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Advances in oocyte cryopreservation - Part II: rapid cooling using vitrification

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Summary

Purpose: The need for freezing oocytes has been established for females undergoing potential therapy that could damage their ovarian egg reserve, for ethical or religious reasons (not having excess embryos frozen) or for women nearing the age of lower fecundity but not married and not ready to use donor sperm. Applying cryopreservation techniques for oocytes used for embryos resulted in very poor pregnancy results. A rapid flash freezing technique has rekindled interest in oocyte freezing known as vitrification. **Methods:** Certain modifications, especially minimizing the volume, have resulted in marked improved pregnancy rates with vitrified thawed oocytes. The lower volume allows decreased exposure to the toxic cryoprotection. Commercial interests have developed an effective device called cryotop but some concerns about microorganism contamination exist because it is an open system. Modifications have been made to make available the cryotip, a closed device which addresses the contamination issue. **Results:** Frozen oocyte survival rates upon thawing fertilization rates and subsequent pregnancy rates after embryo transfer have been reported comparable to data with frozen thawed embryos. **Conclusions:** Because of the uncertainty of the programmable freezer used for the slow cool method and because there has been more commercial interest in the vitrification method, the “flash” freeze protocol seems to have an edge over the slow cool method for oocyte freezing.

Key words: Cryopreservation; Oocytes; Vitrification; Slow cooling.

The Vitrification Method of Cryopreserving Oocytes

Rall and Fahy described vitrification as a potential alternative to slow-cooling. Vitrification involves exposure of the cell to about twice as high cryoprotectant concentrations compared to the slow-cool procedure for brief periods of time usually at or near room temperature followed by rapid cooling in liquid nitrogen [1]. The high osmolarity of the vitrification solution rapidly dehydrates the cell and the submersion into liquid nitrogen quickly solidifies the cell so that the remaining intracellular water does not have time to form damaging ice crystals.

The initial poor success with oocyte freezing using the slow-cool technique that had worked well in many in vitro fertilization (IVF) centers with embryos led researchers to try to modify this old technique of vitrification which was considered prior to the modification as not likely to be effective because of the toxicity of the highly concentrated cryoprotectants and the temperature at which they were used [2, 3].

The first modified technique of vitrification leading to successful deliveries was first described with cows [4]. The first live healthy delivery of a little girl involving fertilization of a vitrified thawed egg and subsequent embryo transfer using a similar technique as the bovine studies was reported in 1999 by Kuleshova *et al.* [5]. It is my belief that it was the relatively poor success of the slow freezing rapid thaw technique (the first live human birth with the slow technique was reported in 1997 by Porcu *et al.*) that led to the commercial push to try to modify the vitrification method [6].

The modification of the vitrification method included decreasing the length of time of exposure to toxic cryoprotectants so that the exposure to the final and highest concentration was 30 seconds or less. This faster cooling increases survival [7-9].

The concept of vitrification proposes that if a cell is dehydrated and then cooled fast enough everything will “freeze” in place and damage will not have time to occur as a vitrified amorphous glass-like solid will instead form of crystals. Similarly thawing must take place at a relatively fast rate to prevent crystal organization upon rewarming. If cells die during vitrification it may or may not be because the cryoprotectant concentration was toxic, or ice did in fact form, or the cooling rate was too slow.

One method to allow an increased rate of cooling and subsequent thawing is to minimize the volume of the vitrification solution which allows bovine concentration of cryoprotectants [10, 11]. The most critical time period is the initial cooling [12]. Minimizing the volume of the vitrification solution containing oocytes also decreases the chance of ice crystal nucleation formation in the small sample [13]. Furthermore, minimum volume vitrification may also help to

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avoid zona pellucida damage and embryo fracture which frequently occurs when oocytes are cryopreserved in standard insemination straws and warmed rapidly afterwards.

Commercialism has led to the cryotop method, a minimum volume vitrification method. A special tool consisting of a narrow film strip attached to a hard plastic holder has been developed to protect the tool from mechanical damage during storage. A 3-cm plastic tube cap can be attached to cover the film part. The vitrification solution contains ethylene glycol, dimethyl sulfoxide and sucrose.

Cryotop is an open method where direct contact between liquid nitrogen and the solution containing oocytes is required. Though an open system would theoretically allow contamination by microbes, those advocating open vitrification systems argue that at such a low temperature proliferation of any serious pathogens is unlikely. Nevertheless some studies show that certain bovine viruses can infect the embryos in an open system but not in a closed system [14, 15]. Some countries will not approve an open vitrification system because of the theoretical contamination of embryos by pathogens. A closed system known as Cryotip has thus been produced. Cryotip is a narrow capillary that can be sealed after loading with a minimum volume solution [16]. There is no direct contact between the biological solution and the liquid nitrogen.

Kuleshova *et al.* reported the first birth from vitrified human oocytes in 1999 after vitrification of 17 oocytes by using ethylene glycol (40%) and 0.6M/l sucrose in open pulled straws [5]. The first large series of human oocyte vitrification was published by Yoon *et al.* in 2003 [17]. They cryopreserved 474 cumulus-oocyte complexes (mature and immature oocytes) by using vitrification with 5.5 M ethylene glycol and 1.0 M sucrose as cryoprotectants. To maximize cooling rates, the oocytes were loaded on an electron-microscope grid. These investigators reported a survival rate of 68.7%, a fertilization rate of 71.7%, an implantation rate of 6.4%, and a clinical-pregnancy and live-birth rate per transfer of 6/21 (21.4%). Chian *et al.* [18] used a combination of ethylene glycol, PROH, and sucrose to vitrify 180 oocytes in an open container called a Cryoleaf [18]. They reported a survival rate of 20.4% and a clinical pregnancy rate per patient of 7/15 (46.7%) [18].

Both Kuwayama *et al.* and Antinori *et al.* showed the best success rates following thawing of cryopreserved oocytes and subsequent embryo development and pregnancies [11-19]. Kuwayama *et al.* reported with the Cryotop method that 148 of 153 vitrified oocytes had normal morphology upon thawing, 91 of 153 oocytes cleaved into embryos and 35 (22.9%) developed into blastocysts (comparable data for fresh oocytes were cleavage of 118 of 153 eggs (77.6%) and 68 (44.7%) became blastocysts. Pregnancies were achieved in 12 of 29 (41.3%) of embryo transfers involving fertilization of vitrified thawed oocytes [11].

Lucena *et al.* also used the Cryotop method for oocyte vitrification and reported an overall pregnancy rate of 13/23 (56.5%) per patient [20]. These high pregnancy rates may be attributed partly to the fact that the majority of transfers used donor oocytes and involved the transfer of a high number of embryos (mean of 4.5) [20].

Antinori *et al.* tried to independently assess the protocol of vitrification described by Kuwayama *et al.* using Cryotop [19]. Antinori *et al.* [19] found that 328 of 330 vitrified oocytes (99.4%) survived upon warming. Following ICSI 305 of 328 (93.0%) fertilized and 295 of these 305 2PN embryos cleaved. These 295 cleaved embryos resulted in 120 embryo transfers. There were 39 (32.5%) clinical pregnancies and 28 ongoing pregnancies (23.3%). The implantation rate per transferred embryo was 13.2% and the implantation rate per thawed oocyte was 11.8% [19].

Ri-Cheng Chian and colleagues at the 14th World Congress on in vitro fertilization in Montreal, 2007, presented their experience involving vitrification of oocytes with fertilization upon thawing with ICSI. There were 38 women (mean age 31.5) in the trial and there were 463 oocytes vitrified. The survival rate post-thaw was 82.7% (383/462) following insemination by ICSI, and 75% (287/383) were fertilized normally. The pregnancy rate per transfer (mean of 3.7 ± 1.1 transferred) was 44.7% (17/38). To date one has had an ectopic pregnancy, 13 have delivered, and there are two ongoing pregnancies. All babies were normal. The implantation rate was 18.1% (24/133).

Conclusions and Caveats

It is clear that modern technology has allowed the development of cryopreservation techniques for oocytes that can approach the success of embryo freezing. The myriad of potential uses for oocyte freezing have been discussed in part I of this editorial [2, 22]. A woman who is considering cryopreserving the only fertilizable eggs she will have for the rest of her life prior to ablative therapy, e.g., for cancer, has to carefully review all options. Even a single young woman without a current partner could still consider fertilizing those oocytes retrieved with donor sperm and freeze them at the 2 pronuclear stage.

Young women or somewhat reproductively older women must realize that the present optimistic conclusion that oocyte freezing and embryo freezing are "about" comparable based on limited studies by highly experienced IVF centers with the best subjects. Just because an IVF center is claiming to use the vitrification method using cryotop does not mean that the center should show equal success to that reported by Kuwayama *et al.*, especially if that given IVF center has limited experience with the procedure [11]. There is apparently a significant learning curve with vitrification that may take five months or more to master even in a center performing the procedure frequently. Some IVF centers willing to perform oocyte freezing have never even tested whether the oocytes that have been cryopreserved will result in live babies or what the chance of success is.

Thus one option until more extensive experience is achieved (unless the young woman can actually have the procedure performed at one of the experienced centers) would be to fertilize the eggs with donor sperm but advise the woman that a future husband could also continue his genes in his progeny by the fertilization of donated eggs. She should also be advised of the future use of a younger sister's eggs or even the use of her brother's sperm with anonymously donated eggs.

There are not enough data, especially with the slow freeze rapid thaw method, to make a determination as to whether one technique is superior to the other for oocyte cryopreservation [21]. There seem to be more publications lately concerning vitrification especially with the cryotop or cryotip but this may be commercially stimulated. What is needed is more very good IVF centers skilled in both techniques to freeze half of the oocytes retrieved with the slow-freeze rapid-thaw procedure and the other half with vitrification, and compare the outcomes. These centers should include not just ideal patients but others, e.g., slightly older women.

Oocyte freezing should still be considered experimental and should be under the supervision of an institutional review board (IRB). Such an IRB, in my opinion, should require that a given IVF center able to cryopreserve oocytes should first demonstrate in an experimental group (given perhaps some financial considerations), that the fertilization of frozen thawed oocytes by that given IVF center results in an adequate live delivery rate after embryo transfer before they should be allowed to freeze oocytes for young women about to undergo therapy that could jeopardize their future egg supply.

Since oocytes present much more of a challenge to successful cryopreservation than embryos, it seems logical that if technology advances with vitrification so that a successful oocyte freezing program can be established, embryos should follow suit and successful embryo vitrification should also be found.

The majority of IVF centers today use the slow cool rapid thaw method for freezing of embryos. If cryopreservation of oocytes proves superior by vitrification than slow cool, even if the two techniques prove equal for embryo freezing, the IVF programs planning on freezing oocytes would likely switch to vitrification of embryos, especially once skilled in the latter technique in order to unify procedures.

If the two cryopreservation procedures prove to have equal efficacy for oocyte and embryo freezing, new IVF centers may prefer the vitrification procedure because they would not have to invest in an expensive programmable freezing machine. Furthermore, since the slow cool rapid thaw technique is very time consuming, whereas vitrification can be completed by one embryologist within minutes, this factor could also influence the majority of neophyte IVF centers to choose vitrification even if success rates prove similar.

As mentioned, one of the things needed to determine if slow cool or vitrification of oocytes results in higher success rates is to have an expert cryobiologist experienced in both techniques to perform comparative studies. Such data is available with human embryos. One of the most respected cryobiologists in the world is Kuwayama. Using slow-cool vs the cryotop method of vitrification for freezing 2 pronuclear (2PN) embryos, 89% (1730/1944) 2PN embryos survived with slow-cool vs 100% (5881/5881) with vitrification [10]. The cleaved survival rate was 90% (1557/1730) with slow-cool vs 93% (5409/5881) with vitrification. The blastocyst cleaved rate was 51% (796/1557) with slow-cool vs 56% (3058/5469) for vitrification and the blastocyst/cryopreserved rate was 41% (796/1944) vs 52% (3058/5881). Thus vitrification of 2PN embryos resulted in higher survival developmental rates than slow-cooling [10]. The author did not provide comparative pregnancy rates per transfer for this stage of embryo freezing.

For 4-cell two human embryos the survival of rate cryopreservation was 91% (857/942) with slow cool vs 879/897 (98%) with vitrification. The pregnancy transfer rate was 32% (172/536) vs 27% (136/504) for vitrification [10].

For blastocysts the survival of rate vitrification was 84% (131/156) with slow cool vs 90% (5695/6328) with vitrification [9]. The pregnancy rate per transfer was 51% (50/98) vs 53% (2516/4745) and the live birth rate per transfer was 41% (40/98) vs 45% (2138/4745) [10].

If the slight advantage seen with vitrification vs the slow-cool method for various stages of embryo freezing remains similar in other studies by other authors, the differences may not be sufficient for IVF centers doing well with slow-cool techniques and already having invested in the expensive programmable freezer to invest the time and possibly risk lower pregnancy rates initially until the learning curve has been satisfied. These IVF centers could be interested in switching if they become interested in oocyte freezing and if the vitrification methods show an even more impressive outcome with oocytes than slow cool techniques. However it is well known that some IVF centers do not seem to have very good results with their present slow cool procedure for embryo freezing. Clinical success may depend on many factors including patient age and stimulation protocol, quality of embryos selected for freezing, developmental stage at freezing, media formulation including type of cryoprotectants used, and parameters of cooling and warming. Another very important factor, however, is the type and quality control of programmable freezing unit employed. This problem with slow cooling would be completely eliminated with vitrification since this procedure does not require sophisticated equipment of questionable reliability of certain makes and types. Thus, there seems to be enough data available at present to entice those IVF centers without great success with their slow cool embryo freezing procedures to switch to vitrification.

Our division of infertility and reproductive endocrinology is a university medical center which has a very large multidisciplinary oncology center. Our IVF center is the largest in the southern part of New Jersey and is one of the largest in the state. Nevertheless only a few times at most per year does a case of potential egg ablation following cancer ther-

apy come up where there is a request to preserve future fertility with a couple's own gametes. In every instance to date the single young women were content to fertilize the eggs with a boyfriend's sperm or donor sperm. Though we advised them of other centers freezing the oocytes they chose to merely freeze the embryos. I suspect that some would have preferred egg freezing if we had it available. Possibly time constraints may have precluded them from contacting these other centers.

There is some variation in success rates among various IVF centers with pregnancy rates following fresh embryo transfer but there are much greater differences in the success rates following frozen embryo transfer. In fact some IVF centers with the best success with fresh embryo transfer do not fare nearly so well with their pregnancy rates following frozen embryo transfer.

My suspicion to explain this apparent paradox is that the poor success with frozen but not fresh embryo transfer is not likely related to a substandard embryology laboratory or poor transfer technique. Instead my hunch has been that the weak point may be the quality of the programmable freezer. Thus we modified a technique that had been used in cattle where a simplified freezing protocol that required a Biocool freezer was used instead of the Planer programmable freezer and a one-step removal of the cryoprotectant 1,2 propanediol [22]. With this simplified freezing protocol we have attained similar pregnancy rates following frozen ET as with our fresh ET pregnancy rates in women who underwent hyperstimulation [23].

This simplified protocol on egg freezing could be attempted using some of the media changes recommended by Porcu *et al.* [6]. However, the lack of requests have prevented my group from performing such a study since, as mentioned, I believe that the ethical thing to do would be to first try the technique on women wanting immediate transfers which would require the normal financial rewards of participating in a research study, i.e., purposely not transferring the portion of embryos formed from fertilization of fresh eggs but instead the portion formed from fertilization of frozen thawed oocytes.

Since most of the commercial efforts have centered on vitrification methods, if a company funded a study, e.g., as proposed above, or even funded a study comparing our modified slow-cool fast-thaw method to vitrification, and we found an advantage to the latter, then I would consider switching.

For a new IVF center just starting because of much less expense and space occupying equipment to start with and because of avoiding the Achilles heel for some freezing programs, i.e., the programmable freezer, I would advise the neophyte center to consider vitrification from the outset. I would also advise an IVF center not doing well with their present slow cool technique to switch to vitrification.

I do not believe that there is enough need for oocyte freezing to justify every IVF center offering the service. Instead I think the companies selling equipment (e.g., the Kitazato Co., Fujinomiya, Japan which makes the cryotop and cryotip) should designate certain IVF centers to cover certain geographical areas to learn the vitrification method and fund a study to evaluate the proficiency of that center in egg freezing for women who desire immediate replacement of embryos. It would be important to check pregnancy rates of sibling frozen oocytes.

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Sex steroidal modulation of collagen metabolism in uterine leiomyomas

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Summary

Uterine leiomyoma is a fibrotic disease that contains abundant extracellular matrix (ECM) components, particularly collagen fibrils. Aberrant ECM metabolism has been thought to contribute to the pathogenesis of uterine leiomyomas. However, it remains poorly understood whether ovarian sex steroid hormones modulate collagen metabolism in uterine leiomyomas. More recently, a few articles have demonstrated the differential effects of ovarian sex steroids, selective estrogen receptor modulators (SERMs), and selective progesterone receptor modulators (SPRMs) on the induction of the ECM-remodeling enzymes and collagen synthesis in uterine leiomyoma cells. Sex steroids may act to up-regulate collagen synthesis, whereas SERMs and SPRMs down-regulate collagen synthesis. Further study will be needed to clarify the precise mechanism underlying steroidal regulation of collagen synthesis in uterine leiomyomas.

Key words: Collagen; Leiomyoma; Estrogen; Progesterone; Estrogen receptor modulator; Progesterone receptor modulator.

Introduction

Uterine leiomyomas are characterized by aberrant metabolism of the extracellular matrix (ECM) components, including collagen and glycosaminoglycans [1-3]. Compared with the myometrium, types I and III collagen mRNAs were shown to be elevated in uterine leiomyomas in the proliferative phase of the menstrual cycle [1], and pro- α 1 (III) and pro- α 1 (I) collagen mRNAs were up-regulated in uterine leiomyomas [4]. Uterine leiomyomas contain an abnormal collagen fibril structure and orientation [5]. The aberrant metabolism of the ECM has been thought to contribute to the pathogenesis of uterine leiomyomas.

Collagens are the major elements of the ECM and play a vital role in the maintenance of the structural integrity of the tissues [6]. Collagen molecules that are secreted into the extracellular space self-assemble into fibrils in the ECM [7]. The turnover and homeostasis of the ECM is controlled by the action of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) [8, 9]. MMPs degrade the ECM, whereas TIMPs inhibit the activity of MMPs [9]. Extracellular matrix metalloproteinase inducer (EMMPRIN) is known to regulate the levels of MMPs [10].

The possibility of sex hormonal regulation of MMPs and TIMPs has been speculated in uterine leiomyomas [11]. However, little is known about the direct action of sex steroid hormones on the ECM metabolism in uterine leiomyomas. This article provides recent evidence showing the regulatory activity of ovarian sex steroids and their steroid receptor modulators on collagen metabolism in uterine leiomyomas.

We have recently demonstrated that cultured human uterine leiomyoma cells have significantly lower EMMPRIN, MMP-1, and membrane type 1-MMP (MT1-MMP) protein contents, but significantly higher TIMP-1, TIMP-2, and types I and III collagen protein contents compared with cultured myometrial cells [12]. Treatment with selective progesterone receptor modulators (SPRMs) significantly increased EMMPRIN, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MT1-MMP at mRNA/protein levels and the enzymatic activities of MMP-1, MMP-2, MMP-3 and MMP-9 in the culture medium [12, 13]. In contrast, SPRMs significantly decreased TIMP-1, TIMP-2, and types I and III collagen protein levels in cultured leiomyoma cells compared with untreated control cultures. Moreover, RNA interference of EMMPRIN abrogated SPRM-mediated induction of MMPs and SPRM-mediated reduction of TIMPs and collagens in cultured leiomyoma cells. Thus, SPRMs disrupt the MMPs/TIMPs balance and reduce collagen synthesis in uterine leiomyoma cells, possibly resulting in the reduced deposition of collagens in the extracellular spaces. Thus, it is tempting to speculate that the antifibrotic action of SPRMs would cause the loss of tissue integrity and the reduced expansive activity of uterine leiomyomas *in vivo*. The shrinkage of uterine leiomyomas observed in patients treated with SPRMs [14, 15] may be partly attributed to the SPRM-induced inhibitory action on collagen synthesis.

Estrogen has recently been shown to modulate collagen metabolism in culture uterine leiomyoma cells. Collagen biosynthesis was demonstrated to be stimulated by low doses of estradiol (5 nM) in uterine leiomyoma cells, but high doses of estradiol concentration (10 nM) inhibited the process, while selective estrogen receptor modulator (SERM), raloxi-

fene and tamoxifen, inhibited collagen biosynthesis in uterine leiomyoma cells [16]. In addition, estrogen and SERMs inhibited MMP-2 levels in uterine leiomyoma cells. The authors suggested that estrogen may contribute to the accumulation of collagen in the ECM of uterine leiomyomas.

Estrogen metabolite 2-methoxyestradiol was shown to induce apoptosis and inhibit cell proliferation and collagen production in human uterine leiomyoma cells [17]. The authors demonstrated that 2-methoxyestradiol inhibited [³H]-proline incorporation in uterine leiomyoma cells, suggesting that collagen synthesis-inhibiting action of 2-methoxyestradiol decreases the leiomyoma size.

Collectively, it seems likely that both estrogen and progesterone act to up-regulate collagen synthesis in uterine leiomyomas, thereby increasing collagen accumulation in the ECM and promoting the expansion of leiomyoma tissues. By contrast, SERMs and SPRMs down-regulate collagen synthesis in uterine leiomyomas and may cause the suppression of tumor growth. The antifibrotic potential of SERMs and SPRMs may be promising for the treatment of uterine leiomyomas. Further study will be needed to clarify the precise mechanism underlying ovarian steroidal regulation of collagen synthesis in uterine leiomyomas.

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Three successful pregnancies following natural conception over an 8-year time span despite serum follicle stimulating hormone level greater than 15 mIU/ml

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Summary

Purpose: To demonstrate that the concept that a level of serum follicle stimulating hormone (FSH) of > 15 mIU/ml on day 3 of a younger woman's menstrual cycle suggests that the remaining eggs are of very poor quality consistent with a woman of very advanced reproductive age is a fallacy. **Methods:** A woman with a serum FSH > 15 mIU/ml on day 3 was studied over an 8-year time period. **Results:** Despite the absence of therapy with follicle maturing drugs, and at the latter part of the study the development of oligomenorrhea, the woman had three successful conceptions over eight years without ART techniques. Though two of her pregnancies were treated with progesterone starting in the luteal phase, her last pregnancy was without any treatment. **Conclusions:** The policy of certain physicians to advise couples that their eggs are extremely unlikely to develop into a normal pregnancy because of increased day 3 serum FSH and that they should immediately proceed to using donor oocytes (even if that is not their desire) is wrong. These women should be given an attempt to achieve a pregnancy with their own eggs. However, the treating physician should avoid the use of high-dosage follicle maturing drugs.

Key words: Elevated serum FSH; Natural conception; Egg quality vs quantity.

Introduction

Some studies suggest that when a woman has an elevated serum follicle stimulating hormone (FSH) on day 3 not only does she have a diminished egg reserve, but these eggs are of poor quality [1-7]. Evaluation of in vitro fertilization (IVF) statistics published by The Center for Disease Control shows extremely poor success rates despite transfer of normal appearing embryos universally among various IVF centers in women \geq age 45. As age advances with less egg reserve, and therefore less antral follicles selected, there is less inhibin B produced by these follicles and thus less of an inhibitor of pituitary release of FSH. The explanation for poor pregnancy rates in these reproductively older women is that over the years there has been a natural selection for the best eggs and when a woman reaches age 45 there are very few good eggs left.

Those IVF centers finding poor outcome in younger women with elevated day 3 serum FSH hypothesize that these younger women for some reason have had a more rapid rate of atresia leaving them with lower quantity and quality of eggs. Thus some have stated that even younger women with elevated day 3 serum FSH levels should proceed immediately to donor egg programs without even giving their own eggs a try [6, 7].

However, other studies make it clear that the dismal prognosis given by these studies is not related to poor egg quality but possibly related to an adverse effect of the high-dose gonadotropins used to try to stimulate these women with diminished egg reserve [8-15].

Instead of the theory that diminished egg reserve in younger women is related to a more rapid atresia process leaving lower number and quality of eggs (i.e., equivalent in quality to women of advanced reproductive age), the data showing good pregnancy rates as long as high-dose gonadotropins are not used suggest that areas of the ovary become damaged leading to less ovarian egg reserve but those remaining have the same quality as their age peers with normal egg reserve [12-15].

With the theory of ovarian damage rather than rapid atresia, the process could be ongoing or may have happened in the past. Thus, the woman may take several years before entering into menopause. One woman who achieved three successful deliveries in four IVF-ET cycles over eight years supports the concept of past damage leading to fewer eggs but the ones remaining having quality equal to their age peers [16]. To date this was the longest known interval between successful pregnancies in a woman with elevated day 3 serum FSH [16]. The present case describes another woman with three successful pregnancies over an eight-year time span that did not require IVF-ET.

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Case Report

A 30-year-old woman presented with six months of primary infertility. On evaluation her day 3 serum FSH was 17 mIU/ml in November, 1999. She was found to have regular menses and normal fallopian tubes by hysterosalpingogram and her husband had a normal semen analysis. At mid-cycle she attained a mature follicle of 19.5 mm with a serum estradiol (E2) of 274 pg/ml. A post-coital test was normal. Three days later the follicle collapsed by 8 mm evidence of egg release. She was treated with progesterone vaginal suppositories, 100 mg twice daily in the luteal phase. She conceived and the progesterone dosage was increased to 200 mg twice daily. She delivered a healthy full-term baby boy.

Her first conception was in December 1999. She then spontaneously conceived in August, 2002 but had only a chemical pregnancy. Her serum FSH was 21 mIU/ml. She then conceived again three months later and had a miscarriage at seven weeks. She had not taken progesterone supplementation for either of these pregnancies.

For pregnancy number 4 she conceived again spontaneously in February 2003 but was placed on supplemental progesterone once she had had a positive pregnancy test. However she still had another miscarriage at six weeks. Her day 3 serum FSH was 22 mIU/ml.

With her next pregnancy (no. 5) progesterone was started in the early luteal phase. She conceived after three treatment cycles in July, 2003 and delivered a full-term healthy girl.

In November, 2006 she conceived again but was not on any progesterone and miscarried at seven weeks.

