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Antisperm antibodies and human reproduction

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Summary

Purpose: To present strategies in diagnosing and treating infertility related to antisperm antibodies. *Methods:* Antisperm antibodies (ASA) were detected on sperm using the direct immunobead (IBD) test. Treatments included intrauterine insemination (IUI) with pretreatment with chymotrypsin/galactose vs in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI). *Results:* Intrauterine insemination with protein digestive enzyme treatment was much more effective than IUI without enzymatic therapy. However IVF with ICSI provided three times the pregnancy rate for males with sperm coated with ASA than IUI with chymotrypsin treated sperm. *Conclusions:* It is advisable to include measurement for ASA on the initial semen analysis. However, another option is to perform it initially only with an abnormal post-coital test. The decision for IUI with chymotrypsin pretreatment of the sperm vs IVF with ICSI may depend on insurance and financial issues.

Key words: Antisperm antibodies; Post-coital tests; Intracytoplasmic sperm injection; Protein digestive enzyme; Intrauterine insemination.

Variable effect of antisperm antibodies on fertility

There is no question that in some instances the presence of antisperm antibodies (ASA) coating the sperm can cause infertility such that intercourse, intrauterine insemination (IUI) with or without treatments of sperm aimed at neutralizing or eluting the ASA or even conventional in vitro fertilization (IVF) (where approximately 50,000 sperm are incubated with each egg) are associated with a very low chance of fertilization [1-8]. Sometimes the only way that fertilization can occur is through IVF with intracytoplasmic sperm injection (ICSI) [9-12].

However it is also known that ASA is present in 1-2.5% of fertile males [13, 14] and possibly in 4% of fertile females [15]. There are many reasons why in some people the presence of ASA requires IVF with ICSI to allow fertilization of eggs whereas in some patients there is no adverse effect and pregnancies occur after natural intercourse. To better understand why some antibodies, but not all, inhibit the achievement of pregnancy it is first important to understand how ASA impairs fertilization.

ASA may inhibit sperm from progressing through the cervical mucus, thus preventing them from reaching the oocyte [16-20]. We evaluated post-coital tests in women whose male partners had > 50% of their sperm coated by ASA [18]. Only 31% (4/13) of males with ≥ 50% ASA by the direct immunobead test demonstrated sperm with progressive linear motion following post-coital testing. All four of these males' wives achieved a pregnancy within six months with just intercourse.

The reason why some males can have > 50% ASA coating their sperm and yet have normal post-coital tests could be related to various factors.

1) Though at least 50% of the sperm may have some antibody attached the immunobead does not detect the concentration of ASA per sperm. Thus possibly the antibody load is not sufficient to cause immobilization of the sperm when coming in contact with the complement in the cervical mucus (ASA typically does not immobilize the sperm when testing the semen analysis because of the absence of complement in the normal ejaculate) [20].

2) The antibodies are not those that lead to immobilization in the mucus. One should be careful though of making the assumption that if ASA does not lead to poor post-coital tests that they do not cause infertility. There are various sperm antigens to which antibodies can be made [21]. There are data showing that ASA may contribute to infertility by disrupting sperm-oocyte recognition and fusion [22, 23], inhibiting sperm from undergoing capacitation [24], inhibiting the acrosome reaction [24, 25], or inhibiting the binding of sperm to the zona pellucida [26-28], and possibly ASA may be directed to specific sperm cell membrane antigens that are essential for oocyte division and thus can inhibit the ability of a fertilized oocyte to cleave to an embryo [29].

3) There is a possibility that the antibodies can be directed against antigens that are not involved in sperm motility or in the fertilization process but are merely inert. This could explain how the four women with normal post-coital tests achieved pregnancies with intercourse [18].

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In the aforementioned study, 44 of 59 women who had intercourse 8-16 hours previously demonstrated some sperm with progressive forward motion in cervical mucus obtained at the time of a mature follicle and before the luteinizing hormone (LH) surge [18]. Only 9% (4/44) of women with adequate post-coital tests had male partners with sperm with ≥ 50% ASA. In contrast in 15 women not demonstrating sperm with progressive forward motion nine (60%) had male partners with ASA > 50% [18].

Over six months of therapy, IUI achieved a pregnancy in five of nine (56%) positive for ASA vs five of six (83%) of those negative for ASA [18]. These findings could suggest that when ASA are present and cause immobilization of sperm in the mucus there may be ASA also present that are directed against antigens that are needed for the process of fertilization. Merely bypassing the mucus by doing an IUI is therefore not sufficient to allow pregnancy to ensue.

Testing for antisperm antibodies

Understanding the type of limitation of immune testing and the source of material helps in determining strategies in diagnosing the possible association of ASA and infertility. Though many types of assays have been developed the two most common assays used are the immunobead (IBD) assay and the mixed antiglobulin reaction (MAR) test [30-34].

The IBD assay is composed of polyacrilimide beads that are coated with a specific anti-immunoglobulin. The coated beads are then mixed with fresh, viable washed or unwashed sperm samples and ultimately bind to sperm-bound ASA. Using the light microsome one can detect the percentage of sperm that have beads attached so that the percentage of sperm coated with ASA can be determined. Furthermore by using antibodies specific for head, tail, and tail tip the location of the antibodies can be detected. Also by using IgA, IgG and IgM antibodies the immunoglobulin class that is involved can be detected.

The MAR assay is similar to IBD testing. Blood group O – Rh-positive ethyrthrocytes are coated with human IgG or IgA and subsequently mixed with washed or unwashed viable sperm. Antibodies specific to the immunoglobulin used to coat the erythrocytes is added and sperm agglutination occurs in the presence of ASA.

Both tests detect what percentage of sperm are coated with ASA but neither detect the concentration of ASA per sperm. There is probably a correlation such that the higher the percentage of sperm coated with ASA the higher the concentration of ASA per sperm. However, there may be some instances when ASA is directed to a key sperm antigen needed for mobilization or fertilization and a high percentage of sperm are positive and yet the concentration of the ASA per sperm is not sufficient to affect fertility. Some of the variability in conclusions as to the significance of ASA as a cause of infertility may be related to what percentage of sperm coated with ASA is considered a positive test. Some studies have considered a positive IBT test as > 20%, some > 50% since some normal fertile sperm donors have ≤ 50% ASA, and some consider ≥ 80%. Some studies have evaluated 100% of sperm showing ASA.

The effect of ASA isotype and location of the binding of ASA in achieving pregnancies

Some studies have suggested that sperm coated with IgG reduces fertilization rates more than sperm coated with IgA or IgM [34]. Another study found that only IgG in the sperm reduced fertilization rates with IVF whereas only IgM in female sera reduced fertilization rates [35]. IgA in the sera was associated with lower pregnancy rates possibly by impairing progression of sperm through the cervical mucus [35]. Some studies have concluded that the combination of IgG and IgA ASA have a synergistic negative effect on oocyte fertilization [36, 37]. It should be noted that not all studies agree that ASA of any particular isotype reduces fertilization rates [38, 39].

There are several studies dealing with the location of the antibody isotype [40-42]. One study found a significant reduction in fertilization when IgA was present on the sperm head [40]. Another study using sperm complement mediated immobilization tests found that a high degree of immobilization was found only when IgG ASA was bound to the distal 2/5 of the principal piece of the tail [42].

Nevertheless there are other studies that fail to show any connection between the location of IgG or IgA ASA [43-45]. Thus it is hard to make "head or tails" of the significance of ASA isotype and location and these extra measurements markedly increase the cost of performing the IBD or MAR assay. Since there are no clear cut data as to modifying treatment protocols based on these parameters, I have eliminated these extra parameters and I am content to merely measure IgG and IgA without location and I do not measure IgM. Perhaps measuring IgG alone may be sufficient.

Philosophy of evaluation and treatment

Though a positive ASA test cannot with certainty diagnose an infertile male, I believe the information provided even with some limitations can be very helpful in management of the infertility and can save the couple time and money in the long run. Thus I believe the slightly extra cost is justified.

Though the presence of ASA is not always an etiologic factor in infertility, a higher percentage of sperm bound with ASA is more likely than not to be a contributing factor either by impeding sperm to progress through the cervical mucus or by preventing fertilization of the oocyte.

One theoretical way to avoid the immobilization of sperm coated with ASA is to avoid the cervical mucus by performing an IUI. Since the sperm coated with ASA is immobilized by the complement in cervical mucus, by washing the sperm and placing it directly into the uterine cavity this deficit could theoretically be overcome. However though the ASA that immobilizes the sperm in mucus (as determined by a properly timed post-coital test) may be obviated by this procedure, pregnancy rates are not very high - especially when there is a high percentage of sperm coated with antibody [46, 47].

A study was performed involving 16 couples where all infertility factors were corrected except a poor properly timed post-coital test (no sperm with progressive linear motion) despite what appeared to be appropriate quality cervical mucus [46]. Furthermore the male partner was found by IBD testing to have > 50% of sperm with ASA. Intrauterine insemination was performed with all the males ejaculating into 5 ml of equal parts of modified human tubal fluid buffered with HEPES solution and 7.5% bovine serum albumin in an attempt to dilute the sperm to theoretically negate the attachment of antibodies at the time of ejaculation. Another group ejaculated into media with the protein digestive enzyme chymotrypsin with galactose in order to cleave part of the immunoglobulin molecule to neutralize function prior to sperm washing [46-51]. The 16 couples were randomly assigned one of the two sperm preparations and if no pregnancy was achieved, the other preparation was used for the second cycle of treatment [46]. With each failure they were switched to the other protocol for the next treatment [46]. There were 65 treatment cycles – 32 with chymotrypsin/galactose and 33 with albumin. Pregnancies were achieved in eight of 32 (25%) cycles following IUI with chymotrypsin galactose vs only one of 33 (3%) performed with sperm ejaculated into albumin fortified media [46]. When 100% of sperm was found to be coated by ASA Francavilla *et al*. found no live pregnancies following 119 IUI cycles [47]. In contrast, a pregnancy rate of 25% per cycle was found following IUI with chymotrypsin-treated sperm including couples whose male partner had 100% of the sperm coated with ASA [46].

These data suggest that when immobilizing antisperm antibodies are present merely bypassing the source of complement, i.e., the cervical mucus, is not very effective in correcting the infertility [46, 47]. The data also suggest that diluting the effects of sperm attaching at the time of ejaculation is not very effective either [48, 49]. The fact that merely bypassing the complement-laden cervical mucus by an IUI did not overcome the problem of ASA coating the sperm strongly suggests that when sperm immobilizing auto antibodies are present they probably co-exist with ASA that also inhibit fertilization. Thus improved efficacy would be gained by either neutralizing the ASA by enzymatic cleavage [46, 50] or possibly eluting the ASA from the sperm, e.g., with fertilization antigen-1 [51]. Possibly this treatment renders less toxic the ASA that are inhibiting oocyte fertilization.

Since the presence of ASA on sperm does not generally have an adverse effect on motility when performing the semen analysis [52] and since a normal post-coital test does not preclude the presence of ASA that can inhibit oocyte fertilization, I recommend that ASA be measured by one of these two simple inexpensive sperm tests (direct IBT or MARS) rather than allowing expensive inappropriate treatments to be rendered.

In 1990 a meta-analysis was published stating that performing a post-coital test was not cost-effective [53]. Though the suggestion to abandon the test was criticized by several clinicians including this author, since that meta-analysis was published many infertility specialists do not perform this simple inexpensive test [54]. As previously mentioned, it is our policy to perform screening for ASA on the male partner's first semen analysis. To cut costs we merely eliminate IgM and eliminate localization of ASA. I could understand an argument that only a small percentage of males will have ASA that merely effect fertilization of the oocyte but do not inhibit mobilization in cervical mucus. So why not perform only ASA in males with apparently normal semen parameters but who fail to demonstrate sperm with progressive movement in cervical mucus of apparent good quality collected at the proper time. Thus measurement of ASA could be reserved for those failing to conceive despite what seems to be an adequate number of treatment cycles for that woman's age. However, a poor post-coital test should immediately prompt the measurement of ASA by a test, e.g., the direct IBT on the sperm and if negative, then the cervical mucus should be evaluated for ASA by the indirect IBT.

Some fertility specialists argue that they perform IUI every cycle to improve the odds of conception. Thus if a poor post-coital test did exist, the IUI would "correct" the problem. It is true that there are some circumstances, e.g., poor quality cervical mucus (especially following clomiphene citrate therapy) where IUI would correct the problem [55-57]. However, Griffith and Grimes argue that $50,000,000 is wasted yearly on a one-time post-coital test. Imagine the amount of money wasted on far more expensive IUI procedures performed monthly for many cycles! There are no clear-cut data to suggest that IUI improves pregnancy rates in women with normal post-coital tests to justify the immense extra expense and time lost from work [58]. What would be even more inexcusable would be to undergo the expenditure of far less effective "plain" IUI if ASA were present when some type of sperm treatment, e.g., chymotrypsin galactose, should precede the IUI. Thus if a couple-physician still choose to empirically try clomiphene citrate therapy/IUI despite normal ovulation or use clomiphene because of not attaining a mature follicle, if the post-coital is poor it certainly could be a side-effect of the drug. However, in this circumstance the sperm should still be checked for ASA to be sure a less efficacious IUI procedure are not performed.

One question that arises is when should a woman be checked for ASA and should the specimen that is tested be mucus or serum? Antisperm antibodies could be present in the serum but not secreted into the mucus. Except for lowering fertilization rates when used as a protein source in IVF media (which is not done much any more by IVF facil-

ities), antibodies exclusively in serum only should not negatively affect pregnancy rates. Bypassing the cervical mucus by performing an IUI (in this case no special sperm treatment is needed) should obviate the problem if ASA in cervical mucus is impeding sperm progression. Based on these assumptions there is little need other than curiosity to determine if ASA in mucus is the cause of an unexplained poor post-coital test. One study showed that only ASA in the cervical mucus as determined by the indirect IBD test (which is more expensive than the direct IBD because donor sperm is needed) occurred in only 7% of female partners [57]. Thus testing for ASA in cervical mucus is not nearly as important as testing sperm for ASA.

In vitro fertilization with intracytoplasmic sperm injection vs IUI for sperm coated with ASA

The first publication claiming an adverse effect of ASA bound to sperm on fertilization rates was published in 1986 [59]. However, subsequently there were some small studies finding no reduction in fertilization rates with IVF-ET using conventional oocyte insemination [60, 61]. Nevertheless, the majority of studies did find that ASA bound to sperm does reduce the fertilization rate [1-7].

In contrast to conventional oocyte insemination with sperm coated by ASA, most data show that ICSI allows normal fertilization rates [9-12].

At the Cooper Center for IVF when sperm coated with ASA seem to be at least partially responsible for the infertility of a given couple, the couples are given the choice of IUI with chymotrypsin/galactose or IVF with ICSI. A priori, the latter would probably be more successful since IVF is generally more successful per cycle than IUI but at a much greater cost.

Considering insurance coverage and personal income over the last ten years there have been slightly more IUI cycles (60.7%) than IVF with ICSI (in women ≤ age 42 whose male partners had > 80% ASA). The clinical pregnancy rate per cycle of IUI with chymotrypsin galactose was 13.1% (34/258) with a miscarriage rate of 15%. In contrast the clinical pregnancy rate per embryo transfer with IVF and ICSI was 40.7% (68/167) with a miscarriage rate of 19% (unpublished data).

Obviously IVF with ICSI will give a couple a three-fold increased chance of achieving a pregnancy compared to IUI. However, three IUI cycles are still a lot cheaper than one IVF-ET cycle. Obviously insurance coverage and finances will help guide a couples' decision. The decision for IUI vs IVF with ICSI would be made easier if it was known whether the ASA was only of the immobilization type, as evidenced by a poor post-coital test, and not one that would inhibit fertilization. Similarly if upon routine testing of a semen analysis ASA is detected despite a normal post-coital test, it would be very helpful to know if these ASA are those that can adversely effect oocyte fertilization or not. Research is presently ongoing with proteomics to try to identify specific immunogenic antigens that are important in the fertilization process [62].

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Editorial Articles

Recurrent aneuploidy - fact or fiction

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Summary

Purpose: To evaluate the likelihood that some women are more prone to forming chromosomally abnormal embryos unrelated to age. *Methods:* The literature involving studies suggesting that predisposition to aneuploidy does exist was reviewed. In addition a new anecdotal unpublished report on a tendency to form trisomies is presented and a couple of case reports dealing with the possibility of predisposition to polyploidy are discussed. *Results:* The results of in vitro fertilization and pre-implantation diagnoses confirm the suspicion that some women are more prone to trisomies or polyploidy. *Conclusions:* In vitro fertilization with pre-implantation genetic diagnosis may help in preventing miscarriage from recurrent polyploidy but is not so valuable for recurrent trisomies.

Key words: Trisomies; Recurrent pregnancy loss; Polyploidy; Pre-implantation genetic diagnosis.

Introduction

The risk of chromosome abnormalities as a cause of miscarriage is approximately 50% [1, 2]. Approximately 50% of cytogenetically abnormal spontaneous abortuses are found to have autosomal trisomies [1]. The most frequent autosomal trisomy involves chromosome 16, then 22, 21, 15, 13, and 14 in descending order of frequency. However, trisomy 16 is rarely, if ever, observed in liveborns [1]. Seventy percent of trisomies involve these six trisomies [1].

The three trisomies compatible with life involve chromosomes 13, 18, and 21 [1]. Fetuses with these three chromosomes grow faster than when lethal trisomies exist, even in those who abort in the first trimester suggesting that they either live longer or have less intrauterine growth retardation [3].

When there are more than two haploid chromosomal compliments present it is termed polyploidy. Triploidy (3n = 69) has three haploid complements and tetraploidy (n = 92) has four. Polyploidy is found in approximately 10% of abortuses. Trisomies are twice as likely to be present in abortuses than polyploidy [1].

Monosomy X is about as frequent as trisomies and 80% of the time it is the paternal x that is missing [1]. There has been a suggestion that recurrent aneuploidy occurs more often than expected by chance alone. Fifty-percent of abortuses are chromosomally normal. Thus only one of eight women with three consecutive miscarriages should show chromosomal abnormalities by chance alone. Several studies found that if aneuploidy existed in the first abortus the risk of aneuploidy, especially trisomy, was more likely in the second abortus than expected by chance alone [4-6].

Some studies adjusted for age have found a correlation with a higher prevalence of aneuploidy with prenatal genetic diagnosis (PGD) in women with progressively more miscarriages [7, 8]. The exception may be those with more than four losses [9]. Since not every embryo created has aneuploidy, if the etiology is increased risk of aneuploidy then the odds are that eventually a normal fetus will be formed. When there have been ≥ 4 previous losses the etiology will favor an abnormality that will be persistent each time [9, 10]. Another study of PGD found the prevalence of trisomy to be 50% higher in women with previous trisomies vs women having PGD performed for other reasons [11]. The same conclusions were reached by Munne *et al*. in women aged ≤ 35 but no differences were found in women > 35 [6].

