assistance of antibiotherapy against the microorganisms isolated from the myometrial abscess.

Intramyometrial abscess can masquerade as degenerating fibroids, necrotic tumours, and can exist without overt signs or symptoms of an infection [13]. To the present authors’ knowledge, the previously reported myometrial abscess cases were of solitary lesions and the present patient seems to be the first multiple myometrial abscess case managed by a conservative surgical approach. Detailed medical history, questioning the possible risk factors and appropriate usage of imaging techniques improves differential diagnosis. In appropriate cases, conservative surgery accompanied by medical interventions may be life-saving and preserve fertility.

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