Her menses had become irregular by that time and she thought that she would no longer be able to conceive because a repeat serum FSH was 24 mIU/ml and on ultrasound (US) the ovaries were small and no follicles over 1 mm were seen. However, because of pregnancy symptoms she attained a serum beta-hCG test in November 2007 which was 2412 mIU/ml and her serum progesterone was 55.5 ng/ml. Since the progesterone level was sufficient she was not supplemented with any additional progesterone. Her seventh pregnancy resulted in the delivery of a full-term healthy baby.

It should be noted that in her last two successful pregnancies she ovulated on day 7.

Discussion

As mentioned there has been a previous case report of three successful pregnancies despite elevated serum FSH over an 8-year time span following IVF-ET [16]. The case described here is the first case report of three successful pregnancies over the same 8-year time span despite elevated serum FSH involving natural conception.

Based on a recent study from a highly reputable IVF center that claimed no live pregnancies despite the transfer of normal appearing embryos in women of any age when the day 3 serum FSH was > 15 mIU/ml, there are many reproductive endocrinologists who will not even try to help a woman to conceive with her own eggs if this level of serum FSH is found but automatically suggest donor eggs [6]. Though successful pregnancies have been recorded in women still menstruating and even in women in apparent menopause with serum FSH levels > 100 mIU/ml, some infertility specialists may consider these cases as miracles that could not ever happen again [17-21].

The present case describing so many pregnancies including three successes over such a long time span suggests that many women with diminished egg reserve, have egg quality more consistent with their age peers rather than women of advanced reproductive age so that successful pregnancies are common if treated properly, i.e., without high-dose gonadotropins.

All of this woman's pregnancies were without the use of follicle maturing drugs and this supports the concept that the use of high-dose FSH stimulation by raising the serum FSH further, down-regulates the FSH receptor in granulosa theca cells for an FSH-dependent key protein needed for implantation, which in turn, may lower pregnancy potential [8-10, 12, 15]. It is our belief that most women who ovulate despite elevated serum FSH have luteal phase defects and benefit from taking extra progesterone in the luteal phase [11, 12]. It is interesting that our patient's last pregnancy was totally without progesterone supplementation. Also of interest is that the woman had two successful pregnancies ovulating on day 7. There seems to be a lower pregnancy rate when there is a short follicular phase possibly by not allowing sufficient estrogen exposure to induce adequate progesterone receptors in the endometrium [22, 23].

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Septoplasty allows successful delivery in a primary aborter with six previous first trimester miscarriages

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Summary

Purpose: To demonstrate that septoplasty can correct a problem of recurrent miscarriage. **Methods:** The patient was a primary aborter with a history of six consecutive miscarriages. Septoplasty by hysteroscopy/laparoscopy was performed. The septum was diagnosed by 3D sonohysterography. **Results:** She delivered a healthy live baby at 36 weeks. **Conclusion:** This case clearly demonstrates that sometimes the uterine septum plays an important role in recurrent miscarriage and that septoplasty can improve the chances of successful delivery. The case also shows that a definitive diagnosis of a bicornuate uterus by HSG and/or MRI can not be made.

Key words: Septoplasty; Miscarriage; 3-D sonohysterogram.

Introduction

When women present with a history of recurrent miscarriage most clinicians will perform uterine evaluations to determine if there are any uterine defects, e.g., adhesions, submucosal fibroids, polyps or congenital uterine anomalies. The main remediable congenital anomaly is the uterine septum. The best method for detection of the septum is either 3-dimensional (3D) ultrasound [1-4] or sonohysterography [5-8].

There are no randomized studies evaluating whether hysteroscopic resection of a septate uterus improves the miscarriage rate. However there are several observational studies suggesting that removing the septum can decrease miscarriage rates [9, 10]. A meta-analysis by Homer *et al.* of published retrospective data comparing pregnancy outcome before and after hysteroscopic septoplasty found a marked decreased miscarriage rate after surgery [11].

However not all studies agree that resection of the septum improves pregnancy outcome [12]. Even Homer *et al.* whose meta-analysis concluded that surgical repair was beneficial stated that the data did not support elective hysteroscopies [11]. In fact the data from Homer *et al.* suggested that after a single miscarriage 80-90% with a septate uterus will have a live birth in the next pregnancy [11].

The mechanism believed to be the etiologic factor for miscarriage with the subseptate uterus is that the embryo can implant on the septum which has a considerably reduced vascular supply relative to the rest of the uterus [12, 13]. Thus it would seem that the subseptate uterus should not be the sole cause of multiple repeated miscarriages in a primary aborter.

A case is presented where a primary aborter did have six consecutive miscarriages but was successful on her seventh pregnancy following hysteroscopic septoplasty.

Case Report

A 34-year-old woman, gravida 6, para 0-0-6-0, had the sixth miscarriage between five and eight weeks. The fetal products were evaluated in three of the losses and showed 46XY in two and 46XX in the other.

As part of the patient's evaluation by her preceding physician she had a hysterosalpingogram which suggested a bicornuate uterus. Since a subsequent magnetic resonance imaging (MRI) study also confirmed the likelihood of a bicornuate uterus, no further uterine evaluations were performed.

Other testing from the patient's previous physicians included: free thyroxin 0.9 ng/dl (nl 0.7-1.8), triiodothyronine 103 ng/dl (nl 60-181), thyroxin 6.3 ug/dl (nl 4.5-10.9), thyroid stimulating hormone 5.49 uIU/ml (nl 0.35-5.5), anticardiolipin antibody – IgG 15.0 GPL (nl < 23.0), ANA 0.6 (negative), lupus anticoagulant negative, prolactin 6.7 ng/ml (nl 3.0-20.0), testosterone < 20 ng/dl (nl 0-80), dehydroepiandrosterone sulfate 64 ug/dl (nl 35-430), Factor II – no mutation, Factor V Leiden – no mutation found, antiphosphatidyl serine IgG 3 GPS (nl 0-15), antithrombin activity 99% (nl 75-135), antithrombin antigen 121% (nl 75-130), homocysteine 9.2 umol/l (nl 3.3-10.4), protein S functional 69% (nl 60-145), protein C functional 75% (nl 74-151). She was subsequently treated with levo-thyroxin 112 mcg daily.

To further evaluate the possibility of a septated uterus a sonohysterogram was performed with 3D ultrasound. A septum was suggested rather than a bicornuate uterus.

The woman was given 3.75 mg depot-leuprolide acetate intramuscularly four weeks prior to diagnostic laparoscopy. Since the laparoscope did not show evidence of a bicornuate uterus, an operative hysteroscopy was performed. The septum was then transected by the Holmium laser. Postoperatively an intrauterine stent was placed for one week. She was then treated with 2.5 mg of conjugated estrogen twice daily for one month.

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A hysterosalpingogram was performed eight weeks following surgery and the uterine cavity was nearly normal with just a slightly arcuate fundus. Four months after surgery the patient conceived naturally. She delivered by cesarean section at 36 weeks a healthy 5 pound 14 ounce baby.

Discussion

As mentioned there are no controlled studies proving the efficacy of surgical removal of the septum to prevent subsequent miscarriages, just observational studies [3, 10, 14]. In fact the latest study suggested that transection of a septate uterus does not help prevent subsequent miscarriages [12]. In lieu of another observational study attempting to refute the aforementioned study [14] sometimes one extreme case report can present a convincing argument that at least in some instances a septate uterus can be a cause of recurrent miscarriages. Furthermore this case illustrates that at least in some cases transection of the septum may help to allow a successful outcome.

Chromosome analysis was performed on the fetal contents three times and each time it was normal. For sure the two with normal males proved no evidence of aneuploidy and probably the normal female also (though maternal contamination was possible). One can not say for sure that some of the six pregnancies may have implanted somewhere other than the septum but were lost for some other reason, e.g., a chromosome abnormality or progesterone deficiency.

There is no proof that the mechanism involved in miscarriage related to the uterine septum involves implantation on a relatively avascular septum. It is merely a hypothesis. The possibility exists that the uterine septum leads to some other mechanism for miscarriage even when the implantation is in the uterine wall and not the septum.

This case illustrates one other clinical point. A hysterosalpingogram and MRI combination is not sufficient to diagnose a bicornuate uterus and exclude a septate uterus. With multiple losses laparoscopy and hysteroscopy might be utilized directly, or maybe as illustrated in this case, the use of hysterosonogram with 3D ultrasound would provide a better method to diagnose a septate uterus [5-8].

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Pregnancy outcome after treatment of cervical intraepithelial neoplasia by the loop electrosurgical excision procedure and cold knife conization

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Summary

Purpose of investigation: The aim of this study was to evaluate the effect of LEEP and cold-knife conization on the outcome of subsequent pregnancy in a tertiary public hospital. *Methods:* One hundred and ninety-nine patients met the inclusion criteria (age between 18 and 45 years old). Cold-knife conization, LEEP, and both (conization and LEEP) were performed in 102 (51.3%), 95 (47.7%) and two (1%) women, respectively. Average ages were respectively, 33 ± 7.3 ; 25 ± 6.73 and 30 ± 2.8 . *Results:* Pregnancies occurred 2.6 and 4.8 years after LEEP and conization, respectively. Miscarriages and preterm pregnancies were more frequent in conization cases versus LEEP, 26% and 5.2%, 23% and 5.5%, respectively. *Conclusion:* If patients express a desire for pregnancy, LEEP should be the procedure of choice.

Key words: : Cervical intraepithelial neoplasia; Conization; Loop electrosurgical excision procedure; Pregnancy, outcome.

Introduction

The most common treatment of cervical intraepithelial neoplasia is the loop electrosurgical excision procedure (LEEP) and cold-knife conization. LEEP requires local anesthesia, whereas cold-knife conization requires hospitalization and general or spinal anesthesia [1-2]. Hemorrhage is a main intra- and early postoperative complication, and cervical stenosis is more frequent in cold-knife conization than LEEP [1-3].

Today pregnancy has become a late event in many women's lives. Thus, a desire for pregnancy after CIN treatment is a relatively common solicitation. In terms of pregnancy outcome, cryosurgery and laser have been found to be safer procedures, but they destroy the tissue making it unavailable for further histological examination. This is important for treatment because an invasive lesion is not diagnostic, and an important marker of recurrence, CIN in surgical margins, is not evaluated. Thus, LEEP and cold-knife conization are illegible treatment for CIN.

Previous studies on pregnancy outcome after treatment of CIN have shown conflicting results. Cold-knife conization [4, 5], and laser [6, 7] are associated with preterm delivery and low birthweight. The effect of LEEP in pregnancy results also showed discordant data [8-12]. Nonetheless, one study affirms that diagnosis of precancerous changes in the cervix, regardless of the treatment, was associated with increased risk of preterm birth and that the preferential use of ablative treatment should be given [12].

Contradictory results on pregnancy outcome have been described after LEEP and cold-knife conization. The aim of this study was to evaluate the effect of LEEP and cold-knife conization on the outcome of subsequent pregnancy in a tertiary public hospital.

Patients and Methods

Patients

A retrospective study was conducted on surgical specimens of women submitted to LEEP and cold-knife conization due to CIN, from 1 January 1981 to 31 December 2004, in the Gynecologic and Obstetrics outpatient service of our Institution (Research Institute of Oncology - IPON/UFTM). The project was approved by the Research Ethics Committee of UFTM.

A total of 338 women were referred because of the presence of CIN I-III in a biopsy specimen. One hundred and ninety-nine patients met the inclusion criteria (age between 18 and 45 years old). Patients submitted to hysterectomy or proposed bilateral tubal ligation for sterilization were excluded. All patients were previously submitted to both triple collection of material for cytological examination and colposcopically directed biopsies. Cold-knife conization, LEEP, and both (conization and LEEP) were performed in 102 (51.3%), 95 (47.7%) and two (1%) women, respectively. Average age was respectively, 33 ± 7.3 , 25 ± 6.7 , and 30 ± 2.8 .

LEEP, conization and hysterectomy management, and follow-up criteria for LEEP, conization and hysterectomy were performed by residents supervised by board-certified attending obstetrician-gynecologists. LEEP was performed in women with the LEEP WEM machine, using a power setting of 50 W. After application of Lugol iodine, cervical anesthesia was performed with 2% lidocaine containing a solution of 1:1000 epinephrine (4-6 ml, approximately 1 ml per cervical quadrant). Loop size was 10 mm x 10 mm, and current was blended to cut and coagulate. After the procedure, all cases underwent roller ball coagulation (50 W) with the aim of hemostasis. The main

criteria for LEEP in the majority of cases were small lesions, visible squamocolumnar junction (SCJ), and desire for future pregnancy.

Cold-knife conization was performed on patients in the operating room after spinal anesthesia. After Schiller's test, a surgical margin of 2 mm was done by bistury and the cone specimen was extirpated. A hystrometer was placed in the endocervix and at least 1 cm of endocervix from the internal cavity was left. The sturmdorf procedure was performed.

Follow-up criteria after the above procedures consisted of cytology and colposcopy each six months for five years, and after annually. The minimum time of follow-up was 16 months. The presence of CIN I-III confirmed by colposcopically directed biopsies was considered as recurrence. The median time of patient follow-up for diagnosis of pregnancy ranged from one to 23 years (median 10 years).

Cytohistological techniques and colposcopy

The cytological material was processed by Papanicolaou's technique and reading was performed by trained cytologists. Biopsies were guided by colposcopic exams carried out by residents under teaching supervision, and the material was fixed in 4% formaldehyde. Colposcopy was considered unsatisfactory when the SCJ was not visible. There was no standard number of histological cuts for each biopsy, varying from 1-10 successive cuts as judged necessary by the pathologist for each case.

Cone biopsy specimens were marked with sewing thread at the 12 o'clock position. Hysterectomy and cone biopsy specimens were fixed in 4% formaldehyde. The cone biopsy or uterine cervix were cut into pieces of about 1 mm in thickness, perpendicular to the surface of the endocervical mucosa and the material was processed for inclusion in paraffin. One histological cut of each block was stained with hematoxylin-eosin. Additional cuts were made when necessary.

Statistical analysis

Comparisons between groups were made with the chi-square test with Yates' correction or the Fisher's exact test, depending on the conditions of validity of the chi-square test. The differences were considered significant with $p < 0.05$.

Results

One hundred and ninety-nine patients met inclusion criteria and were evaluated (102, 95, and two were submitted to cold-knife conization, LEEP, and LEEP and cold knife conization, respectively). Histological results of CIN I, II and III were found in one, 25, and 76 cases of the LEEP procedure; 0, 2, and 93 in the cold-knife conization. The two cases of CIN III in patients undergoing LEEP and conization, first had CIN I with recurrence after LEEP.

Table 1 shows the frequency of full-term pregnancy and miscarriages in patients who underwent conization and LEEP. Miscarriages were most frequent in conization cases. Preterm pregnancy was more frequent in conization cases (Table 2). Pregnancies occurred 3.5 ± 3.09 and 3 ± 3.9 years after LEEP and conization, respectively.

Discussion

This study was conducted in a tertiary service of gynecology and obstetrics that examines pregnancy outcomes

Table 1. — Total number of full-term pregnancies and miscarriages in patients submitted to conization and/or LEEP.

	Full term pregnancy		Miscarried		
	n	%	n	%	
LEEP (n = 95)	18	18.9	1	1	(5.2)
Conization (n = 102)	17	16.6	6	5.8	(26)
LEEP and conization (n = 2)	1	50	0	0	(0)

(): % in relation to number of pregnancies. X^2 test: $p = 0.0240$.

Table 2. — Distribution of preterm birth and low birthweight in patients undergoing conization and/or LEEP.

	Preterm pregnancy		Low birth-weight	
	n	%	n	%
LEEP (n = 18)	1	5.5	2	11.1
Conization (n = 17)	4	23.5	2	11.7
LEEP and conization (n = 1)	—	—	—	—

X^2 test: $p =$ not significant.

following histological diagnoses and treatment for CIN, thus, the same group of physicians are involved. The aim of this study was to compare pregnancy outcome after conization and/or LEEP for CIN in our service where a standard treatment is utilized and the median age of patients is similar. The results showed that miscarriages occurred more frequently in patients with previous conization than in patients who underwent LEEP.

Preterm birth was more frequently found in patients treated for CIN (standardized prevalence ratio 2.0, 95% CI 1.8-2.3) but also in untreated patients (standardized prevalence ratio 1.5, 95% CI 1.4-1.7) than the general population, suggesting that the treatment is not the only factor that plays a role in increased risks for preterm patients [12]. In other studies on untreated patients, preterm birth was reported in 10.7 to 12.2% [10], but none has been compared with the general population. In our series, a 13.8% prevalence was found (5 out of 36 pregnancies > 20 weeks).

Our results showed that preterm birth is a more frequent event in patients submitted to conization than LEEP (23.5 vs 5.5%). Inconsistent findings on the association between conization and preterm birth have been shown in previous studies. A retrospective analysis showed that after conization women had an increased odds ratio of preterm birth after adjusting for maternal smoking, race, parity, marital status and history of induced terminations (OR 1.6, 95% CI 1.2-2.0) [13].

Conization is the most cumulative experience in the treatment of CIN, thus the possible data on its influence in fertility is insufficient for a definitive conclusion [14]. Delivery before 37 weeks and a birthweight lower than 2,500 g had a relative risk, respectively, of 3.4 and 2.5 compared to a control group [15]. An increased rate of induced abortion and a significantly higher risk of preterm delivery in a group that had undergone conization (OR 4.13, 95% CI 2.53-6.75) were found in another study [5, 16]. Cone height is an important point to discuss. One study showed that a cone height of at least 10 mm is an independent risk factor for the duration of pregnancy, and for the occurrence of preterm delivery in

a subsequent pregnancy [16]. In our study, we performed conization with a hysterometer and at least 1 cm of endocervix was left. However with this practice miscarriages were more common than for the LEEP procedure.

Although the number of LEEP being performed has increased, the data regarding pregnancy outcome is more scant than for conization data [14]. A study in Norway demonstrated that LEEP in women with CIN did not significantly increase the risk of preterm birth or low birth-weight in subsequent pregnancies in comparison with controls, nevertheless, the size of the electrosurgical loop was relatively large [11]. Apparently, the maximum diameter of loop that will not affect pregnancy outcome is 18 mm [11, 17], but a loop diameter of 25 mm is correlated with increased risk of preterm birth and low birth-weight [11, 16, 18]. We used a loop of 1 x 1 cm. The small diameters of loop that we utilized explain the low finding of miscarriages in comparison with conization. Nevertheless, when we used this type of loop, more than two fragments of cone specimen were removed, which can be a problem for accurate histological studies, but the fragments were small and could explain the low rate of miscarriages in our results.

One review showed all excisional procedures to treat CIN present similar pregnancy-related morbidity without apparent neonatal morbidity [19]. Another study demonstrated that the diagnosis of precancerous changes in the cervix (regardless of the treatment) was associated with an increased risk of preterm birth. The results showed that both treated and untreated women were at a significantly increased risk for preterm birth compared with those in the general population: treated - standardized prevalence ratio (SPR) 2.0, 95% CI 1.8-2.3 and untreated - SPR 1.5, 95% CI 1.4-1.7. Within the cohort, the treated women were significantly more likely to give birth preterm (adjusted OR 1.23, 95% CI 1.01-1.51). Cone biopsy, LEEP and diathermy were associated with preterm birth. After adjusting for possible confounding factors, only diathermy remained significant (adjusted OR 1.72, 95% CI 1.36-2.17). Women treated using laser ablation were not at an increased risk for preterm birth (adjusted OR 1.1, 95% CI 0.8-1.4), but consideration should be given to the preferential use of ablative treatments [12].

Conization and LEEP are safe procedures for CIN treatment. Histological study of the specimen is important to exclude invasion [1, 2]. Expectant observation is conducted in pregnant women with CIN, but a surgical procedure is indicated in non pregnant women. In our point of view, histological study is important and the procedures must be preferable to others that do not permit histological analysis. Taken together, our results showed that the frequency of miscarriages is minor in women who undergo LEEP. Thus, if patients express a desire for pregnancy, LEEP should be the procedure of choice.

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The possible influence of increased body mass index on the clinical efficacy of standard human chorionic gonadotropin dosage

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Summary

Objectives: To evaluate whether the efficacy of standard (10,000 IU) hCG dosage is BMI dependent. **Patients & Methods:** During the study period, body mass index (BMI) was recorded in 261 consecutive women enrolled in our ICSI program. Women in the 90th BMI percentile were compared with those in the 10th percentile. The number and percent of mature metaphase-II (M-II) oocytes were considered as the outcome measure. **Results:** Mean BMI of the 10th and 90th percentile groups were 18.2 ± 0.7 kg/m² (n = 26) and 32.8 ± 2.2 kg/m² (n = 27), respectively. There were no differences between the groups in mean patients age, number of gonadotropin ampoules used, mean number of oocytes retrieved or the number and percentage of mature M-II oocytes. **Conclusions:** Standard (10,000 IU) hCG dosage is adequate to induce final oocyte maturation in IVF patients regardless of their BMI. This may imply that this hCG dosage is much higher than the dosage that is actually required.

Key words: BMI; ART; ICSI; hCG; M-II oocyte.

Introduction

Obesity is an increasing major health problem [1]. The magnitude of its associated health detriments increases with increasing body mass index (BMI). While being overweight or obese (BMI > 30 kg/m²) may reduce a woman's fertility and increases pregnancy loss [2] and late pregnancy complications (preeclampsia, gestational diabetes, etc.) [3], data regarding the impact of obesity on IVF cycle outcomes are controversial.

Moreover, while several reports have shown no effect of increasing BMI on in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) success rates, except for higher cancellation rates [4-7], others have demonstrated that excess weight is associated with lower cumulative live birth rates, lower implantation rates, increased gonadotropin requirement, and the possibility of decreased follicle development and oocyte numbers [8-14].

Controlled ovarian hyperstimulation (COH) is apparently a key factor in the success of in vitro fertilization-embryo transfer (IVF-ET). During IVF treatment, human chorionic gonadotropin (hCG) is usually used as a surrogate LH surge to induce final oocyte maturation and resumption of meiosis. The presence of metaphase-II (MII) oocytes reflects the resumption of meiosis of the oocyte and can be objectively assessed after the oocytes have been denuded before ICSI. The number and the percentage of MII oocytes are therefore better indicators of

final maturation of the oocyte than the total number of oocytes retrieved.

Recently, in an attempt to explore whether the mechanism is ovarian or extraovarian, Bellver and colleagues [15] studied the effect of BMI on the reproductive outcome in the oocyte donation model, thus excluding potentially confounding ovarian hormonal environment. Their results showed similar implantation, pregnancy, and ongoing pregnancy rates among the four studied BMI groups (< 20 kg/m², 20-24.9 kg/m², 25-29.9 kg/m², and > 30 kg/m²), with negative trends as BMI increased. They therefore concluded that the ovary is not the only factor responsible for the poor reproductive outcome in obese patients; the endometrium or its environment also contributes to this discouraging prognosis, but in a more subtle manner.

Carrell *et al.* [16] evaluated the relationship between intra-follicular hCG concentration and BMI. They observed a significant inverse correlation between the BMI and intra-follicular hCG concentration, which could be attributed to the concurrent decrease in embryo quality and pregnancy rates. Salha *et al.* [17] while examining the effect of adiposity on serum concentrations of hCG found that patients with high BMI had a significantly lower mean serum hCG concentration, required a higher dosage of gonadotrophin and had significantly fewer oocytes aspirated.

Prompted by these findings, and in a further attempt to clarify the mechanisms by which BMI affects the reproductive outcome, we decided to evaluate whether the effect of standard (10,000 IU) hCG dosage on resumption

of meiosis and final maturation of the oocytes are BMI dependent.

Patients and Methods

We reviewed the computerized files of all consecutive women admitted to our IVF unit during a one-year period, who had reached the ovum pick-up (OPU) stage. Exclusion criteria included use of donor oocytes or transfer of frozen-thawed embryos, and use of any other procedure which does not include ICSI. All the usual indications for ICSI and accepted protocols for ovarian stimulations were included.

All patients received 10,000 IU to induce final oocyte maturation. The criteria for hCG administration were identical in all of these patients, namely, at least three follicles with the diameter of 18 mm with appropriate peripheral estradiol levels. Oocytes were retrieved 35 to 38 hours after hCG administration. Following OPU, and before the ICSI procedure, oocytes were denuded and classified according to their intactness and nuclear maturational stages, e.g., germinal vesicle, metaphase-I and mature metaphase II (M-II) oocytes.

Data on patient age and infertility-treatment-related variables, with emphasis on the patients' BMI, ovarian stimulation characteristics, number of oocytes retrieved and the number of M-II oocytes, were collected from the files.

Results are presented as means \pm standard deviations. Differences in variables were statistically analyzed with the nonparametric Wilcoxon signed rank test, Student's t-test and chi-square test, as appropriate. A p value of less than 0.05 was considered significant.

Results

Two hundred and sixty one patients with a mean age of 32.8 ± 5.9 years and mean BMI of 23.7 ± 4.4 kg/m² (range: 16.6-38.1 kg/m²), were evaluated. Patients were divided into three groups according to their BMI. Underweight, BMI \leq 10th percentile (10th percentile group); normal; at risk of overweight, BMI $>$ 10th and $<$ 90th percentile (10-90th percentile group); obese, BMI \geq 90th percentile (90th percentile group). The clinical characteristics of their ICSI cycles are shown in Table 1.

Mean BMI of the 10th and 90th percentile groups were 18.2 ± 0.7 kg/m² (n = 26) and 32.8 ± 2.2 kg/m² (n = 27), respectively (p < 0.0001). Patients in the 90th percentile group required significantly longer stimulation compared to the two other groups, and had significantly lower peak E2 levels as compared to the 10th percentile group. Moreover, we observed non-significant trends toward increase in the length of stimulation and the number of gonadotropin ampoules used, with increasing BMI. There were no differences between the groups in mean patient age, number of gonadotropin ampoules used or mean number of oocytes retrieved. Moreover, the number and percentage of mature M-II oocytes were similar among the three groups (Table 1).

Discussion

In the present study of patients undergoing ICSI, standard (10,000 IU) hCG dosage was adequate to induce

Table 1. — Comparison between IVF cycles according to the different BMI percentile groups.