Even if one concedes to the argument that some women may be more prone to produce embryos with trisomies, this may have little clinical significance. Even with in vitro fertilization given the frequency of trisomic embryos even in those with a history of one or two previous miscarriages with documented trisomies, the odds are that with two or three embryos transferred that there should be at least one normal embryo. Thus it does not seem warranted to add the extra expenditure of pre-genetic diagnosis of the embryo prior to transfer for the reason of a previous loss or two of a trisomic fetus. Since a trisomic embryo occurs in about one-fourth of the embryos created one may expect one in 16 women to experience by chance alone two miscarriages related to trisomies.

The more significant question is the role of aneuploidy as a cause of recurrent miscarriages of three or more. At first glance the data from Stephenson *et al*. would suggest that a predisposition to trisomies is not an important factor in women with recurrent miscarriage because 54% of the cytogenetic diagnosis of another abortus in a group of women with recurrent miscarriage were normal euploid [12]. However to reiterate a point made above, the more miscarriages

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in a row the more likely there could be causes that could repeat in all or most pregnancies, e.g., hormonal (especially progesterone deficiency), immunological, or structural uterine defects which would favor more euploid embryos. Perhaps the 54% euploidy rate would have been higher if it had not been somewhat negated by an increased predisposition to aneuploidal embryos. In fact as previously mentioned the expected distribution between trisomy and monosomy x would be equal. However the study by Stephenson *et al*. found that 67% of the embryos were trisomies vs only 9% with monosomic x [12]. The polyploidy rate of 9% of women was consistent with the rate in abortuses of an unselected population.

These data suggest to us that the purposeful use of in vitro fertilization-embryo transfer (IVF-ET) and PGD for most women with recurrent miscarriage is not warranted based on its expense and risk of ovarian hyperstimulation. If certain genetic factors make a 32-year-old woman as prone to aneuploidy as a 42-year-old, strategies to prevent another miscarriage would not change. The knowledge might merely influence the treating physician to more strongly advise antenatal testing.

The question still exists however as to whether there still may be some women who for some reason are prone to having trisomies in the large proportion of the embryos formed. If so, that knowledge could influence the couple to change gametes if the partner causing the problem is identified or to consider donor embryos if the source of the problem is not identified. Since such cases would be rare the establishment of the existence of such problems may have to depend on anecdotal experience.

The first author of this editorial had an oral presentation at the 2008 American Society for Reproductive Medicine meeting in San Francisco entitled "The effect of the 46XX dup [8] (p23 p23) and 46XY, inv [9] (p11 913) on recurrent pregnancy loss". These chromosomal structural rearrangements are generally considered to be benign karyotypic variants. The case described was a 35-year-old primary aborter with four consecutive first trimester miscarriages. Following the first two miscarriages (in which chromosome analysis of fetal products were not performed) both male and female partners had karotyping performed.

The female partner, who did not have any phenotypic abnormalities, was found to be 46XX dup [8] (p23 p23). This karytope would be consistent with either a true 8 duplication or a euchromatic variant, i.e., variation in the number of copies of the 8p 23.1 chromosomal segment. Since the former, i.e., true duplication is associated with an increased risk of developmental delay or cardiac defects and the latter is not associated with any phenotypic defects it becomes important to distinguish these entities. Blood was sent to John Barber at Wessex Regional Genetics Laboratory in the United Kingdom and the results of molecular genetic analysis indicated that it was just a euchromatic variant and should not have any clinical significance.

The male partner was found to have a pericentric inversion of chromosome 9 which is also considered as a benign variant. Despite the fact that the chromosome abnormalities in male and female partners were considered benign there was consideration given that these chromosomal variants could possibly lead to meiosis errors leading to aneuploidy. Thus the couple elected to have this procedure performed after discussing the benefits and deficits of IVF-ET with PGD. Fluorescent in situ hybridization (FISH) was used to evaluate chromosomes 13,15,16,17,18,21,22 X and Y. Only one of five embryos with blastomere biopsy and PGD was normal and it did not survive for a day 5 transfer. A second IVF-ET PGD cycle was attempted and this time none of the three embryos evaluated were normal. The chromosome abnormalities for these seven abnormal embryos included trisomies 13, 15, 16, 18 and monosomies for chromosomes 15, 16 and 18.

Though the possibility exists that the miscarriages in this couple were related to non-genetic reasons the much higher frequency of aneuploid following PGD suggests that some women may be markedly prone to meiosis errors. In this case the question is whether the normally "benign"chromosome variations may have been responsible for this apparent predisposition to aneuploidy. This case could possibly also suggest that the problem could be related to a normally benign karyotypic variant in the chromosomes of the male partner.

As mentioned earlier, for those who think there can be some women with a predisposition to aneuploidy and that this predisposition mainly is represented by triploidy as based on evaluation of a large series of PGD, there are some anecdotal reports also suggesting a predisposition to polyploidy. Two cases have been described supporting this concept [13, 14]. One report described a woman with two previous miscarriages with chromosome analysis of fetal products revealing triploidy [13]. Subsequent IVF-ET with intracytoplasmic sperm injection (ICSI) and PGD found two embryos out of 13 with triploidy [12]. In the other case a woman had a spontaneous miscarriage at age 24. Her second pregnancy ended in a first trimester miscarriage and it was triploidy. The third pregnancy she completed the first trimester but aborted in the second trimester and again triploidy was found [14]. In vitro fertilization with ICSI and PGD were performed and 13 embryos were biopsied and six were normal. There was one tetraploidy among the seven abnormal embryos [14].

Triploidy occurs in 2% of all conceptuses [15]. Maternal origin of triploidy occurs in only 10% of the cases [1]. Thus it is estimated that one in 100 oocytes (0.2%) has a failure of maternal meiosis resulting in triploidy [15]. Tetraploidy is even less common.

These two cases strongly suggest that some women may have a predisposition for meiosis errors leading to triploidy. In these two cases there were no chromosome abnormalities in the male or female partners [13, 14]. However as illus-

trated in case 2, if it is assumed that all the abnormal fetuses had lethal chromosome abnormalities so that only the six normal embryos and the polyploidy embryo could implant was it just a coincidence that the two documented abortuses were trisomy 21 with odds of one in 50 of this happening? Another possibility is that when it comes to polyploidy for some reason the oocyte with at least one extra set of chromosomes is more likely than even the chromosomally normal oocyte in becoming the dominant follicle.

For this second case IVF with ICSI and PGD resulted in a live normal delivery. The mere development of multiple embryos may have been sufficient even without the IVF and PGD if the theory of the egg with more than one set of chromosomes becoming the dominant egg is correct. The question is whether there is some property about the polyploidy embryo that allows its dominance for implantation compared to the other embryos. If so then merely controlled ovarian hyperstimulation on IVF-ET with transfer of three or four embryos may not be sufficient and PGD could be necessary.

On the other hand, the woman with the recurrent trisomies had two IVF-ET cycles without the transfer of a single normal embryo. Thus a huge financial burden was accrued without any benefit. Thus whereas a possible role for IVF-ET and PGD exists for recurrent polyploidy it does not seem appropriate for recurrent trisomies.

For the couple with recurrent triploidy they could keep trying naturally hoping for a lucky break. The next cheapest option would be to just try donor sperm hoping the male was responsible. Donor oocytes with fertilization of half the husband's sperm and half the donor's sperm with the transfer of the embryos fertilized by the husband first is the most expensive option, but giving hope to the couple of at least carrying some of their own genetic information. Finally for this couple the use of donated embryos could be considered as it is relatively inexpensive [16, 17].

The role of fetal karytyping for repeated pregnancy loss related to structural chromosomal abnormalities, e.g., balanced or reciprocal translocation or inversion in paternal or maternal karyotyping, will be discussed in more detail in a subsequent editorial.

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Original Articles

Reproductive Biology Section

Evidence that high serum progesterone (P) levels on day of human chorionic gonadotropin (hCG) injection have no adverse effect on the embryo itself as determined by pregnancy outcome following embryo transfer using donated eggs

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Summary

Purpose: To determine if too high of a level of progesterone at the time of peak follicular maturation of donors adversely affects pregnancy or implantation rates of recipients. *Methods:* A retrospective cohort analysis was performed on donor egg recipients. Pregnancy rates were calculated according to ranges of five serum progesterone (P) levels based on two standard deviations before and above the mean. *Results:* No adverse effect was found in recipients whose donors had serum P levels between 2.47 and 3.41 ng/ml. There may have been a slightly lower pregnancy rate in recipients whose donors had seen P levels over 3.41 but there were only seven patients in that group and there still was a live delivered pregnancy rate of 28.6% per transfer. *Conclusions:* The main adverse effect of a premature rise of progesterone in women making multiple follicles with gonadotropin stimulation seems to be on the endometrium. There appear to be enough follicles not affected by the progesterone to recommend proceeding with oocyte retrieval in the donor so as not to waste money on expensive medication and monitoring.

Key words: Serum progesterone; Late follicular phase; Oocyte donor, recipients.

Introduction

Premature luteinization in which luteinizing hormone (LH) surges result in a rise in progesterone before follicular maturation is achieved, is a recognized problem in women in natural cycles, or milder stimulation protocols aimed at inducing single follicular maturation [1-3].

It is not clear whether the adverse effect of the production of progesterone (P) by the granulosa-theca cells in natural cycles is on the oocyte contained within or on the endometrium. However even in women making multiple follicles premature rise of P has been associated with failure to achieve pregnancies.

When multiple follicular maturation is induced premature rise in progesterone also adversely effects the achievement of a successful pregnancy [4]. It is not clear if all of the follicles are producing P or one or some but not all of them are the source. If the adverse effect of a premature increase in P is on the oocyte within and the endometrium then it would be merely an academic exercise rather than a clinically important distinction to make in a woman trying to conceive naturally since whatever the mechanism pregnancy is not likely to happen.

However, the knowledge as to whether some of the eggs within the follicles, especially those that have not luteinized during in vitro fertilization (IVF) cycles, have potential to allow normal pregnancies, has clinical value. This has a special value for women who are egg donors. The recipient has spent a great deal of money on the medication and cost of follicular monitoring of the donor. Thus if the majority of eggs from the donor can still produce viable pregnancies it would be cost effective for the recipient not to cancel the cycle but to proceed with giving human chorionic gonadotropin to the donar when the follicles are mature and then do egg retrieval.

The present study evaluated the pregnancy rates in recipients according to the P levels of their respective donors to see if this is a certain level where pregnancy rates are significantly lower and thus should suggest that the recipient cancels the donor's cycles.

Materials and Methods

A retrospective cohort analysis was conducted. The serum P levels of oocyte donors were recorded on day of hCG injection. Recipients were told the serum P levels and informed of the existing evidence of low pregnancy rates associated with higher P levels in women who themselves are having oocyte retrieval and embryo transfer but that these effects may be on the endometrium. Only transfers with at least two embryos were included.

The data were stratified into five ranges of P based on standard deviation from the mean, and the pregnancy rates were determined.

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The recipients were advised that we were not sure whether the adverse effect of a rise of P adversely effects the egg or the endometrium so they were given the option of canceling the cycle or not.

Results

The pregnancy and implantation rates according to five serum P level ranges is given in Table 1. Interestingly, the highest clinical and live delivered and implantation rates were found in the group where the P ranged from 2.47 to 3.41 ng/ml.

The data suggest that if an adverse effect associated with higher P levels does exist (especially > 2 ng/ml), this does not effect the pregnancy rates of recipients. Possibly serum P levels > 3.41 in oocyte donors may adversely effect subsequent implantation and pregnancy rates in recipients but there were only seven (3.4% of the donor cycles) with serum P levels that high. If this small data set proves to be truly reflective of a larger population these data could suggest that with very high serum P levels more follicles have luteinized thus possibly affecting the egg itself.

Table 1. — *Pregnancy and implantation rates according to five serum progesterone (P) levels*.

Donor P level day of hCG	≤ .59	.59-1.53	1.53-2.47	2.47-3.41	3.41-4.35
# transfers ≥ 2 ET	16	105	60	17	7
Avg. age of donor	31.5	31.6	30.7	30.9	29.8
Mean E2 level day of hCG donor	2831.5	3058.3	3862.5	4082.8	3166.1
Mean P level day of hCG donor	0.4	1.0	2.0	2.9	3.8
# clinical pregnancies	7	62	33	12	4
% clinical pregnancy/ transfers	43.8	59.0	55.0	70.6	57.1
# viable	5	55	29	10	2
% viable/transfers	31.3	52.4	48.3	58.8	28.6
Implantation rate (%)	30.0	31.8	29.8	38.6	19.0

Discussion

Previous studies have found a correlation with lower pregnancy rates and high P levels on the day of hCG injection following controlled ovarian hyperstimulation [5, 6]. Though other studies have failed to confirm the importance of very low serum progesterone levels prognosticating higher pregnancy rates, certain higher progesterone levels that would prognosticate low chances of conception are still considered [7]. This level is frequently considered over 2 ng/ml.

By not finding a lower pregnancy rate in recipients receiving eggs from the donor with higher serum P levels these adverse effects seen in women undergoing IVF-ET themselves appear to be on the endometrium and not on the embryos per se.

These results have practical importance since some IVF centers would cancel the retrieval and not give the hCG injection to advance meiosis if the serum P of the donor exceeded a certain level. Gonadotropins are expensive and the money spent (usually by the recipient) would be wasted if the cycle was cancelled – not to mention the expense and inconvenience of monitoring serum hormone levels and follicular development by ultrasound.

The conclusions of this study could extend to women undergoing IVF-embryo transfer (IVF-ET). For IVF centers with good frozen ET pregnancy rates a women with elevated serum progesterone at the time of peak follicular maturation could consider – instead of canceling the cycle – to take the hCG injection and freeze all the embryos since it seems the main adverse effect of increased progesterone is on the endometrium.

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Effect of the length of time that donated embryos are frozen on pregnancy outcome

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Summary

Purpose: To determine if the longer length of time that embryos donated to an anonymous couple have been frozen has a negative effect of subsequent successful pregnancy following thawing and transfer to the recipients. *Methods:* Retrospective determination of pregnancy rates according to the length of cryopreservation time has on pregnancy outcome following transfer of embryos designated for donation. *Results:* Longer time of freezing did not adversely affect subsequent pregnancy rates following frozen embryo transfer. *Conclusions:* Donated embryos frozen for over five years (the time when some countries demand that the embryos be discarded) contributed to one-fourth of the donor embryo pool and one-third of the live deliveries.

Key words: Cryopreservation; Donated embryos; Length of freezing; Pregnancy rates.

Introduction

When couples undergoing in vitro fertilization (IVF) complete their families, some choose to donate their remaining cryopreserved embryos to other couples. The donation is anonymous and patients receive no financial compensation for their generous donation.

However, the donation may not occur for many years, due to many factors. One is the length of time it takes the donor couple to complete their family, plus the time needed to ultimately decide to donate the remaining embryos. Added to this is the entire process of "adopting" the embryos, including selection of a desirable batch by the recipient patient or couple, paperwork and preparation, and eventually cycling through for a frozen embryo transfer (ET). By this time, the embryos may have been in storage for over ten years.

The following data were collected retrospectively to determine whether duration of storage was detrimental to pregnancy outcomes.

Materials and Methods

Embryos were cryopreserved in 1.5 M 1,2-propanediol (PrOH) either at the 2-pronuclear (2PN) or multicell stage using a simplified freezing protocol in a BioCool alcohol-bath freezer [1]. Within 20 minutes of being placed in PrOH, embryos were loaded into 0.25 ml straws, heat-sealed at both ends, and manually seeded in the alcohol-bath freezer at -6.0°C. The straws were cooled at -0.4°C/min until -40°C was reached; after a 15 minute hold the embryos were plunged into liquid nitrogen for storage.

Thawing involved submersion in a 37°C waterbath and a simple one-step removal of cryoprotectant. Embryos were cultured for one to two days in either HTF (Irvine Scientific) supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific) or in Quinn's cleavage medium containing 10% synthetic protein substitute (Sage BioPharma) under sterile mineral oil (Squibb or Irvine Scientific).

Patients were prepared for the frozen ET using either oral estradiol or leuprolide acetate-estradiol protocols for endometrial preparation. Progesterone supplementation was begun when the endometrial lining was approximately 10 mm thick and embryos were thawed and transferred accordingly. Transfers were performed on day 3 and were preceded by assisted embryo hatching using acid Tyrode's solution [2].

A retrospective evaluation of pregnancy rates following thawing of frozen donated embryos was made according to six time periods.

Results

In the group of patients whose embryos were stored longer than ten years there were two pregnancies and two live deliveries achieved in three transfers (Table 1).

One pregnancy resulted from embryos stored for 11.8 years. A total of six embryos were available for thawing, three at the 2PN stage, two at the 4-cell stage, and one at the 6-cell stage. All of the embryos survived the thaw. Three embryos were transferred into the recipient, and all three had reached the 8-cell stage and were good quality at the time of transfer. The patient conceived twins and delivered a healthy full-term boy and girl. The age of the donor at the time of retrieval was 38.0 years old.

Another delivery resulted from embryos which had been stored for 10.8 years. A total of four embryos were available for thawing, three at the 4-cell stage and one at the 7-cell stage. Three embryos survived, and two were transferred into the recipient. Both had good morphology, and had reached 8-9 cells at the time of transfer. The patient conceived and delivered a healthy full-term boy. The donor's age at time of cryopreservation was 27.9 years old.

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Table 1. — *Pregnancy and implantation rates following transfer of donated frozen embryos according to the length of time frozen.*

Years cryopreserved	≤ 1.9	2.0-3.9	4.0-5.9	6.0-7.9	8.0-9.9	≥ 10
No. of transfers	32	54	50	23	20	3
% clinical pregnancy/transfers	40.6	40.7	42.0	60.9	30.0	66.7
% pregnancies delivered/transfer	28.1	35.2	36.0	60.9	30.0	66.7
Average # embryos transferred	3.7	3.5	3.5	3.3	3.0	2.7
Implantation rate	13.7%	16.0%	20.1%	26.3%	18.6%	37.5%

There did not appear to be any decrease in pregnancy or implantation rates with longer storage duration. In contrast, there seemed to be a trend for the older embryos to do slightly better with a higher pregnancy rate and lower spontaneous abortion rate, though this was not significant. No birth defects were recorded in the deliveries of any of the groups, except one male with a hernia, which technically is not a birth defect but was reported by the patient.

Discussion and Conclusion

A careful literature review revealed that the longest time multicell embryos have been kept frozen before transfer resulting in a delivery was 12 years [3], approximately the same as our recorded delivery after 11.8 years. The longest duration of storage prior to a donated embryo transfer prior to this study was nine years [4]. Information was also presented on the website IVF.net regarding a pregnancy achieved after 13 years of storage, but we could not find any citations in peer-reviewed journals about this anecdotal case report.

These data are important since legislation in some countries allows (or requires) embryos to be destroyed after two to five years of storage. Indeed one-fourth of the donated embryos came from ones stored ≥ 6 years as did about one-third of the live pregnancies. Unequivocally, cryopreserved embryos can produce viable pregnancies and deliveries far beyond this arbitrary cut-off time. When considered in combination with a voluntary embryo donation program, it seems wise to allow IVF patients the option of cryopreserving their supernumerary embryos and donating them when no longer needed. Both pregnancies mentioned in our results were from donated embryos transferred into anonymous recipients, and neither would have been possible if the embryos had to had be destroyed after five years.

It cannot be assumed that embryos stored for increasing lengths of time will have suboptimal quality or reduced survival rates. Machtinger *et al*. [5] did not see a drop in either of these parameters. Original cell stage and quality seem to be more important than duration of storage when predicting survival and implantation [6]. Although we did not include any data on cell stage and quality in this study, the duration of freezing did not seem to have detrimental effects on the implantation or pregnancy potential of thawed embryos, as evidenced by the similar pregnancy and implantation rates in all categories.

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Effect of fertilization by intracytoplasmic sperm injection versus conventional insemination on embryo cleavage rates

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Summary

Purpose: To determine if fertilization by conventional oocyte insemination vs intracytoplasmic sperm injection (ICSI) causes any difference in the maximum number of blastomeres of fresh or frozen thawed embryos transferred. *Methods:* Retrospective evaluation of all in vitro fertilization (IVF) cycles over a 10-year period in cycles having ≥ 2 embryos transferred where the semen analysis was normal except for strict morphology which was allowed to be 2-5% normal. The percentage of the maximum number of blastomeres in any transfers was compared according to the method of insemination. *Results:* There were no differences in the maximum blastomere numbers in cycles where there was conventional insemination vs ICSI. *Conclusions:* Though higher pregnancy rates have been found following the transfer of embryos derived from conventional oocyte insemination vs ICSI, and higher pregnancy rates were found following single embryo day 3 transfers with embryos with more blastomeres, the beneficial effect of conventional insemination does not seem to be related to forming embryos with more rapid cleavage.

Key words: Blastomeres; Intracytoplasmic sperm injection; Oocyte insemination.

Introduction

A recent study of single embryo transfers found similar pregnancy rates with embryos with 6-8 blastomeres [1]. The pregnancy rate with 6-8 blastomeres was much higher than when embryos with 4-5 blastomeres were transferred [1].

Recent studies have demonstrated that at least in younger women the use of ICSI for fertilization of eggs results in embryos with less implantation potential than those fertilized by conventional insemination [2]. The present study evaluated whether ICSI leads to the generation of embryos with fewer blastomeres compared with conventional insemination.

Materials and Methods

A retrospective evaluation of all ICSI and conventional in vitro fertilization-embryo transfer (IVF-ET) cycles over a 10-year period in women ≤ 39 who had ≥ 2 embryos transferred was performed. A requirement for semen parameters was that the motile density was ≥ 8 x 10^6/ml, no antisperm antibodies were present, and the hypo-osmotic swelling test was ≥ 50%. A further requirement was that the normal morphology using strict criteria was 2-5%.

In vitro fertilization-embryo transfer cycles were evaluated according to the maximum number of blastomeres in any of the embryos transferred. The study would compare the relative frequency of 4, 5, 6, 7 and 8-cell embryos on day 3 immediately prior to transfer to the uterus according to whether fertilization was achieved by ICSI vs conventional oocyte insemination. Frozen embryo transfers were similarly evaluated.

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Results

A comparison of the effect of the method of oocyte insemination (intracytoplasmic sperm injection vs conventional insemination) on the maximum number of blastomeres of any one embryo transferred in a given fresh embryo transfer is seen in Table 1.

Sixty-nine percent of the transfers of embryos derived from ICSI had at least one 8-cell embryos vs 71.3% of embryos derived from conventional insemination (p = NS). There were 90.7% of the transfers of embryos derived from ICSI had at least one good prognosis 6-8 cell embryo vs 91.9% of embryos derived from conventional insemination. More details are provided in Table 1. The percentage of fresh embryos transferred with better implantation potential, i.e., 6, 7 and 8-cell embryos were similar whether the eggs were fertilized by ICSI (6-cell 10%, 7-cell 11.9%, and 8-cell 69.0%) or conventional insemination (8.9%, 12.7%, 71.3%).

With frozen embryo transfers an 8-cell embryo was found in 48.6% of transfers with ICSI vs 52.3% with con-

Table 1. — *Comparison of the effect of the method of oocyte insemination (intracytoplasmic sperm injection vs conventional insemination) on the maximum number of blastomeres of any embryo following fresh embryo transfers.*

	ICSI		Conventional	
Total # patients	1670		820	
No. cells	No. of patients	% of cycles with	No. of patients	% of cycles with
4-cell	45	2.6%	21	2.5%
5-cell	71	4.1%	37	4.5%
6-cell	171	10.0%	72	8.9%
7-cell	201	11.7%	105	12.7%
≥ 8-cell	1182	69.0%	585	71.3%

Table 2. — *Comparison of the effect of the method of oocyte insemination (intracytoplasmic sperm injection vs conventional insemination) on the maximum number of blastomeres of any given embryo following frozen-thawed embryo transfers*.

	ICSI		Conventional	
Total # patients	1713		1129	
	No. of patients	% of cycles with	No. of patients	% of cycles with
4-cell	129	7.5%	84	7.4%
5-cell	180	10.5%	95	8.4%
6-cell	246	14.4%	151	13.4%
7-cell	292	17.0%	181	16.0%
> 8-cell	832	48.6%	590	52.3%
morula	23	1.3%	12	1.1%
blastocysts	11	0.6%	16	1.4%

ventional oocyte insemination. There was at least one frozen thawed 6-8 cell embryo transferred in 80.0% of the transfers of ICSI derived embryos vs 81.7% of frozen embryo transfers derived from conventional oocyte insemination. Table 2 shows similar findings for frozen embryo transfers.

Discussion

Though both a higher clinical and live delivery rate were found in previous studies with embryos derived from conventional insemination vs ICSI despite subnormal sperm morphology using strict criteria, this does not seem to be reflected by embryo quality, at least as manifested by blastomere number. In the aforementioned study of single embryo transfer the degree of fragmentation did not seem to have a significant impact on pregnancy outcome following single embryo transfer so this parameter was not evaluated in this study [1].

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Length of time of embryo storage does not negatively influence pregnancy rates after thawing and transfer

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Summary

Purpose: To determine if longer storage of embryos in a cryopreserved state negatively affects the chance of successful implantation following thawing and transfer. *Methods:* A retrospective cohort analysis of frozen-thawed embryos that had been donated to recipients. Four time periods were evaluated. *Results:* No significant decrease in pregnancy or implantation rates was found in the longest freezing group (≥ 6 years). In fact, if there was a trend, it was for improved pregnancy rates with longer storage. One of the successes was from embryos stored about 12 years. *Conclusions:* Hopefully these data and results from other IVF centers will influence those countries having a mandatory discarding policy to reconsider and lift these restrictions, especially to increase the pool of embryos available for donation.

Key words: Cryopreservation; Donated embryos; Length of freezing.

Introduction

Some countries have requirements that specify that frozen stored embryos be destroyed after a period of time. Donated frozen embryos are at a premium. Frequently a couple will hold on to their frozen embryos not sure if they may want another child in the future.

When couples are about to lose their embryos some of them are emotionally forced to attempt another pregnancy before desired or even have another child that they may not be sure they really wanted. Instead others would donate their embryos to an anonymous couple at a later date if they were not "emotionally" forced to transfer them back to themselves or for others, and by waiting until the last minute, it would be too late to consider donating them.

The objective of the present study was to determine if there is any given length of time of being in the frozen state that reduces the chance of implantation potential of these frozen/thawed embryos.

Materials and Methods

The study was a retrospective cohort analysis. Only frozen donated embryo transfers were included in the study. This allowed more uniformity of results, because length of time in storage was not necessarily related to successful fresh transfer, therefore biasing the data.

All embryos were cryopreserved either at the 2-pronuclear (PN) or multicell stage. A simplified freezing protocol was used on all embryos with slow-cooling in a BioCool alcohol bath freezer, and a one-step removal of the cryoprotectant 1,2-propanediol upon thawing [1].

Transfers were performed on day 3, and were preceded by assisted embryo hatching [2].

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Results

There did not appear to be any decrease in pregnancy or implantation rates with longer embryo storage time as seen in Table 1. Although none of the parameters reached clinical significance, there actually seemed to be a trend toward higher implantation rates, and lower spontaneous abortion rates, with longer storage times.

In the "greater than ten years stored" group, the two patients out of three who got pregnant both delivered healthy babies. One was a singleton produced from multicell embryos frozen for 10.8 years [3]. The other was a twin delivery from a mixed batch of 2PN and multicell embryos which had been stored for 11.8 years. There were no birth defects in any of the donated embryo deliveries other than a hernia.

Discussion

From the current data we can conclude that there is no decline in implantation potential of cryopreserved embryos as length of storage time increases, as also found by Machtinger *et al*. [4]. This information is useful in counseling donor embryo recipients who may be concerned about choosing batches of embryos which have been in storage for long periods of time, as well as patients wishing to donate who are afraid that their stored embryos are no longer viable.

Some patients are hesitant to choose embryos which have been stored for long periods of time since little information is available concerning their possible viability, including birth defects [5]. These recipients could be reassured by the present data. Since many countries now have legislation authorizing (or even requiring) IVF clinics to destroy embryos after three to five years, it is important to acquire more information on this topic so legislators and oversight agencies can make informed decisions regarding the potential ramifications of destroying embryos based on arbitrary time limits.

Table 1. — *Effect of length of freezing on pregnancy and implantation rates following frozen embryo transfer*.

	Clinical pregnancy rate	SAB rate (failure/ clinical preg.)	Implantation rate	Live/delivered pregnancy rate
< 2 years	40.6% (13/32)	30.8% (4/13)	13.7% (16/117)	28.1% (9/32)
2.0-3.9 years	40.7% (22/54)	13.6% (3/22)	16.0% (30/188)	35.2% (19/22)
4.0-5.9 years	42.0% (21/50)	19.0% (4/22)	20.1% (35/174)	36.0% (18/21)
≥ 6 years	47.8% (22/46)	0.0% (0/22)	23.8% (34/143)	47.8% (22/46)
p value (chi-square analysis)	.887	.071	.135	.325

SAB: spontaneous abortion.

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Pretreatment of sperm with low hypo-osmotic swelling tests with chymotrypsin prior to intrauterine insemination (IUI) and avoidance of unprotectected intercourse results in pregnancy rates comparable to IUI for other male factor problems

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Summary

Purpose: To carry out a retrospectively performed matched controlled study to determine the efficacy of pretreatment of sperm having low hypo-osmotic swelling (HOS) test scores with chymotrypsin galactose prior to intrauterine insemination (IUI) compared to IUI for other types of male factor problems. The women with male partners with low HOS scores were advised not to have unprotected intercourse. *Methods:* All cycles having IUI with chymotrypsin treated sperm for low HOS scores were matched with the very next woman having IUI for sperm with other male factor problems but with normal HOS scores. *Results:* There was a significantly higher clinical pregnancy rate with chymotrypsin treated sperm (32.3% per IUI) vs 21.9% for other male factor cases. The live birth rate per IUI cycle was not significantly different (21.2% vs 15.4%). *Conclusions:* These results now show that pretreatment of sperm with low HOS scores allows very good pregnancy rates following IUI as long as the couple is cautioned about unprotected intercourse. These data support the concept that sperm with low HOS test scores impair fertility by transferring a toxic factor from the sperm to the zona pellucida to the embryo membrane which impairs the embryos from implanting.

Key words: Hypo-osmotic swelling test; Implantation; Intrauterine insemination.

Introduction

In 1989, a study demonstrated that males with normal semen parameters but hypo-osmotic swelling (HOS) scores < 50% did not achieve pregnancies following intercourse [1]. However, several subsequent research publications by other authors have stated that a low HOS score had no adverse effect on fertilization rates following in vitro fertilization-embryo transfer (IVF-ET) [2-5]. Thus, most infertility specialists assumed that this means that a low HOS score has no clinical importance since it was assumed that once the sperm fertilized the egg the role of the sperm ceased.

However, a matched-controlled study performing IVF with conventional oocyte fertilization found no adverse effect of low HOS test scores on fertilization rates similar to these other authors [6]. However, there were hardly any live pregnancies [6]. Another study from the 1990s evaluating single-sperm defects on IVF outcome found a 25% clinical pregnancy rate/transfer with all semen parameters normal and 25% with low motile density, 44% with low strict morphology, but 0% with a HOS test score < 50% [7]. A study where a single pool of oocytes was shared between two male partners with normal semen parameters but one with a normal and the other a subnormal HOS test score was performed. The clinical pregnancy rate/transfer was 50% in the former vs 0% in the latter [8].

The 50% cutoff is critical for the test. Jeyendran *et al.* stated that the grey zone for this test was 50-59% [9]. However no reduced pregnancy rates with male partners with the grey zone HOS scores were found [10]. It has been found that the HOS test abnormality, once it is subnormal, generally tends to stay subnormal [11]. This HOS test abnormality was found in 8% of the male partners aged < 45 in our infertile population, 16% in males aged 45-49, and 33% in males ≥ aged 50 [12].

Fertilization of oocytes by intracytoplasmic sperm injection (ICSI) overcomes the HOS test abnormality [13]. One hypothesis to explain how fertilization is not impaired but pregnancy rates are markedly decreased when a low HOS test score is present is that the impairment of the functional integrity of the sperm membrane, as demonstrated by low HOS test scores, is related to a toxic factor attached to the sperm [14]. This toxic factor can be transferred to the zona pellucida by the supernumerary sperm that attach, which when incorporated into the embryo membrane causes functional impairment of the embryo membrane, which in turn inhibits implantation [14]. The possibility exists that this toxic factor is a protein. If so, then treatment of the sperm with a protein

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digestive enzyme, (e.g., chymotrypsin) might overcome the abnormality.

A pilot study was initiated in 1997 in which eight of 12 men had improved HOS test scores (≥ 50%) after chymotrypsin treatment [15]. Four of these eight couples (50%) conceived in 12 IUI cycles (33% per cycle) [15]. The four whose scores remained < 50% despite chymotrypsin therapy were offered IVF with ICSI, and two of the four conceived.

In this pilot study, the couples were advised not to have unprotected intercourse because otherwise it could not be certain that conception occurred from the treated sperm. On the basis of the pilot study, we recommended intrauterine insemination (IUI) with chymotrypsin as first-line therapy. In-vitro fertilization with ICSI was only suggested if there was no success with IUI. However, a retrospective review of 99 IUI cycles with chymotrypsin therapy found only three live pregnancies (3.0%) [16].

A subsequent study determined whether treating sperm with low HOS test scores with the protein digestive enzyme chymotrypsin before conventional fertilization of oocytes could improve pregnancy rates after ET [17]. Couples in whom the HOS test score was < 50% in two consecutive evaluations were offered free IVF-ET (exclusive of medications) if they would allow half of the retrieved oocytes to be fertilized conventionally with chymotrypsin-treated sperm and the other half to be fertilized by ICSI. The agreement was that the embryos transferred on the retrieval cycle were the ones formed by conventional insemination with chymotrypsin-treated sperm.

There were 28 oocyte retrievals and 28 ETs. The clinical pregnancy rate per transfer was 42.9% (12/28). The ongoing/delivered pregnancy rate was 32% (9/28). The implantation rate was 21.3% (19/89) [17].

The present study attempted to repeat the aforementioned study of treating sperm with HOS test scores < 50% with chymotrypsin prior to IUI but this time cautioning the couples not to have unprotected intercourse prior to ovulation. Since it seems that based on these studies chymotrypsin-galactose treatment may neutralize the "toxic" factor, it was considered that the aforementioned retrospective review showing poor pregnancy rates despite pretreatment of sperm with chymotrypsin prior to IUI could have been related to sperm with this toxic factor reaching the egg through normal intercourse since the patients were not admonished about having unprotected intercourse [16]. The sperm from the control group was not treated by chymotrypsin.

Materials and Methods

A retrospective matched study was performed. All cycles using IUI for male factor related to HOS test scores < 50% treated with chymotrypsin and protected intercourse were evaluated for clinical pregnancy rate (ultrasound evidence of pregnancy at 8 weeks and miscarriage rate at 12 weeks). During this time period the same number of consecutive cycles treated with IUI for male factor with normal HOS test scores were similarly evaluated.

The HOS test was performed by combining 0.1 ml of ejaculate with 1.0 ml hypo-osmotic solution (fructose/sodium citrate) following precisely the technique described by Jeyendran et al [9]. After incubation of the mixture for at least 30 min at 37°C, 100 spermatozoa were observed with a phase-contrast microscope for tail changes typical of a reaction in the HOS test. The HOS tests were performed on unprepared specimens during standard semen analysis.

For chymotrypsin-galactose treatment, 0.1 M galactose was dissolved in 5 ml of Earle's balanced salt solution and added to 5 mg of chymotrypsin. The patient ejaculated directly into this chymotrypsin-galactose mixture. The semen immediately were mixed to break up the coagulum. Bovine serum albumin (30 mg/ml) was added to stop the enzymatic reaction.

The women receiving IUI for low HOS scores were matched to the very next woman having IUI for other types of male factor with normal HOS test scores. The female partner of the males with subnormal HOS test scores were admonished not to have unprotected intercourse prior to ovulation.

Results

A matched controlled comparison of clinical pregnancies (ultrasound evidence of pregnancy at 8 weeks) per IUI cycle and miscarriage rates by the end of the first trimester in women with male partners with low HOS test scores vs women with male partners with problems with sperm concentration, motility, or morphology is shown in Table 1.

Table 1. — *Comparison of clinical pregnancy rates following intrauterine insemination (IUI) for males with low hypo-osmotic swelling test scores compared to pregnancy rates following IUI for other types of male factor.*

	Number of cycles	Number of pregnancies	Percent of pregnancies per IUI cycle	Number of miscarriages	Miscarriage rate
Abnormal HOS scores (< 50%)	155	50	32.3%	17	34%
Normal HOS scores (> 50%)	155	34	21.9%	10	29%

The treatment of sperm with low HOS test scores with the protein digestive enzyme chymotrypsin, with emphasis on protected intercourse, with subsequent IUI resulted in a very adequate clinical pregnancy rate of 32.3% and an ongoing delivered pregnancy rate of 21.2% per IUI cycle. The clinical pregnancy rate per IUI cycle was actually significantly higher with the HOS abnormal sperm than the clinical pregnancy rates of 21.9% for abnormal sperm with normal HOS scores ($p = .04$), but the ongoing/delivered pregnancy rates were not different (21.2% with low HOS vs 15.4% with normal HOS test scores).

Conclusions

The original pilot study of only eight patients found a clinical pregnancy rate per IUI cycle of 33.3%. The new data show very similar pregnancy rates (32.3%) [15].

This is in marked contrast to the aforementioned study of chymotrypsin treatment prior to IUI without cautioning about unprotected intercourse where only a 3% success was found [16].

Thus these data support the hypothesis that the HOS abnormality is related to a toxic effect that occurs when the sperm attach to the zona pellucida. The assumption is that in the previous large study of chymotrypsin treatment of sperm with low HOS scores prior to IUI, the much lower success rate was related to transferring of the toxic factor from sperm to egg by the untreated sperm present in the cervical mucus that attached to the zona pellucida at ovulation.