	BMI - 10 th percentile	BMI - 10 th to 90 th percentile	BMI-90 th percentile
Number of cycles	26	208	27
Patient age (yrs)	31.3 \pm 5.9	33.1 \pm 6.0	31.9 \pm 4.8
BMI (kg/m ²)	18.2 \pm 0.7 ^a	23.2 \pm 2.9 ^b	32.8 \pm 2.2 ^c
Day 3 FSH (IU/l)	6.5 \pm 2.3	6.5 \pm 3.1	5.9 \pm 2.5
Length of stimulation (days)	10.1 \pm 2.0 ^d	10.5 \pm 2.0 ^e	11.0 \pm 2.7 ^f
Number of gonadotropin ampoules used	43 \pm 25	46 \pm 26	50 \pm 25
Peak E2 levels on day of hCG administration (pg/ml)	2480 \pm 1428 ^g	2093 \pm 1171	1666 \pm 1065 ^h
Progesterone levels on day of hCG administration (ng/ml)	0.8 \pm 0.4	1.0 \pm 0.9	0.5 \pm 0.3
Number of oocytes retrieved	11.6 \pm 7.4	10.6 \pm 6.6	12.3 \pm 8.8
Number of oocytes stripped for ICSI	10.4 \pm 7.1	8.9 \pm 6.2	10.6 \pm 7.6
Ratio of M-II oocytes per oocytes stripped for ICSI (%)	72 \pm 23	78 \pm 21	77 \pm 21

BMI - body mass index.

p < 0.05 between ^aand ^band ^c, between ^dand ^eand ^f, between ^gand ^h.

Standard (10,000 IU) hCG dosage is adequate to induce final oocytes maturation in IVF patients regardless of their BMI.

final oocyte maturation regardless of their BMI. This probably implies that the current hCG dosage is much higher than the dosage actually required for the resumption of meiosis of the oocyte. This observation is in agreement with previous studies demonstrating that oocyte maturation can be achieved with hCG doses of lesser amplitude than those required for follicular rupture [18]. Moreover, a low serum hCG level before oocyte recovery was shown to be as equally potent as the higher levels at initiating oocyte maturity and as reflected by a similar percentage of MII oocytes recovered [19].

We also observed a trend toward increase in the length of stimulation and the number of gonadotropin ampoules used and a decrease in peak E2 levels, with increasing BMI. These later observations are in accordance with other studies showing an association between an increased BMI and an increased gonadotropin requirement during COH [8, 12] or decreased serum estradiol concentrations [5, 7].

Recently, several editorials have addressed the role of BMI in IVF outcome [20, 22]; all of which referred to the study by Bellver and colleagues [15], who attempted to delineate the mechanism for the observed decrease in the reproductive outcome of obese patients, by the oocyte donation model. In the later study, an extraovarian mechanism was shown to contribute to the poor reproductive outcome in obese patients. Exploring the effect of obesity on folliculogenesis will therefore complete this puzzle.

In the present study, we added further information to this puzzle by demonstrating no effect of obesity on final oocyte maturation and resumption of meiosis. Further large studies are therefore required to assess the effect of obesity on COH variables and embryo quality and to clarify the pathophysiologic alterations responsible for the associated poor reproductive outcome in obese patients.

These studies may help fertility specialists in individualization and careful tailoring of the COH protocol, for optimizing IVF success in obese patients.

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Serological testing for celiac disease in women with endometriosis. A pilot study

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Summary

Purpose of investigation: Celiac disease (CD) involves immunologically mediated intestinal damage with consequent micronutrient malabsorption and varied clinical manifestations, and there is a controversial association with infertility. The objective of the present study was to determine the presence of CD in a population of infertile women with endometriosis. *Methods:* A total of 120 women with a diagnosis of endometriosis confirmed by laparoscopy (study group) and 1,500 healthy female donors aged 18 to 45 years were tested for CD by the determination of IgA-transglutaminase antibody against human tissue transglutaminase (t-TGA) and anti-endomysium (anti-EMA) antibodies. *Results:* Nine of the 120 women in the study group were anti-tTGA positive and five of them were also anti-EMA positive. Four of these five patients were submitted to intestinal biopsy which revealed CD in three cases (2.5% prevalence). The overall CD prevalence among the population control group was 1:136 women (0.66%). *Conclusion:* This is the first study reporting the prevalence of CD among women with endometriosis, showing that CD is common in this population group (2.5%) and may be clinically relevant.

Key words: Celiac disease; Endometriosis; Infertility; Serologic screening; Pelvic pain.

Introduction

Celiac disease (CD) is characterized by chronic intolerance to gluten ingestion in genetically susceptible individuals, leading to immunologically mediated intestinal tissue damage [1, 2]. The disease may occur in different forms, with patients being asymptomatic or having the classical clinical forms characterized by diarrhea with or without intestinal malabsorption. This broad spectrum of manifestations often impairs the diagnosis of this disease. CD has been described in several world regions including Europe, North America, South America, Africa, India, and New Zealand, and is rare among African blacks and Asians such as Chinese and Japanese subjects. The disease predominates among white individuals and the female to male ratio is 2:1.

Some clinical conditions are related to CD, such as diabetes mellitus, selective IgA deficiency, autoimmune thyroiditis, primary biliary cirrhosis, neuropsychic changes, herpetiform dermatitis, autoimmune disease, osteoporosis, and reproductive disorders [1]. Several studies have indicated a higher prevalence of reproductive disorders among women with CD, such as spontaneous abortion, late menarche, early menopause, amenorrhea, and infertility [3-5].

One of the major causes of infertility is endometriosis, whose incidence in infertile patients ranges from 10-25%, with a prevalence increasing to 60-70% in cases of chronic pelvic pain, and it may be present in 1-20% of asymptomatic women. Although the pathogenesis of

endometriosis is not completely understood, endometriosis is associated with changes in both cell-mediated and humoral components of innate and acquired immunity with an increase of auto-antibodies and circulating immunocomplexes [6]. Celiac disease is also an immunologically mediated disease with production of auto-antibodies and association with a series of other autoimmune diseases such as IgA deficiency, diabetes mellitus, autoimmune thyroiditis, and herpetiform dermatitis [2]. Although there are many reports of CD among infertile patients, there are no reports specifically evaluating a group of women with endometriosis. The present study was undertaken to evaluate the prevalence of celiac disease in a cohort of Brazilian women with endometriosis. This was achieved by means of serological screening based on anti-t-TGA, confirmed by anti-endomysial antibody (EMA) IgA, which showed a sensitivity of 95-100% and 85-100%, and a specificity of 94-100% and 95-100%, respectively, and final confirmation was obtained with a jejunal biopsy and by patient follow-up.

Material and Methods

The present cohort consisted of 120 consecutively selected women with endometriosis attending a tertiary university service due to pelvic pain and/or infertility between 2000 and 2003. Inclusion criteria were age between 18 and 45 years and a laparoscopic and histological diagnosis of endometriosis made up to six months before blood collection. As a population control, blood samples were collected from 1,500 apparently healthy female blood donors aged 18 to 45 years at the Blood Center of Ribeirão Preto, State of São Paulo, Brazil, from September 2001 to October 2002 (same city for all patients). Data about these individuals have been published previously [7]. The

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studies were approved by the internal Institutional Review Board and patients gave written informed consent to participate.

Serological screening was applied to blood samples collected during the early follicular phase from the study group. After separation, serum was stored at -70°C and the samples were tested in duplicate in the same assay. The blood samples were tested for the presence of t-TGA antibodies by ELISA and when a sample was positive for this antibody, it was tested for EMA-IgA by indirect immunofluorescence. The INOVA commercial kit (San Diego, CA, USA) was used for the detection of t-TGA. Serum samples were diluted 1:100 and ELISA readings were obtained at 450 nm wavelength. A value of less than 20 IU was considered to be negative, a value of 20 to 30 IU was considered to be weakly positive, and a value of more than 30 IU was considered to be moderately/strongly positive. The sensitivity and specificity of this test were 95-98% and 94-95%, respectively. Another INOVA kit was used for the detection of EMA-IgA by indirect immunofluorescence using slides with monkey esophagus sections as substrate. Sera were diluted 1:5, 1:10 and 1:20 with phosphate buffered saline (PBS), pH 7.2. A result was considered to be positive when immunofluorescence was observed starting from the 1:10 dilution. The sensitivity and specificity of this test were 90-100% and 97-100%, respectively. An intestinal biopsy was proposed when both tests were positive. The biopsy was obtained by jejunal aspiration capsule and/or by upper digestive endoscopy and analyzed histologically according to Marsh criteria [8]. Subjects diagnosed as having CD underwent a standard clinical assessment aimed at detecting symptoms or signs of digestive tract disease or manifestations suggestive of extra-intestinal involvement.

Data were analyzed statistically using the GraphPad Prism® 2.01 32 Bit Executable program (GraphPad Software Inc., San Diego, CA, USA). Paired variables with normal distribution were analyzed by the Student's *t*-test and analysis of variance was carried out using the F test for comparison of the prevalence of CD, with the level of significance set at $p < 0.05$. The chi-square test was used to compare the prevalence of CD among the three groups studied (endometriosis x population control x endometriosis control), and the Fisher exact test was used for 2 x 2 comparisons.

Results

Mean age was 29.2 ± 5.6 years for the endometriosis group and 27.9 ± 4.5 years for the population control ($p = 0.10$ for the endometriosis group). The symptoms reported by the 120 women with pelvic endometriosis were infertility (81.0%), dysmenorrhea (91.0%), and dyspareunia (57.1%). The patients had Stage I/II (56.2%) or III/IV (43%) endometriosis as defined by the American Society of Reproductive Medicine (ASRM). Of the 120 patients with endometriosis, nine were positive for t-TGA at levels ranging from 25.54 to 190.7 IU (Table 1), with a prevalence of 7.5%. When these nine patients were tested for EMA-IgA, five were found to be positive at 1/10 and 1/20 dilution (5/120 = 4.16%). One patient refused the jejunal biopsy (patient no. 8, Table 1) and three of the four patients submitted to the biopsy (prevalence of confirmed cases: 3/120, 2.5%) fulfilled the histopathological criteria for CD Marsh grade III (destructive pattern) (nos. 1, 4 and 5, Table 1). In a later analysis, the three patients with confirmed CD presented mild gastrointestinal symptoms, with

undefined abdominal discomfort but without persistent diarrhea. The other two EMA-IgA-positive patients without confirmation by the biopsy (nos. 6 and 8, Table 1) had no gastrointestinal symptoms. There were no signs or symptoms of extra-intestinal disease in any of the nine t-TGA-positive patients.

In the population control group 20 women showed moderately/strongly positive t-TGA levels and 12 of them were EMA-IgA positive, while the other eight were EMA negative. These individuals who were positive to both serum tests were submitted to an intestinal biopsy. Of these, two were classified as Marsh type 0, and 10 as Marsh \geq type 1, with a global prevalence of CD among females of 0.66% (10 confirmed cases, 1:136 women) [7]. Statistical analysis revealed that the prevalence rates of positive serology in the study group (12 out of 120) versus the population control group (12 out of 1,500) was significant ($p = 0.001$), odds ratio (OR) = 5.4 with a confidence interval (CI) = 1.8 – 15.5 and a test power = 60%. The prevalence rates of biopsy-confirmed CD were: three out of 120 versus the population control group (10 out of 1,500), with the difference being non significant ($p = 0.065$), OR = 3.8 with CI = 1.03 – 14.08 and a test power = 40%.

Table 1. — Serologic results for the patients with an initial positive screening for t-TGA.

Patient	t-TGA(IU)	EMA-IgA	Biopsy
1	138.9	1:20	Type 3
2	81.03	Negative	ND
3	25.54	Negative	ND
4	102.5	1:20	Type 3
5	190.7	1:20	Type 3
6	63.34	1:20	Type 0
7	26.5	Negative	ND
8	86.71	1:20	ND
9	28.01	Negative	ND

ND: not done.

Discussion

Celiac disease is an enteropathy that occurs in genetically susceptible individuals and is characterized by permanent lesion of the intestinal mucosa triggered by the ingestion of gluten, with full recovery occurring when gluten is removed from the diet. Dietrich *et al.* [9] showed that the enzyme tissue transglutaminase is a target of immunological reaction. The deamidation activity of this enzyme seems to generate a negative charge in the prolamine bound to the HLA molecule and this complex is recognized by T helper cells, which activate other lymphocytes in the intestinal mucosa, causing an inflammatory response by the production of interleukins, interferon-gamma and tumor necrosis factor [1].

Clinically, the classical form is characterized by chronic diarrhea (malabsorption syndrome) and malnutrition, and the atypical form by various other symptoms, including manifestations of thyroid disease, epilepsy and infertility [1]. Individuals with the silent form are asymptomatic but have positive serology for CD and alteration

of the intestinal mucosa, and individuals with the latent form have positive serology but do not present histological changes of the intestinal mucosa [10]. CD is probably much underdiagnosed.

Several studies have tried to relate a higher incidence of infertility to the presence of CD or a higher incidence of CD to the presence of infertility. Collin *et al.* [11] studied women of reproductive age with primary or secondary infertility and women with repeated abortion. Anti-reticulín and anti-gliadin antibodies were used to screen for CD and positive cases were confirmed by an intestinal biopsy. Four of 150 infertile patients (2.7%) and none of the 150 control women had a diagnosis of CD. In the group with sterility of no apparent cause, the incidence of CD was 4.1%. Other studies have reported an incidence of CD ranging from 1.6% to 3.3% among infertile couples, with a higher prevalence (8%) being observed when patients with sterility of no apparent cause are evaluated separately [5, 12]. However, there is no consensus about a systematic survey of this affection in infertile patients.

Since endometriosis is one of the main causes of infertility and since there are no studies specifically evaluating a group of women with endometriosis, we conducted a study of CD in women with proven endometriosis regardless of the type of symptoms presented, although most of them were infertile. Our interest was to determine the prevalence of disease (CD) in a group of patients with clinical manifestations of confirmed endometriosis. As reported in surveys of CD among infertile women, we observed that CD is frequent among women with endometriosis and that this frequency is higher than that for the general population, although we did not detect a statistically significant difference, probably due to the small number of cases, as also reported in other studies on the association of infertility and CD [5]. We used a significant control of the same ethnic composition as the study population since there are race-related differences in the prevalence of CD. Although blood donors cannot be considered to be representative of the general population, we selected them because these are apparently healthy individuals, especially regarding nutrition and the absence of serious diseases. The population of the State of São Paulo presents important miscegenation with Europeans, which was now found to be associated with a higher prevalence of CD than previously recorded. This miscegenation mainly occurred during the first 50 years of the last century (specifically from 1920 to 1930), after the immigration of huge numbers of Italian and Spanish citizens to São Paulo, where these immigrants came to work on coffee plantations and in the incipient Brazilian industry.

This is the first report on CD prevalence in women with endometriosis, demonstrating that CD is common (2.5%) in this patient population and may be of clinical relevance. If the presence of CD had been confirmed in the patient who refused the intestinal biopsy, the prevalence of CD in the endometriosis group would have been 3.3%, a value that would be statistically significant ($p = 0.01$),

and that would support the possibility that CD is common among women with endometriosis.

If we assume that there is a causal link between CD and endometriosis, independently of the presence of infertility, we can speculate about a common genetic/immunologic link more than about a possible nutritional deficit. Although the pathogenesis of endometriosis is not completely understood, endometriosis is associated with changes in both the cell-mediated and humoral components of innate and acquired immunity [6]. Celiac disease also is an immunologically mediated disease, with the production of autoantibodies and an association with other autoimmune diseases [2].

The present study is the first to associate CD with endometriosis, although the clinical relevance of this increase may be questioned. As reported in surveys of CD among infertile women, we observed that CD is frequent among women with confirmed endometriosis and that this frequency is higher than that in the general population. Further studies will probably elucidate the clinical relevance of these findings.

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Microcolposcopy in the diagnostic evaluation of abnormal cervical cytology: when and why to do it

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Summary

Microcolposcopy is an *in vivo* cytological examination of the uterine cervix allowing the localization of exoendocervical precancerous lesions. The aim of this study was to assess the diagnostic reliability of microcolposcopy by means of correlation with histology, colposcopy and Pap test results. For the study, 256 patients with abnormal Pap test results were selected and subjected to colposcopy and microcolposcopy with the aim of evaluating the presence of any intraepithelial lesions. One hundred and nine of these patients were subjected to a biopsy. Colposcopy, histology and cytology results were compared with those obtained by microcolposcopy. In low-grade squamous intraepithelial lesion (LSIL) cytology cases, the percentage agreement on lesion grade between Pap test and microcolposcopy results was 74%, while in high-grade squamous intraepithelial lesion (HSIL) cytology cases, it was equal to 80%. The comparison between colposcopy and microcolposcopy showed a level of agreement of 72% for lower grades and 68% for higher grades. Finally, histology was in agreement with microcolposcopy in 73% of cervical intraepithelial grade 1 neoplasia (CIN 1) cases and reached 71% for CIN 2-3. Microcolposcopy proved to be accurate with regard to the diagnosis of lesion grade, and showed to be definitive in patients where cytology was positive for HPV infection and colposcopy was not able to identify any lesions.

Key words: Pap test; Abnormal cervical cytology; Colposcopy; Microcolposcopy; Squamous intraepithelial lesions.

Introduction

Microcolposcopy is a rarely used diagnostic technique, introduced in 1981 by Hamou, who invented the microcolpohysteroscope [1]. This device allows magnification of up to 150 times for performing *in vivo* cytological examinations of the uterine cervix. It is possible to obtain a panoramic view to study tissue structure, and a contact microscopic view to evaluate individual cellular components. Lugol's solution (2%) and Waterman blue are used to stain the squamous epithelium during the examination [2].

Since the 1980s, various authors have conducted studies in which this method was compared with both histology and colposcopy results. In the literature, the reported microcolposcopy-histology level of agreement varies between 74.4% [3], and 88.5% [4], to even 93% [5]. In another study, the level of agreement was 78.7% although in 47.5% of cases, colposcopy was judged unsatisfactory and, of these, 51.7% were better studied using microcolposcopy [6]. In other studies, it has been claimed that in 46% of cases, colposcopy highlighted aceto-white areas that, when subjected to biopsy, showed no intraepithelial lesions [7]. It has also been reported that colposcopy failed to diagnose any lesions in 7.1% of patients with positive Pap test results [8].

Indeed, it is well known that colposcopy has certain limitations, including not being able to satisfactorily examine the cervical canal and not being able to identify

the squamocolumnar junction (SCJ) in certain cases, above all in menopausal women, where the junction starts towards the inside of the canal [9]. It is precisely in such circumstances where colposcopy is lacking, that microcolposcopy can make a valid contribution to diagnosis.

However, microcolposcopy also has limitations in terms of diagnostic accuracy: in the literature, there are reports of false-negative percentages of 5.6% [5] and lesion grade identification error percentages of 21.4%, with 4.6% grade overestimation and 16.8% underestimation [3]. On the other hand, the false-positive percentage was reported as 11.3% in two cases of acute cervicitis, classified as severe dysplasia by microcolposcopy, and in one case of basal cell hyperplasia, classified as moderate dysplasia [4].

The main limitation of microcolposcopy is the "superficiality" of observation, i.e., that it is impossible to examine glandular crypts. In spite of this, studies agree on the efficiency of microcolposcopy for examinations of the cervical canal, deemed superior to endocervical curettage, especially in cases where the colposcopy is in doubt (abnormal cytology, negative or unsatisfactory colposcopy) [5].

Thus, it is believed that the main indications for conducting a microcolposcopy examination are: unsatisfactory colposcopy examination (SCJ not visualized), discrepancies between the cytology and colposcopy reports, topography of the lesions extending into the endocervical canal (microcolposcopy map), in order to perform a "personalized" cone biopsy, and finally, post-cone follow-up.

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The aim of this study was to assess the diagnostic reliability of microcolposcopy by means of correlation with histology, colposcopy and Pap test results.

Materials and Methods

Two hundred and fifty-six microcolposcopy examinations were requested for patients with abnormal Pap test results in the period between January 2005 and July 2006 in our colposcopy and lower genital tract pathology unit of the Department of Gynecological Sciences, Perinatology and Child Health, University of Rome "Sapienza".

Microcolposcopy examinations were conducted within our facility by a single operator, using the Hamou I microcolposcope in panoramic view and contact mode, and using 2% Lugol's and Waterman blue as stains. Microcolposcopy examinations were reported using the following terminology: viral cytopathic effects (VCE) for cellular alterations compatible with human papillomavirus (HPV) infection, for low-grade (LG) lesions and for high-grade (HG) lesions. In addition, the presence of any mature or immature metaplasia and keratosis was always recorded.

All Pap tests were reported in accordance with the 2001 Bethesda System [10].

Colposcopy examinations, conducted in all cases, were reported in accordance with SICPCV (Società Italiana di Colposcopia e Patologia Cervico-Vaginale - the Italian Colposcopy and Cervico-Vaginal Pathology Society) criteria [9], using the colposcopy report form comprising the abbreviations TA1 for a grade 1 abnormal transformation and TA2 for a grade 2 abnormal transformation. The term "unsatisfactory colposcopy" was used for all colposcopy examination cases that were non-diagnostic at the time of evaluation, and thus referred for subsequent examination.

Targeted biopsy was conducted in cases deemed indicated. At the time of data evaluation, 109 patients had been subjected to targeted biopsy. Histology reports were classified using the CIN (cervical intraepithelial neoplasia) nomenclature, introduced by Richart [11].

Results from microcolposcopy examinations were compared with colposcopy, cytology and histology results, and the percentage agreement and Pearson index calculated.

Results

Overall, 17.6% (45/256) of the cytology examinations showed evidence of atypical squamous cells of undetermined significance (ASCUS), 2.3% atypical squamous cells (ASC) (6/256), 56.3% (144/256) low-grade squamous intraepithelial lesions (LSIL), 17.2% (44/256) high-grade (H)SIL, 6.6% (17/256) atypical glandular cells (AGC) (Table 1), and finally there was a single case with high-grade atypical glandular cells (AGC-H).

Table 1. — *Pap test frequency.*

	Frequency	Percentage
ASCUS	45	17.6
ASC-H	6	2.3
LSIL	144	56.3
HSIL	44	17.2
AGC	17	6.6
Total	256	100.0

ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells cannot exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells.

All patients enrolled with positive Pap tests underwent second level colposcopy examinations and microcolposcopy (MCH). Over 30% of the examinations conducted were non-diagnostic or negative (Tables 2 and 3).

Table 2. — *Colposcopy frequency.*

	Frequency	Percentage
Unsatisfactory	93	36.3
TA1	107	41.8
TA2	56	21.9
Total	256	100.0

TA1: abnormal transformation zone (grade 1); TA2: abnormal transformation zone (grade 2).

Table 3. — *Microcolposcopy frequency.*

	Frequency	Percentage
HG	40	15.6
LG	46	18.0
Negative	86	33.6
VCE	84	32.8
Total	256	100.0

HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

From data reported subsequently, it emerged that MCH and Pap tests, both cytological examinations, showed an interesting level of agreement.

Among the total number of patients classified as HSIL, the MCH (HG) results agreed with the cytology results in 80% of cases (35/44), while among the low cytological grades (LSIL) the corresponding MCH results (VCE and LG) were observed in 74% of cases (107/144) (Table 4a). This level of agreement is increased if just the histologically confirmed cases are considered: 33/39 (85%) for high grades and 48/56 (86%) for low grades (Table 4b).

Table 4a. — *Microcolposcopy and Pap test level of agreement for the entire patient sample.*

		MCH				Total
		Negative	VCE	LG	HG	
Pap	AGC	16	1	0	0	17
	ASCUS	33	11	5	2	51
Test	HSIL	3	2	4	35	44
	LSIL	34	70	37	3	144
Total		86	84	46	40	256

MCH: microcolposcopy; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells can not exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

Table 4b. — *Agreement between microcolposcopy and Pap test results from the sample of patients subjected to biopsy.*

		MCH				Total
		Negative	VCE	LG	HG	
Pap	AGC	7	0	0	0	7
	ASCUS	2	2	1	2	7
Test	LSIL	5	25	23	3	56
	HSIL	1	1	4	33	39
Total		15	28	28	38	109

MCH: microcolposcopy; ASCUS: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells can not exclude HSIL; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; AGC: atypical glandular cells; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect.

In our case histories 109 colposcopy-guided targeted biopsies were conducted.

The MCH patterns classified as LG or VCE in 73% (18+15/45) of cases correspond to CIN1 histology, while those classified as HG to MCH in 71% (7+25/17+28) of cases correspond to CIN2 or CIN3 (Table 5).

Table 5. — *Absolute microcolposcopy and histology frequency.*

		Histology			Total	
		Negative	CIN1	CIN2		CIN3
MCH	Negative	6	7	2	0	15
	VCE	6	18	4	0	28
	LG	6	15	4	3	28
	HG	1	5	7	25	38
Total		19	45	17	28	109

MCH: microcolposcopy; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

Colposcopy results classified as TA1 in 80% (36/45) of cases correspond to CIN1 histology. The observation of grade 2 abnormal transformation zone (TA2) by colposcopy in 68% (11+20/17+28) of cases corresponds to CIN2 or CIN3 (Table 6). The greater diagnostic “accuracy” becomes evident when considering just the cases of CIN3 biopsy, where the percentage agreement was 89% (25/28) for microcolposcopy and 71% (20/28) for colposcopy (Tables 5, 6 and 7).

MCH examination in nine positive histology cases, seven of which CIN1 and two CIN2, showed no signs of

Table 6. — *Absolute colposcopy and histology frequency.*

		Histology			Total	
		Negative	CIN1	CIN2		CIN3
Colposcopy	TA1	15	36	6	8	65
	TA2	4	9	11	20	44
Total		19	45	17	28	109

TA1: abnormal transformation zone grade 1; TA2: abnormal transformation zone grade 2; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

Table 7. — *Absolute colposcopy and histology frequency.*

Histology			MCH			Total	
			Negative	VCE	LG		HG
Negative	Colposcopy	TA1	4	6	5	0	15
		TA2	2	0	1	1	4
		Total	6	6	6	1	19
CIN1	Colposcopy	TA1	5	17	13	1	36
		TA2	2	1	2	4	9
		Total	7	18	15	5	45
CIN2	Colposcopy	TA1	1	2	3	0	6
		TA2	1	2	1	7	11
		Total	2	4	4	7	17
CIN3	Colposcopy	TA1			1	7	8
		TA2			2	18	20
		Total			3	25	28

MCH: microcolposcopy; HG: high-grade lesion; LG: low-grade lesion; VCE: viral cytopathic effect; TA1: abnormal transformation zone grade 1; TA2: abnormal transformation zone grade 2; CIN1: cervical intraepithelial neoplasia grade 1; CIN2: cervical intraepithelial neoplasia grade 2; CIN3: cervical intraepithelial neoplasia grade 3.

any neoplastic intraepithelial lesions (Table 5). The level of agreement between colposcopy and MCH was 72% for low-grade lesions (TA1 vs VCE and LG) and 68% for the high-grade (TA2 vs HG) lesions (Table 8).

Table 8. — *Agreement between colposcopy and microcolposcopy on degree of histologically conformed lesions.*

	Colposcopy	MCH	%
Level of agreement among the lower grades	47	65	72.3
Level of agreement among the higher grades	30	44	68.2

MCH: Microcolposcopy.

Analysis of the correlation between histological tests on biopsy samples and the other three variables (cytology, colposcopy and microcolposcopy) in the 109 cases subjected to biopsy in the sample population considered shows an intermediate level of positive correlation (Table 9).

Table 9. — *Analysis of the correlation between histology, cytology, microcolposcopy and colposcopy in the 109 cases subjected to biopsy.*

	Pearson correlation
Pap test	0.453
Colposcopy	0.447
MCH	0.623

MCH: Microcolposcopy.

The value of the correlation is an index comprised of between -1 and 1, hence, there is a moderate positive correlation between the pairs of examinations considered, with rather modest differences.

From the evaluation of the paired correlations, the highest value was observed with the MCH examination.

Discussion

In comparison to the literature, the overall percentage agreements between microcolposcopy and histology (73% for the higher grades, with a peak value of 89% for CIN3 cases, and 71% for the lower grades) were in accordance with the data from the literature [3-6].

From the percentage agreements between colposcopy and histology on the one hand, and microcolposcopy and histology on the other, improved diagnostic accuracy may be seen for the microcolposcopic examination of higher grade lesions with respect to colposcopy, while the opposite situation is true for the lower grades, for which colposcopy remains, in our case study, more predictive.

From the Pearson index values it may be claimed that none of the examinations (Pap test, colposcopy and microcolposcopy) alone can identify the grade of lesions diagnosed by histological examination.

One point worthy of note is the distribution of results conditioned by the CIN3 biopsy category, where MCH shows a higher tendency for correct identification of the seriousness of the lesion compared to colposcopy (25/28 vs 20/28) (Tables 5 and 6).

From analysis of the absolute frequency results (Tables 6, 7 and 8) it is possible to extrapolate the main contribution of colposcopy examinations. Indeed, this method is more reliable compared to MCH in negative result histology cases, since MCH classified a total of nine examinations in the CIN1 (7 cases) and CIN2 (2 cases) categories as “false-negatives”. Microcolposcopic false-negatives are also reported in the literature (5.6%) [5, 6]. This result can be explained by colposcopy-guided targeted biopsy sampling in all cases and the deep intraglandular localization of certain endocervical lesions, which not even microcolposcopy is capable of detecting.

The negative histology results in 19 cases of lesions detected by colposcopy, 15 of which were grade 1 abnormal transformations, confirms the low specificity of this examination technique (Table 6). Thus, it is possible to hypothesize an error in the targeted biopsy site, both in terms of grade of lesion and site of the same, or in the histological interpretation of certain borderline situations, such as chronic cervicitis or immature metaplasia, the latter often being indistinguishable from a low-grade abnormal transformation. Indeed, the literature reports false-positive colposcopy cases, where biopsy analysis of aceto-white areas did not correspond to histologically detectable lesions [7, 14].

MCH overestimated lesion grade in five CIN1 cases and underestimated it in eight CIN2 cases and three CIN3 cases, data most likely correlated with biopsy sampling conducted in all cases with the aid of colposcopy and not MCH. Indeed, the literature reports percentages of 4.6% for overestimation and 16.8% for underestimation [3].

It should be noted that the greatest percentage of histology-microcolposcopy disagreement was observed in CIN2 biopsy cases, where the outcome of microcolposcopy examinations showed evidence of LG lesions in 8/15 subjects with positive MCH results (Table 5). This disagreement may be partly explained by the incompletely defined natural history of this clinical phenomenon, which can represent an intermediate condition between moderate dysplasia (CIN1) and severe dysplasia (CIN3), frequently much closer to a low-grade lesion [13].

In negative biopsy cases, the lack of agreement between histology and MCH diagnoses was much more evident. Indeed, a microcolposcopic “false-positive” number equal to 13/19 was recorded (Table 5). However, it is evident that all the cases, with the exception of one, involved low-grade microcolposcopic lesions. It is essential to once more remember that biopsies are always conducted with the aid of colposcopy, which can be less accurate in identifying clinical situations of VCE (Table 7).

With regard to the level of agreement between colposcopy and microcolposcopy in terms of the grade of lesion identified, it was 72% for low-grade (TA1 vs VCE and LG) and 68% for high-grade (TA2 vs HG) lesions (Table 8).

Conclusion

Microcolposcopy demonstrated its usefulness in the biological characterization of preneoplastic lesions in patients with abnormal cytology results, particularly in HSIL and LSIL cytology cases. The method proved to be sufficiently accurate in relation to the diagnosis of lesion grade. Furthermore, in cases where cytology was positive for HPV infection and colposcopy did not identify any lesions, MCH was definitive in identifying microscopic VCE phenomena, not always identifiable with a pathognomonic colposcopy pattern.

Having thoroughly considered the results, it may be deduced from the sample that microcolposcopy cannot be used in place of colposcopy (due to the presence of “false negatives”), but is capable of providing additional information on “grade” of the lesion (with greater accuracy for higher lesion grades).

Thus, it may be concluded that microcolposcopy is a useful diagnostic tool, to be used in a manner complementary to colposcopy. From the results that emerged from our study, and in agreement with the literature (15-20), its main indications in the pretreatment examination of cervical lesions remain as follows: unsatisfactory colposcopy due to SCJ not visualized or endocervical, the pre-surgical topographic localization of exocervical lesions, and early HPV infections not yet clinically identifiable by colposcopy examination.

On the other hand, the main limitation of the method remains the number of “false-negatives” in cases of glandular localization of lesions.

In consideration of these results, greater knowledge and more widespread use of the method, for the purpose of improving the diagnosis and treatment of intraepithelial lesions, would seem desirable.

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The accuracy of frozen section analysis at hysterectomy in patients with atypical endometrial hyperplasia

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Summary

Purpose of investigation: To evaluate the accuracy of frozen section analysis in patients with atypical endometrial hyperplasia. **Methods:** Women who underwent hysterectomy with frozen section analysis for atypical endometrial hyperplasia were identified. Frozen section evaluation aimed to give information about the presence of malignancy. Also, myometrial or cervical involvement was assessed in cases with malignancy to reveal the need for staging. Final pathological evaluation results were compared with intraoperative frozen section analyses. **Results:** Twelve patients (34.3%) had endometrial cancer on final pathologic examination and eight required a staging procedure due to either myometrial invasion or cervical involvement; 75% of patients with endometrial cancer were successfully detected by frozen section analysis. Moreover, among women with cancer, frozen section examination revealed 75% of cases who required surgical staging. **Conclusion:** Frozen section analysis of hysterectomy specimens in patients with atypical endometrial hyperplasia is necessary to determine the presence of cancer and the need for surgical staging.

Key words: Frozen section; Endometrial hyperplasia; Atypical endometrial hyperplasia; Endometrial cancer; Surgical staging.

Introduction

Endometrial cancer (EC) is the most common gynaecological malignancy in developed countries [1]. Of the two major forms of EC with different clinicopathologic and prognostic characteristics, type I tumours are estrogen-related and are preceded by endometrial hyperplasia (EH) [2, 3].

EH is characterized by the nonphysiological proliferation of the endometrium that results in irregular shaped glands of varying sizes [4]. The diagnosis of EH is usually achieved through diagnostic evaluation of women with abnormal uterine bleeding by endometrial biopsy [5]. EH is categorized according to the architectural disruption and the presence or absence of cytological atypia. Thus, there are four types of EH in the World Health Organization classification including simple or complex hyperplasia with or without atypia [6]. The risk of progression of EH to EC is closely related to the presence of cytological atypia and to architectural crowding; 1.6% of patients with EH without atypia develop EC in a mean duration of nearly ten years while 23% of patients with atypical EH develop EC in four years [7]. Moreover, up to 50% of patients with atypical EH were reported to have coexistent EC with considerable rates of invasion beyond the endometrium [8]. Therefore, both preoperative pathological identification and intraoperative frozen section analysis are important since they closely guide clinical management which may be quite different depending on the presence of atypia and presence or absence of coexistent EC [9, 10].

The aim of this study was to retrospectively evaluate the accuracy of frozen section diagnoses of patients treated with total abdominal hysterectomy who had a preoperative diagnosis of atypical EH.

Materials and Methods

A review of the Hacettepe University Faculty of Medicine Gynaecology and Pathology database was performed to identify patients diagnosed with EH between January 2002 and June 2008.

Among 92 women with a preoperative diagnosis of atypical EH, 35 patients who subsequently underwent hysterectomy with frozen section analysis at our institution within four weeks following diagnosis were identified. The clinicopathologic characteristics of these patients were obtained using medical records.

All patients had undergone endometrial sampling via Karman aspiration or curettage with a diagnosis of abnormal uterine bleeding. After diagnosis, all patients initially underwent total abdominal hysterectomy with or without salpingo-oophorectomy and the specimen was sent for frozen section evaluation. The pathologist evaluated the specimen grossly and sampled abnormal endometrial areas for microscopic evaluation. During the frozen section examination, it was reported whether the specimen contained any areas consistent with EC or not. In cases detected with EC, it was also reported whether myometrial invasion greater than 50% or cervical involvement was present or not to guide the extent of surgery. In patients with myometrial invasion and/or cervical involvement, surgery was extended to include bilateral pelvic and paraaortic lymph node dissection and infracolic omentectomy in order to determine the surgical stage of disease. Further surgery was not performed in cases in whom endometrial cancer was reported to be confined only to the endometrium.

The results of final pathological evaluation were compared with intraoperative frozen section analyses to document the success of frozen section examination in diagnosing endometrial cancer in patients with atypical EH and in detecting the patients who required comprehensive surgical staging procedure.

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Table 1. — *Clinical and pathological characteristics of patients with atypical endometrial hyperplasia.*

Characteristic	
Age (mean, range):	
All patients	51.4 (35-77)
Patients without EC	48.5 (35-75)
Patients with EC	56.8 (42-77)
Presenting symptoms (n, %):	
PMB	13 (37.1)
Heavy and/or irregular menses	22 (62.9)
Preoperative diagnoses (n, %):	
Simple EH with atypia	2 (5, 7)
Complex EH with atypia	33 (94.3)
Surgery (n, %):	
TAH	3 (8.6)
TAH + BSO	26 (74.3)
Staging	6 (17.1)
EC at frozen (n, %):	
Present	9 (25.7)
Absent	26 (74.3)
EC at final pathology (n, %):	
Present	12 (34.3)
Absent	23 (65.7)

EC: Endometrial carcinoma; PMB: postmenopausal bleeding; EH: endometrial hyperplasia; TAH: total abdominal hysterectomy; BSO: bilateral salpingo-oophorectomy; Staging: total abdominal hysterectomy + bilateral salpingo-oophorectomy + bilateral pelvic and paraaortic lymph node dissection + infracolic omentectomy.

Results

The mean age of the study group was 51.4 years (range 35-77). Endometrial sampling was performed for postmenopausal bleeding in 13 patients and for heavy and/or irregular menstrual bleeding in 22 premenopausal patients. The preoperative diagnoses were simple hyperplasia with atypia in two patients (5.7%) and complex hyperplasia with atypia in remaining 33 patients (94.3%) (Table 1).

Initially, 32 patients in the study group underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and three underwent total abdominal hysterectomy. After frozen section evaluation, 29 patients (82.9%) did not undergo further surgical intervention while six (17.1%) were subjected to a comprehensive surgical staging procedure including bilateral pelvic and paraaortic lymph node dissection in addition to total abdominal hysterectomy and bilateral salpingo-oophorectomy.

Twelve patients (34.3%) had EC on final pathologic examination. All EC cases were diagnosed as having complex hyperplasia with atypia preoperatively. Therefore, 36.4% of patients with a biopsy diagnosis of complex hyperplasia with atypia had EC at final pathology. Of patients with EC, eight were postmenopausal. EC was more common in postmenopausal patients (61.5% vs 18.2%, $p = 0.024$). Additionally, mean age of patients with EC was significantly higher compared with patients without EC (56.8 vs 48.5, $p = 0.014$).

Thirty-two of 35 frozen section diagnoses were consistent with final pathological diagnoses in detecting the presence of EC in patients with a preoperative diagnosis

of atypical EH. In this respect, the accuracy of frozen section examination was 91.4%.

On the other hand, among 12 patients with EC, nine were successfully detected by frozen section analysis with an accuracy of 75.0%. Six of these nine patients underwent staging surgery following EC diagnosis at frozen section. Interestingly, in two of these cases the tumour was found to involve the whole myometrium and uterine serosal surface which meant FIGO Stage 3a tumour, and therefore they were given adjuvant radiation. One patient in this group also had cervical stromal involvement and was given radiotherapy as well. The remaining three patients had well-differentiated EC with only superficial myometrial invasion and they were not given adjuvant therapy (Table 2).

Three patients who had a frozen section diagnosis of EC were not subjected to staging surgery because all were reported to have well differentiated EC confined only to the endometrium without apparent myometrial invasion on frozen section examination. Final pathologic examination also confirmed the frozen section diagnosis in two of these patients. However, one patient was diagnosed as having cervical stromal involvement and was given adjuvant radiotherapy since a restaging procedure was rejected by the patient (Table 2).

Table 2. — *Clinical and pathologic characteristics of patients with endometrial carcinoma.*

Patients	Age	EC at Frozen Section	Details Frozen Section Analysis	Operation	Final Stage	Final Grade	Additional Therapy
Case 1	46	Absent	No EC	TAH+BSO	2a*	1	RT
Case 2	62	Absent	No EC	TAH+BSO	1a*	1	None
Case 3	64	Absent	No EC	TAH+BSO	1a*	1	None
Case 4	48	Present	G1 EC confined to endometrium	TAH+BSO	2b*	2	RT
Case 5	52	Present	G1 EC confined to endometrium	TAH+BSO	1a*	1	None
Case 6	42	Present	G1 EC confined to endometrium	TAH+BSO	1a*	1	None
Case 7	57	Present	G1 EC with DMI	Staging	3a	1	RT
Case 8	67	Present	G1 EC with DMI	Staging	3a	2	RT
Case 9	55	Present	G1 EC with MI	Staging	1b	1	None
Case 10	62	Present	G1 EC with cervical involvement	Staging	2b	2	RT
Case 11	77	Present	G1 EC with MI	Staging	1b	1	None
Case 12	50	Present	G1 EC with MI	Staging	1b	1	None

*According to findings in hysterectomy specimen without a comprehensive surgical staging procedure.

G: grade; EC: endometrial carcinoma; MI: myometrial invasion; SMI: superficial myometrial invasion; DMI: deep myometrial invasion; TAH + BSO: total abdominal hysterectomy + bilateral salpingo-oophorectomy; Staging: total abdominal hysterectomy + bilateral salpingo-oophorectomy + bilateral pelvic and paraaortic lymph node dissection + infracolic omentectomy; RT: radiotherapy.

Frozen section analysis failed to diagnose EC in three of 35 cases (8.6%). The endometrium was reported to be free of malignancy at frozen section examination. However, well differentiated ECs were diagnosed at final pathologic examination. The disease was confined to endometrium in two patients who did not receive adjuvant treatment. The third patient had EC with cervical glandular involvement and received adjuvant radiotherapy because the patient was a poor candidate for a restaging procedure due to uncontrolled medical problems (Table 2).

In 12 patients with EC at final pathology, a staging procedure was required in eight due to either myometrial invasion or cervical involvement. Frozen section successfully documented six of them. In this respect, the success of frozen section examination was 75%. One of the missed cases had cervical glandular involvement and the other had cervical stromal involvement.

Discussion

EH is the precursor lesion of EC which is the most commonly seen malignancy of the female genital tract [1-3]. The management of women with EH is mostly influenced by the age and fertility desire of the patient and presence or absence of cytological atypia. Nevertheless, the principal concern in these patients is the risk of having a coexistent EC or progression to EC [11]. Cytological atypia is the most important feature in EH for the development or coexistence of EC [12]. Up to 50% of patients with atypical EH were reported to have coexistent EC [8]. Given the noteworthy risk of detecting a coexistent EC in women with atypical EH, some may consider a routine staging surgical procedure for all of these patients. However, it is estimated that routine surgical staging in all patients with atypical EH would be associated with a 20% complication rate and a 6% rate of serious complications which is a higher percentage than the risk of lymph node involvement [13]. In our series, 34.3% of patients with atypical EH and 36.4% of patients with complex atypical EH had EC at final pathology. Therefore, although the surgical approach is the more commonly preferred treatment method when atypical EH is diagnosed, surgical management of women, namely the extent of surgery, is frequently influenced by intraoperative frozen section analysis. The decision to perform a comprehensive surgical staging including bilateral salpingo-oophorectomy and lymph node dissection can only be determined by the presence or absence of malignant endometrial disease on frozen section analysis [10, 11]. Otherwise, the surgeon might be obliged to perform a restaging surgery exposing the patient to the risk of a second invasive procedure. Also, some legal and ethical issues related with the over-treatment or under-treatment of patients may arise.

The limitations of frozen section appear to be highly important from this point of view but in the current literature the data on the accuracy of frozen section at time of hysterectomy is limited. In one series of 46 women who

underwent hysterectomy for atypical EH, frozen section failed to diagnose 50% of EC [14]. In another series, the accuracy of frozen section diagnosis for endometrial adenocarcinoma in patients with complex atypical EH was 65%. According to their results, the authors characterised frozen section as an unreliable tool in determining the definitive decision of management [10]. Nevertheless, the availability of a pathologist specifically experienced in gynaecological pathology is an important issue in frozen section analysis due to the fact that there are a range of abnormalities seen in hyperplastic endometrium and differentiation between them may be quite difficult [11]. There is considerable interobserver and intraobserver variation in the diagnosis of EH even in biopsy specimens [15-17]. Only pathologists with frequent exposure to such specimens are likely to be proficient in reporting results of these specimens [11]. In the current study, the accuracy of frozen section diagnoses was 91.4% in detecting the presence or absence of EC. Moreover, frozen section examination detected 75% of cases with EC. To the best of our knowledge, such a high detection rate has not been reported in the English literature up to date. The frozen section analysis in our study group was performed by a gynaecological pathologist studying specifically specimens originating from gynaecology clinics who is often exposed to such specimens. The high detection rate of EC in our series of patients with atypical EH may essentially be attributed to the proficiency of the pathologist.

It was reported that EC confined to the endometrium may be missed during frozen section [13]. However, gross evaluation of hysterectomy specimens was reported to accurately predict myometrial invasion in 88.2% of patients [18]. Two of four cases with grade 1 EC confined only to the endometrium were missed in our series. However, neither restaging nor adjuvant therapy is required in such patients. Therefore missing these women during frozen section analysis may be acceptable since lymph node involvement was detected in less than 1% of patients. Nonetheless, detecting the presence of myometrial invasion at frozen section is much more important because lymphatic spread is considerable in this situation. In cases with myometrial invasion, the risk of lymphatic involvement is 5% or higher and a staging surgery including lymph node dissection should be performed [19]. Myometrial invasion was detected in 75% and 89% of patients with atypical EH having coexistent EC in two different studies [8, 20]. In the current series, all five patients with myometrial invasion of different degrees were successfully diagnosed at frozen examination.

Eight cases with EC in our series required surgical staging due to either myometrial invasion or cervical involvement. Frozen section successfully detected six of them. In this respect, the success of frozen section examination was 75%. Both the missed cases had cervical involvement. In fact, two of three cases with cervical involvement could not be detected. Therefore, in addition to the myometrium, the uterine cervix should also be inspected carefully in such cases during frozen analysis.

Also, in patients with abnormal uterine bleeding, endocervical sampling should be performed in addition to endometrial sampling to detect cervical invasion of an endometrial tumour. When cervical involvement is documented preoperatively, the patient should undergo radical hysterectomy to eliminate the necessity of postoperative adjuvant radiotherapy.

Moreover, the missed cases in the current study were under-diagnosed on frozen examination; none of the cases were over-diagnosed. Therefore, the surgeon may safely make a decision of performing a staging procedure when the frozen section diagnosis is EC with myometrial or cervical spread.

In patients with a preoperative diagnosis of atypical EH, the clinician should also remember that some patients will have tumors with higher grades. Janicek *et al.* reported grade 2 or 3 lesions in 21% of patients [20]. In our study group, although none of the cases had grade 3 lesions, three (25%) had grade 2 tumours. Thus, advanced stages and higher grades may be expected frequently in patients with atypical EH.

Conclusion

The diagnosis of atypical EH carries a considerable risk of a coexistent EC. Therefore, frozen section analysis of hysterectomy specimens in such patients is necessary to determine cases having EC and cases with EC requiring surgical staging. The accurate detection of EC may be possible especially in the presence of a pathologist specifically experienced in gynaecological pathology. Thus, at least, cases with myometrial invasion who have a risk of extrauterine spread are detected and staging surgery is completed in the same setting. Additionally, it should always be kept in mind that advanced stage and high grade diseases may be detected more than expected in patients with a biopsy diagnosis of atypical EH. Therefore, the patients should undergo surgery in centers where a gynaecological oncologist is available and a staging surgery can be completed. This approach can prevent morbidities resulting from over-treatment and medical issues resulting from under-treatment.

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Transvaginal sonography of cervical length and Bishop score as predictors of successful induction of term labor: the effect of parity

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Summary

Objective: To evaluate the predictive value for successful labor induction of transvaginal ultrasound (TVS) of cervical length according to parity. **Method:** TVS of the cervix was performed before term labor induction. Induction was considered successful if vaginal delivery was achieved within 24 hours; 231 women were available for final analysis. **Results:** Analysis of the receiver operator characteristics curve showed an optimal cut-off for cervical length of ≤ 20 mm for successful induction. Following multivariate logistic regression analysis, a sonographic short cervix (AOR 5.6; $p < 0.001$) was an independent predictor of successful induction but not a favorable Bishop score ($p = 0.47$). Among multiparas with a short cervix, positive and negative predictive values for successful induction were 98% (95% CI 90-100%) and 21% (95% CI 13%-32%) and among nulliparas, predictive values were 69% (95% CI 53%-82%) and 77% (95% CI 64%-87%) respectively. **Conclusion:** In nulliparas, cervical length can usefully predict labor induction outcome.

Key words: Transvaginal ultrasound; Cervical length; Bishop score; Labor induction; Parity.

Introduction

Since its inception in 1964, the Bishop score [1] has remained the gold standard for assessing cervical favorability for labor induction [2]. In contemporary practice, induction of labor is done for about 20% of term pregnancies [3]. Transvaginal ultrasonic measurement of cervical length has been shown to be a better predictor of shorter induction to delivery interval when compared to Bishop Score by some investigators [4-7] but this is disputed by others [8-11]. A recent meta-analysis concludes that transvaginal sonography (TVS) is not demonstrably superior to the Bishop Score and calls for further research [2].

Parity is a strong predictor of successful induction following labor induction at term [12, 13]. Cervical length by TVS but not Bishop score has been shown to be an independent predictor of cesarean delivery following labor induction at term in nulliparas [14].

A prospective study was performed to compare TVS of cervical length and Bishop score in predicting labor induction outcome at term in a mixed population of nulliparas and multiparas. This information is useful for women contemplating labor induction to help manage their expectations of the induction process.

Material and Methods

Women from 37 to 42 weeks of gestation with a singleton fetus, intact membranes and cephalic presentation were recruited when they presented to the induction bay of the delivery suite for labor induction. Recruitment was carried out by the

investigators. Women with intrauterine fetal death or known gross fetal anomaly were excluded.

Recruitment took place from January 2003 to October 2005. Two hundred and fifty-three women were approached. One woman declined TVS. Another woman with a previous cesarean scar opted for elective cesarean delivery after TVS but before commencement of labor induction. Two other women who were preterm (at 35 and 36 weeks of gestation) were mistakenly recruited. These four women were excluded. We had previously reported on tolerability of TVS versus digital assessment for Bishop score in this group of 249 women and we used all-cause cesarean delivery as the primary outcome measure [13]. Eighteen women delivered by cesarean section for non-reassuring fetal status within 24 hours of labor induction were excluded for this analysis [5] these women were excluded as having non-reassuring fetal status soon after induction of labor likely due to a pre-existing condition e.g., uteroplacental insufficiency. We felt such cases were potential confounders in the analysis of assessment methods of cervical favorability for labor induction. Following these exclusions, 231 women were left for the final analysis.

We defined successful labor induction as vaginal delivery within 24 hours of labor induction [15]. Cesarean delivery indicated by failure to progress after labor induction that took place within 24 hours of labor induction was considered as a failed induction, as failure to progress could be considered a failure of current methods of labor induction to overcome an unfavorable cervix. All other deliveries after 24 hours of commencement were also considered to be failed labor inductions.

Women were categorized into two groups based on their parity: nulliparas (no previous births) and multiparas (at least 1 previous birth). We further defined short stature as maternal height of < 150 cm, post date as gestational age > 40 weeks and categorized maternal age into under 35 years old and 35 years and above to assess the effect of these parameters on successful labor induction.

Study women were asked to empty their bladder before TVS.

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Ultrasound was performed in a specially equipped room separate from the induction bay. The sagittal image of the entire cervical canal was acquired sonographically as previously described [16]. Measurement of cervical length was made from the internal os to external os in a straight line [17]. The cervical images were acquired three times in succession and cervical length was measured for each image. The shortest length of the three obtained was used for analysis [5]. Funneling was defined as funnel shape appearance at the internal cervical os due to dilatation of the internal os of at least 5 mm as detected by TVS. Ultrasound findings were concealed from providers.

The ultrasound examination was performed by investigators who had at least a year's experience with sonography. Sonography was performed using a Toshiba Eccocee or Toshiba Capasee ultrasound machine (Toshiba Medical Systems, Tokyo, Japan) with a 6 MHz endovaginal probe.

The Bishop score was obtained by induction bay providers blinded to the ultrasound findings.

The method of labor induction was decided upon by providers. Labor was induced by dinoprostone (3 mg) pessary or amniotomy in our institution as previously described [13]. Universal electronic fetal monitoring was performed at labor induction.

After dinoprostone pessary insertion, a planned assessment was made after six hours and depending on cervical favorability, another dinoprostone pessary (maximum 2 doses in 24 hours) might be inserted or amniotomy performed. The women were then assessed again after six hours: if the cervix remained unfavorable and immediate delivery was not indicated, the women usually rested overnight for the induction to be repeated the following morning. If labor induction was by amniotomy, oxytocin was given within two hours depending on contractions. When labor was established, vaginal assessment was done at least every four hours.

The study was approved by our institution review board and written consent was obtained from participants. This study was done at a university hospital conducting about 5,000 deliveries per year.