Though ICSI is a highly effective treatment for low HOS scores, chymotrypsin treatment and IUI provides an effective alternative that is much less expensive and much less risky [13]. However, the couple must be cautioned about unprotected intercourse prior to ovulation.

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General Section

Evaluation of the feasibility of a new method for performing chorion villus sampling

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Summary

Objective: This study aimed to evaluate the usefulness and safety of a new method for taking a placental biopsy. *Methods:* The procedures were performed using the traditional single needle technique (group 1) or the new method (group 2). In group 2, the piston was fixed in a simple metallic clip and the negative pressure was maintained in a continuous manner which was controlled with a three-way stopcock. *Results:* Multiple uterine insertion was necessary in 14 cases (32.6%) in group 1 and five (11.9%) in group 2 ($p < 0.05$). The amount of chorionic tissue obtained was significantly higher in group 2 (19.1 ± 15.0 mg vs 33.9 ± 17.4 mg $p < 0.05$). The abortion rates did not differ in either group. *Conclusion:* While using this technique, the operator is capable of performing the procedure without any assistance and of applying constant negative pressure only in the placenta. The advantageous outcomes are probably related to the size as well as the incessant fashion of the vacuum force.

Key words: Chorionic villus sampling; Prenatal diagnosis; Ultrasound; Invasive prenatal procedure; Aspiration; New technique.

Introduction

Prenatal diagnosis of fetal genetic abnormalities as early as possible is quite important. While the pregnancy progresses the psychological linkage gets stronger, termination becomes dangerous and may be restricted in some countries or by religions. Although theoretically early amniocentesis and chorion villus sampling (CVS) are the techniques for assessment of the fetal karyotype in the first trimester, the former is no longer proposed because of its high rate of abortion and talipes equinovarus [1]. However, recent studies have demonstrated that CVS at ten weeks or later had similar abortion rates with second trimester amniocentesis which was estimated at 1% and did not increase the risk of limb abnormalities [2, 3].

Recently Danish data on CVS and amniocentesis over the last 11 years has been published. The authors demonstrated that the number of invasive procedures is decreasing, but the percentage of CVS is continuously increasing [4]. However, the technique has not developed since the first applications and the procedure is performed heterogeneously between operators and institutions. Carlin and Alfirevic conducted a questionnaire on a group of subspecialists to evaluate their attitude on invasive prenatal diagnoses. It revealed that while they usually performed CVS transabdominally, there were some nuances regarding the diameter of the needle and technique (single or double needle) [5]. Recently, we published an upgraded technique of CVS in which we tried to render the assistance redundant [6]. In this study, we aimed to evaluate the feasibility and safety of this technique.

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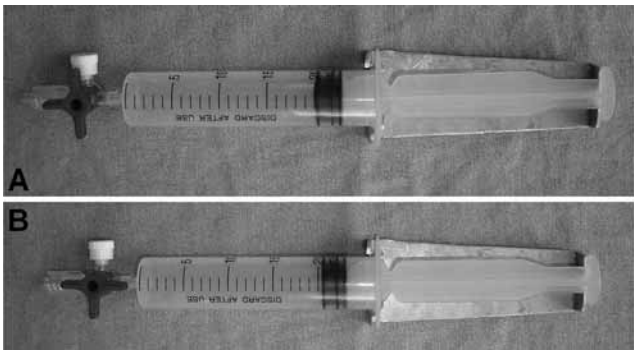
Methods

This was a randomized study which was conducted in the prenatal diagnosis unit of Cukurova University from April 2009 to September 2009. Inclusion criteria of the study were viable singleton pregnancies between week 11^{+0} and week 13^{+6} of gestation. The patients were placed into two groups according to the last number of their national identification number. If the last number was odd they were entered in group 1 and if it was even in group 2. In group 1 women had CVS with the traditional single needle technique and in group 2 they had the invasive procedure according to the technique that we have proposed [6]. Before the procedure, women were counseled about the disease which necessitated invasive testing and complications of the test. Women were also informed that some would undergo the procedure with a new technique. The women in each group gave written consent to participate in this trial.

The invasive tests were performed by one operator. Localization of the placenta was recorded and gestational age was also determined by measuring the crown-rump length in the saggital plan. Skin disinfection was performed with 10% povidone-iodine solution. Before the procedure, a 20-gauge needle was washed out with heparin and a quarter of the 20 ml syringe was filled with culture medium. The use of the biopsy line tool or biopsy needle guide was left to operator choice, and was necessary in a minority of cases.

In group 1 the procedure was performed traditionally with the help of an assistant. The procedure is carried out as follows: when the operator determines that the tip of the needle is in the chorion, the assistant removes the mandrin and attaches the syringe to the needle. While he/she holds the transducer, the operator handles the syringe. The operator moves the needle back ward and forward while pulling back the piston of the syringe to create negative pressure. The procedure in group 2 was performed with a simple metallic clip, designed in our institution, which takes over the assignment of creating negative pressure. The system is fixed to the needle when the operator detects that the tip of the needle is in the placenta. The three-



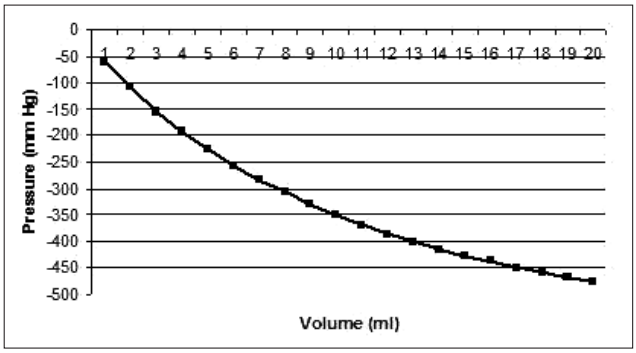


Figure 1. — To create negative pressure in the syringe, the piston is pulled and installed in the metallic clip while the three-way stopcock is turned off (A). When the system is fastened to the needle the three-way stopcock is turned on for aspiration (B).
Figure 2. — Relation between the negative pressure and syringe volume.

way stopcock, which is the control point of the negative pressure, is turned on and the needle is thrust into the chorionic tissue consecutively. This device assures the continuity of the negative pressure, allowing the operator to hold the transducer with one hand and the other is used to control the needle without needing any assistance (Figure 1). The three-way stopcock should be turned off before pulling back the needle to prevent any maternal tissue contamination.

The adequacy of the collected tissue quantity for both groups was visually evaluated in the operating room by the laboratory technician. Precise measurement was done in the laboratory with a microbalance enabling measurement of 0.001 mg. The women were followed for four weeks after the CVS for procedure-related complications.

Data were analyzed using the Statistical Package for the Social Sciences (version 11, SPSS Inc., Chicago, Ill., USA). Normality of each continuous variable was checked. The Student's t-test was used to compare the normally distributed variables and Mann Whitney U test was used to compare the non normal (skewed) distributed variables between the groups. The chi square test was used for categorical data analyses. Data are expressed as mean ± SD and median (min-max). A p-value less than 0.05 was considered statistically significant. This study was approved by the ethical committee of the Medical School of Cukurova University.

Results

The relationship between the negative pressure and volume obtained in the syringe was evaluated with a sensitive electronic calibrator. While the piston was pulled back step by step the amount of negative pressure was recorded every 0.1 ml (Figure 2).

Eighty-five women were eligible and agreed to participate in the study in which 43 (50.5%) were in group 1 and 42 (49.5%) in group 2. In the majority of cases the placenta was located anteriorly. Distribution of the placental localization was the same in each group. There was not any statistical difference between the groups regarding maternal age and gestational age (Table 1). Indications of CVS were advanced maternal age in 46 (54.12%), positive first trimester screening test in 13 (15.29%), and jeopardy of hemoglobinopathy in 26 (30.59%).

Table 1. — *Descriptive data of the patients in each group*.

	Group 1	Group 2	*p*
Placental localization			
• Anterior (n = 55)	28 (65.1%)	27 (64.3%)	0.936
• Posterior (n = 30)	15 (34.9%)	15 (35.7%)	
Gestational age*	12.5 ± 1.1	12.3 ± 0.9	0.298
	12.1 (11.0-13.7)	12.2 (11.0-13.9)	
Age*	33.7 ± 4.5	34.0 ± 5.0	0.726
	32.0 (27-44)	33.0 (27-44)	

*: Data expressed as Mean ± SD in the first line and Median (min-max) in the second line.

Table 2. — *Comparison of procedural results for both groups*.

	Group 1 (n = 43)	Group 2 (n = 42)	*p*
Attempt of uterine insertion			
• 1: n (%)	29 (67.4%)	37 (88.1%)	
• ≥ 2: n (%)	14 (32.6%)	5 (11.9%)	0.022
Tissue weight (mg)			
• Mean ± SD	19.1 ± 15.0	33.9 ± 17.4	
• Median (min-max)	13.1 (5.8-60.6)	33.5 (6.6-62.2)	0.001

The number of the women who needed more than one uterine needle insertion was higher in group 1. The amount of the chorionic tissue was particularly low in group 1 compared to group 2. Comparisons of the procedural results of the both techniques are shown in Table 2. Cytogenetic evaluation of chorionic tissue was successful in all cases and did not reveal inadequate material or failed culture in any case.

Two of the women from group 1 and one from group 2 elected termination of the pregnancy due to the pathological results which were sickle cell anemia and β-thalassemia for group 1 and β-thalassemia for group 2. Only one pregnancy loss was detected following CVS in group 1 and there was not any abortion in group 2 ($p = 0.314$).

Discussion

Invasive procedures during prenatal diagnosis have been performed all over the world since ultrasound became an essential part of obstetric units. Amniocentesis

is still the most performed invasive prenatal diagnostic procedure. However the rate of CVS has been increasing since the introduction of the first trimester trisomy screening test. The method that we are proposing provides a new way to retrieve placental tissue with no need for an assistant and with the of aim facilitating the procedure.

It is a well known fact that the number of insertions and the experience of the operator are the most important factors influencing the procedure-dependant abortion rates [7]. The rates of multiple insertions have been quite heterogeneously reported in previous publications. Mujezinovic and Alfirevic published in a review that multiple insertion rates in CVS range from 1.4% to 26.6%. They calculated the pooled risk to be 7.8 (95% CI 3.1-14.2) [8]. When compared to many previous publications the multiple insertion rate is higher in our study. This may be attributed to the small size of groups or the experience of the operator. All procedures were performed by a single operator which permits us to uniquely evaluate the performance of this technique.

Traditional methods constitute the negative pressure in a discontinuous fashion. The continuous aspiration of chorionic tissue was previously reported in two studies [9, 10]. The authors used a commercial blood collection tube, which is available in all wards. The ability to control the negative pressure is the main difference between these techniques and ours. The three-way stopcock permits negative pressure to be created only in the chorionic plate, thereby protecting the maternal tissue from contamination. There are also some differences in the setting of these studies. Calda and Brestak used an 18-gauge needle with a 10 ml vacutainer [10], whereas Battagliarin *et al*. used a 20-gauge needle with a 4 ml vacutainer [9]. According to our in vitro experiment, the corresponding pressures for each one were approximately 190 and 350 mm Hg consecutively. However the model that we have proposed offers nearly 475 mm Hg. Battagliarin *et al*. reported an increased second needle insertion rate while Calda and Brestak we found that the need of multiple needle insertion diminishes when negative pressure is sufficiently strong [9, 10]. We suppose that the continuous manner of the negative pressure is as important as the force created by the aspiration systems.

This device design was inspired by the widely used pregnancy termination tool: Karman cannula. We found that it apparently diminishes the risk of multiple insertions and assures that a sufficient amount of tissue can be collected. Subcuticular adipose tissue thickness and placental localization may additionally affect the performance of CVS. Larger studies are needed to evaluate and confirm the effect of these factors.

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Expression of matrix metalloproteinase-9 (MMP-9) in human midpregnancy amniotic fluid and risk of preterm labor

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Summary

Object: This work stands as a pilot study in assessing the reliability of metalloproteinase-9 (MMP-9) as a marker for intraamiotic infection and preterm birth already in early pregnancy. *Subject:* 100 amniotic fluids taken at the Midwife Obstetrics and Gynaecological Clinic of the University of L'Aquila (Italy). *Results:* Our results show that MMP-9 is a sensitive marker of intraamionic infection (an important risk factor for preterm delivery) already in early pregnancy, because only women with a significant elevation were subsequently exposed to preterm birth. *Conclusions:* Early identification of women at risk of preterm birth is of important clinical significance. In- deed exposing women to deep diagnostic and therapeutic protocols could possibly reduce the incidence of preterm birth in the near future and have a positive impact on fetal prognosis related to unknown intraamniotic infection.

Key words: Preterm birth; Metalloproteinases; Intraamiotic infections.

Introduction

Remodeling of the extracellular matrix is a fundamental event in both physiological and pathological conditions [1]. The metalloproteinase array (MMPs) are a family of enzymes involved in many physiological and pathological processes that affect the extracellular matrix. The activity of MMPs in the extracellular space is strictly controlled by endogenous inhibitors (TIMPs) [2, 3].

The balance between gelatinase and inhibitors is crucial in the evolution of pregnancy, and an alteration of this balance underlies physiological rupture of the membranes during labour and obstetrical complications such as PROM and preterm birth [4].

A condition of subintrauterine/fetal inflammation during early pregnancy can be the basis for subsequent preterm birth, since the physiopathological processes that contribute to preterm rupture of membranes and /or to preterm birth may already be triggered in the first/second trimester of pregnancy.

Numerous studies state the role of MMP-9 in the mechanisms responsible for term or preterm breaking of the amniotic membrane and stress the importance of microbial infection of the amniotic cavity for the production and release of MMP-9, which was thus confirmed as a sensitive and specific marker in the case of sub-corioamnionitis and prediction of preterm birth [5, 6].

Given this, the purpose of this work was to analyze MMP-2 and MMP-9 in the amniotic fluid of women who had undergone amniocentesis at 17 weeks of gestation and in others that were instead subjected to caesarean, to confirm the literature data about the expression in those gestational stages and to relate these results with the evolution of pregnancy and or specific complications.

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Materials and Methods

One hundred samples of amniotic fluid (marked with a serial number from 1 to 100) were taken at the Gynaecology and Obstetrics Clinic of the University de L'Aquila between October 2005 and February 2006. The samples of amniotic fluids were donated by randomly selected patients with their explicit and informed consent. Amniotic fluids were all taken with transabdominal amniocentesis between 16 + 5 and 18 + 4 weeks of pregnancy, except for fluids 60, 81, 82, 83 and 100 which belonged to patients undergoing caesarean in a period between 28 +5 and 41 +1 weeks of pregnancy.

Patients whose amniotic fluid was taken by amniocentesis at 17 weeks were contacted again after the expected time of delivery to gather news about the subsequent evolution of the pregnancy. For determination of metalloproteinases MMP-2 and MMP-9 (Laboratory of Biochemistry and Molecular Biology of the University of L'Aquila) the zymogram technique was used; this procedure requires the preparation of a polyacrylamide gel with subsequent revelation of gelatinase activities.

For protein balance an indirect method was used - comparison of the protein content of samples with one of a standard protein (bovine serum albumin -BSA- at a concentration of 1 mg/ml).

We used the method proposed by Bradford [7] in 1976, which requires a reactive BIO-RAD where proteins bind to a dye (Coomassie Blue).

The acrylamide-bisacrylamide gel (40% w/v) was prepared with an anionic detergent, sodium-dodecyl sulfate (SDS), according to the method reported by Laemmli [8].

Results

Obstetric complications in the third trimester developed in our sample. Specific conditions are listed in Table 1. Figure 1 shows zymography analysis results.

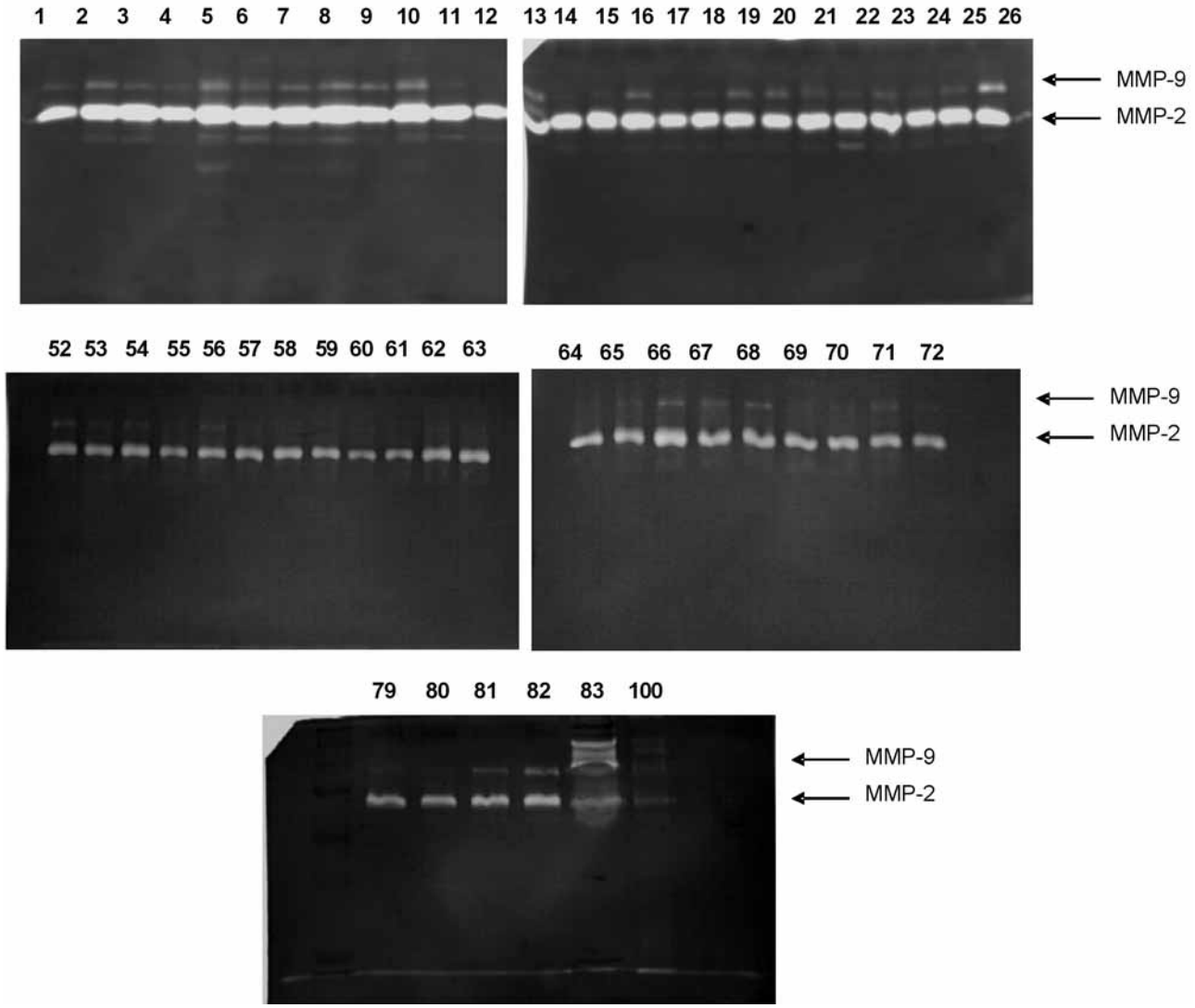

Figure 1. — Most representive expressions of MMPs in amniotic fluid.

Table 1. — *Pregnancy complications in our sample*.

Complication	No. of cases	ID number
Preterm birth	6	5, 10,16, 17, 26, 83
pPROM	2	16, 26
PROM	5	9, 34, 54, 81
Gestational hypertension	2	17, 31
Sepsis during pregnancy	1	83
Urogenital tract infections	11	5, 7, 9, 10, 20, 26, 30, 59, 60, 72, 82

The activity of MMP-2 was uniformly expressed in all samples, while the activity of MMP-9 showed variability.

The nearly constant expression of MMP-2 perfectly reflects the physiological expression of the enzyme, representing the most abundant gelatinase in the amniotic fluid from the second trimester of pregnancy and remaining relatively stable up to increase when labor starts [9, 10].

Figure 9.1 shows that MMP-9 was expressed, albeit at low levels, in almost all samples but shows a significant increase of its activities in the fluid 5.8, 10, 16, 26, 81 and 82. The expression of MMP-9 instead had an intermediate value between the standard of fluid analysed and those just mentioned, in samples 7, 9.

Physiologically the expression of MMP-9 in the amniotic fluid was minimal through the whole gestation period and underwent a significant increase around the first two days of labor, which helps in triggering contractile activities and breaking the membranes. Analysis of the zymography also confirms this fact (see increased expression in 81 and 82 from caesareans with contractile activity and low contractile activity in 60 and 100 from caesareans without any activity).

MMP-9 plays a key role not just during physiological delivery, but also in conditions such as PROM, pPROM, endoamniotic infections and preterm birth [9, 10].

Checking the medical history of patients in whom gelatinase activity (MMP-9) is more expressed has shown that they are linked to a single theme: each had a bacterial infection during pregnancy and, more precisely, during the first trimester:

– Patients of samples 5 and 26 developed vaginitis by *Gardnerella vaginalis*;

– The patient of sample 10 developed asymptomatic bacteriuria;

– The patient of sample 16 reported at 15 weeks positivity of a vaginal swab for beta haemolytic streptococcus (group B);

– The patient of sample 8 voluntary interrupted the pregnancy for therapeutic reasons - *Cytomegalovirus* first infection - the results of PCR on amniotic fluid confirmed the presence of viral RNA;

– The patient of sample 82 showed infection from *E. coli* and *Ureaplasma urealyticum*.

The pregnancy of patients 5, 10, 16 and 26 were complicated with preterm birth between 33 and 36 gestational weeks.

We also noted intermediate gelatinase activity MMP-9 in samples 7 and 9. Both patients belonged to the group of women who contracted urogenital infections during the first quarter but their pregnancies concluded with spontaneous birth in the long term, albeit with PROM (39 weeks) in patient 9.

Discussion

The role of urogenital infections in induction of preterm birth is now clear. Among most significant urogenital infections we want to highlight the role of bacterial vaginosis in the first quarter, which is in fact responsible for 21.9% of preterm births and 43.8% of PROM [11, 12].

In the observed sample patients 5 and 26 reported a diagnosis of *bacterial vaginosis* in the 1st trimester. For bacterial vaginosis there is a significant association of risk factors in the determination of preterm birth [13]. Significant in this regard is low socio-economic level, poor prenatal care and the state of chronic hypothyroidism in patient 5 and smoking during pregnancy and fetal death in the previous patient 26. Also confirming the literature data, patient 5 had early interrupted treatment with oral clindamycin and patient 26 had followed topical therapy with the same drug which does not significantly reduce the risk of preterm birth compared to oral therapy [14].

Patient 10 reported history of recurrent pre-pregnancy urinary infections, asymptomatic bacteriuria in urine culture tests during the first obstetric visit, genital blood loss in the first trimester and development of a pyelonephritis during the third trimester. Recently, the role of asymptomatic bacteriuria as a risk factor for preterm birth has been reevaluated by many authors who associate this condition especially with the risk of a subsequent pyelonephrite [15].

Patient 16, who at 15 weeks had a positive vaginal swab for beta haemolytic streptococcus (group B), was hospitalised at 36 weeks for pPROM and threat of preterm birth that led to spontaneous delivery in the same day [16]. Currently, the role of that etiologic agent in the onset of preterm birth has been quite reduced even though it is found in the vaginal secretions of women who give birth at term - much more rarely than those who give birth prematurely. As for the patient in question, we should point out the simultaneous presence of other risk factors such as black race, smoking during pregnancy and low socio-economic level.

Conclusions

The aim of this work was to confirm the reliability of MMP-9 as a marker for preterm birth and intraamniotic infection, even in early periods of pregnancy. The methodology was mainly based on the possibility of finding markers that could identify an early state of endouterine infection; development of such markers could certainly derive important therapeutic implications. Although results certainly seem encouraging, they must be further studied with the analysis of a larger sample and a quantitative analysis, and not just qualitative expression of the enzyme. The idea stems from the study of recent literature which suggests that at-term pregnancies complicated by corioamnionitis and PROM show important collagenolytic activity (MMP-9), usually absent in normal at-term pregnancies [17]. Since among the different metalloproteinases MMP-9 is the one whose production and release is inducible by certain conditions, and especially by endoamniotic infections, it was investigated and confirmed as a marker in the case of subclinical endo amnionitis and prediction of preterm birth.

The dosage in such conditions reaches values of sensitivity, specificity, positive predictive value and negative predictive value respectively, 83%, 100%, 100% and 90%.

The fact that enzyme quantification methods on the maternal serum or even on saliva (which reflects the expression at the level of amniotic fluid) are being developed will exceed the limit and the fair objection as to whether it is legal to practice amniocentesis, an invasive diagnostic technique, which could result in preterm childbirth [18].

An analysis of zymographies concluded that the only patients in whom MMP-9 expression showed a higher than normal expression were those who had urogenital infections during the first quarter, and that preterm birth occurred only in those where the enzyme expression was greater. In this regard we should point out that early and adequate (oral) therapy could reduce the risk of subsequent complications.

We would like to stress that enzyme expression at 17 weeks did not allow a precise time interval to be determined between enzyme dosage and the time of the subsequent preterm birth. It is not clear whether with a dose quantity of MMP-9 it would be possible.

Patients with subsequent preterm birth typically showed a combination of etiologic factors where proba-

bly only the endoamniotic infection, without such competition, would never have led to a subsequent preterm birth or PROM.

In conclusion early identification of women at risk of preterm birth certainly has important clinical significance. Indeed, submitting a woman to thorough diagnostic investigations and maybe treatment protocols could in the near future allow the incidence of preterm birth to be reduced, positively impacting on fetal prognosis related to unrecognised states of intraamniotic infection.

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Diabetes supersedes dobutamine stress echocardiography in predicting cardiac events in female patients

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Summary

Background: The many available choices for testing for coronary artery disease (CAD) brought about several questions regarding suitability of certain tests for different groups of patients and the prognostic value of obtained results in predicting events and mortality. The aim of this study is to describe the prognostic value of dobutamine stress echocardiography (DSE) results in predicting cardiac events and mortality in ≥ 60-year-old females. *Methods:* 49 women (≥ 60 years old) who were referred for DSE were included in the study. Data including CAD risk factors, and results of tests and a follow-up of events (MI, unstable angina, progression of CHF) and death. *Results:* Eleven patients were considered to have a positive DSE result. There was no difference between DSE (+) and DSE (-) patients in cardiac events and cardiac death. However when interventions were included to events, analysis showed DSE (+) to have more overall events. Non-cardiac deaths and "all deaths" were 11 and 8 times more common among DSE (+) patients compared with DSE (–) patients $p < 0.01$. Multivariable logistic regression showed that diabetics and DSE (+) patients were 32 ($p = 0.01$) and 23 ($p = 0.02$) times more likely to have an event compared with non-diabetics and DSE (–) patients, respectively. *Conclusion:* DSE is a safe procedure to be used in ≥ 60-year-old female patients and can provide informative prognostic information regarding all-cause deaths and cardiac events (including interventions) over a 4-year period. In addition we find that diabetes is a strong predictor of events regardless of DSE result.

Key words: Dobutamine stress echocardiography; Coronary artery disease; Diabetes.

Introduction

The proliferation of cardiology technology assisting in early detection of coronary artery disease (CAD) has provided the physician with a bouquet of choices [1]. Concomitantly, because of worldwide-improved health care more patients of advanced ages are presenting with angina [2] and subsequently for non-invasive CAD detection. This fact has posed several questions regarding suitability of tests offered to older patients and the prognostic value of information received from each test. Physicians well appreciate this once they consider the physical limitations of treadmill exercising among the elderly for example, where dobutamine stress echocardiography (DSE) is presented as an acceptable alternative [3]. Moreover, for years CAD remained to be perceived as a male disease until a change in this perception was founded [4]. This change was driven by the discovery of differences in the prognostic value of certain noninvasive tests in females as in the case of elevated false-positive rate of treadmill exercise ECG in premenopausal women [3]. Therefore the aim of this study is to describe the suitability and prognostic value of DSE results in predicting cardiac events and mortality in a specific group of elderly (≥ 60 years old) females in a Middle East tertiary care center.

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Materials and Methods

Patient population

The study group consisted initially of 50 females who are greater than or equal to 60 years old. One patient had a non-diagnostic DSE so she was excluded from the analysis. DSE and wall motion scoring was performed in accordance with standard protocol [6-8].

The Institutional Review Board approved the study, and the participants signed an informed consent.

Baseline and follow-up characteristics

Follow-up data were obtained for almost all patients. At the time of DSE, baseline information regarding medical history and coronary risk factors were recorded for each patient and included age, tobacco usage, diabetes, hypertension (HTN), angina, family history of coronary artery disease (FHx), hypercholesterolemia, congestive heart failure (CHF), previous myocardial infarction, cerebrovascular accident (CVA), percutaneous transluminal coronary angioplasty (PTCA) intervention and baseline left ventricular ejection fraction.

A follow-up questionnaire was used to record the data on each subject. Follow-up data were collected after a review of the patient's hospital chart, private clinics or Out Patient Department records; the referring physicians were identified and contacted, and telephone interview with the patient or patient's relatives was done. The clinical events recorded during the follow-up were cardiac and non-cardiac deaths, acute coronary syndrome (STEMI, NSTEMI), pulmonary edema, malignant arrhythmias and coronary revascularization (surgery or angioplasty). The diagnosis of acute myocardial infarction was made on the basis of symptoms, electrocardiographic changes, and cardiac enzyme level increases. Revascularization was considered as a clinical end point reflecting new or progressive symptoms.

Statistical Analysis

Categorical data were reported as percentages, and continuous data were reported as mean ± standard deviation (SD). Continuous variables were compared with the Student independent *t-test* whereas differences of categorical variables were assessed by the chi-square test. DSE was considered positive if the cardiac tissue was found to be ischemic, viable or non-viable, and negative in case of non-ischemic tissue. Statistical significance was considered if $p < 0.05$.

Results

The final patient population consisted of 49 female patients. The average age was 67.8 ± 6.5 years (range 60 to 90 years). The DSE was done to test for the presence of viable myocardium (4 patients) and for the presence of ischemia (45 patients). These patients were not considered as two different populations and were used in the same analysis. DSE revealed the presence of ischemia in seven patients, viable tissue in two patients, and non-viable tissue in two patients. Thus, the test was considered to be positive in 11 patients (22.4%). During the test four patients (8.2%) complained of nausea, three (6.1%) of dyspnea, and one (2.0%) of dizziness.

Comparing DSE (+) to DSE (-) patients, there was no significant difference between the two groups with respect to age and except for history of a myocardial infarction (MI), there was no significant difference with respect to past medical history. Patients with positive DSE were respectively eight and ten times more likely to have a history of MI and coronary artery bypass grafting (CABG) as compared to patients with negative DSE. Among the medications, only statins were significantly associated with DSE result (Table 1). With respect to electrocardiographic and echocardiographic parameters, patients with positive DSE were more likely to have resting or peak ST segment/T-wave changes (Table 2).

Forty-five patients {10 DSE (+) and 35 DSE (-)} had a two-year follow-up and one of them died in these two years. There was no significant difference between the two groups with respect to having a cardiac event or the type of event if they had any. Cardiac event was defined as having a MI, unstable angina (UA), and deterioration of congestive heart failure (CHF). Of the ten DSE (+) patients four underwent PTCA with stent deployment and six underwent (CABG) subsequent to the DSE result, which was significantly higher than DSE (–) patients (Table 3). Thirty-one patients [9 DSE (+) and 22 DSE (–)] were followed-up for an additional two years. Again, there was no significant difference between the two groups with respect to having a cardiac event and the type of the event.

To investigate the difference in overall mortality, the 31 patients (followed-up for four years) and the one patient who died in the first two years were combined in one sample. There was no difference between the DSE (+) and DSE (-) patients with respect to cardiac death within four years. Events of non-cardiac deaths and "all deaths" were 11- and 8-times more common among DSE (+) patients compared with DSE (-) patients (Table 3). The same was done to investigate overall occurrence of events. Patients with an event in the first two years and no further follow-up were added to the 31 patients with four years follow-up. There was no significant association between the DSE result and having at least one cardiac event during the four years despite higher occurrence in the DSE (+) group. However, if performed interventions are considered among cardiac events, then the DSE (+) patients will be significantly more likely have a cardiac event (Table 3).

Table 1. — *Clinical characteristics for the DSE (+) and DSE (–) groups*.

	DSE+ n = 11	DSE- n = 38	*p* value
Mean age (years)	67.3 ± 8.3	67.9 ± 6.0	0.78
Diabetes	4 (36.4)	15 (39.5)	0.85
Hypertension	4 (36.4)	25 (65.8)	0.08
Hypercholesterolemia	7 (63.6)	14 (36.8)	0.11
Smoking	4 (36.4)	11 (28.9)	0.64
COPD	1 (9.1)	6 (15.8)	0.58
Carotid stenosis	0 (0.0)	1 (2.6)	0.44
PVD	2 (18.2)	5 (13.2)	0.68
CHF	1 (9.1)	2 (5.3)	0.64
Stroke	1 (9.1)	3 (7.9)	0.90
CRF	2 (18.2)	5 (13.2)	0.68
Old MI	6 (54.5)	5 (13.2)	**< 0.01**
Use of antiplatelets	8 (72.7)	16 (42.1)	0.07
Use of beta blockers	4 (36.4)	10 (26.3)	0.52
Use of ACEI/ARB	4 (36.4)	12 (31.6)	0.77
Use of statins	4(36.4)	2 (5.3)	**0.01**
Prior PTCA	0 (0.0)	3 (7.9)	0.33
Prior CABG	4(36.4)	2 (5.3)	**0.01**

Data is presented as mean value ± SD or number (%) of patients.
DSE +/–,Positive/negative echocardiographic result of dobutamine stress testing; *COPD,* Chronic obstructive Pulmonary Disease; *PVD*, Peripheral Vascular disease; *CHF,* Congestive heart failure; *CRF*, Chronic renal failure; *ACEI/ARB*, Angiotansin Converting enzyme Inhibitor/Angiotensin Receptor Blocker. PTCA, percutaneous transluminal coronary angioplasty; *CABG,* coronary artery bypass grafting.

Table 2. — *Electrocardiographic, baseline, and stress echocardiography characteristics for the DSE (+) and DSE (-) groups*.

	DSE+ n = 11	DSE- n = 38	*p* value
Previous myocardial infarction	2 (18.2)	4 (10.5)	0.50
Resting ST segment/ Twave changes	2 (18.2)	0 (0.0)	**0.01**
Peak exercice ST segment/ T wave changes	2 (18.2)	1 (2.6)	**0.058**
VT or AF during the test	0 (0.0)	1 (2.6)	0.59
Hypotension	0 (0.0)	0 (0.0)	–
Bradycardia	0 (0.0)	0 (0.0)	–
RWMSI > 1.15	7 (70.0)	5 (13.9)	< 0.001
PEWMSI > 1.15	6 (100.0)	4 (11.4)	< 0.001

Data is presented as mean value ± SD or number (%) of patients.
DSE+/DSE-, Positive/negative echocardiographic result of dobutamine stress testing; VT, Ventrivular Tachycardia; AF Atrial Fibrillation; RWMA, Resting Wall Motion Abnormalities; NWMA, New Wall Motion Abnormalities; RWMSI, Resting Wall Motion Score Index, PEWMSI, Peak Exercise Wall Motion Score Index.

Table 3. — *Events according to the result of stress echocardiography in the DSE (+) and DSE (-) groups.*

	DSE+	DSE-	*p* value
Two years FU			
PTCA	4/10 (40.0)	3/35 (8.6)	**0.02**
CABG	6/10 (60.0)	3/35 (8.6)	**< 0.01**
Any cardiac event	3/10 (30.0)	4/35 (11.4)	0.15
Type of cardiac event			
UA	1/10 (10)	2/35 (5.7)	0.24
MI	1/10 (10)	2/35 (5.7)	
CHF	1/10 (10)	0/35 (0)	
Four years FU			
Any cardiac event	3/9 (33.3)	3/22 (13.6)	0.21
Type of cardiac event			
UA	1/9 (11.1)	2/22 (9.1)	0.36
MI	1/9 (11.1)	1/22 (4.5)	
CHF	1/9 (11.1)	0/22 (0)	
All events[1]			
At least one cardiac event	3/9 (33.3)	6/25 (24.0)	0.59
At least one cardiac event (including intervention)	8/10 (80.0)	8/26 (30.8)	**0.01**
Cardiac deaths	2/9(22.2)	2/23 (8.7)	0.30
Non cardiac deaths	3/9 (33.3)	1/23 (4.3)	**0.03**
All deaths	5/9 (55.5)	3/23 (13.0)	**0.01**

DSE+/DSE–, Positive/negative echocardiographic result of dobutamine stress testing; 1) Grouping patients with 4 years follow-up and those who had an event in the first two years with no additional follow-up.