Data was entered into a statistical software package SPSS version 13.0 (SPSS Inc., Chicago IL) and GraphPad InStat software (GraphPad Software Inc., San Diego CA) was also used for data analysis. The Student's *t* test was used to analyze means, Fisher's exact test was used for categorical datasets, relative risk (RR) and its 95% confidence interval (CI) were calculated using GraphPad InStat. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (and their 95% confidence interval, CI) and likelihood ratio of a test were obtained using GraphPad InStat. A receiver operating characteristic curve was used to determine the best cut-off for cervical length and Bishop score for successful labor induction, and area under the curve was derived. Multiple logistic regression analysis to determine independent predictors of successful labor induction was performed and all variables with crude $p < 0.05$ were included in the analysis; $p < 0.05$ at 2-tailed was taken as a significant level.

Results

Data from 231 women were analyzed. Labor induction was successful (vaginal delivery within 24 hours of commencement of induction) in 157/231 (68%) comprising 149 spontaneous vaginal deliveries and eight vaginal instrumental deliveries. Seventy-four women failed induction comprising 11 women who were delivered by

cesarean section for failure to progress within 24 hours and another 63 women (37 vaginal deliveries: 24 spontaneous and 13 instrumental vaginal deliveries and 26 cesarean sections: 19 for failure to progress and 7 for fetal distress) who delivered more than 24 hours after induction. Table 1 showed the characteristics and outcomes of 231 study women stratified according to vaginal delivery within 24 hours of labor induction (successful induction).

Receiver operating characteristics (ROC) curve indicated that the best cut-off for cervical length as predictor for successful labor induction was ≤ 20 mm (sensitivity 54%, specificity 81%, PPV 86% and NPV 45%). The optimal cut off for Bishop score was a score of ≥ 6 (sensitivity 57%, specificity 69%, PPV 80% and NPV 43%). Although cervical length ≤ 20 mm had better positive and negative predictive values for successful labor induction than Bishop score ≥ 6 , their 95% confidence intervals overlapped. ROC area under the curve analyses and Fisher's exact test both showed that cervical length and Bishop score were useful predictors of successful labor induction. Cervical length of ≤ 20 mm had better predictive values for success of induction compared to Bishop Score ≥ 6 (Table 2).

Table 1 also shows the interaction of sonographic findings and pre-induction clinical variables on successful labor induction. On univariate analysis parity, cervical length, funneling at the internal cervical os, maternal age, Bishop score and mode of induction were all associated with successful induction. After adjustment, controlling for the significant variables stated above, only parity ≥ 1 [(adjusted odds ratio (AOR) 12.4; 95% CI 5.6-27.3; $p < 0.001$), cervical length ≤ 20 mm (AOR 5.6; 95% CI 2.3-13.8; $p < 0.001$) and labor induction by amniotomy (AOR 6.5; 95% CI 1.3-32, $p = 0.023$) were independently associated with successful labor inductions.

We performed a further analysis stratified by parity. Among nulliparas and multiparas, the PPVs and NPVs for successful labor induction again favored cervical length ≤ 20 mm compared to a Bishop score ≥ 6 (Table 3) though their 95% CIs overlapped. For short cervical length ≤ 20 mm in nulliparas, the PPVs and NPVs for successful labor induction were 69% and 77%, respectively as compared to 98% and 21% among the multiparas.

Discussion

Our finding added to the available evidence [4-7] that TVS is better than Bishop score in predicting successful labor induction. Our study had also shown that Bishop score was no longer independently associated with successful labor induction once parity, cervical length and mode of labor induction were taken into account in a multivariate logistic regression model as has previously been demonstrated [12].

The cut-offs for cervical length of ≤ 20 mm and Bishop score ≥ 6 by ROC for predicting vaginal delivery within 24 hours determined in this analysis is identical to that

Table 1. — Characteristics. Number (%) or mean ± standard deviation.

	Successful labor induction n = 157	Failed labor induction n = 74	Crude p value	Adjusted Odds Ratio (AOR) (95% confidence interval) and p value
Age (years)	30.5 ± 4.9	29.7 ± 4.3	p = 0.26	
≥ 35 years	32 (82.1)	7 (17.9)	p = 0.04	AOR 1.2 (0.4-3.4): p = 0.73
< 35 years	125 (65.1)	67 (34.9)		
Parity	1.4 ± 1.2	0.4 ± 1.0	p < 0.001	
Nulliparas	42 (42.4)	57 (57.6)	p < 0.001	AOR 12.4 (5.6-27.3): p < 0.001
Multiparas	115 (87.1)	17 (12.9)		
Ethnicity				
Malay	105 (69.5)	46 (30.5)	p = 0.57	
Chinese	29 (72.5)	11 (27.5)		
Indian	19 (52.8)	11 (47.2)		
Others	4 (100)	0 (0)		
Height (cm)	156 ± 6	156 ± 6	p = 0.84	
< 150 cm	20 (76.9)	6 (23.1)	p = 0.38	
≥ 150 cm	136 (67.3)	66 (32.7)		
Gestation age (weeks)	39.9 ± 1.2	39.9 ± 1.4	p = 0.77	
> 40 weeks gestation	69 (63.3)	40 (36.7)	p = 0.16	
≤ 40 weeks	88 (72.1)	34 (27.9)		
Indications for induction of labor‡				
Diabetes mellitus	63 (74.1)	22 (25.9)	p = 0.24	
Prolonged pregnancy	54 (66.7)	27 (33.3)		
Non reassuring fetal status§	26 (66.7)	13 (33.3)		
Hypertension	19 (52.8)	17 (47.2)		
Others	16 (72.7)	6 (27.3)		
Cervical length (mm)	21 ± 9	26 ± 7	p < 0.001	
Cervix > 20 mm	73 (54.9)	60 (45.1)	p < 0.001	AOR 5.6 (2.3-13.8): p < 0.001
Cervix ≤ 20 mm	84 (85.7)	14 (14.3)		
Funneling at internal cervical os				
No	116 (63.0)	68 (37)	p = 0.001	AOR 1.3 (0.4-4.3): p = 0.69
Yes	41 (87.2)	6 (12.8)		
Bishop score	6.0 ± 1.8	4.8 ± 1.4	p < 0.001	
Bishop score ≤ 5	68 (57.1)	51 (42.9)	p < 0.001	AOR 1.3 (0.6-3.0): p = 0.47
Bishop score ≥ 6	89 (79.5)	23 (20.5)		
Mode of labor induction				
Vaginal dinoprostone	104 (59.1)	72 (40.9)	p < 0.001	AOR 6.5 (1.3-32.0): p = 0.023
Amniotomy	53 (96.4)	2 (3.6)		
Induction to delivery interval (hours)	9.5 ± 5.2	34.0 ± 13.1	p < 0.001	

*Successful labor induction defined by vaginal delivery within 24 hours of labor induction.

†Multivariate logistic regression analysis using all variables with crude p < 0.05. Adjusted odds ratio shown where variable is used in the model.

‡Total of 263 indications as 30 women had 2 indications and one had 3 indications listed for induction of labor.

§Non reassuring fetal status included oligohydramnios, suspected intrauterine growth restriction, reduced fetal movement and suboptimal umbilical artery Doppler or cardiotocograph.

Table 2. — Comparison of transvaginal sonography for cervical length ≤ 20 mm and Bishop score ≥ 6 for predicting successful labor successful induction (vaginal delivery within 24 hours) in 231 women. 95% confidence interval within brackets (95% CI).

	Prediction of vaginal delivery within 24 hours of induction of labor*	
	Cervical length ≤ 20 mm vs > 20 mm	Bishop score Score > 5 vs Score ≤ 5
Relative risk and p value	RR 1.6 (1.3-1.9): p < 0.001	RR 1.4 (1.2-1.7): p < 0.001
Sensitivity	54% (45%-61%)	57% (49%-65%)
Specificity	81% (70%-89%)	69% (57%-79%)
Positive predictive value	86% (77%-92%)	80% (71%-86%)
Negative predictive value	45% (37%-54%)	43% (34%-52%)
Likelihood ratio	2.8	1.8
Area under receiver operator characteristics curve	0.68 (0.61-0.75): p < 0.001	0.70 (0.63-0.77): p < 0.001

*Eighteen cesarean deliveries indicated by fetal distress within 24 hours of labor induction censored and excluded from analysis.

reported in our previous report on all-cause cesarean delivery as outcome which has also found sonographic cervical length but not Bishop score to be an independent predictor [13]. This finding suggests that cervical length is a reliable independent predictor over a range of definitions of failed inductions with consistent cervical length cut-offs.

In this analysis, funneling at the internal cervical os on ultrasonic assessment was not an independent predictor of cesarean delivery in contrast to an earlier finding [6] due to the strong and dependent association between funneling and short cervical length (p < 0.001) within our study.

Multiparity [12, 18] and mode of induction [1, 10] as anticipated were significant predictors for successful labor induction after adjusted analysis.

Our data indicated that there was a distinct difference in the interpretation of cervical length as a predictor of

Table 3. — Comparison of transvaginal sonography for cervical length ≤ 20 mm and Bishop score ≥ 6 for successful labor induction (vaginal delivery within 24 hours) in labor induction at term stratified according to parity in 231 study women. 95% confidence interval within brackets (95% CI).

	Prediction of successful induction: Nulliparas		Prediction of successful induction: Multiparas	
	Cervical length ≤ 20 mm v > 20 mm	Bishop score Score > 5 v Score ≤ 5	Cervical length ≤ 20 mm v > 20 mm	Bishop score Score > 5 v Score ≤ 5
Relative risk and p value	RR 3.0 (1.8-5.1): p < 0.001	RR 1.9 (1.2-3.0): p = 0.014	RR 1.2 (1.1-1.4): p = 0.001	RR 1.2 (1.1-1.4): p = 0.008
Sensitivity	69% (53%-82%)	62% (46%-76%)	47% (38%-57%)	55% (45%-64%)
Specificity	77% (64%-87%)	65% (51%-77%)	94% (71%-100%)	82% (57%-96%)
Positive predictive value	69% (53%-82%)	56% (41%-71%)	98% (90%-100%)	95% (87%-99%)
Negative predictive value	77% (64%-87%)	69% (56%-82%)	21% (13%-32%)	21% (12%-33%)
Likelihood ratio	3.0	1.8	8.1	3.1

*Eighteen women who had emergency cesarean sections for fetal distress within 24 hours of labor induction censored and excluded leaving 231 women for final analysis.

successful labor induction at term between multiparas and nulliparas. The PPV of short cervical length ≤ 20 mm for successful labor induction of 98% (95% CI, 90-100%) in multiparas was very high: 98% of these women might deliver vaginally within 24 hours. However, in contrast, the NPV was only 21% (95% CI, 12-33%) which was very poor: even with long cervical length > 20 mm, 79% were expected to deliver vaginally within 24 hours. Among nulliparas, short cervical length ≤ 20 mm had a PPV of 69% (95% CI 53-82%) and a NPV of 77% (64-87%). This implied that 69% of nulliparous women with a short cervix might expect vaginal delivery within 24 hours of labor induction and 77% of nulliparas with a long cervix might encounter failure to deliver vaginally within 24 hours.

A previous study has shown TVS cervical length < 27 mm to have a sensitivity of 76% and specificity of 75.5% for predicting successful labor induction and vaginal delivery in nulliparas [14]. Our sensitivity and specificity at 69% and 77%, respectively, for cervical length ≤ 20 mm in predicting successful labor induction among nulliparas were comparable to the earlier study.

A potential problem with the use of TVS cervical length in assessing cervical favorability for labor induction is the different cut-offs suggested by previous studies ranging from 24 mm to 30 mm for the cervical length [4, 5, 7, 14]. Our study cut-off at 20 mm is similar to 18-20 mm cut-off of a recent large study [19].

Interestingly, a recent randomized study comparing TVS assessment and Bishop score to guide pre-induction cervical ripening with prostaglandins has shown a reduction in prostaglandin use with TVS without affecting the success rate of labor induction [20].

Previous studies have also shown that TVS is better tolerated compared to digital assessment for Bishop score [10, 13, 21].

Conclusion

TVS for cervical length may be a better alternative to assess favorability for labor induction for nulliparous women in the office setting provided expertise and ultrasound equipment are available. Multiparas respond well to labor induction regardless of cervical favorability.

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Integrating cervical length measurement into routine antenatal screening and only emergency cerclage when indicated

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Summary

Purpose: To integrate cervical length measurement into antenatal screening and apply emergency cerclage when indicated to prevent spontaneous deliveries at < 34 weeks of gestation. **Methods:** Cervical length measurements of 400 pregnant volunteers were obtained at gestational weeks 12-14, 18-20 and 28-32. Whenever a cervical measurement < 30 mm was observed, vaginal cultures and bacterial vaginosis were investigated, and weekly cervical length measurements were performed thereafter. Emergency cerclage was performed whenever complete cervical effacement and ≥ 3 cm cervical dilatation were observed before 32 completed weeks of gestation. We adopted and tested a strategy of only emergency cerclage application when clinically indicated after ultrasound screening and microbial monitoring of short cervixes. Patients were given cyclooxygenase-inhibitors, progesterone, and antibiotics in the postoperative period. **Results:** Spontaneous preterm births at < 34 weeks of gestation occurred in 15 women (3.8%). We performed five emergency cerclages according to the presented screening strategy between 20-28 weeks of gestation all of which reached > 34 weeks. We successfully postponed 62.5% (5/8) of deliveries before 32 completed weeks and 33.3% (5/15) of deliveries before 34 completed weeks. **Conclusion:** Routine cervical length measurement combined with serial transvaginal sonograms and vaginal microbial monitoring of the short cervixes will avoid unnecessary prophylactic cerclages while increasing the success of emergency cerclages performed upon solid clinical findings.

Key words: Preterm birth; Cervical insufficiency; Cervical length measurement; Cervical cerclage; Bacterial vaginosis

Introduction

Preterm birth before 37 weeks of gestation is the leading cause of neonatal morbidity and mortality. One of the risk factors for preterm birth is cervical insufficiency. The traditional obstetric dogma considered cervical insufficiency as a dichotomous variable. Recently newer models have proposed cervical competence as a continuum and insufficiency may result from loss of connective tissue due to operations, congenital disorders of the uterus and cervix, infection/colonization, local or systemic hormonal effects, inflammatory processes or genetic predisposition [1, 2].

During the last decade, transvaginal ultrasound (TVS) screening of the cervix has been used to predict preterm deliveries [3-5]. Different studies used cervical length screening at 11-14 weeks of gestation, 18-22 weeks of gestation, and 24-28 weeks of gestation and calculated variable sensitivities for cervical length cut-off values of 15 mm, 25 mm, and 30 mm [3, 4, 6-8].

As the prevalence of preterm birth is low, cervical length screening would generate either a false-positive rate or a low sensitivity [9]. This has constituted the main pitfall of intervention studies using prophylactic or emergency cerclage placement depending on the results of cervical length screening, because cerclage application may result in unintended complications like preterm labor, premature rupture of the membranes, chorioamnionitis or abruption. Not surprisingly inconclusive or conflicting

results have been reported from studies conducted on different pregnant populations with variable screening strategies, cerclage techniques, and follow-up methods after cerclage placement [5, 7, 8, 10-12].

To overcome the low sensitivity and positive predictive value (PPV) of cervical length screening, several other variables have been used including cervical funneling, degree of cervical funneling > 25%, cervical dilatation > 5 mm, visible fetal membranes, and number of prior preterm births [4, 5, 12-14]. Despite these efforts, the ideal selection criteria remain to be determined.

Increasing data points out the importance of microbial invasion of the amniotic cavity by microbial flora of the lower genital tract and discriminates short cervixes as a predisposing factor for this invasion [1, 15].

In our study, we aimed to test whether integrating cervical length measurement into antenatal screening can help identify patients at risk for preterm delivery. We also used this data to test an evidence-based algorithm including microbial screening and ultrasound follow-up for the management of short cervixes and evaluation of the outcome of emergent cerclage placement when indicated.

Materials and Methods

This prospective follow-up study was performed on patients who underwent an ultrasonographic examination between 12-14 weeks of gestation in an obstetric unit of a tertiary center at Kocaeli University School of Medicine, Kocaeli, Turkey between June 2003 and April 2005. The local ethics committee approved the study. The study population consisted of 400 consecutive pregnant women who gave written consent.

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In our study, our primary outcome measure was to test whether integrating cervical length measurement to antenatal screening would help identify patients at risk for preterm delivery. Our secondary outcome measure was to assess ultrasound (US) follow-up for the management of short cervixes and evaluation of the outcome of emergent cerclage placement only when indicated.

Criteria for enrollment included all patients who underwent US examination between 12-14 weeks of gestation. Gestational age was determined by the last menstrual period and/or by first trimester US if the patient was unsure of the date of her last menstrual period. Exclusion criteria included pregnancies complicated by multiple gestations, induced abortions due to fetal anomaly or women who had their first antenatal visit after 14 weeks of gestation.

Ultrasonographic examinations were performed with a 6.5 MHz transvaginal transducer (Siemens Medical Systems, Inc). A single specialist performed the measurements in order to eliminate the possibility for interobserver variability in measurement technique. The specialist was blinded to the woman's previous cervical length records. After the patient emptied her bladder, she was placed in the lithotomy position. TVS measurements of the cervix were made with a standard technique, as previously described by Iams *et al.* [6]. The internal cervical os was identified by a sagittal plane view, and the calipers were placed at the furthest points between the internal and external cervical os. When funneling was present, the distance over which endocervical walls were juxtaposed was measured. Three measurements were recorded for each and the shortest measurements were used.

According to the trial protocol, cervical length was measured first at 12-14 weeks corresponding to routine first trimester nuchal translucency screening, second at 18-20 weeks corresponding to triple test and abnormality screening and lastly at 28-32 weeks of gestation. Spontaneous deliveries and cerclage placements until 36 completed gestational weeks formed Group 1, iatrogenic inductions of labor for maternal and fetal indications until 36 completed gestational weeks formed Group 2, and term deliveries at ≥ 37 weeks formed Group 3.

Whenever a cervical length measurement < 30 mm was observed, weekly cervical length measurements were performed thereafter. Patients with a cervical length < 30 mm were scheduled for vaginal examination three to five days following TVS examination. All vaginal samples obtained were inoculated into a selective Todd-Hewitt broth with 8 $\mu\text{g/ml}$ gentamicin plus 15 $\mu\text{g/ml}$ nalidixic acid and transported immediately to microbiology laboratory. After 18 to 24 hours of incubation selective broth medium was subcultured onto 5% sheep blood agar. All plated media were incubated overnight at 35°C in 5% carbon dioxide. After 24 hours both beta-hemolytic and non-hemolytic colonies resembling Group B hemolytic streptococcus were evaluated according to morphology and the presence of hemolysis. Facultative gram-positive coccus that grows on blood agar, small, gray-white flat colonies, and beta-hemolysis characteristics were used to select colonies for identification. Presumptive identification of GBS includes Gram's stain, a negative catalase test and the Christie-Atkins-Munch-Peterson test with synergistic hemolysis. All negative subculture plates were reincubated for an additional 18-24 hours and reexamined.

Patients were also screened for bacterial vaginosis. A polyester swab taken from the junction of the upper third and lower two-thirds of the lateral vaginal wall was rolled on a glass slide. The slides were Gram stained and interpreted according to the criteria of Nugent *et al.* [16]. Bacterial vaginosis was diagnosed if the Gram stain score was 7 to 10. Patients with bacterial vaginosis were treated with ornidazole 500 mg/5 days and povidone iodine

0.2 g/7 days. Control vaginal samples were obtained a week after completion of therapy and repeated whenever necessary.

Whenever a cervical length ≤ 15 mm was observed on TVS, a speculum examination was performed for vaginal cultures, bacterial vaginosis screening and presence of visible membranes [14]. Patients were offered "emergency" cerclage placement in the presence of a fully effaced cervix and a dilatation ≥ 3 cm with visible protrusion of intact fetal membranes at or below the external cervical os. We assumed full effacement and 3 cm cervical dilatation as an irreversible end-point of forthcoming delivery due to the following evidence: i) only 21% of instantly diagnosed women with advanced cervical dilatation remained undelivered after one week despite tocolysis between 24-35 gestational weeks [17], ii) < 15 mm cervical length at midtrimester refers to the 1st percentile of all population-based large studies and carried a 50% delivery rate before 32 completed weeks irrespective of dilatation [4, 6, 7] iii) our cut-off point is a follow-up finding which indicated a progression rather than a single screening reflecting an indicator of forthcoming preterm delivery [3, 18].

All cerclage procedures were of the McDonald type with Mersilene tape (Ethicon, Summerville, NJ) used as sutures placed a minimum of 3 cm above the most distal part of the cervix, together with two no. 2.0 polyglactin sutures (Vicryl, Ethicon) placed circularly 1-2 cm above the most distal part of the external cervical os closing the cervix tightly. None of the patients in the cerclage group had any evidence of chorioamnionitis such as fever $> 38^\circ\text{C}$, uterine tenderness, fetal tachycardia, marked leukocytosis ($> 15,000 \times 10^9/\text{l}$) or elevated C-reactive protein (> 15 mg/l). Results were available at the time of cerclage. All patients with cerclages received sulbactam and ampicillin (3 g/day for 7 days), and metronidazole (0.5% for a day). Women who were allocated to emergency cerclage were given indomethacine (300 mg/day for 3 days) and progesterone (300 mg/day for 10 days). Indomethacine was given because of its inhibitory effect on prostaglandins (prostaglandins are upregulated in inflammation via interleukin-1 and cause uterine contractions in the myometrium and fetal membranes) [19]. Progesterone treatment was prescribed to cerclage cases as it was found to decrease recurrent preterm delivery and suppress genes necessary for uterine contractility [20].

Hospitalization was maintained and the women were restricted to bed rest for 48 hours. They were discharged from the hospital within 10-15 days of cerclage operation. Daily US follow-up of amniotic fluid level and vaginal speculum examination for amniotic fluid flow were performed until the patients were discharged from the hospital. Irregular uterine contractions were observed in one of the five cerclage patients which disappeared with the addition of nifedipine (60 mg/day for 7 days). Transient oligohydramnios developed in four of five cerclage patients following indomethacine treatment but all recovered within two weeks of follow-up. Patients underwent TVS weekly after discharge and bed rest was advised until delivery. Cerclages were removed at the 35th week of gestation due to high magnitude of uterine contractions and bleeding from the cerclage side in one case and electively at 37 weeks of gestation in the remaining four cases.

The statistical analysis of the data was performed using the Statistical Package for Social Sciences for Windows (SPSS, Chicago, IL, USA). Our hospital is a reference center for a population of about two million people. The rate of preterm deliveries of patients that are followed up in our center from the beginning of their pregnancies was 11.5% during the year preceding our study. This rate is twice the 5.6% rate found in a population-based study in our country [21]. This is due to our

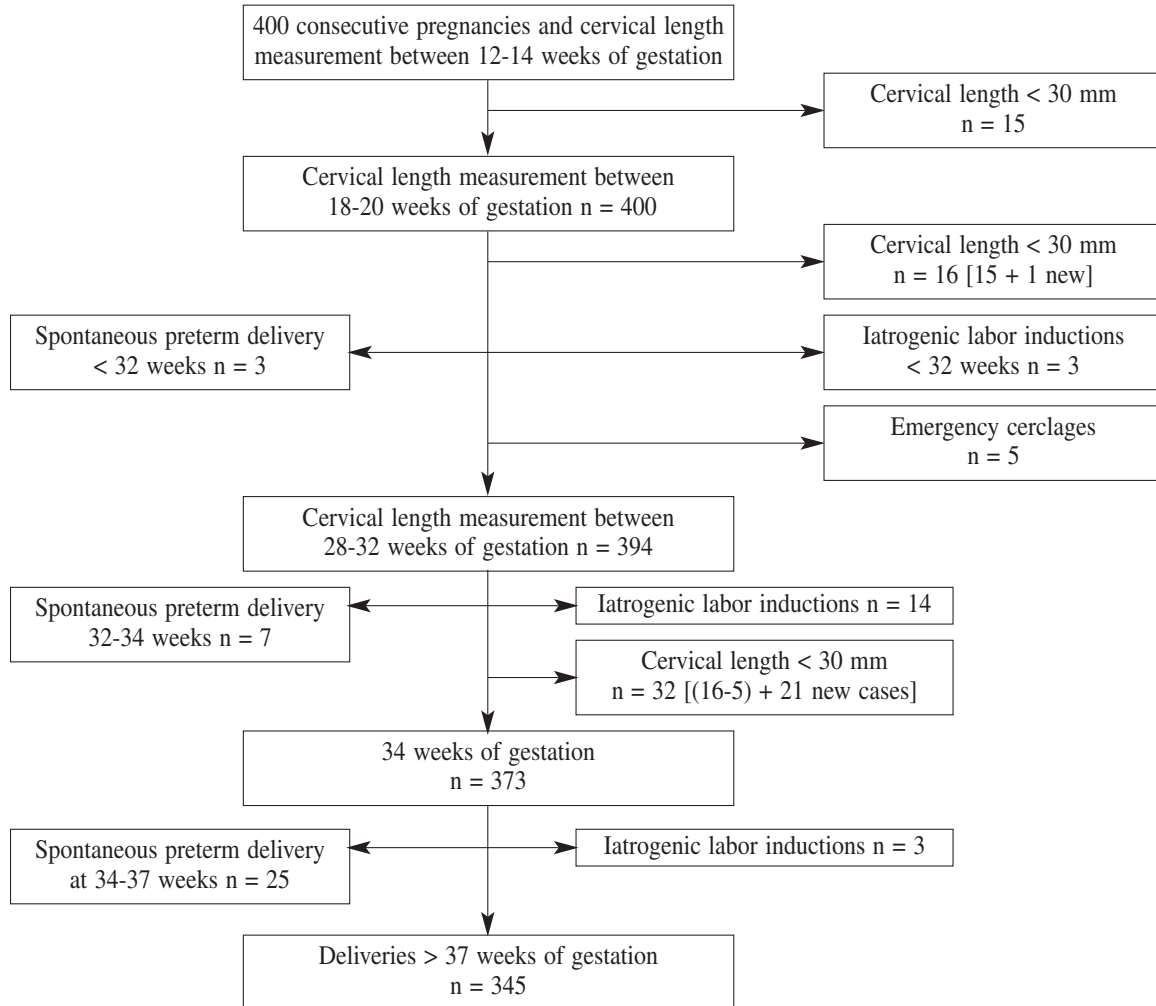


Figure 1. — Flow-chart of the study.

patient population which is mainly formed from high-risk women and women with previously poor maternal and fetal outcomes. Using the 11.5% rate, the number of patients that will represent our population with a 3% error at $\alpha = 0.05$ was calculated to be 393. Accordingly we recruited 400 consecutive women who consented to the study protocol in order to compensate any lost to follow-up or protocol deviations.