Table 4. — *Predictors of having at least one cardiac event (including intervention) within 4 years.*

	Cardiac event (including intervention)			
	No n = 20 (%)	Yes n = 16 (%)	OR (95% CI)	*p* value
Demographics				
Age ≥ 70 years	10 (50.0)	13 (81.3)	0.3 (0.94-20.0)	**0.08**
Medical History				
Diabetes	4 (20.0)	11 (68.8)	8.8 (1.9-40.3)	**0.01**
Hypertension	11(5.0)	10 (62.5)	1.4 (0.4-5.2)	0.65
Dyslipidemia	9 (45.0)	9 (56.3)	1.6 (0.4-5.9)	0.50
Smoking	6 (30.0)	5 (31.3)	1.1 (0.2-4.4)	0.93
COPD	4 (20.0)	1 (6.3)	0.3 (0.03-2.7)	0.24
PVD	1 (5.0)	5 (31.3)	8.6 (0.9-83.7)	**0.07**
CHF	0 (0.0)	2 (12.5)	–	0.10
Stroke	0 (0.0)	2 (12.5)	–	0.10
CRF	3 (15.0)	1 (6.3)	0.4 (0.04-4.0)	0.94
Old MI	4 (20.0)	6 (37.5)	2.5 (0.6-11.1)	0.24
Medications				
Use of antiplatelets	6 (30.0)	11 (68.8)	5.1 (1.2-21.4)	**0.04**
Use of beta blockers	6 (30.0)	6 (37.5)	1.4 (0.3-5.6)	0.63
Use of ACEI/ARB	6 (30.0)	7 (43.8)	1.8 (0.5-7.2)	0.40
Use of Statins	1 (5.0)	5 (31.3)	8.6 (0.9-83.7)	**0.07**
Prior PTCA	3 (15.0)	0 (0.0)	–	0.11
Prior CABG	1 (5.0)	5 (31.3)	8.6 (0.9-83.7)	**0.07**

Data is presented as mean value ± SD or number (%) of patients.
COPD, Chronic obstructive Pulmonary Disease; PVD, Peripheral Vascular disease; CHF, Congestive heart failure; CRF, Chronic renal failure; ACEI/ARB, Angiotansin Converting enzyme Inhibitor/Angiotensin Receptor Blocker. PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass grafting.

The 34 patients (31 patients with 4-year follow-up and three patients with an event in the first two years) were categorized based on whether they had an event or not and compared with respect to their past medical history. Compared to those with no cardiac event, patients with at least one event were 32 times more likely to be diabetics, and 10.2 times more likely to have history of hypertension. The same analysis was repeated but after considering those with an intervention as having an event. In addition to DSE (Table 3), diabetes and antiplatelet use were significantly associated with having an event (Table 4). The three variables were used in a multivariable logistic regression. Diabetics and DSE+ patients were 32 (p = 0.01) and 23 (p = 0.02) times more likely to have an event compared with non-diabetics and DSE- patients, respectively. Antiplatelets were not found to be statistically significant in the final analysis.

Discussion

It is required for exercise stress testing that the patient is capable of performing the test with no risk of harm infliction. With aging the risk of harm or limitations precluding from exercise testing (osteoarthritis, joint problems, muscular deconditioning) increase and alternatives such as DSE are offered [3]. Clearly the results above indicate that DSE can be performed safely with no fear of serious complications (nausea, dyspnea, and shortness of breath 2-8%) in ≥ 60-year-old females. This is in line with the reported literature for other age groups and both males and females, thus eliminating potential restrictions for performing the test in this group specifically [3, 5].

Furthermore, the above-listed results show that a DSE (+) result indicates unfavorable all-death and non-cardiac death prognosis over four years, and though not statistically significant but also an increased incidence of cardiac death. This could be explained by the fact that as DSE (+) patients undergo subsequently coronary angiography and intervention, cardiac death rate decreases because of intervention [9]. On the other hand as a DSE (+) result indicates coronary atherosclerosis, which by itself is a marker of generalized ill-health; it is not surprising that all-death is elevated in this group in comparison to DSE (-) patients [5].

Concerning the prognostic value of DSE in predicting cardiac events, only after including PTCA or CABG to the cardiac events (MI, UA or progression of CHF) was this endpoint shown to occur more in a statistically significant manner in DSE (+) patients in comparison to DSE (-). This could very well be attributed to the small sample size, and also to the inclusive indication of DSE (+) i.e., presence of coronary atherosclerosis and thus need for intervention. On the other hand these results indirectly suggest that identifying CAD using DSE and intervening is decreasing the number of events – excluding interventions – over a 4-year period, though not eliminating event occurrence. The latter is explained when the CAD risk factor profile of our cohort is analyzed and not surprisingly patients who suffered from cardiac events were 30- and 10-times more likely to be diabetic and hypertensive, respectively [5, 10]. It is worth noting here that diabetes among the female gender appears to be a very risky factor reflected in the multivariable logistic analysis showing that diabetic patients regardless of DSE

result were 32 times more likely to have an event. This is in line with the literature that shows that cardiac mortality and morbidity of diabetic females is an area of minor – if any – success. Thus whereas cardiovascular mortality among male diabetics has decreased it has remained stable or some suggested that it has even increased by 23% in female diabetics [10, 11]. Moreover in a recently published article, a similar result showed that a normal DSE predicted a less favorable outcome in diabetic in comparison to non-diabetic patients overall [10].

In conclusion, we find that dobutamine stress echocardiography is a safe procedure to be used in females ≥ 60 year old and can provide informative prognostic information regarding all-cause deaths and cardiac events (MI, UA, progression of CHF, PTCA and CABG) over a 4-year period. In addition we find that diabetes is a strong predictor of events regardless of DSE result in our cohort and needs to be addressed aggressively in this group of patients.

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What kind of care and support do infertile women undergoing fertility treatment in Greece expect? A questionnaire survey

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Summary

The aim of this study was to identify infertile women's expectations and perceived importance of professional psychosocial services and to identify the predictors of their expectations. The study included 404 infertile women. Most women sought more medical information and more emotional support than what was offered, mainly by the hospital staff. Less than half the women rated psychosocial services as important. The main predictors of the importance of ratings were high fertility-related stress, low provision of social support, low social class and male infertility factor. A provision for information regarding the medical and psychosocial aspects of infertility should be included in routine care in fertility clinics. Although it seems possible to meet the emotional and psychosocial needs of less distressed women through information and support, it is necessary to offer professional psychosocial services to more distressed women.

Key words: Expectations; Infertility; In vitro fertilization; Psychosocial services.

Introduction

Infertility is the inability to conceive or carry a pregnancy to a live birth. It is estimated that one in six couples seeks help because of problems in conceiving [1]. In addition, 1.3-4.2% of babies born in different European countries were conceived after assisted reproduction treatment [1]. Infertility and its treatment can be a very stressful experience. Since parenthood is perceived in most cultures as having a central role in society, infertile couples seek a solution to their childlessness by using medical interventions. During the past few years in vitro fertilization (IVF) has become one of the standard infertility treatments and provides the hope of pregnancy for infertile women, but does not always turn this hope into reality. Infertility treatment is often experienced as a psychological strain [2]. While dramatic progress has been achieved in relation to the diagnosis and treatment of the organic components of infertility, less attention has been paid to the emotional dimensions of this life crisis. The psychological impact of new reproductive treatments should not be understated. The provision of psychosocial interventions for infertile couples has been recommended since Eck Menning [3] directed research attention to emotional burden as a consequence of infertility rather than, as had been the emphasis until then, a cause of infertility. Some countries have legislation governing the provision of counselling for assisted conception treatments. According to the Human Fertilization and Embryology Authority (HFEA), which regulates assisted reproduction in the UK, psychosocial counselling must be offered to any patient seeking IVF [4]. As it has been described in the HFEA code of practice, the purpose of psychosocial counselling is to provide patients with emotional support and help with decision-making. Today, all licensed IVF clinics in the UK are required to offer patients counselling [5].

Background: The results of previous studies on patient satisfaction with IVF centres, suggest that many patients are dissatisfied with the psychosocial services offered before, during and after treatment [6-9]. Moreover, the HFEA recommendation is consistent with the infertile couple's expectations in receiving more psychosocial help and professional psychological counselling [8]. In a study by Laffont and Edelmann [7] it was found that both men and women feel that a routinely provided information booklet about the practical aspects of IVF would improve knowledge of and passage through an IVF cycle. In the same study it was found that women expressed a desire for some form of counselling or support during IVF treatment. Glover and colleagues [10] investigated the expectations and motivations of infertile men who participated in an IVF program. The majority of men (75%-88%) expected an information provision about their specific problem and possible therapeutic alternatives and help with decision-making processes. Fifty-two percent of them considered it important to discuss their feelings about infertility as well as the way infertility was treated. In a French study [11], results showed the need for psychological counselling after a diagnosis of infertility. Post-treatment counselling seemed to be particularly important. Lack of support at that time influenced the way couples regarded the whole support assistance provided during treatments. Similar conclusions have come

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from other researchers [2, 12]. Dyer *et al*. [13] recognized the importance of health education and counselling as well as the integration of these services into fertility management, especially in the developing world. In a study by Schmidt *et al*. [14], it was shown that the majority of infertile couples considered important the provision of information, regarding test results and potential treatment options. However, fewer patients rated the provision of professional psychosocial services as important. In a previous Greek study [15] it was found that infertile women who were undergoing fertility treatment asked for more emotional support and medical information.

Thus, it seems particularly important to identify the factors that predict women's expectations concerning psychosocial services. Past research has shown that infertile couple's expectations regarding IVF were influenced by their psychological status; particularly those who attended support groups [16] or expressed a wish for counselling services [7] seemed to experience more personal and/or marital stress than those who did not. Additionally, patients who drop out of counselling tend to experience less stress than those who continue [17]. In a study by Boivin [18], it was found that less distressed patients reported that the coping resources available to them were sufficient to manage the strains of infertility. Boivin *et al*. [19] found that the core predictor of a greater need for psychosocial care was high infertility-related stress. A recent study found that the main predictor of perceived importance regarding patient-centred care and psychosocial care was high infertility related stress in the marital, personal and social domain [14].

Study aims: The aims of the study were: a) to identify infertile women's expectations and perceived importance of professional psychosocial services and b) to identify the factors that predict women's expectations and perceived importance of professional psychosocial services. The considered factors were demographic (age, social class), medical (duration of infertility, number of previous therapies, etiology of infertility) and psychosocial (state and trait anxiety, fertility related stress, family/friend support). Selection of factors was based on the fact that these factors have been found to impact women's expectations and perceived importance of professional psychosocial services in previous studies [7, 18-20]. The study hypotheses were that: a) women would seek more information regarding medical and psychosocial aspects of infertility and more emotional support by the hospital staff b) would perceive the participation in support groups as the most important psychosocial service and c) the selected factors would influence women's expectations and perceived importance of professional psychosocial services.

Materials and Methods

This study is a descriptive, cross-sectional survey, which involved collecting data from the participants by using two questionnaires.

Study setting: The study took place in a public fertility clinic in Athens, Greece. This clinic is one of the largest clinics in Greece and covers many geographical regions (capital city and some rural areas). The staff working in infertility clinics in Greece include obstetricians, midwives, laboratory personnel and secretaries. There are no psychologists, social workers or sex therapists employed at public IVF clinics. Psychological counselling regarding infertility and fertility treatment is not mandatory in Greek public clinics.

Study procedure and participants: A random sample of infertile women undergoing fertility treatment in the fertility clinic of the hospital recruited. According to the inclusion criteria, the participants chosen: a) were able to read and write in the Greek language in order to have the ability to complete the questionnaires, b) were married, and c) have unsuccessfully tried to conceive a child with natural methods for more than one year. Eligible participants received an envelope immediately before their fertility treatment. The envelope contained an information letter which explained the aim and expected benefits of the study and two questionnaires. The questionnaires were returned to the researcher who was not an employee of the clinic. Data were collected over an 11-month period, from November 2005 to September 2006. During the recruitment period, 452 women were asked to participate in the study. A total of 410 women (90%) agreed to take part and finally 404 women (89%) returned completed questionnaires.

Study instruments: The research instruments were two self-administered questionnaires. The participants completed the COMPI questionnaire which was developed and validated by Schmidt *et al*. [14] and the Greek version of the State-Trait Anxiety Inventory (STAI) questionnaire [21].

The STAI was used to measure anxiety in women undergoing fertility treatment. The STAI assesses both 'state' and 'trait' anxiety. *State anxiety* is defined as an unpleasant emotional condition that emerges in case of threatening demands or dangers. *Trait anxiety,* on the other hand, reflects the stable tendency of an individual to respond with state anxiety in the anticipation of threatening situations. The state scale consists of 20 items that ask people to describe how they feel at a particular moment in time rated on a 4-point scale ranging from *not at all* to *very much so*. The trait scale consists of 20 statements describing how people generally feel (e.g., confident) rated on a 4-point frequency scale ranging from *almost never* to *almost always*. Total scores for state and trait anxiety range from 20 to 80 [22], whereas the published normative score by non-pregnant women for state anxiety it is 35.2 (SD 10.6), for trait anxiety it is 34.8 (SD 9.2) and for people with diagnosed anxiety disorder it ranges between 47 and 61 [22]. The COMPI questionnaire was adapted from a previous Danish study [14, 23]. Details about the development of this measure are available in other studies [14, 24]. However, some information about the COMPI questionnaire is also presented in this article. The COMPI questionnaire booklet contains questions about reproductive history, psychosocial aspects of infertility (including fertility problem stress, ways of coping, communication and social relations), health and well being. Only those questions relevant to the present study are described in this article.

A total number of 14 items from the COMPI questionnaire were used to assess sociodemograpfic profiles of participants. Sociodemographic background information included variables concerning age, years of marriage, occupational social position and educational level. Education level is described by three categories: low, medium and high. Low education level includes

primary education, medium education level refers to secondary education and high educational level to university/polytechnic school degree or higher. A measure of occupational social position was used. Based on this measure, social position was recoded into three levels: from social class I (high level) to social class III (low level). High social level includes professionals and executives, medium social level refers to white-collar employees and skilled workers, and low social level to all unskilled workers and participants supported by the Social Benefit Program. Medical background information included information regarding duration of infertility, former children, diagnosis of infertility and past fertility treatment. A total number of 16 items from the COMPI questionnaire were used to measure fertility problem stress. Fertility problem stress was measured by using three subscales referring to personal, social and marital domains. These subscales are described in detail by Schmidt *et al*. [14]. Infertility-related stress in the personal domain (subscale of six items) reflected the stress that infertility had produced on the person's physical and mental health. Infertility-related stress on the social domain (subscale of four items) assessed the extent to which infertility had caused strain on social relations with friends, family and colleagues. Infertility-related stress on the marital domain (subscale of four items) assessed the stress that infertility had produced on the marital and sexual relations. The response categories from the subscales of personal stress, social stress and two items from marital stress was a four-point Likert response scale from (1) none at all to (4) a great deal. The response categories from the remaining two items of marital stress were a five-point Likert response scale from (1) strongly disagree to (5) strongly agree. The range differed according to the subscale: personal stress (range 0-20), social stress (range 0-12) and marital stress (range 0-14). Total scores were calculated by summing the relevant items. Higher scores indicated higher personal, social and marital stress. Four items assessed the importance of medical care, four items assessed the importance of patient-centred care and four items assessed the importance of a provision of professional psychosocial services. The responses for all items about importance ratings were (1) important, (2) less important and (3) not important. Although importance ratings were rated on a 3-point scale, they were finally dichotomized (important versus less important and not important) for statistical analysis purposes. Reliability of the subscales of COMPI questionnaires were assessed by Cronbach's alpha. In this study the alpha coefficient was 0.71 for the personal stress subscale, 0.70 for the social stress subscale and 0.72 for the marital stress subscale. These values were within acceptable limits. The psychometric properties of the STAI questionnaire have been evaluated and it has been demonstrated that the STAI questionnaire is a reliable and valid measure.

Translation and questionnaire pilot: The questionnaires in English were translated into Greek by two independent bilingual persons and then translated back to English by two other bilingual persons. After the translation was conducted, the researcher checked the translation in order to minimize misunderstandings concerning especially the terminology. In this study, the questionnaires were piloted using cognitive interviewing methods with the objective of examining the understanding of the questions, in order to eliminate any ambiguities in questions and to predict the timing for completion. The sample of the cognitive testing consisted of 40 women with different demographic characteristics to ensure the representation of the main sample. The returned questionnaires were fully and appropriately completed and the response choices were adequate and understandable.

Ethical considerations: Permission to complete this study was obtained from the ethical and scientific committee of the hospital. The researcher approached each participant who wanted to participate in the study. Participants were given the opportunity to ask for clarification and were assured about the anonymity and confidentiality of their responses and about their right to withdraw at any time, even if they decided to take part in the study. Participants were also assured that the collected data would be used only for the purpose of the study. The clinic staff did not know whether or not a woman participated in the study. It was assumed that completing the questionnaires equated with consent.

Statistical analysis: Quantitative data were analyzed using SPSS version 13.0. Data analysis involved descriptive statistics to calculate percentages, frequencies, means and standard deviations. Logistic regression analysis was used to determine the predictors of importance ratings (important versus less important and not important) and of intentions to use psychosocial services (yes versus other responses). The predictor variables that were used for each logistic regression analysis were: age, social class, infertility duration, number of previous therapies, infertility etiology, personal stress, social stress, marital stress, marital benefit, state anxiety, trait anxiety, friend and family support. The anxiety- and fertility-related stress scores were entered into the regression analysis as continuous variables. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from the logistic regression analysis for each predictor variable.

Results

Characteristics of participants: During the recruitment period, 452 women were asked to participate in the study and finally 404 (89% response rate) completed the questionnaires. The mean age of participants was 36.9 years (SD 4.1 and range 25-47). Thirty-six percent of women had tertiary education (high educational level), 48% of women had high school education (medium level) and 16% of women had less than a high school education (low level). Most women (72%) were working and 28% were housewives. Forty-nine percent of women had high social class, 27% had medium social class and 13% had low social class. Participants reported a mean duration of infertility of two years (SD 0.9 years) and a mean number of previous treatments of 2.4. The majority of participants (88%) had prior experience with fertility treatment. Diagnosis of infertility was recoded into female infertility, male infertility, mixed infertility (both female and male infertility) and idiopathic infertility (unknown etiology). One hundred and two women had female factor infertility, 150 women had male factor infertility, 90 women had combined infertility and 62 women had unknown factor infertility. Table 1 shows the sociodemographic, medical and treatment characteristics of the participants.

Descriptive results

Expectations about medical and patient-centred care: Almost all women rated receiving medical information (test results and potential treatment options) from medical staff as important and only 35% of women rated receiv-

Table 1. — *Sociodemographic, medical, and treatment characteristics of the participants.*

Participant characteristics	Participants (n = 404)
Sociodemographic characteristics	%
Age (years)	
≤ 30	6.0
31-35	27.0
≥ 35	67.0
Occupational social class	
High	49.0
Medium	27.0
Low	13.0
Outside classification	11.0
Medical characteristics	
Diagnosed female infertility	25.0
Diagnosed male infertility	37.0
Diagnosed mixed infertility	22.0
Unknown infertility factor	16.0
	Mean (SD)
No. of previous treatments, mean (SD)	2.4 (2.0)
Duration of infertility, mean (SD)	2.1 (0.9)

ing information about adoption as important. The majority of women found it important to receive written information. The vast majority of women found the provision of patient-centred care important and specifically women sought the offer of emotional support (concern and understanding) by the hospital staff and a provision of written information about psychosocial aspects of infertility as important. Although 76% of women stated that a provision of information about psychosocial aspects of infertility would be important, only 30% of women stated that a provision of information about associations which support infertile couples was important for them. Table 2 shows the expectations about medical and patient-centred care that was rated as important (versus less important and not important) by women.

Table 2. — *Reasons for seeking treatment and expectations about medical and patient-centred care rated as important by women (n = 404).*

Variable	Women (%)
Reasons for seeking treatment	
To find a cause	31
To get pregnant	99
To have a child	86
For having tried everything	50
For my self	41
For my partner	37
Expectations about medical care	
Offer information for test results	87
Offer information for treatment options	95
Offer written information (leaflets)	81
Offer information about adoption	18
Expectations about patient-centred care	
Show more concern	83
Show understanding	90
Offer written information about psychocosial aspects of infertility	76
Offer contact information for infertility associations	30

The importance of a provision of professional psychosocial services: Women were asked to rate the importance of specific professional psychosocial services that were not offered at the fertility clinic at the time of data collection. The proposed psychosocial services were: participation in seminars about infertility, participation in support groups, and attendance in sessions with psychologists and with sex therapists. Table 2 shows the proportion of women who rated the provision of professional psychosocial services as important and the proportion of women who stated that they would participate if these services were available at the fertility clinic. From our results it was demonstrated that less than half the women rated a provision for psychosocial services as important.

Anxiety- and fertility-related stress: It was found that the mean level of participants' state anxiety was 44.5 (SD 9.5) and the mean level of trait anxiety was 41.8 (SD 7.1). These were higher in comparison to published normative scores of state and trait anxiety (mean 35.2 and 34.8, respectively) [22]. Evaluating the results of this study within the ranges for low and high levels for each subscale of fertility problem stress as suggested by Schmidt *et al*. [14, 20], the levels of personal (range 0-20, mean 7.95), social (range 0-12, mean 1.9) and marital stress (range 0-14, mean 3.1) were low.

Predictors of women's expectations for medical care, patient centred care and psychosocial services: Logistic regression analysis was computed in order to examine whether demographic (age, social class), medical (duration and etiology of infertility, number of previous therapies and duration of therapy) and psychosocial (state and trait anxiety, personal, social and marital stress and marital benefit, social support) variables were associated with women's expectations for medical care, patient-centred care and psychosocial services.

Table 3. — *Expectations and intentions to use professional psychosocial services by women (n = 404).*

Variable	Women (%)
Consideration of professional psychosocial services as important	
Participation in seminars about infertility	37
Participation in support groups	44
Consultation with psychologist	41
Consultation with sex therapist	19
Intention to use professional psychosocial services	
Seminars about infertility	34
Support groups	42
Psychologist	36
Sex therapist	17

Table 3 illustrates the women's expectations about medical and patient-centred care that are provided in the fertility clinic. The major findings that have emerged concerned the provision of medical and psychosocial information, staff's supportive attitude and the provision of information concerning adoption. In most cases, higher levels of stress and anxiety and lower social support were

Table 4. — *Odds ratios for demographic, medical, and psychosocial predictors of importance ratings for medical and patient-centred care*.

Predictors	*Medical care*					*Patient-centred care*	
	Information for results	Written information	Adoption information	Staff concern	Staff understanding	Psychosocial information	Infertility associations
Demographic							
Age	1.08 (0.98-1.20)	1.02 (0.94-1.10)	**1.08 (1.01-1.15)**	1.04 (0.95-1.14)	1.11 (0.99-1.25)	0.93 90.86-1.00)	0.97 (.01-1.03)
Social class I	0.64 (0.16-2.51)	1.25 (0.76-1.78)	0.78 (0.32-1.85)	1.78 (0.48-6.58)	1.05 (0.92-1.08)	2.35 (0.71-7.82)	1.65 (0.70-3.84)
Social class II	1.40 (023-8.63)	0.95 (0.88-1/04)	2.12 (0.80-5.60)	1.85 (0.45-7.56)	0.78 (0.54-1.34)	2.02 (0.56-7.25)	**3.13 (1.23-7.99)**
Social class III	2.53 (0.38-16.7)	1.43 (0.95-2.34)	**10.35 (1.88-56.7)**	**7.84 (1.79-34.2)**	0.89 (0.76-1.55)	**4.24 (1.07-16.6)**	1.01 (0.35-2.91)
Medical							
Infertility duration	**0.73 (0.60-0.89)**	1.04 (0.96-1.14)	0.96 (0.90-1.03)	**0.85 (0.74-0.99)**	0.93 (0.80-1.07)	0.96 (0.87-1.06)	1.05 (0.97-1.13)
No of therapies	1.00 (0.79-1.27)	0.85 (0.71-1.02)	**0.87 (0.76-0.99)**	**0.74 (0.58-0.94)**	0.80 (0.61-1.04)	**0.82 (0.68-0.99)**	**0.75 (0.65-0.87)**
Duration of therapy	0.99 (0.59-1.66)	**0.56 (0.34-0.91)**	1.25 (0.92-1.70)	0.84 (0.48-1.47)	0.87 (0.52-1.47)	0.95 (0.68-1.33)	**2.00 (1.34-2.99)**
Female infertility	0.73 (0.29-1.85)	1.72 (0.35-1.67)	0.49 (0.18-1.32)	1.78 (0.34-9.13)	0.49 (0.18-1.38)	**6.31 (1.20-32.9)**	0.62 (0.22-1.75)
Male infertility	1.02 (0.61-1.08)	0.99 (0.72-1.36)	0.59 (0.21-1.59)	0.99 (0.18-5.31)	1.02 (0.92-1.10)	4.88 (0.93-25.3)	1.06 (0.37-3.03)
Mixed infertility	1.02 (0.99-1.06)	0.89 (0.73-1.08)	1.12 (0.34-3.72)	**8.85 (1.43-54.5)**	0.73 (0.59-1.85)	5.51 (0.88-34.6)	1.97 (0.60-6.44)
Idiopathic infertility	0.95 (0.33-2.75)	0.18 (0.22-1.58)	0.67 (0.45-1.34)	0.95 (0.24-1.67)	1.25 (0.95-1.76)	1.12 (0.76-1.87)	1.17 (0.67-1.35)
Psychosocial							
State anxiety	**1.12 (1.07-1.28)**	0.98 (0.93-1.02)	1.01 (0.98-1.05)	0.96 (0.91-1.05)	1.02 (0.97-1.10)	**1.08 (1.02-1.16)**	**1.06 (1.03-1.11)**
Trait anxiety	0.92 (0.83-1.01)	1.05 (0.95-1.07)	1.05 (0.95-1.05)	0.97 (0.91-1.04)	**1.19 (1.02-1.27)**	0.99 (0.93-1.05)	0.97 (0.92-1.02)
Personal stress	1.09 (0.96-1.25)	0.94 (0.85-1.05)	**1.11 (1.07-1.22)**	0.96 (0.85-1.08)	**1.15 (1.01-1.32)**	1.02 (0.93-1.13)	0.98 (0.89-1.07)
Social stress	**1.15 (1.08-1.20)**	**1.05 (1.02-1.11)**	0.90 (0.78-1.04)	1.02 (0.83-1.08)	**1.29 (1.12-1.43)**	**1.17 (1.06-1.29)**	0.96 (0.83-1.11)
Marital stress	**1.49 (1.20-1.86)**	0.95 (0.79-1.14)	0.89 (0.77-1.03)	0.98 (0.81-1.19)	0.92 (0.71-1.19)	0.84 (0.70-1.01)	**1.17 (1.05-1.23)**
Marital benefit	**1.85 (1.36-2.53)**	1.01 (0.84-1.22)	**0.80 (0.66-0.96)**	**0.73 (0.59-0.90)**	1.01 (0.84-1.22)	1.04 (0.86-1.25)	0.91 (0.77-1.08)
Family support	1.00 (0.65-1.55)	0.58 (0.38-1.23)	1.09 (0.80-1.46)	0.76 (0.50-1.15)	0.97 (0.63-1.47)	0.67 (0.46-0.98)	1.00 (0.75-1.31)
Friend support	**0.34 (1.19-0.61)**	**1.58 (1.06-2.36)**	**0.62 (0.45-0.85)**	**0.61 (0.41-0.91)**	**0.71 (0.45-0.88)**	**1.48 (1.03-2.12)**	**0.68 (0.50-0.92)**

Odds ratios with p value < 0.05 in bold.

associated with greater expectations regarding the provision of information and staff support. Higher importance ratings for medical information were observed among women with fewer years of infertility, higher state anxiety, higher social and marital stress, and lower support from friends. Moreover, higher importance ratings for psychosocial information were observed among women with female infertility, lower social class, smaller number of therapies, higher state anxiety and social stress and lower family support. It has also been found that lower social class, fewer years of infertility, higher levels of trait anxiety, personal and social stress, lower marital benefit and lower support from friends were the predictors of higher importance ratings for staff support. Older women of lower social class with fewer number of therapies, having higher levels of state anxiety, personal and marital stress, and lower levels of social support were more likely to attach importance to information regarding adoption and infertility associations. Table 4 illustrates the OR for women's perceived importance of psychosocial services in relation to the demographic, medical and psychosocial predictors. The major findings that have emerged concerned the perceived importance of sex therapist and psychologist consultations. In most cases, higher stress and lower social support were associated with higher importance ratings of psychosocial services. Higher importance ratings concerning infertility seminars were observed among women with higher personal stress and lower family support. Higher importance ratings of psychologist consultations were observed among women with higher state and trait anxiety, lower family or/and friend support and longer duration of infertility. The predictors of higher importance ratings of sex therapist consultation were low social class, male infertility factor, the higher state anxiety and higher marital stress. It was unexpectedly found that women with fewer therapies rated sex therapist counselling as important.

Discussion

The study has three limitations. Firstly, although the great efforts to be comprehensive and to appraise all predictors of women's expectations, it is possible that related domains were omitted. The second limitation of the current study is that it involves only one public hospital in Athens. A further limitation of the study is that the sample consisted of only patients who did not have to pay for their treatment. It is possible that patients who attend private clinics have different expectations of services. Our findings need to be replicated in samples from private clinics. The study has several strengths: the questionnaires that were used in this study were evaluated and it was demonstrated that they are reliable and valid measures, the response rate was high (97%) ensuring a large sample size (n = 404), all items in the questionnaires were answered by almost all participants and all questionnaires were validated through pilot studies. These strengths ensure the reliability of study findings.

The cardinal findings of our study showed that most women sought more medical information (both written and verbal) and desired more emotional support which is offered mainly by the hospital staff and not by external sources (associations). From our results it was also demonstrated that less than half of the women rated the provision of psychosocial services as important. Similarly, in a recent study [5], in patients who did not

receive counselling, the main reasons cited were: 'felt I can cope on my own' (37%), and 'did not think it would be beneficial' (15%).

In our study, the main predictors of importance ratings of psychosocial services were the high fertility-related stress and the low provision of social support. Other factors, such as women's social class, etiology of infertility and infertility duration were also associated with women's expectations.

The hypothesis that women would seek more information regarding medical and psychosocial aspects of infertility was supported by this study. Many women expressed their need for an information provision. Almost all women asked for more medical information (test results and alternative therapies) and 76% of them expressed their need to receive more information about psychosocial impact of infertility. Therefore, it can be hypothesized that women's expectations regarding an information provision were not fulfilled. This could influence the degree of their satisfaction concerning fertility treatment. Previous studies have also reported low levels of satisfaction about information given to infertile couples [6, 8, 12, 13, 25]. Laffont and Edelmann [7] in their study reported that the use of information booklets about the practical and psychological aspects of IVF, improved acceptability of infertility treatment and care as well as patient knowledge. Results of this study are in keeping with the above findings, as respondents stated their need for a provision of written information and pamphlets about the medical and emotional consequences of childlessness. A provision of information was particularly important to women with higher levels of anxiety and fertility-related stress, lower social support, lower social class and infertility due to female factors. This finding was expected since women who experienced extensive infertility stress, did not get enough social support, and had lower social class seem to need more information in order to cope with infertility stress.

The hypothesis that women would seek more emotional support by the hospital staff was supported by the findings of the study. The results demonstrate that almost all participants, expected the medical and nursing staff of the fertility clinic to have a supportive attitude towards them. They wished that the hospital staff would ask about their feelings and show understanding as has been previously found [9, 15, 26, 27]. It has also been found that the supportive attitude of staff was important for those women who were of lower social class, had fewer years of infertility, and experienced higher levels of anxiety and fertility-related stress and lower marital benefit and social support. This finding was expected since women who did not get enough social and marital support and experienced extensive infertility burden seem to need more staff support in order to cope with infertility strains. One key predictor of adjustment to fertility treatment is the strength of the marital relationship, probably because of the need of support among spouses. It has been suggested that medical and nursing staff may be called upon to provide this support when there is marital strife [28]. Hirsch and Hirsch [29] found that people experience more support as their period of childlessness increases. Possibly, in the longer term involuntary childlessness people have learned how to deal with their infertility and how best to involve their social environment in that situation. Therefore, infertile women with shorter duration of infertility may ask for more staff emotional support because they have not learned how to involve their social environment into their fertility problem. Social support seems to have a protective effect, resulting in less clinical distress [30, 31]. In a recent study, it was shown that infertile couples seeking psychological help are characterized by high levels of psychological distress, primarily in women and that the women's distress seems to be more important for attending infertility counselling than that of the men [32].

It is noteworthy that although the vast majority of women felt that it was important to have a patient-centred approach in the fertility clinic, less than half the women rated the provision of psychosocial services as important. When participants were asked about the perceived importance of psychosocial services, 44% of them rated participation in support groups as important, 41% consultation with psychologists, 37% participation in infertility seminars and 19% consultation with a sex therapist. While findings with respect to women's importance ratings were consistent with those of previous infertility studies [8, 9, 12, 14, 33], they were unexpected since great emphasis is given by the clinic staff on the provision of professional psychosocial services. This finding seems to suggest that it is possible to meet women's emotional and psychosocial needs without professional psychosocial services but through a supportive staff attitude. However, it has been found that even if women do not seek psychosocial support and counselling they are reassured to know that these services are available to them [7, 12, 34].

One possible explanation for the low importance ratings about a provision of professional psychosocial services could be that women may not consider themselves sufficiently distressed or they probably received enough social support from informal sources (family and friends) in order to cope with their fertility problem. Although infertility can be very distressing for women, external support from informal sources (family and friends) could mitigate emotional and psychological burdens such that only a few women will need professional psychosocial care [33]. The predictors of importance ratings for professional psychosocial services were similar to those of patient-centred care and as expected were linked to high levels of anxiety and fertility-related stress, low grade of family and social support and low social class. These data suggest that anxiety, stress and social support mainly determine who will ask for professional psychosocial support. Therefore, it could be concluded that women may not consult with psychologists/counsellors when the support that they receive from their own network of family and friends is sufficient for the level of stress they experience. As Boivin [18] suggested, patients consult psychologists because they cannot manage their distress and not because they experience it. This finding is in

accordance with findings of comparative research [7, 14, 16, 19] in which it has been found that women attending support groups or/and counselling experience more fertility-related stress and less social support.

The hypothesis that women would perceive participation in support groups as the most important psychosocial service was supported by this study. The most preferred professional psychosocial service was participation in a support group. The group format seems to be beneficial for a number of reasons. People who experience the same problems understand each other better than anyone else. The main advantages of support groups, the common experience, and the exchange or sharing with other people with fertility problems have been reported through other studies as well [35]. No one can better understand your experiences than people having similar problems. In this type of intervention people make themselves feel better by seeing their problem as not being as bad as the problem of other infertile people [19].

Participants of advanced age and not adequately supported by their family and friends were more likely to rate participation in seminars, support groups and psychologist consultations as important. These findings were expected and suggest that the level of stress and anxiety partly determines who will participate in seminars, support groups and psychologist consultations. If childless people are not getting any social support or if they are dissatisfied with the support given, this may result in even more distress [36] and consequently may lead them to participate in support groups and psychologist consultations.

Low social class, male fertility factor, higher state anxiety and higher marital stress were predictors of higher importance ratings of sex therapist consultation. It seems that male factor infertility is more stressful for couples compared with the diagnosis of female infertility [37] and consequently increases participants' need to participate in consultations with a sex therapist. This finding was expected since the Greek society that places great emphasis on male fertility and manhood. However, in couples undergoing assisted reproductive treatment, men only reported marginally elevated depression scores compared to their controls [38].

It can be hypothesized that couples of high social class are usually well educated and can have more frequent and deeper discussions between partners about the intimate aspects of their relationship as a couple.

Conclusions

Several recommendations can be made on the basis of the findings from this study. Clinics could offer information regarding medical and psychosocial aspects of infertility and could increase women's desire to 'take home' information by providing patients with pamphlets, booklets and other formats with information [33]. A provision of clear and sufficient information on the medical and psychosocial aspects of fertility treatment is fundamental for women to be able to make informed decisions about fertility treatment. It is also recommended that information be provided repeatedly through the course of fertility treatment and not only at the beginning as it has been found that the ability to retain information varies significantly, and that information processing may be restrained by anxiety [39]. Health care professionals should dedicate more time to informing women who experience high infertility stress, and provide appropriate and understandable information tailored to the educational level of women. Such information has to be delivered in a sensitive way. Complicated medical terms unfamiliar to patients may confuse them and contribute to their stress. Based on the results of the present study, it can be recommended that the staff of a fertility unit must approach their patients in a supportive way. The team should be prepared to provide psychosocial care at each step of the fertility therapy. During treatment infertile people may feel the need of support towards continuing treatment (keeping hope of success, not giving up). Patient-centred care is the psychosocial care that must be provided by all members of medical and nursing staffs, as a part of their routine services at a fertility clinic. Conversely, psychological interventions based on definite theoretical frameworks (counselling) should be used, and trained mental health professionals should be the providers. Both types of care are essential and should be equally offered to all patients. Clinics could adopt a two-tier approach to psychosocial services aiming to provide written information to less distressed patients and counselling to more distressed patients [18]. Written psychosocial documentation and emotional support by hospital staff may meet the needs of most less distressed patients but may not be sufficient for the more distressed patients [33]. In such cases the assistance of professionals trained in infertility counselling and psychology should be enlisted. On the other hand, developing and evaluating different options such as education of fertility clinic staff in the psychosocial field can partly meet the psychosocial needs of less distressed patients. However, fertility clinic staff must recognize their limitations and try to avoid discussing subjects outside their competence. Another issue seems to be related to the question of who would perceive the use of professional psychosocial services as important. High levels of fertility-related stress may not be the best predictor of professional psychosocial needs, as stress is expected in response to infertility and fertility treatment [40]. It would seem that women with poor coping resources (internal and external) are not able to cope with the distress they experience. Such patients would be more likely to use professional psychosocial services. Fertility clinics must be proactive in identifying patient needs and fulfill them in the most appropriate way. However, clinics should be aware that patients might hesitate to use professional psychosocial services, even if they recognize the need for them. Concerns about privacy, fears that they may be perceived as emotionally and/or mentally unstable, impotent or abnormal in some way if they consult a counsellor/therapist may prevent infertile people from using professional psychosocial services. Therefore,

counsellors need to make every effort to contact such patients individually [33].