Results were reported as mean \pm standard deviation and percentages. Differences between the groups were assessed using the chi-square test for categorical data. To detect the differences of continuous variables between the groups, analysis of variance (ANOVA) and Tukey tests were used. For all comparisons $p < 0.05$ was considered statistically significant.

Results

From June 2003 to April 2005, a total of 400 women were recruited in the follow-up. The algorithm of the study is presented in Figure 1.

Selected maternal variables according to the groups are presented in Table 1. Maternal education, multiparity, occupational status, tobacco usage, presence of previous abortions and curettages were similar among the groups. Mean maternal age of group II was significantly higher

than group III ($p = 0.01$). Previous preterm delivery was significantly more frequent in group I compared to group II and III ($p < 0.001$). On the other hand, the rate of

Table 1. — Maternal variables according to the groups. Data are presented as mean \pm SD or numbers (percentages).

	Group I† (n = 39)	Group II (n = 20)	Group III (n = 341)	p
Maternal age (years)	28.8 \pm 6	30.2 \pm 5.1*	27.3 \pm 4.8	0.01*
Education (years)	9.6 \pm 4.2	7.4 \pm 4	10 \pm 3.9	0.06
Multiparity	20 (51.3)	12 (60)	176 (51.6)	0.7
No occupation	25 (64.1)	12 (60)	180 (52.8)	0.3
Tobacco use	3 (8.8)	0	20 (7.4)	0.5
Previous abortion \geq 1	10 (25.6)	6 (30)	69 (20.2)	0.4
Previous D&C \geq 1	4 (10.3)	4 (20)	40 (11.7)	0.5
Previous preterm delivery at 14-34 weeks \geq 1	8 (20.5)	0	16 (4.7)	< 0.001**
Mullerian anomaly	2 (5.1)	1 (5)	3 (0.9)	0.049**
Threatened abortion in studied pregnancies	9 (23.1)	3 (15)	17 (5.0)	< 0.001**
Infertility treatment in studied pregnancies	5 (12.8)	4 (20)	9 (2.7)	< 0.001*

*Significantly higher than group 3 ($p = 0.03$), ANOVA, Tukey tests.

**Significantly different, $p < 0.05$, chi-square test.

†All patients with emergency cerclage were analyzed under this group.

Table 2. — Flow-chart of the study until 32 completed weeks of gestation.

Cervical length screening	Findings	Intervention	Outcome and medical treatment
400 consecutive pregnancies at 12-14 weeks of gestation	Cervix < 30 mm (n = 15)	- Culture screening and weekly ultrasound follow-up (n = 15)	- Cervical shortening progressed in three cases - Four bacterial vaginosis, one group B streptococcus, and one E.coli colonization treated
400 pregnancies at 18-20 weeks of gestation	Cervix < 30 mm (n = 16) (15 previously diagnosed cases plus one new case)	- Culture screening and weekly ultrasound follow-up (n = 16) - Three labor inductions - 5 emergency cerclages at 20, 21, 23, 24, 27 weeks	- Antibiotics, progesterone and indomethacine treatment in 5 cerclage cases - One recurrent bacterial vaginosis treated - Three spontaneous deliveries < 32 completed weeks
394 consecutive pregnancies at 28-32 weeks of gestation	Cervix < 30 mm (n = 32) (16 previously diagnosed cases minus four cerclage cases plus 20 new cases)	- Culture screening and weekly ultrasound follow-up (n = 32)	- Four bacterial vaginosis, one group B streptococcus and two E. coli colonization treated

Table 3. — Summary of obstetrical outcomes at selected gestational weeks and grouping.

	Iatrogenic inductions	Spontaneous deliveries	Cerclage cases
28-32 weeks of gestation	n = 3	n = 3	n = 5
28-34 weeks of gestation	n = 17	n = 10	n = 5
28-37 weeks of gestation	n = 20	n = 35	n = 4
Deliveries ≥ 37 weeks	Iatrogenic deliveries	Spontaneous deliveries and cerclage placements < 37 weeks	
Group 3*	Group 2	Group 1	
n = 341	n = 20	n = 39	

*Cerclage cases were included in group 1 to compare cervical length follow-up of cases with a preterm delivery risk to other cases.

Mullerian anomaly ($p = 0.049$), threatened abortion ($p < 0.001$) and infertility treatment ($p < 0.001$) in the studied pregnancies were significantly lower in group III compared to group I and II.

Cervical length screening, interventions and treatments of the study groups are presented in Table 2. Obstetric outcomes are presented in Table 3.

Cervical length follow-up according to groups is presented in Figure 2. The mean cervical length was significantly different in the groups at 12-14 weeks ($p = 0.007$), at 18-20 weeks ($p < 0.001$) and at 28-32 weeks of gestation ($p < 0.001$).

A total of 3.7% (15/400), 4% (16/400) and 8.1% (32/394) of the women were diagnosed with a cervical length < 30 mm at 12-14, 18-20, 28-32 weeks, respectively. The predictive value of a < 30 mm cervical length at different gestational weeks is presented in Table 4. Although the specificity of this cut-off value was high, the sensitivity and PPV was unacceptably low to justify an invasive procedure like cervical cerclage.

Using the weekly cervical screening strategy until 32 weeks of gestation in case of a cervical length < 30 mm, we performed 424 TVS scannings in addition to cervical length measurements at 12-14, 18-20 and 28-32 weeks and also, 63 vaginal cultures were performed. The total cost of extra tests after detection of a short cervix was calculated to be 2220 Euros which is much less than the cost of 8300 Euros for one surviving neonate managed in our hospital after delivering at the 25th week of gestation. On the other hand, we were able to treat five cases of cervical

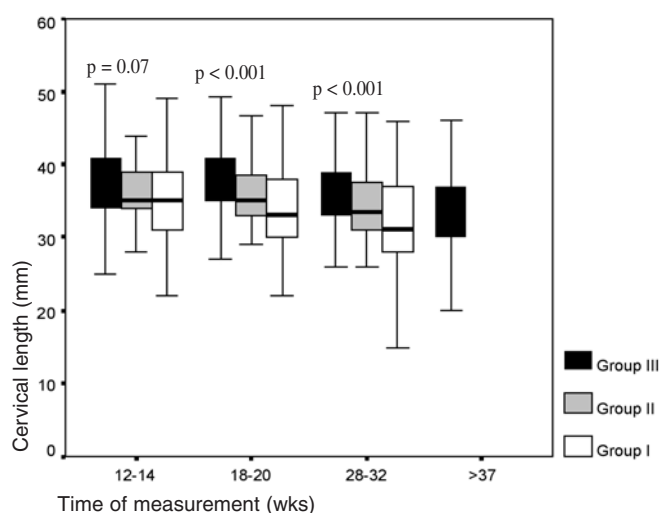


Figure 2. — Cervical length follow-up according to time and indication of delivery.

insufficiency successfully out of eight possible spontaneous deliveries < 32 weeks and 15 possible spontaneous deliveries < 34 weeks. Among the five cases with emergency cerclages performed at 20, 21, 23, 24, 27 weeks of gestation, one case (cerclage performed at 23 weeks) delivered at 35 weeks of gestation and was diagnosed as having a partial uterine septum while the other four cases delivered ≥ 37 weeks of gestation. Three of the cerclage patients were nulliparas while the patient with a partial uterine septum had two mid trimester losses and the last patient was a multipara with one live preterm delivery at 33 weeks of gestation. Preterm premature rupture of membranes with anhydramnios and intrauterine growth restriction were the indications for iatrogenic labor induction in three cases < 32 weeks of gestation. Intrauterine growth restriction, anhydramnios, preeclampsia, preterm premature rupture of membranes, and maternal heart disease were indications of iatrogenic induction of labor in 17 cases between 32 to 36 completed weeks.

Newborns of women that had cerclage were followed for 12 to 18 months of life and were completely healthy.

Table 4. — Predictive value of a < 30 mm cervical length at different gestational weeks.

Measurement time	Spontaneous preterm birth < 34 weeks (n = 15)				Spontaneous preterm birth < 37 weeks (n = 39)			
	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
12-14 weeks (n = 400)	26.7	97.1	26.7	97.1	15.4	97.5	40	91.4
18-20 weeks (n = 400)	26.7	96.9	25	97.1	17.9	97.5	43.8	91.7
28-32 weeks (n = 394)*	11.1	91.9	3.1	97.8	39.4	94.7	40.6	94.5

Sens = Sensitivity; Spec = Specificity; PPV = positive predictive value; NPV = negative predictive value; *Predictive values of 28-32 weeks screening were calculated for 9 and 33 cases for birth < 34 and < 37 weeks, respectively, as four cerclage and two spontaneous deliveries occurred until screening.

Discussion

Using assessment of cervical length measurement at the 12-14th, 18-20th and 28-32nd weeks of gestation strategy, we were able to postpone five inevitable extreme premature preterm deliveries until after 34 weeks of gestation without fetal morbidity and mortality.

Our data supports the notion that short cervixes do not necessarily indicate the risk of preterm delivery. Prophylactic cerclages based solely on this finding would be an over-treatment in a considerable number of patients as the sensitivity and the PPV of a diagnosed short cervix as the above-mentioned screening intervals are low. Also, different than most cerclage studies conducted in the literature we screened all women including nulliparas which constituted 48% of our study population. As a result of that three of the five emergency cerclage cases were nulliparas. Several prior case control studies, reviews, and metaanalyses have suggested that only high-risk women with one or two mid trimester losses should be screened for cervical length measurement, and prophylactic cerclage placement should be performed after these losses [2, 5, 22]. Although this might be a statistical fact it underscores the physiological burden of one or more prior mid trimester losses on both the couple and their obstetrician.

Cervical length is a continuum and might be influenced by sociodemographic factors like race, stress, and occupation across the gestation [23]. Bacterial vaginosis, microbial invasion of the amniotic cavity and intra-amniotic inflammation might also cause cervical insufficiency through IL-8 mediated degradation of the cervical matrix [24]. Whether a predisposing factor or a result, women with cervical length < 25 mm have 9% microbiologically proven intraamniotic infection, < 20 mm have a higher rate of placental inflammation and if cervical dilatation is present, microbial invasion of amniotic cavity might be as high as 51.5% [1, 15, 25]. These findings formed the background of our vaginal culture and bacterial vaginosis screening of short cervixes which might be the contributing factor for the high success rate of emergency cerclages placed. A study published after the start of this study showed that vaginal povidone iodine, also used in our study, normalizes IL-8 concentrations in 23.2% of patients and decreases preterm delivery rates before 34 weeks of gestation [26].

A high rate of surveillance after emergency cerclage in a small number of patients in this study might be due to the attempt of controlling vaginal microbial flora via antibiotic use before and after the operation or use of agents that suppress uterine contractility and enhance cervical remodeling such as cyclooxygenase inhibitors and progesterone. Cerclage in our population was performed on pregnant women with well identified poor prognostic factors for successful operations such as visible membranes, cervical length < 5 mm, cervical dilatation \geq 3 cm, and nulliparity [14, 27]. Fetal salvage in these cases of emergency cerclage is reported to be 46-50%, most of which are delivered preterm due to short operation delivery interval [14, 28].

Our study indicates two important measures: 1) follow-up of short cervixes until clinically advanced cervical dilatation is a safe procedure after which a successful cerclage operation can be performed. This is proven by the fact that prophylactic cerclage placement for incidental detection of cervical length < 25 mm in the early second trimester does not improve pregnancy outcome [29], and 2) cervical length is probably influenced by factors not yet defined as women with poor obstetric performances have shorter cervixes since the end of first trimester across the gestation. This latter proposal is evidenced by shorter cervixes identified in cases of iatrogenic induction of labor due to early rupture of membranes, chronic maternal illness and evidence of placental insufficiency.

Conclusion

The management algorithm proposed in this study resulted in prevention of one very early premature delivery for every 85 additional US measurements performed and 13 vaginal cultures obtained in addition to ideal current antenatal US screening. Routine cervical length measurement combined with serial TVS and vaginal microbial monitoring of the short cervixes will avoid unnecessary prophylactic cerclages while increasing the success of emergency cerclages performed upon solid clinical findings.

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Case Reports

Complete remission of OC-resistant catamenial shoulder joint pain and inguinal pain associated with extraperitoneal endometriosis following personalized GnRH agonist therapy

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Summary

Background: Patients with severe extraperitoneal endometriosis require rapid remission and cannot wait for the effects of oral contraceptive hormones (OCs) to appear. **Case:** We successfully achieved personalized gonadotropin-releasing hormone agonist (GnRHa) therapy for a patient with catamenial right shoulder joint pain and right inguinal pain associated with extraperitoneal endometriosis, which was completely unable to be suppressed by OCs. A total of 15 subcutaneous GnRHa depot injections over a period of 19 months was performed according to the serum estradiol and LH levels, in order to maintain long-term amenorrhea without any estrogen-deprivation effects. No recurrence of the catamenial symptoms has been observed for more than 35 months after the final GnRHa depot injection. **Conclusion:** Personalized GnRHa therapy should become the first-choice therapy for OC-resistant inoperable extraperitoneal endometriosis.

Key words: Endometriosis; GnRH agonist; Leuprolide acetate; Oral contraceptive; Shoulder joint pain.

Introduction

Anti-endometriosis therapy must be determined for each individual patient according to the severity of their signs and symptoms, desire to become pregnant, and localization and size of the endometriotic lesions. There are still no standard therapeutic protocols for extraperitoneal endometriosis, despite the fact that surgical and medicinal therapies for intraperitoneal endometriosis are almost established. Extraperitoneal endometriosis often results in severe and lethal symptoms [1]. Recently, oral contraceptives (OCs) have been commonly applied to patients with extraperitoneal endometriosis. However, OCs are not applicable for severe and emergency cases of endometriosis that require rapid therapeutic effects, since their effects may occur several months after the initiation of the therapy. Gonadotropin-releasing hormone agonist (GnRHa) therapy can certainly induce complete remission within a short time. However, if GnRHa depots are injected every month over a prolonged period of time, the patients develop severe menopausal malaises due to estrogen deprivation and require estrogen supplementation [2, 3]. Here, we describe a very rare case of catamenial shoulder joint pain and inguinal pain complicated with inguinal endometriosis that was successfully and completely treated by personalized GnRHa therapy without any estrogen-deprivation effects, rather than by OC therapy.

Case Report

The patient visited the gynecology outpatient clinic of our university hospital complaining of a right inguinal mass with increasing catamenial right inguinal pain every month for the previous six months. She was a 47-year-old multiparous woman with dysmenorrhea and hypermenorrhea due to pelvic endometriosis and adenomyosis. She had a past history of two cycles of 6-month GnRHa depot therapy for abdominal endometriosis when she was 40 and 42 years of age, respectively. She was initially treated with three cycles of OCs, but the catamenial inguinal pain remained unrelieved. Histopathological examination of a surgically resected inguinal nodule confirmed that the right inguinal pain was due to inguinal endometriosis (Figure 1). After the surgical resection, the patient received a further three cycles of OCs. Although her dysmenorrhea and hypermenorrhea were completely suppressed by the OC therapy, the right inguinal pain remained. Moreover, right shoulder joint pain appeared and increased with every menstruation, despite the OC treatment (Figure 2). Since OCs did not show any suppression of the catamenial shoulder joint pain or inguinal pain, personalized GnRHa therapy was carried out. After initial administration of two 3.75-mg leuprolide acetate depots to the patient with a 4-week interval in order to avoid flare-up phenomena, 1.8-mg GnRHa depots (leuprolide acetate or goserelin acetate) were injected subcutaneously at five to seven week intervals. The inter-injection intervals were modulated to achieve stable endocrine conditions with 15-50 pg/ml serum estradiol and 0-5 IU/l serum LH, in order to maintain stable long-term amenorrhea without any estrogen-deprivation effects. Three months after the final depot injection (15th injection), regular menstruation reappeared, but no catamenial shoulder pain, inguinal pain or dysmenorrhea have been observed for over 35 months (Figure 2). From 11.3 pg/ml serum estradiol and 84.33 IU/l serum FSH levels, a diagnosis of spontaneous menopause was made ten months after the last GnRHa injection.

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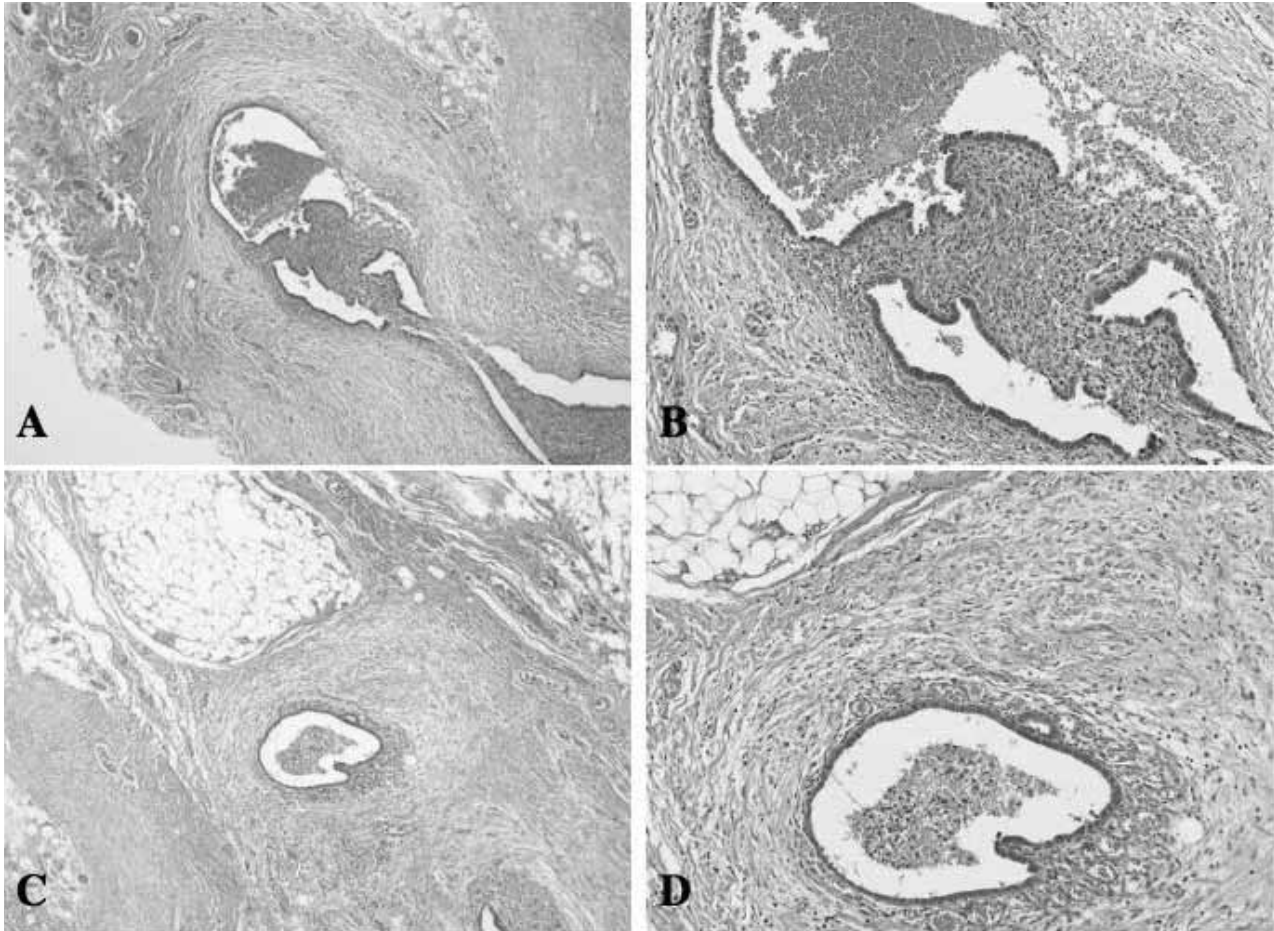


Figure 1. — Histological findings of the resected inguinal lesions.

Inguinal nodule containing typical endometriotic lesions in the subcutaneous tissue. Endometrium-like epithelium on stromal cells with hemorrhage is present in the inguinal subcutaneous tissue (A, B). Endometriotic lesions invading the subcutaneous adipose tissue (C, D). B and D show magnified views (x100) of A and C (x60), respectively.

Discussion

The present case may represent the first report of possible endometriosis in a shoulder joint. Although the catamenial shoulder joint pain could not be examined histopathologically since no superficial tumorous lesions were detected around the joint, this shoulder joint pain was considered to be one symptom associated with endometriosis for the following reasons: the inguinal lesion was histopathologically proven to be extraperitoneal endometriosis as shown in Figure 1; the shoulder joint pain appeared simultaneously with inguinal pain due to endometriosis only during menstruation; the shoulder joint pain was only observed in the menstrual period; during the amenorrhea period induced by the GnRH α therapy, the patient had neither inguinal pain nor shoulder joint pain. Although several previous papers have reported that catamenial shoulder tip pain associated with endometriotic pneumothorax can be cured after treatment of diaphragmatic endometriosis [4-6], endometriotic pneumothorax was excluded in the present case because the

patient had never complained of chest pain or dyspnea and a chest X-ray examination did not show any signs of pneumothorax.

Although solitary intraperitoneal endometriotic lesions may be radically cured by surgery, medicinal therapy, rather than surgical therapy, is theoretically the first choice for radical therapy of endometriosis, since most endometriotic patients have multiple lesions. For patients with severe extraperitoneal endometriosis who cannot be cured radically by surgery, the best medicinal therapy must be a long-acting, safe and curative treatment that can reduce the endometriotic lesions for a prolonged period of time. Therefore, OCs are currently the first-choice drugs for remission-maintenance therapy after remission-induction, since they are very safe medicines with few adverse effects [7]. However, for patients with severe inoperable endometriosis, such as our present case, OC therapy is too slow-acting for the required rapid induction of complete remission. In fact, several cycles of OC therapy induced complete remission of the dysmenorrhea in the present case, but had no effects on her catamenial

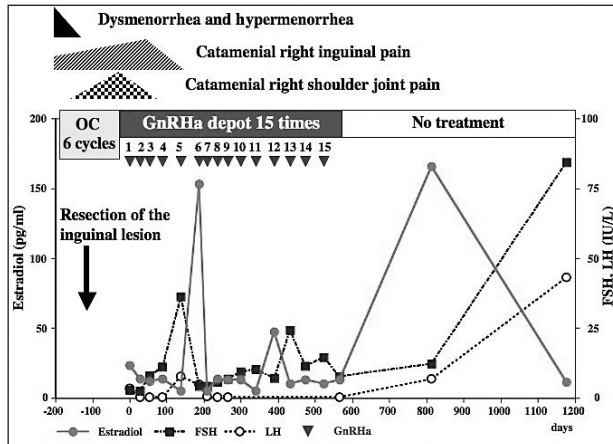


Figure 2. — Personalized GnRH α therapy and catamenial symptoms.

Closed triangles: GnRH α depot injections; closed circles: serum estradiol levels; closed squares: serum FSH levels; open circles: serum LH levels. During the OC therapy, the catamenial lower abdominal pain and hypermenorrhea were completely suppressed. However, the catamenial shoulder joint pain and inguinal pain were only suppressed by the GnRH α therapy.

right shoulder joint pain or right inguinal pain. Therefore, in order to avoid any adverse effects associated with long-term GnRH α therapy, we performed personalized GnRH α therapy that was regulated according to the serum LH and estradiol levels of the patient. Personalized GnRH α therapy is considered to be a safe and long-acting therapy that can efficiently and completely inhibit menstruation, and radically cure endometriotic lesions. At the present time, there has been no recurrence of the catamenial shoulder

joint pain or inguinal pain for 35 months since the cessation of the personalized GnRH α therapy, indicating that the patient's extrauterine endometriotic lesions may have been almost radically suppressed by GnRH α therapy. The results for the present case suggest that personalized GnRH α therapy should become the first-choice therapy for OC-resistant inoperable extraperitoneal endometriosis.

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Near lethal endometriosis and a massive (64 kg) endometrioma: case report and review of the literature

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Summary

A 51-year-old morbidly obese, hypertensive, anemic, and amenorrheic female presented with anuria and respiratory symptoms. The patient had a distinctly massive abdomen with necrotic anterior abdominal wall, and laboratory findings revealed a leukocytosis, profound anemia, coagulopathy and renal failure. An abdominal sonogram showed a large, complex intra-abdominopelvic mass and ascites. At surgery, a massive, cystic left ovarian mass, 37 l of ascitic/cyst fluid, and several peritoneal nodules were removed—a total of 64 kg of tumorous tissue. Histopathological evaluation of the mass revealed an endometrioma. We present this rare case of severe endometriosis in a morbidly obese patient who presented with an exceptionally large endometrioma (64 kg), multifactorial respiratory and renal failure, coagulopathy, and profound anemia.

Key words: Ascites; Endometriosis; Endometrioma; Renal failure.

Introduction

Endometriosis is the presence of ectopic endometrial tissue, particularly in the peritoneal cavity and adnexa. Ovarian endometriotic tissue may organize into a complex cyst—an ovarian endometrioma. Variable in size, endometriomas may very rarely exceed > 20 cm in diameter. We report an exceptionally large endometrioma (64 kg) in a 51-year-old female who presented with rare symptoms of the disease. Multiple theories have proposed likely pathophysiology of endometriosis, but the growth and development of endometriotic implants appear multifactorially regulated. In the majority of women endometriosis is asymptomatic, but some may present commonly with complaints such as dyspareunia, pelvic pain and infertility. Dyspnea secondary to ascites and/or pleural effusions is very rare in endometriosis. Characteristic ultrasound (US) findings – homogeneous, echolucent fluid-filled cyst – may suggest the diagnosis of an endometrioma, however magnetic resonance imaging (MRI) serves the same purpose with higher sensitivity, specificity and accuracy. Malignant transformation of endometrioma is extremely rare but a possibility. Hence, precise histopathological scrutiny of endometriotic tissue is essential. Both medical and/or multi-step surgical therapy may be implemented in endometriosis to alleviate symptoms, and most importantly restore fertility and prevent near-lethal complications of the disease.

Case Report

A 51-year-old morbidly obese (264 kg) African-American female was admitted with dyspnea, productive cough and anuria of three days duration. Her past medical history included hypertension treated with hydrochlorothiazide-losartan combination and hypochromic microcytic anemia secondary to a two-year

history of a suspected leiomyomatous uterus that was being treated with subcutaneous erythropoietin and occasional intravenous iron. Casarean section was the only surgical procedure she underwent in the past. In addition to a leiomyomatous uterus, she reported 12 months of amenorrhea at admission as part of her gynecologic history. She refused surgical intervention and blood products on religious grounds in the past. There was no significant family history except for diabetes and hypertension. The patient denied a history of cigarette smoking, alcohol consumption or illicit drug use.

On admission, she was pale in appearance, afebrile, tachycardic (100-110 beats/min) and tachypneic (22 breaths/min). Chest examination revealed diffuse bilateral rhonchi with decreased bibasilar breath sounds. The abdomen was massive, distended and tense with significant abdominal varices. The skin over her lower abdomen was edematous and necrotic with significant seepage of serous fluid. The umbilicus was everted and necrotic over an area of 60 x 35 cm (Figures 1 and 2). Laboratory findings revealed a leukocytosis (WBC 17,200/mm³), microcytic hypochromic anemia (Hgb 7.0 g/dl & Hct 22.9%), coagulopathy (INR 1.5), and elevated serum urea-nitrogen and creatinine levels (BUN 51 mmol/l & Cr 4.44 μmol/l). An electrocardiogram revealed sinus tachycardia. Chest roentgenography demonstrated bilaterally hypoinflated lungs with a probable right-sided infiltrate. Except for a limited abdominal US, radiological testing to evaluate intraabdominal pathology or rule out pulmonary embolism could not be performed due to her morbid obesity (264 kg). Sonogram revealed moderate, loculated ascites throughout the abdomen in addition to a large, complex, heterogenous, intraabdominal mass arising from the pelvis. Although very limited, the study revealed no evidence of apparent hydronephrosis. She was treated with hemodialysis for her acute renal failure. Broad spectrum antibiotics were started for a presumptive pneumonia/bronchitis and a necrotic anterior abdominal wall. The treatment of her coagulopathy and profound anemia was initially supportive only.

An abdominal paracentesis was performed which revealed no malignant cells in the peritoneal fluid. The patient's body habitus disqualified cystoscopic evaluation for post-renal uropathy. Though initially deferring blood products, the patient and her family decided to accept transfusion and invasive interven-

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Fig. 1

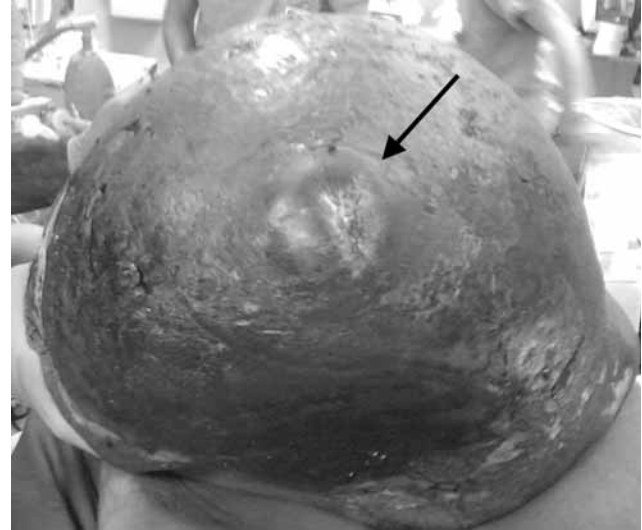


Fig. 2

Figure 1. — Right anterolateral view of the patient's massively distended abdomen with ulcerated, necrotic skin spread circumferentially over an area of 60 x 35 cm. A hard necrotic everted umbilicus is noticeable at the black arrow.

Figure 2. — Caudal to cranial view of the patient's abdomen exhibiting circumferential necrotic skin, and an everted umbilicus (black arrow) at the center.

tion. Worsening multifactorial respiratory failure, renal failure, and persistent ascites in the presence of worsening anemia led to a steep deterioration in her clinical status. Severe sepsis culminating in respiratory failure and shock led to endotracheal intubation and mechanical ventilation, as well as the administration of pressor support. Worsening acidosis (pH = 7.30), leukocytosis (WBC 48,000/mm³) and the development of profuse vaginal bleeding, in the wake of a necrotic abdominal wall and unknown intraabdominal/pelvic pathology led to surgical exploration.

At surgery, the patient underwent a panniculectomy to excise the necrotic skin areas over the abdominal wall that extended down to the fascia (measuring 60 x 35 x 3.5 cm and weighing 4,500 g). Exploratory laparotomy revealed a massive extraperitoneal cystic mass arising from the left ovary. Extirpation of the mass without decompression was not feasible. At decompression, approximately 37 l of thick grey-brown ascitic/cyst fluid was removed. Multiple irregular, tan-red, necrotic, tumorous peritoneal nodules were excised (measuring 50 x 30 x 15 cm and weighing in excess of 9 kg). In total, tumoral tissue including ascitic fluid removed weighed 64 kg. The stage of endometriosis was thought to be IV based on R-AFS (revised-American Fertility Society) classification (Table 1).

Histopathological examination identified the mass as an ovarian cyst (endometrial glandular epithelium and stroma) with organized hemorrhage, suggesting an endometrioma. The skin, fascia and peritoneal specimens revealed inflamed, necrotic tissue with no evidence of malignancy.

Postoperatively, the patient followed a critical course complicated by a novel episode of hemorrhagic shock secondary to a bleeding duodenal ulcer requiring a second laparotomy, oversew of the ulcer bed and pyloroplasty. At that time, 7 l of new ascitic fluid was aspirated from the abdominal cavity. Right thoracostomy drainage for pleural effusion, tracheostomy, percutaneous endoscopic gastrostomy, and multiple dialysis catheter insertions and abdominal wound debridements were also performed. She was eventually discharged on tracheostomy collar tolerating full-strength tube feeds and purée diet on hospital day 57. Vacuum assisted closure (VAC) therapy was applied to the abdominal wound and a sacral decubitus.

Table 1. — American Fertility Society's scoring system for staging endometriosis. Cumulation of points based on descriptive findings of endometriosis and adhesions determines the stage of the disease. Stage I (minimal): 1-5; Stage II (mild): 6-15; Stage III (moderate): 16-40; and Stage IV (severe): > 40. Highlighted numbers in the table represent findings in our patient.

Endometriosis		< 1 cm	1-3 cm	> 3 cm
Peritoneum	Superficial	1	2	4
	Deep	2	4	6
Ovary	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20
Posterior Culdesac obliteration		Partial 4	Complete 40	
Adhesions		< 1/3 cm Enclosure	1/3-2/3 Enclosure	> 2/3 Enclosure
Ovary	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
Tube	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16

Discussion

Endometriosis is the presence of ectopic endometrial tissue, particularly in the peritoneal cavity or adnexa. Although extrapelvic endometriosis is possible, it is very rare. The incidence of endometriosis is unknown, but in women undergoing evaluation for infertility and for dysmenorrhea/pelvic pain, it is approximately 30% and 40-50%, respectively [1]. Like uterine endometrium, endometriotic tissue responds to ovarian cycles. Under hormonal influence, ovarian endometriotic tissue may

organize into a complex hemorrhagic mass known as an ovarian endometrioma or commonly referred to as "chocolate cyst". Out of the two-thirds of women with endometriosis who have ovarian enlargement, only 5% are detectable on pelvic exam [2]. Endometriomas vary from 1-5 mm in diameter, superficial, blue-black implants to 5-10 cm in diameter, multiloculated cysts. Endometriomas > 20 cm in diameter are extremely rare.

The two widely accepted theories explaining the pathophysiology of endometriosis are the Sampson theory and the metaplasia theory [3]. The Sampson theory suggests that viable endometrial cells shed by retrograde menstruation into the peritoneal cavity, implant and grow within the peritoneum, whereas metaplasia explains endometriosis in an amenorrhic state. Neither of these two theories explains the growth and development of endometriotic tissue. Compartmentalized peritoneal fluid with higher than plasma concentrations of steroid hormones, along side cellular elements and their secreted cytokines, growth factors and angiogenic factors suggests a tumor mechanism – mass formation, extracellular matrix degradation and immunologic defense – in the growth of endometriotic implants. Similarly, high intraovarian concentrations of steroid hormones may be vital in the development of ovarian endometriosis via direct membrane effects of estrogens and immunosuppressive effects of progesterone. Also, enhanced expression of proteolytic enzymes, fibronectin receptors, and angiogenesis by endometriotic cells, in addition to the local ovarian and peritoneal fluid milieu may suggest a genetic contribution. Hence, development of endometriotic disease is likely multifactorial.

The majority of women with endometriomas are asymptomatic. When patients are symptomatic, dyspareunia, pelvic pain, and infertility are the most common complaints. Despite its antagonistic effects on fertility, endometriosis or endometrioma may nevertheless yield a successful pregnancy. Birken *et al.* reported a successful conception, pregnancy, and vaginal delivery of a third child by a mother who underwent right ovarian cystectomy (10 x 12 cm endometrioma) in the second trimester as well as left salpingo-oophorectomy for severe endometriosis in the past [4]. Severe symptoms such as abdominal distention and dyspnea are very rare with endometriomas. Dyspnea, if persisting, is usually due to pleural effusions caused by direct extrapelvic endometriotic implants and/or from abdominal distention caused by ascites in severe endometriosis. Elevated intraabdominal pressure causing transudation of transdiaphragmatic lymphatics also may result in pleural effusions. Cytological diagnosis of pleural or peritoneal endometriosis can be difficult since non-specific hemosiderin-laden macrophages may be the only cells identified [1].

Endometriomas may be solid, mixed or cystic. A homogeneous, echolucent fluid-filled cyst is a characteristic sonographic finding of an endometrioma. Although complete radiological imaging was difficult in our case, Togashi *et al.* reported that MRI enabled accurate diagnosis of 77 of 86 endometrial cysts and exclusion of diag-

nosis in 263 of 268 other gynecologic masses, with or without internal hemorrhage. Thus, MR imaging is an acceptable modality for endometriomas with diagnostic sensitivity, specificity, and accuracy of 90%, 98% and 96%, respectively [5]. On histopathology, endometrial columnar glandular epithelium, stroma, and hemosiderin-laden macrophages may be identified in endometriotic tissue [6]. Fibrosis from inflammation may also be seen and is usually present in symptomatic cases when the ovaries adhere to surrounding structures resulting in tender, fixed ovaries.

In many ways, symptoms of severe or extrapelvic endometriosis may mimic neoplastic disease, especially advanced ovarian malignancy. However, tumor markers such as CA-125, CEA and CA19-9 are normally not helpful in distinguishing between those entities. Interestingly, Goumenou *et al.* reported a case of endometriosis, in which the patient presented with massive ascites, pleural effusions and extremely elevated CA-125 and CA19-9 levels. Chemotherapy (carboplatin and taxol) was administered based on the presumptive diagnosis of an ovarian neoplasm, however subsequent total abdominal hysterectomy, bilateral salpingo-oophorectomy, lymphadenectomy, omentectomy, peritoneal biopsy and appendectomy revealed no evidence of malignancy [1].

Malignant transformation of endometrioma is rare but is a recognized event, with the ovaries being the most common site. Criteria for establishing the diagnosis requires 1) coexistence of carcinoma and endometriosis at the same site, 2) similar histological pattern, 3) exclusion of another malignancy elsewhere, and 4) morphologic demonstration of continuity between benign and malignant endometrioid epithelium. Moll *et al.* were among the first few to report a case of invasive clear-cell adenocarcinoma three years after diagnosing and treating an endometrioma containing a focus of severe epithelial atypia in the same ovary [7]. Hence, precise histopathological scrutiny of endometrioid tissue for atypia is essential.

Medical or surgical intervention, or a combination of both may be applied to the management of large ovarian endometriomas. Medical therapy involves ovarian estrogen suppression and induction of amenorrhea by using gonadotropin-release hormone (GnRH) agonists. The surgical approach may involve radiologically-guided cyst drainage, and/or open or laparoscopic cystectomy with ablation of the residual tissue as well as excision of endometriotic implants from other tissue surfaces. Brosens *et al.* have proposed a 2-step laparoscopic technique of ovarian reconstruction in large endometriomas based on the concept that typical endometriomas are extraovarian pseudocysts formed by invagination of the ovarian cortex [8]. The first step involves ovarioscopy, biopsy and pseudocyst exposure allowing spontaneous regression and eversion, followed by a second laparoscopy at two to three months involving adhesiolysis and coagulative destruction of neovascularization and implants on the exposed pseudocyst cortex. Of the (n = 18) patients (age range 26-41 years) who underwent both

steps, no recurrences were noted between 26 and 42 months of follow-up except for the development of a contralateral ovarian endometrioma in a single patient. Donnez *et al.* reported on patients with endometriosis who were initially treated with laparoscopic cyst (> 3 cm) drainage, followed by a second laparoscopy to assess endometriosis and perform CO₂ laser vaporization of the internal ovarian cyst wall (site of active implants). In between these two procedures, patients were administered a 12-week regimen of GnRH agonist. They reported a significant decrease in the number and size of endometriotic implants, and a pregnancy rate of > 51% (majority in first 10 months after surgery) in patients followed-up for a period of two to 13 years [9].

As mentioned earlier, endometriomas > 20 cm in diameter are extremely rare. Our extensive literature search revealed only a handful (n = 3) of super-sized endometriomas. Roth *et al.* reported on a large left ovarian endometrioma (18 x 11 x 5 cm) in an adolescent girl [10], and Reuter *et al.* reported a large right ovarian endometrioma (measuring 20 x 30 cm and weighing 2,750 g) in a premenopausal woman [6]. A right ovarian endometrioma (measuring 24 x 15 x 15 cm and weighing 3,121 g) in a postmenopausal woman published by Bellina *et al.* was the largest until now [11]. In the case presented, the endometrioma measured 89 x 76 cm prior to decompression and weighed a mammoth 64 kg. No similar instance of such an oversized – both in weight and size – endometrioma has ever been reported.

Conclusion

Endometriosis although asymptomatic in the majority may exhibit severe, progressive symptoms in rare instances. In our morbidly obese patient, an enormous endometrioma and associated ascites that initially presented with dyspnea and anuria led to extreme clinical deterioration. The result was almost lethal-septic shock, multifactorial respiratory failure, renal failure, anemia and coagulopathy. Although infeasible in our case, a precise diagnosis of endometriosis with radiological diagnostic modalities (US or MRI) and biopsy is essential since it most commonly affects women of reproductive age. Despite its rarity, malignant degeneration of an endometrioma is also a possibility and must be excluded. Hence, accurate histopathological scrutiny is vital. Defining the extent of endometriotic progression may help stage the disease and categorize the patients (Table 1).

Accordingly then, implementing appropriate and timely therapeutic interventions – GnRH agonist administration or multi-step surgical therapy, or a combination of these – may not only prevent lethal complications, but also decrease disease recurrence and restore fertility. We believe self-neglect and the reluctance to accept medical and surgical intervention previously led to an aggressive and severe progression of endometriosis in our patient. We recommend early diagnosis followed by necessary treatment to avoid severe, near-lethal complications that may arise from this disease.

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The development of placenta increta following pelvic transcatheter artery embolization for postpartum hemorrhage

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Summary

Objective: Pelvic transcatheter artery embolization (TAE) has been widely used for the management of postpartum hemorrhage (PPH). However, the adverse effects of TAE on the subsequent pregnancy remain poorly understood. **Case:** A 30-year-old woman, gravida 2, para 1, developed PPH due to atonic bleeding and underwent TAE. Thereafter, her menstrual cycle became irregular with less blood volume. Three years later, she became pregnant despite a thin endometrial thickness of 6 mm during the ovulatory period. She delivered a healthy baby at 39 weeks of gestation. No signs of placental separation were obtained, and an attempt at manual extraction of the placenta failed, followed by massive PPH. She underwent emergent TAE. The placenta was not spontaneously delivered even on day 8 postpartum. A supracervical hysterectomy was performed due to a worsening intrauterine infection. Pathological examination revealed findings compatible with placenta increta. **Conclusion:** A TAE-associated thin endometrium may be attributable to the development of placenta increta. Pregnant women undergoing TAE should be managed carefully because the information about pregnancy outcomes after TAE remains scanty.

Key words: Placenta increta; Transcatheter artery embolization; Postpartum hemorrhage.

Introduction

There is little information regarding the severe adverse effects in pregnant women who undergo pelvic transcatheter artery embolization (TAE), while a pregnancy subsequent to TAE for postpartum hemorrhage (PPH) has been reported to have a risk of recurrence of PPH [1]. We report a case of the development of placenta increta in a woman who underwent TAE for PPH in a previous pregnancy.

Case Report

The patient was 30-year-old woman, gravida 2, para 1. At the age of 27, she had undergone bilateral pelvic transcatheter artery embolization (TAE) of the internal iliac arteries due to atonic bleeding. Thereafter, her menstrual cycle had become irregular with less blood volume. Three years later, she became pregnant despite a thin endometrial thickness of 6 mm during the ovulatory period. The course of pregnancy was uneventful until 20 weeks of gestation when an episode of uterine bleeding was noted. The episode repeated several times in the latter half of the second trimester. Ultrasonography (US) demonstrated many anechoic areas with convoluted margins around the placental and maternal interface (Figure 1). These findings of placental lacunae were suggestive of placenta accreta.

The patient gave birth to a healthy male infant weighing 3,248 g at 39 weeks, but considerable bleeding began 10 min after delivery without any signs of placental expulsion. An attempt at manual removal of the placenta and treatment to cease massive hemorrhage failed to succeed. Total blood loss amounted to 3,490 g, and her hemoglobin level dropped to 3.5 g/dl. She underwent

an emergent bilateral TAE of the internal iliac arteries, and the refractory bleeding was successfully controlled.

Gadolinium-enhanced magnetic resonance imaging (MRI) displayed a thin or absent retroplacental myometrial zone in the anterior uterine wall where placental fragments were attached, suggesting placenta accreta. Conservative treatment with antibiotics and uterotonic agents was given because of the patient's strong desire to preserve the uterus. On day 3 postpartum, severe intrauterine infection manifested. Five days later, the patient's body temperature rose to greater than 39°C with a white blood cell count of 19,200/μl and C-reactive protein of 10.1 mg/dl. She underwent a supracervical hysterectomy due to the worsening uterine infection. At laparotomy, the placenta was visible through the thin uterine wall, suggesting placenta increta (Figure 2). A foul-smelling specimen of the placenta was tightly adhered to the uterine wall. Total blood loss amounted to 1,075 g and six units of red blood cell were transfused. The pathological diagnosis was consistent with placenta increta. The postoperative course was uneventful, and the patient was discharged home on day 15 postoperation.

Conclusion

TAE has been thought to contribute to the pathogenesis of abnormal placentation such as placenta accreta. Pron *et al.* [2] reported two cases of placenta accreta among 18 pregnancies undergoing uterine artery embolization (UAE) for leiomyomas. El-Miligy *et al.* [3] also reported a case of placenta accreta following bilateral UAE for leiomyomas. Although it is uncertain whether an abnormal placentation in the present case was directly related with TAE, it is speculated that the uterus damaged by TAE might have caused endometrial atrophy.

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Fig. 1



Fig. 2

Figure 1. — Placental lacunae around the placental and maternal interface early in the second trimester. The white arrows show anechoic areas with convoluted margins.

Figure 2. — The uterine surface of placenta increta. The placenta is visible through the thin uterine wall.

The thinness of the endometrium may in turn lead to the defect of an appropriate development of the decidua with subsequent abnormal placental attachment. The mechanism underlying endometrial atrophy associated with TAE remains unknown. Nonetheless, it is tempting to speculate that a reduced endometrial blood supply by TAE may cause endometrial atrophy. A relevant study has been reported by Tropeano *et al.* [4], who described a case of permanent amenorrhea associated with endometrial atrophy which occurred following UAE for uterine fibroids. In the present case, we observed placental lacunae around the placental and maternal interface early in the second trimester. A recent report has indicated that visualization of placental lacunae can predict a high possibility of placenta accreta as early as 15 to 20 weeks of gestation [5].

In conclusion, it is suggested that the thin endometrium following pelvic TAE may be involved, at least in part, in the development of placenta increta. The present case warns that a subsequent pregnancy following TAE should be carefully monitored in the second and third trimesters of pregnancy.

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Pregnancy-induced symptomatic pelvic and extra-pelvic cavernous hemangiomas

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Summary

Background: Pregnancy-induced pelvic and extra-pelvic cavernous hemangiomas is a serious condition, associated with considerable maternal and fetal risks. This report describes the ultrasound (US) features and the clinical management of such rare condition in a young caucasian woman. **Case:** A 20-year-old woman was referred to our department following the occurrence of swelling in her inguinal and vulvar area together with lipotimic episodes. Diffuse cavernous hemangiomas of the pregnant uterus associated with vaginal, inguinal and vulvar varicosities was diagnosed clinically and by 2D and 3D US. The patient underwent expectant management of the gestation, under close US monitoring of both superficial and inner varicosities, careful surveillance of the maternal and fetal condition and prophylaxis for thrombotic complications with medical therapy. A cesarean section was performed at 37 weeks of gestation because of the worsening of her lipotimic episodes and her unfavorable Bishop's score. **Conclusion:** This is the first report in which pregnancy-induced varicose disease involved contemporarily uterine, vaginal, inguinal, and vulvar veins totally sparing the lower extremities. This case suggests that, under close monitoring, a conservative approach can be adopted in such conditions. Vaginal delivery is to be preferred, but if cesarean section is required, the surgery should be performed under general anesthesia and packed red cells and plasma units should always be available.

Key words: Cavernoma; Pregnancy; Ultrasound.

Introduction

Primary varicose veins usually involve the greater saphenous system (75%) or the short saphenous system (20%) with the remaining 5% arising from perforating veins [1]. More rarely, in parous female patients of reproductive age, primary varicose veins arise within the pelvis and may secondarily involve the legs.

Pregnancy has been repeatedly presumed to be a major contributing factor in the pathophysiology of pelvic varicosities in female patients [2]. However, despite such strong evidence, relatively few cases of pregnancy-induced severe varicose disease are described in literature and only little is known about the management of such condition [3].

We describe a case of symptomatic, diffuse cavernous hemangiomas of a pregnant uterus associated with vaginal, inguinal and vulvar varicosities. The pregnancy course, the pathophysiologic and diagnostic characterization as well as the delivery management are described in this report. Being apparently the first such case to be reported, the employed management may be of help should such a condition be encountered.

Case Report

A 20-year-old woman, gravida 2, para 0 (+ 1 CS), was referred to our Department at 12 weeks of gestation because of the sudden occurrence of varicosities involving her right inguinal and vulvar superficial veins. Her familial and general

histories were negative for either varicose or coagulatory diseases. Her obstetric history revealed an uneventful course of a prior gestation which was terminated by cesarean section due to fetopelvic disproportion. Since the time of the referral she had been treated with low-dose aspirin (100 mg daily; Aspirinetta®, Bayer; Milan). The second trimester ultrasound (US) examination failed to detect any fetal or uterine anomaly. In particular, the search of ileo-pelvic venous congestion did not reveal any significant varicosities, beyond those usually visible in pregnant uteri. The patient's prenatal course was otherwise uneventful until 27 weeks of gestation when she presented complaining of a sudden swelling in her right groin accompanied by sensations of burden and discomfort. Clinical examination revealed a 15-cm lump in her right groin involving both the right inguinal area and the labium majora, which appeared grossly distorted (Figure 1a). The swelling was irreducible, soft and non-tender, suggesting a voluminous varicose plexus. Speculum pelvic examination confirmed the presence of large, tortuous varices surfacing the right vaginal wall and the uterine cervix. Two-dimensional and three-dimensional US showed an extensive vascular lesion involving the whole right paracervical area with numerous vascular channels largely replacing the right vaginal wall and flowing into a large cavernoma of the right labium maiora (Figures 1b and 2a). Power Doppler examination in the upright position revealed slow blood flow in the affected areas (Figure 2b). A loud reflux during Valsalva's maneuver was evidence of pelvic venous reflux. Except for moderately increased amniotic fluid, all other biometric and anatomic fetal parameters were unremarkable, as was the uteroplacental Doppler velocimetry.

Also pertinent blood tests, including coagulation parameters (prothrombin time, partial thromboplastin time, fibrinogen and anti-thrombin III) were in normal ranges.

Premature contractions arising at 29 weeks of gestation were successfully treated with bed rest and oral tocolytic agents (ritodrine hydrochloride) according to ACOG guidelines [4].

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Figure 1. — A) Voluminous varicose plexus involving the right inguinal area and the labium majora B) Transvaginal ultrasound showing severe varicosities of the subcutaneous vessels.

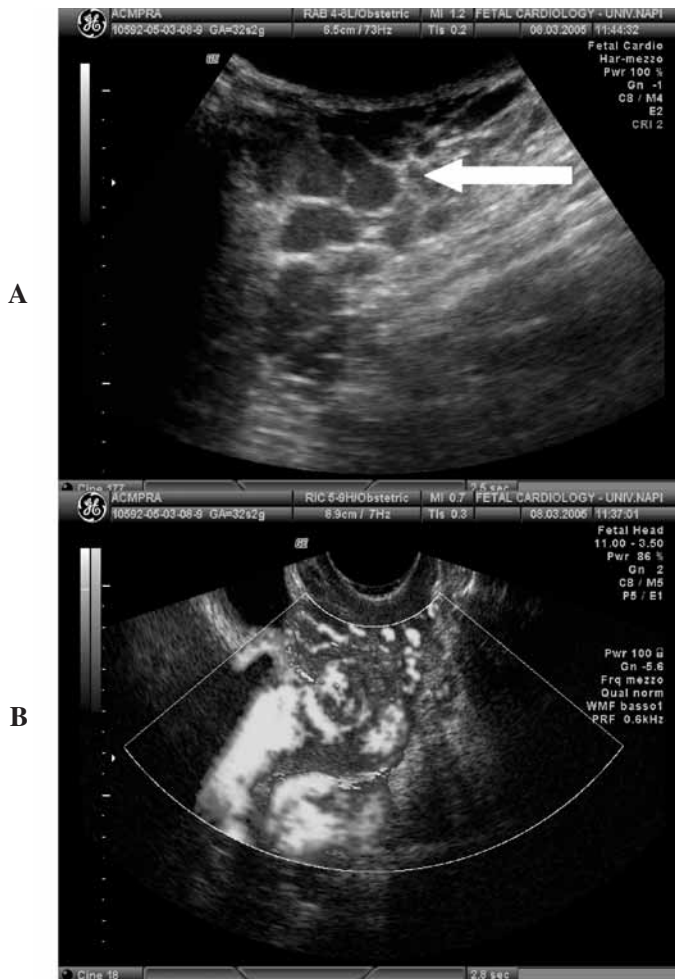


Figure 2. — A) Transvaginal ultrasound showing the microcystic structure (arrow) of the right paravaginal ectatic venous plexus. B) Transvaginal power Doppler examination confirming diffuse dilatation of the right paravaginal venous plexus.

At 30 weeks of gestation, the patient started complaining of headaches, dizziness and fainting. Taking into account the hypothesis of an emergency abdominal delivery, the treatment with low-dose aspirin was replaced with subcutaneous administration of low molecular weight heparin (4000 IU daily, Clexane; Aventis Pharma, Milan).

Since the lipotimic episodes became progressively more frequent and intense and the Bishop score was 0, the patient was scheduled for elective cesarean section at 37 weeks of gestation. An accurate informed consent about the increased risk of hemorrhage and hysterectomy associated with her varicose disease was obtained from the patient. Packed red cells and plasma units were made available before the surgery and compressive stockings were provided to the patient.

Cesarean section was performed under general anesthesia and through a transversal supra-pubic incision. At laparotomy, the inspection of the pelvis revealed extensive varicosities covering the right anterior surface of the uterus and involving the paracervical and iliac homolateral areas. A transverse asymmetric hysterotomy incision was performed, carefully avoiding the numerous, tortuous varices and the baby was delivered. A few seconds after extraction of the neonate and the patient's adnexa, the huge pelvic varicosities regressed almost completely. The final stage of uterine and wall sutures was uncomplicated. The healthy female infant weighed 3,650 g and had an Apgar score of 8 and 9 at 1 and 5 min, respectively. Estimated blood loss during the operation was 720 ml. Uterotonic agents administered intraoperatively contributed to an effective uterine contracture. Within one hour after delivery, also the conspicuous vulvar varicosities regressed almost completely. The patient was discharged in stable conditions five days later. Her puerperium was uneventful. She came back for a follow-up visit three months later. Clinical and US examinations showed a nearly complete regression of the vulvovaginal varicose lesions.

Conclusion

The anatomy of the pelvic venous system is extremely complex. Moreover a high inter-individual variability in venous valve number and distribution, as well as in vein trunk compliance, course, duplications and interconnections has been described.

Published studies on the epidemiology and natural history of pregnancy-induced pelvic and lower extremity venous disorders show a prevalence ranging from 10% to 20% of all pregnancies.

This is likely to be due to a number of hemodynamic and anatomic changes occurring during pregnancy which might contribute to the development of venous distention and varicose veins.

First, during pregnancy there is a significant increase in blood volume, which is primarily due to a plasma volume expansion. Second, low uteroplacental resistances act as true arteriovenous fistulas causing venous dilation and tortuosity. Third, the compression of the iliac veins (especially the left common iliac veins) and the inferior vena cava by the pregnant uterus produces hypertension and dilation of the proximal pelvic veins. Fourth, the hypercoagulability state characteristic of pregnancy predisposes to venous thrombosis and secondary superficial venous insufficiency. Finally, it has been speculated that elevated sex steroid concentrations induce venous dilation and blood vessel wall weakening.

Because of the resulting pressure and compliance excesses, pregnancy may induce the development of varices in the genital veins which usually regress almost completely after delivery.

In selected cases, pregnancy-induced venous hypertension results in superficial vein incontinence which, in turn can be a source of reflux and varices involving the superficial venous network of the perineum, vulva and lower extremities.

Likewise, after childbirth, varices may or may not regress: the time required for regression as well as the degree of regression may vary depending on one self's capacity to recover competency.

The most common varices identified during pregnancy are those in the legs, vulva and hemorrhoidal plexus [2]. Uterine [5], cervical [6, 7], vaginal [8] and round ligament varices have rarely been described. A MEDLINE literature review using the terms *cavernous hemangioma*, *inguinal cavernoma*, *vulvar varices*, *pelvic congestion syndrome* and *pregnancy* as key words failed to identify any other reported cases of such a pregnancy-induced severe pelvic and extra-pelvic varicose disease.

To our knowledge, this is the first report in which pregnancy-induced varicose disease involved contemporarily uterine, vaginal, inguinal and vulvar veins totally sparing the lower extremities.

In the present case, the clinical and US characterization of the venous disorder showed the involvement of both the gonadal plexus (bilateral parametrial and cervical varices) and the tributaries of the right hypogastric vein (uterine, ipsilateral vaginal and vulvar varices). Such findings are consistent with evidence in literature suggesting the association of vascular gonadal insufficiency with incompetence of the hypogastric vein tributaries to produce severe symptoms ("pelvic dumping syndrome"). However, as the lower extremities were totally spared in our patient it can be hypothesized that gonadal venous compromise was only partial.

Pregnancy-induced varicose disease is associated with considerable maternal and fetal risks, as shown by the life-threatening obstetric complications reported in some published cases [8]. Little is known about diagnosis and management of such condition during pregnancy. In the cases reported in the English literature, the diagnosis in pregnant patients was made by clinical examination and confirmed by US, magnetic resonance imaging (MRI) or computed tomography (CT) scan imaging.

The reported case is the first one in which the diagnosis was made by 2D and 3D US, which is a very accurate, non-invasive technique, thus extremely suitable for pregnant patients.

We decided on expectant management of the gestation, under close US monitoring of both the superficial and inner varicosities, careful surveillance of the maternal and fetal condition and prophylaxis of thrombotic complications through medical therapy.

Despite the considerable risk of hemorrhage, amniotic fluid embolism and consumptive coagulopathy associated with surgical delivery in case of varicose syndrome, in consideration of both the patient's worsening symptomatology and the unfavorable obstetric conditions we decided on an elective abdominal delivery.

Pregnancy-induced uterine, vaginal, inguinal and vulvar hemangiomas is associated with considerable maternal and fetal obstetric complications including increased hemorrhagic risks [6, 8]. Thus a close surveillance of affected patients is needed. Vaginal delivery is to be preferred, but if cesarean section is required, given the increased risk of bleeding complications, the surgery should be performed under general anesthesia [9] and packed red cells and plasma units should always be available.

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Serous cystadenoma with massive ovarian edema. A case report and review of the literature.

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Summary

Background: Massive ovarian edema is an usual tumour-like condition. It may be confused with an ovarian neoplasm. **Case:** A 13-year-old female in premenarche was referred as emergency case to a local hospital due to acute, severe and persistent hypogastric pain. She had noticed a gradual abdominal enlargement, mainly on the right abdomen during the last months. Ultrasound revealed a mass of a non-echogenic cystic compartment of 13 cm maximum diameter, and an area of mixed echogenicity of 11 cm maximum diameter at the anatomic site of the right ovary. The CA-125 levels were increased. An unilateral salpingo-oophorectomy was performed. The pathology examination revealed serous cystadenoma with massive ovarian edema. **Conclusions:** Conservative treatment and ovarian suspension may be more appropriate, when histology on frozen section suggests a benign lesion.

Key words: Massive ovarian edema; Serous cystadenoma; Ovarian neoplasm.

Introduction

Massive ovarian edema (MOE) is a rare, benign entity. It affects one or, rarely, both ovaries and is characterised by accumulation of edema fluid in the stroma. Although the exact mechanism of this condition is not known, at least two well-justified hypotheses have been set forth. It is supposed that MOE is usually a result of partial torsion due to a predisposing factor causing the underlying pathology. Few cases of ovarian lesions, benign or malignant, associated with MOE have been reported. In this article, we present the case of MOE with serous cystadenoma in a 13-year-old female, along with the pathologic, radiologic and laboratory findings and a review of the literature.

Case Report

The patient was a 13-year-old premenarcheal female. She was admitted to the local hospital due to acute, severe and persistent hypogastric pain. She had noticed a gradual asymmetric abdominal enlargement for a few months and described it as a firm and well outlined mass. She had no other symptoms up to that time. Her past medical and surgical history was unremarkable. There was no significant family history, except for a trait of thalassemia. Physical examination revealed a large, firm, abdominal mass tender on palpation, extending from the pubis to 2-3 cm above the umbilicus. The patient's abdomen was peripherally soft and painless in palpation. There was no rebound sign or guarding on the right lower hypogastrium. The liver and spleen were not palpable. Her secondary sex characteristics were well developed. Clinical diagnosis was that of a large adnexal mass. Abdominal ultrasound (US) revealed pelvic masses, probably

arising from both ovaries. The patient was referred to "Attikon" University Hospital for further evaluation and treatment, while her symptoms were in remission.

The following examinations were performed:

Complete blood count and blood analysis, blood coagulation tests, serum electrolytes, urine analysis and liver, thyroid and kidney function tests, electrocardiogram and chest X-ray. As the possibility of malignant neoplasia could not be excluded, tumor markers and hormone examinations were performed, according to the protocols of our department. Transabdominal sonography and magnetic resonance imaging (MRI) of the upper and lower abdomen were performed to clarify the texture, structure and location of the masses.

The blood count, biochemical examinations and thyroid hormones were within normal values. Sex hormone profile and tumour markers were also within normal values except for a slightly raised CA-125. Chest X-ray was normal. Extrarenal pelvis was diagnosed by US in the left kidney.

US of the lower abdomen revealed a mass with a cystic area 13 cm in diameter and a mixed area of 11 cm in the maximum diameter at the anatomic site of the right ovary. This particular mass dislocated the uterus to the left. The uterus appeared normal in shape and size.

MRI examination revealed a mass 13 x 9 x 10.5 cm in size, located in the pouch of Douglas, arising from the right ovary. It displaced the uterus to the front-right and the rectum backwards. The mass had a thin wall, intermediate signal intensity on T1-weighted MRI and slightly high signal intensity on T2-weighted MRI, with many internal diaphragms, without amplification. It was presumed that it was full of protein and possibly mucous content. Near the lesion, the space towards the right hypochondrium was occupied by a second cystic mass, 13 x 9 x 10.5 cm in size, pressing the vena cava. This lesion was full of aqueous content. The uterus appeared normal in shape and size. Multiple ovarian follicles were noted at the cortex of both ovaries. The left ovary was enlarged (almost 5 cm in maximum diameter) compared to normal size, according to the patient's age. There was a small quantity of free fluid in the pouch of Douglas. No

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Fig. 1



Fig. 3

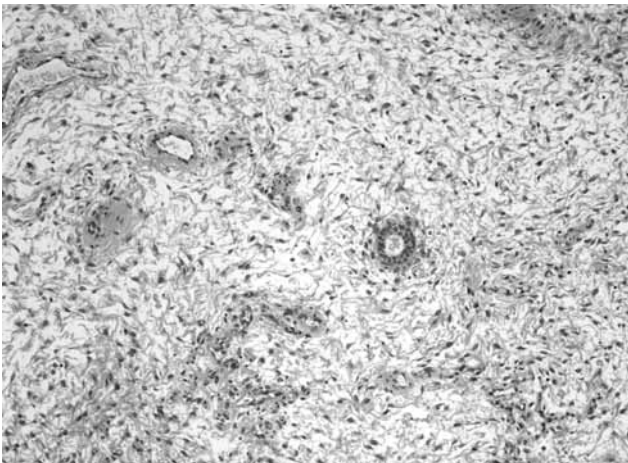


Fig. 2



Figure 1. — Serous cystadenoma with massive ovarian edema.
 Figure 2. — Gross sections of the tumor.
 Figure 3. — Stromal edema separating the vessels. A follicle can be seen at the centre (H&E x10).

nodal infiltration was noted. The abdominal organs were normal.

Exploratory laparotomy was decided on. The incision was middle vertical hypo-hyper umbilical. After entering the peritoneal cavity and before any further procedure, 60 cc of peritoneal fluid were aspirated and the cavity was washed with 120 cc of serum which was also aspirated for cytological examination. A large two-lobed mass occupying the pelvis, resembling an ovarian tumour in torsion was found. The cystic and solid lobes were well outlined (Figure 1).

Right salpingo-oophorectomy was performed. Frozen section histology was negative for malignancy and consistent with MOE. The left ovary was found enlarged (almost 5 cm) and was preserved.

The patient was re-examined three months postoperatively. By that time, menarche had been established. After the first menstrual cycle, she had no menses for two months and progesterone was prescribed. She responded well to this treatment. Six months later she had regular menses and was in good health.

Pathologic findings

A 15 cm large, encapsulated, partly sectioned tumour was received at the Department of Pathology. Upon further sections, it had a yellowish colour and a soft “humid” consistency with fluid oozing from the sectioned surfaces. It was connected to a 4.8 cm long fallopian tube and a 13.5 cm large unilocular cyst,

with smooth outer and inner surfaces containing clear serous fluid (Figure 2).

The frozen section report was that of MOE; the diagnosis was confirmed with tissue fixed in buffered formalin solution. The cyst was diagnosed as a serous cystadenoma (Figure 3).

Discussion

MOE is a rare but distinct clinicopathological entity. Serov *et al.* [1] defined it as “marked enlargement of one or both ovaries by an accumulation of edema fluid in the stroma, separating normal follicular structure”. The World Health Organization’s Histological Classification [1] defines this tumor-like condition as “an accumulation of edema fluid within the ovarian stroma separating normal follicular structures. In some cases the stroma contains lutein cells and the patient is virilised”. This entity was first noted by Gustafson *et al.* (2) but it was not characterised until Kalstone *et al.* [3] described it in 1969.

About 90 cases have been reported worldwide [4]. Usually MOE concerns adolescents and young women aged 5-33 years [5] with a mean age of 21 years. Similar cases have also been reported in menopausal and pregnant women [6, 7]. In almost half of the cases, there is

evidence during surgery indicating ovarian torsion [7]. Usually, 85% of the cases are unilateral, 75% affecting the right ovary and only 15% are bilateral [7]. The predisposition for the right ovary is supposed to be due to elevated right ovarian vein pressure, caused by the anatomic drainage from the right ovary directly to the inferior vena cava [8]. Alternatively it may be because of the sigmoid colon decreasing the left adnexal mobility [9].

MOE is typically present with acute abdominal pain when there is concomitant torsion and a pelvic mass. Cases of MOE also present as an incidental finding at laparotomy. Menstrual irregularity is a quite common sign, affecting post-pubertal women, but it subsides after treatment. Acute virilisation occurs in 25% of the cases [10]. Precocious puberty or early puberty and infertility have also been reported [11, 12]. It has also been associated with polycystic ovary syndrome (POS), ovarian fibrothecoma, twin pregnancy, ovarian capillary hemangioma, mucinous cystadenoma, leiomyomatous nodule, diffuse intraabdominal fibromatosis, Meig's Syndrome and metastatic carcinomas [13-16]. Our case is the second report of MOE associated with ovarian serous cystadenoma in a young patient [13].

The exact aetiology is still controversial. Two theories have been suggested to explain the pathogenesis of MOE. It is supposed that MOE is either a primary or a secondary result of partial torsion of the mesovarium, depending on the underlying pathology that led to torsion as a predisposing factor [17]. Torsion is the result of venous and lymphatic obstruction but not arterial occlusion, so there is no hemorrhage or infarction [10]. The resulting lymphedema leads to proliferation of the stromal cells and in some cases to conversion to lutein cells. This luteinisation and stromal hyperplasia result in an increase in ovarian androgen and estrogen production, causing virilisation and pubertal abnormalities [18]. The second hypothesis suggests that stromal proliferation or stromal hyperthecosis can occur, with resultant ovarian enlargement and subsequent torsion and edema [10]. This hypothesis is supported by the fact that there are cases of MOE in patients with a previously surgically fixed ovary [19]. There are also cases of relapse.

Most authors agree that the definitive diagnosis of MOE cannot be established preoperatively [15], because the diagnosis is usually intraoperative [14]. Recommended investigation includes the evaluation of the pituitary-ovarian axis, ovarian tumor markers, US and MRI of the ovaries [20, 21]. Sonographic findings indicating MOE can also be detected in cases of PCOS as multiple ovarian follicles are located at the peripheral cortex of an enlarged ovary [22]. Unlike typical fibroma and fibrothecoma, MRI may reveal a mass with unhomogeneous content and predominantly high signal intensity on T2-weighted MRI, indicating an abundant fluid component [23]. It is commonly accepted that frozen section can confirm the final diagnosis.

The differential diagnosis in cases of MOE is very significant, since benign or malignant ovarian tumours should be taken under consideration preoperatively. The most important entity to exclude is ovarian cancer. In pre-

pubertal girls, ovarian tumours account for approximately 1% of childhood malignancies. They are rare in early childhood with the median age for most ovarian tumours being greater than eight years [21, 24]. Metastatic disease of another malignancy may be a cause of MOE if the ovarian lymphatic vessel is obstructed by carcinoma cells [25, 26]. MOE must be distinguished from fibroma and stromal hyperplasia. Unlike the edematous fibroma, which displaces ovarian structures such as follicles, corpora lutea, and corpora albicantia, the edematous tissue in cases of MOE surrounds these structures [27].

Our patient was a 13-year-old premenarcheal girl, who underwent right salpingo-oophorectomy due to MOE. The cystic compartment had the appearance of serous cystadenoma when examined at the Pathology Department. The patient presented with established menarche three months after surgery. After the first menstrual cycle she missed the two next cycles and was treated with progesterone. Six months later, on regular follow-up, she reported regular menses and was in good health.

The most conservative management should always be considered in younger patients. Ovarian suspension may be more appropriate, when histology on frozen section suggests a benign lesion. Our patient had no possibility of preserving the affected ovary since this large tumour co-existed with serous cystadenoma.

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Acute urinary retention due to a uterine fibroid in a non-pregnant woman

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Summary

Background: Uterine fibroids represent a rare cause of acute urinary retention (AUR) and most cases have been reported in pregnant women. **Case:** We report the case of a non-pregnant woman who presented with AUR due to a uterine fibroid. **Conclusion:** When evaluating patients who present with severe oliguria or anuria, it is important to rule out urinary tract obstruction. Early identification of reversible causes of acute oliguria and institution of appropriate therapy are crucial to prevent the development of protracted acute renal failure. Close collaboration between gynaecologists, urologists and radiological services is required to promptly diagnose and treat uterine fibroid-associated AUR, a rare but reversible cause of acute renal failure.

Key words: Uterine fibroids; Acute urinary retention; Acute renal failure; Oliguria; Anuria.

Introduction

Acute urinary retention (AUR) is a urological emergency characterized by a sudden and painful inability to pass urine [1]. It is estimated that 10% of men in their seventies and a third in their eighties will present with this condition within the next five years [2]. AUR is 13 times less frequent in women, occurring in approximately seven women per 100,000 per year [3]. There are several potential etiologies for this condition, classified as anatomic, neurologic, pharmacologic, psychogenic, functional and myopathic [4]. Large uterine fibroids represent a rare cause of AUR and most cases have been reported in pregnant women [5-7]. We report the case of a non-pregnant woman who presented with AUR due to a uterine fibroid.

Case Report

A 47-year-old woman (gravida 1, para 1) came to the emergency department of our hospital because of worsening dyspnoea, progressive abdominal distention and bilateral swelling of her legs. In addition, she reported that during the last two weeks she was able to pass urine only in the standing position. She was in the perimenopausal period. Her medical history was unremarkable and she had not been taking any medication. The patient was afebrile, her heart rate was 100 beats/min and blood pressure was 125/80 mm Hg. On physical examination, the patient had a distended bladder and bilateral lower-extremity edema (2+). On gynaecological examination, the uterus was increased in size. On admission, the laboratory values included: white blood cell count $9.5 \times 10^9/l$ (with neutrophils 78.5% and lymphocytes 13.0%), hemoglobin 10.8 mg/dl, hematocrit 31.8%, MCV 79.6 fl, MCH 26.9 pg, urea 37 mg/dl and creatinine 1.4 mg/dl. Renal ultrasonography (US) revealed dilatation of both ureters and the pelvis and calices of both kidneys; the

size of both kidneys was normal. Transvaginal US demonstrated an enlarged uterus (measuring 202 x 168 x 128 mm) and multiple uterine fibroids. The biggest fibroid was located on the posterior lower segment of the uterus, measured 103 x 98 x 87 mm and caused anteflexion of the uterine cervix, which in turn compressed the bladder neck. A computed tomography (CT) scan was performed and disclosed an enlarged fibroid uterus and an overextended urinary bladder rising up to the kidneys, and bilateral dilatation of the urinary tract up to the renal calices (Figure 1). Bladder catheterization was performed and yielded 6,900 ml of urine. The next day, a drop in the creatinine level to 0.9 mg/dl was noted. The patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. The histopathological diagnosis was uterine fibroid with no evidence of malignancy. The postoperative course was uneventful.

Discussion

Uterine fibroids (leiomyomas or myomas) are benign tumours arising from uterine smooth muscle cells [8]. They are the most common gynecological tumours during the reproductive age and are clinically apparent in about 25% of women [8]. Moreover, their prevalence in surgical specimens might be as high as 77% [8]. Most myomas are asymptomatic, but many women have significant symptoms warranting therapy, mainly uterine bleeding, pelvic pressure and reproductive dysfunction [8]. Large uterine fibroids may also induce AUR, but this is a rare occurrence [5-7]. AUR due to these estrogen-dependent tumours occurs primarily during pregnancy, due to the enlargement of both uterus and fibroid [5-7]. However, fibroids can cause AUR even in non-pregnant women, as illustrated in our case.

When evaluating patients who present with severe oliguria or anuria, it is important to rule out urinary tract obstruction. Early identification of reversible causes of acute oliguria and institution of appropriate therapy are crucial in order to prevent the development of protracted acute renal failure [9]. The latter is associated with high

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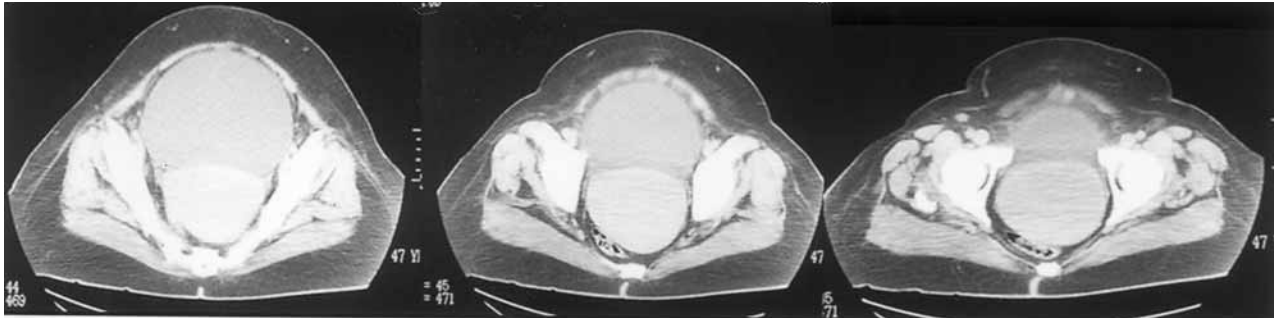


Figure 1. — Computed tomography scan showing overextension of the urinary bladder and an enlarged fibroid uterus compressing the bladder neck and rectum.

morbidity and mortality [9]. In addition, the potential for recovery of renal function is inversely related to the duration of obstruction [10].

Urinary tract obstruction should be first sought by history and physical examination [10]. Bladder catheterization can rule out urethral obstruction [10]. Renal US is the study of choice to detect obstructive uropathy since it is sensitive, non-invasive, does not expose the patient to radiation and is relatively simple and fast to perform [10]. Obstructive uropathy is usually manifested by dilatation of the urinary tract above the obstruction [10]. However, a non-dilated collecting system does not necessarily exclude the possibility of obstruction, especially in cases of recent obstruction (< 24 hours) or in patients with hypovolemia [10]. Encasement of the ureters by malignant tissue or retroperitoneal fibrosis may also result in failure of the urinary tract to dilate even when obstructed [10]. When US does not reveal urinary tract dilatation but there is a high index of clinical suspicion for obstruction, CT, cystoscopy with retrograde ureteropyelography or antegrade pyelography may be used to confirm the diagnosis [10]. Transvaginal US may also provide useful information if a gynaecological condition is suspected as the cause of the obstruction [5].

The treatment of urinary tract obstruction depends on the underlying cause [10]. Bladder outflow obstruction can be relieved by passage of a urethral catheter but relief of upper tract obstruction may require either antegrade (percutaneous nephrostomy) or retrograde (cystoscopy and retrograde ureteral catheterisation) approaches [10]. If a fibroid uterus is strongly suspected as the etiology of her urinary retention, hysterectomy is an appropriate management option [5].

In conclusion, uterine fibroids represent a rare cause of AUR. Close collaboration between gynaecologists, urologists and radiological services is required to promptly diagnose and treat this reversible cause of AUR.

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Book Review

RECOMBINANT HUMAN ERYTHROPOIETIN(rhEPO) IN CLINICAL ONCOLOGY. Scientific and Clinical Aspects of Anemia in Cancer

*11th revised and enlarged edition Springer-Verlag (Vienna) 2008,
886 pages, M.R. Nowrousian (editor).*

Since the introduction of rhEPO in clinical oncology in 1993, considerable insight has been gained into the pathogenesis, prevalence, and incidence of anemia in cancer and its impact on cancer patients' quality of life, course of the disease, effect on treatment modality (chemotherapy, radiotherapy).

Anemia is not only a major factor in patient well-being but also a key factor in the development of tumor resistance against chemo and radiation treatments.

It is well known that anemia and consequent hypoxia contribute to the selection of a more malignant phenotype of tumors cells.

Acute cyclic hypoxia has been shown to be even more deleterious than chronic hypoxia in selecting aggressive, apoptosis-resistant tumors cell.

The volume edited by M.R. Nowrousian aims to be a comprehensive source of information on the clinical and scientific aspects of anemia in cancer and its treatment with erythropoiesis stimulating agents (ESAs).

Outstanding authors, all experts on their topics, have contributed with comprehensive chapters on the present state of knowledge in their fields.

The first part of the book explores the pathophysiology of anemia in cancer cells; the prevalence, incidence of anemia, and its risk factors in cancer patients.

The second part of the book examines the relationship between hemoglobin levels and tumor oxygenation, its impact in therapeutic resistance and relationship with fatigue and QoL in cancer patients.

The third part updates recent scientific knowledge on the indications, advantages and limits of the use of human erythropoietin therapy in specific clinical conditions.

This selection includes chapters on EPO during chemotherapy and radiotherapy in hematopoietic stem cell transplantation, in pediatric oncology, and in surgical oncology.

The last part covers debatable questions such as: the effect of EPO in QoL and survival, thrombosis during therapy, and congestive heart failure. Particular emphasis is dedicated to the last chapter concerning the cost-effectiveness of treating cancer anemia with EPO.

The volume should be considered the book of reference in anemia and cancer and will serve as an essential source of information for any specialists involved in the management of cancer patients.

M. Marchetti