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Comparison of bolus remifentanil-propofol versus bolus fentanyl-propofol for dilatation and sharp curettage

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Summary

Background and Objective: The study was conducted to determine whether bolus administrations of remifentanil-propofol could provide adequate analgesia and similar patient comfort with a faster recovery profile compared with bolus administrations of fentanyl-propofol during dilatation and sharp curettage. *Methods:* The patients were randomized to a remifentanil group (n = 36) or fentanyl group (n = 36). The remifentanil group received an IV bolus dose of 1 μg kg^{-1} remifentanil. The fentanyl group received an IV bolus dose of fentanyl 0.5 μg kg^{-1}. The Verbal Pain Scale (VPS), modified Aldrete scores, blood pressure, heart rate, peripheric oxygen saturation, recovery time from anesthesia and adverse events during or after surgery were evaluated. *Results:* The groups were found to be similar in duration of the surgical procedure, anesthesia time and hemodynamic variables and VPS scores. Patients in the remifentanil group recovered from anesthesia earlier. Modified Aldrete scores were higher in the remifentanil group at 5 and 10 min postoperatively. The frequency of perioperative adverse events did not differ significantly between the groups. *Conclusions:* Bolus injections of remifentanil appear to be a safe and effective alternative to fentanyl, producing faster recovery in providing analgesia during dilatation and sharp curettage procedures.

Key words: : Remifentanil; Fentanyl; Dilatation and Curettage; Propofol.

Introduction

Attaining a faster recovery time from anesthesia is extremely important for brief outpatient surgical procedures such as dilatation and sharp curettage. Dilatation and sharp curettage, a short-lasting procedure, is one of the most frequently performed gynecological surgical procedures. This procedure is performed for the diagnosis and treatment of endometrial and intrauterine disorders. Patients are day-case patients who are usually discharged and able to return to their routine daily activities after a brief hospital rest. Despite its shortness, the procedure generally causes considerable pain due to cervical dilatation that is usually performed by Hegar dilators and tissue extraction. The procedure therefore necessitates rapid-acting intense analgesia [1, 2].

Opioids are generally used during the dilatation and sharp curettage procedure [3, 4]. Fentanyl has generally been used as the first choice to provide analgesia [5, 6]. Remifentanil, which has recently gained popularity, may be a good alternative to fentanyl for dilatation and sharp curettage since remifentanil is a relatively new and ultra-short-acting drug with a half life of 9-11 min and may provide a faster recovery profile [7]. Remifentanil has an ester linkage that makes its metabolism unique compared to other opioids since it is metabolized by blood and tissue esterases [8], independent of hepatic and renal function which may make it also suitable for hepatic and renal patients [9-11].

Therefore the aim of the current prospective randomized study was to determine whether bolus administration of remifentanil-propofol could provide adequate analgesia and similar patient comfort with a faster recovery profile when compared with bolus administration of fentanyl-propofol during dilatation and sharp curettage.

Material and Methods

This prospective study was performed at GATA Academic Military Hospital and Adnan Menderes University Hospital. Women undergoing dilatation and sharp curettage aged 18-60, whose ASA physical status were I or II were asked to participate in the study.

Participation was on a voluntary basis. All participants gave their informed consent. The study protocol was approved by the local ethics committee. Patients with pulmonary, hepatorenal, neuromuscular and neuropsychiatric disease, morbid obesity, and patients undergoing emergency curettage for massive bleeding or hemodynamic instability were excluded from the study. Patients who were unable or refused to give informed consent were also excluded from the study. All patients had undergone dilatation and curettage procedures for evaluation of abnormal uterine bleeding.

Subsequent to transfer to the operating room and before anesthetic induction, IV cannulae were inserted and standard monitoring was initiated, consisting of a five-lead ECG, noninvasive blood pressure, pulse oximetry. Patients were placed supine on the gynecological table with their legs in stirrups. They were randomized to the remifentanil group (Ultiva; GlaxoSmithKline, The Upjohn Company, Belgium) (n = 36) or the fentanyl group (Fentanyl Citrate, USP 50 mcg/ml; Abbott Laboratories, North Chicago, USA) (n = 36) by using a computer-based random number generator program. The remifentanil group received an IV bolus dose of 1 μg kg^{-1} remifentanil over a period

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of 30 sec whereas the fentanyl group received an IV bolus dose of fentanyl 0.5 ug/kg. After obtaining baseline measurements, we administered 1 mg kg^{-1} of lidocaine IV to minimize the burning that accompanies administration of propofol. Then, anesthesia was induced with propofol (Propofol 1% Fresenius, Fresenius Kabi, Australia GmbH]) 2 mg/kg in both groups. Anesthesia was maintained with 60% nitrous oxide (N_2O) in oxygen with a fresh gas flow of 4 l min^{-1} through a facemask. N_2O was discontinued when the gynecologist declared the dilatation and curettage procedure finished.

After the operation, the surgeons were questioned about their subjective evaluation of surgical working conditions during dilatation and sharp curettage (0 = not satisfied, 1 = satisfied, 2 = extremely satisfied). In addition, the patients were questioned at discharge about their anesthetic experience (0 = not satisfied, 1 = satisfied, 2 = extremely satisfied). Recovery of the patients was evaluated using the modified Aldrete scoring system [12].

A verbal pain scale (VPS) was used to evaluate pain intensity, with scores of 0 (no pain), 1 (light pain), 2 (moderate pain), 3 (severe pain). VPS scores were evaluated 5 and 10 min postoperatively. Modified Aldrete scores were evaluated 5 and 10 min postoperatively. VPS scores were queried by a nurse blinded to the opioid administered. Diclofenac sodium (Miyadren 75 mg, Fako Drug Company, Istanbul, Turkey) was administered IM to patients with a score > 1. Blood pressure, heart rate, and oxygen saturation were recorded just before the administration of fentanyl or remifentanil (preinduction), 5-10 min after induction, and five and ten minutes after the end of the dilatation and curettage procedure. Duration of the surgical procedure, duration of anesthesia, awakening time (time from end of the discontinuation of N_2O to spontaneous eye opening), orientation time (time from end of the discontinuation of N_2O to the time the patient is able to recall name and date of birth) and also time from end of the discontinuation of N_2O to the patient's responding to verbal comments were recorded. Also recorded were the frequency of the adverse events during or after surgery (e.g., episodes of nausea, vomiting).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 14.0; p values < 0.05 were considered significant. Data are presented as mean ± standard deviation (SD). Parametric continuous variables were analyzed using the Student's t-test. Differences between categorical variables were analyzed with the chi-square test and Fisher's exact test. Based on a previous study, a priori power analysis was performed using two-sided analysis with an (alpha) error of 0.05 and a power of 0.8 to detect a difference of 60% for recovery times. Thirty patients were calculated to be needed for each group. Assuming possible dropouts, the sample size was increased to 36 patients per group.

Results

Seventy-two women undergoing dilatation and sharp curettage procedures were included in the study. Both the remifentanil and fentanyl group consisted of 36 patients each. Both groups were found comparable in terms of patient characteristics (Table 1).

No statistically significant difference was found between the groups (Tables 1 and 2) in terms of ASA physical status, duration of the surgical procedure, anesthesia time and hemodynamic variables throughout the study period.

Table 1. — *Patient characteristics of the groups*.

	Group R (n = 36)	Group F (n = 36)
Age (years)	40.6 ± 9.5	40.3 ± 10.4
Weight (kg)	70.3 ± 10.3	67.9 ± 10.9
Height (cm)	162.4 ± 10.1	160.9 ± 11.2
ASA physical status (I/II)	28/8	29/7
Duration of surgery (min)	6.7 ± 0.8	6.5 ± 0.9
Duration of anesthesia (min)	8.3 ± 0.6	8.5 ± 0.5

Data are means ± SD, or number of patients.
There were no statistically significant differences between the groups.
R = remifentanil; F = fentanyl; ASA: The American Society of Anesthesiologists.

Table 2. — *Hemodynamic parameters of the groups at selected time points*.

	Preinduction	5 minutes after induction	10 minutes after induction	5 minutes postoperative	10 minutes postoperative
HR (bpm)					
Group R	83.0 ± 9.8	68.9 ± 9.6	72.2 ± 8.7	78.8 ± 5.8	75.2 ± 12.9
Group F	83.4 ± 9.4	72.3 ± 7.4	72.5 ± 7.0	80.5 ± 7.5	71.3 ± 9.5
SAP (mmHg)					
Group R	131.4 ± 18.6	118.7 ± 19.1	116.7 ± 16.7	117.1 ± 10.7	123.8 ± 23.5
Group F	130.9 ± 17.3	120.2 ± 17.4	119.5 ± 20.1	119.0 ± 23.9	129.3 ± 19.2
DAP (mmHg)					
Group R	75.9 ± 14.5	70.9 ± 13.1	70.9 ± 10.1	80.5 ± 18.8	76.8 ± 12.4
Group F	82.6 ± 14.2	75.6 ± 12.6	75.4 ± 7.9	78.8 ± 20.9	82.3 ± 12.8
MAP (mmHg)					
Group R	95.8 ± 14.3	84.7 ± 15.2	82.5 ± 12.5	85.8 ± 8.0	94.3 ± 18.0
Group F	99.7 ± 16.8	89.7 ± 13.2	86.3 ± 13.4	86.7 ± 7.5	98.3 ± 13.3
SpO_2					
Group R	99 ± 1	99 ± 1	99 ± 1	98 ± 2	98 ± 2
Group F	98 ± 2	99 ± 1	98 ± 2	98 ± 2	98 ± 2

Data are means ± SD.
There were no statistically significant differences between the groups.
R = remifentanil; F = fentanyl; Bpm = beats per minute; MAP = mean arterial pressure; DAP = diastolic arterial pressure; SAP = systolic arterial pressure; HR = heart rate; SpO_2 = peripheric oxygen saturation.

VPS scores after the operation did not differ significantly between the groups (Table 3). Modified Aldrete scores were higher in the remifentanil group both 5 and 10 min postoperatively (Table 3).

Patients in the remifentanil group recovered from anesthesia earlier. Awakening time, orientation time, and time from the end of anesthesia to response to verbal commands for the patients enrolled in the study was significantly shorter in the remifentanil group compared with the fentanyl group (Table 3). Both gynecologists and patients in the remifentanil group expressed similar satisfaction as compared with the fentanyl group. The frequency of perioperative adverse events (e.g., episodes of nausea, vomiting) did not differ significantly between the groups (Table 4).

Discussion

Dilatation and sharp curettage, among the most frequently performed gynecological surgeries, is a short procedure. In the current study, mean operation time for dilatation and sharp curettage was 7 min, which is within the 9-11 min [13] systemic half-life of remifentanil. Even though it is a short procedure, the pain related with dilatation and sharp curettage is generally considerable due to

Table 3. — *Verbal pain scale (VPS), modified Aldrete scores of subjects at selected time points, recovery profiles of subjects and satisfaction scores of both subjects and gynecologists.*

	Group R (n = 36)	Group F (n = 36)	*p*
Pain score (VPS)			
PO 5 (min)			
0/1/2/3	28/7/1/0	30/6/0/0	ns
PO 10 (min)			
0/1/2/3	26/8/2/0	28/6/2/0	ns
Modified Aldrete score			
PO 5 (min)	9.5 ± 0.8	7.4 ± 1.6	0.001
PO 10 (min)	10.1 ± 0.6	9.2 ± 0.9	0.001
Satisfaction scores of patients			
Very satisfied (2)	32 (88.8%)	32 (88.8%)	ns
Satisfied (1)	4 (11.2%)	4 (11.2%)	ns
Not satisfied (0)	0 (0%)	0 (0%)	
Satisfaction scores of gynecologists			
Very satisfied (2)	31 (86.1%)	32 (88.8%)	ns
Satisfied (1)	5 (13.9%)	4 (11.2%)	ns
Not satisfied (0)	0 (0%)	0 (0%)	ns
Recovery times			
**Time to spontaneous eye opening (min)	2.0 ± 0.6	4.3 ± 1.1	0.01
**Time to responding to verbal comments (min)	3.4 ± 0.9	5.9 ± 1.1	0.01
**Orientation time (min)	4.9 ± 1.2	8.6 ± 1.2	0.01
Analgesic Requirement (n)	10	8	ns

Data are means ± SD, or number of subjects.
* $p < .05$ (significant difference), ns: not significant.
R = remifentanil; F = fentanyl; PO = postoperative; VPS = verbal pain scale.
** Calculated from discontinuation of nitrous oxide.

Table 4. — *Perioperative adverse events.*

	Group R (n = 36)	Group F (n = 36)
Nausea/Vomiting	1/1 (2.8%)	1/1 (2.8%)
Hypotension (SAP < 90 mmHg)	3 (8.3%)	2 (5.6%)
Bradycardia (HR < 50 bpm)	4 (11.1%)	3 (8.3%)

Data are means ± SD, or number of patients.
There were no statistically significant differences between the groups.
R = remifentanil; F = fentanyl; bpm = beats per minute; HR = heart rate.

cervical dilatation and tissue extraction [14]. Bolus doses of remifentanil, with its short half-life and rapid action, appears to be a good candidate for intraoperative analgesia during such short procedures as dilatation and curettage. Remifentanil infusion for such procedures has been shown to be effective and safe [15]. However when compared with the easy use of bolus fentanyl, remifentanyl infusions necessitated setting up an infusion pump which was not practical for such a brief procedure. Remifentanil in bolus administrations would eliminate the need for setting up an infusion pump apparatus for such a brief surgical procedure, thus making the procedure easier and simpler. However, there is limited data concerning the use of bolus-dose remifentanil. Bolus-dose remifentanil has been studied and found useful in various limited clinical settings, such as preventing unwanted hyperdynamic cardiovascular response during laryngoscopy, intubation, and craniotomy procedures [16-21]. In gynecologic settings, Castillo *et al*. compared different bolus doses of remifentanil in dilatation and sharp curettage but did not compare bolus-dose remifentanil versus the standard drug, fentanyl [22]. To the best our knowledge, the present study is the first study that compares bolus doses of remifentanil with fentanyl for the dilatation and sharp curettage procedure.

We found in the current study that patients in the remifentanil group recovered from anesthesia earlier. Satisfaction scores for both patients and gynecologists were similar between the groups. Adverse effects reported perioperatively were similar in both fentanyl and remifentanil groups. In addition, patients in the remifentanil group reported higher modified Aldrete scores.

Hemodynamic responses in both groups were comparable in the present study. In both the remifentanil and fentanyl groups the frequency of hypotension and bradycardia was consistent with previous studies [22, 23]. Previous researchers have actually reported conflicting results for nausea and vomiting [24, 25] in the use of remifentanil. In the current study, only one case of nausea and vomiting was observed in the remifentanil group, an outcome which was found comparable to the fentanyl group. It should be taken into account in this context that in the current study, propofol, with its antiemetic effect, was co-administered with both remifentanil and fentanyl [26].

VPS scores after the operation did not differ significantly between the groups. Previous studies have recorded conflicting results regarding postoperative analgesic requirements in remifentanil-based intraoperative analgesia. Although some studies suggest a requirement for increased postoperative analgesia [27, 28], others have not found an increased analgesic demand in remifentanil-based intraoperative analgesia [29]. No significant difference in analgesic requirement was found between the groups in the current study. We think that for such a short surgical procedure, the administration of bolus injections of remifentanil is effective. Bolus injections of remifentanil have also been reported to be effective in providing analgesia for extracorporeal shock wave lithotripsy, a very painful procedure [16].

We have further found that awakening time, orientation time, and time of response to verbal comments after anesthesic gas is discontinued were shorter with remifentanil. Attaining faster recovery times from anesthesia is much more important for brief outpatient surgical procedures such as dilatation and sharp curettage. Moreover, patients in both the remifentanil and fentanyl groups expressed similar satisfaction scores. The satisfaction scores of the surgeons also did not differ between the groups and administration of bolus doses of remifentanil did not adversely affect the satisfaction scores of both patients and gynecologists during the dilatation and sharp curettage procedure.

In summary bolus administration of remifentanil would be a good alternative for dilatation and sharp curettage procedures for patients with hepatic and renal diseases as metabolism of remifentanil independent of hepatic and renal function. Moreover when compared with infusion of remifentanil, remifentanil in bolus

administrations would eliminate the need for setting up an infusion apparatus for the dilatation and sharp curettage procedure, thus making the procedure easier and simpler.

Conclusion

In conclusion, remifentanil provided faster recovery times with similar VPS scores and satisfaction scores for both patients and gynecologists. The analgesic requirement also did not increase with remifentanil. Thus, bolus injections of remifentanil appear to be a safe and effective alternative to fentanyl with faster recovery times in providing analgesia during the dilatation and sharp curettage procedures. Further studies are needed.

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Factors affecting maternal and perinatal outcomes in HELLP syndrome: evaluation of 126 cases

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Summary

Objectives: To ascertain the characteristics, clinical features, and maternal fetal outcome in HELLP (hemolysis elevated liver enzymes, low platelets) syndrome at a tertiary referral center. *Material and Methods:* This was a cross-sectional study carried out at Dicle University between January 2004 and December 2008 in which the charts of 126 cases were retrieved retrospectively and data analyzed descriptively. *Results:* Of all deliveries 0.9% were complicated by HELLP syndrome. Of the cases with HELLP syndrome 79 (62.6%) had preeclampsia, 28 (22.2%) had eclampsia and 19 (15.2%) had a diagnosis of HELLP syndrome. The values of significant biochemical parameters (mean ± SD) were documented as ALT (alanin aminotransferase) 224 ± 42 IU/l and ALT[1] (after birth) 140 ± 22, AST 379 ± 23 IU/l and AST[1] 215 ± 51, LDH (lactate dehydrogenase) 1418 ± 67 IU/l and LDH[1] 875 ± 16, together with the hematological parameters as platelet count (86 ± 12 K/Ul), urine protein (3 + in urine test stick) and albumin levels (2 ± 0.9 g/dl). Eighty-six (68.25%) of the patients required albumin replacement. Thirty-one (24.6%) cases were nullipara and 95 (75.4%) multipara; of which 32 women (25.4%) were in Class I, and 94 (74.6 %) in Class II of complete HELLP syndrome. Regular antenatal examination was accomplished in a very small number of patients (12.25%). Fifty-eight (46.03%) patients required transfusions with blood or blood products and 12 (9.5%) underwent laparotomy due to major intraabdominal bleeding. Magnesium sulphate to prevent convulsions and corticosteroids (12 mg betametazone) to enhance fetal lung maturity were administered. Forty-four (34.9%) cases had vaginal delivery and 82 (65.1%) cesarean section; another 18 (14.2%) were with in utero stillbirth. Fifteen babies (11.9%) died, 26 (20.63%) developed placental abruption, 14 (11.11%) acute renal insufficiency, and 13 (10.31%) postoperative subcutaneous hematomas. Maternal mortality occurred in ten cases (7.93%). *Conclusion:* HELLP syndrome is a pathology associated with a high incidence of maternal and perinatal complications. Laboratory parameters in cases with HELLP syndrome are not efficient in detecting perinatal results, but can be used as risk denominators in evaluating maternal complications. Therefore, for patients with HELLP syndrome, standard antenatal follow-up protocols should be applied in order to obtain early diagnosis and improve the speed of transfer to obstetric departments with expertise in this field.

Key words: HELLP; Preeclampsia; Eclampsia; High-risk pregnancy.

Introduction

Hypertensive disorders represent the most common medical complication of pregnancy, affecting 6-8% of gestations in the United States [1]. HELLP (elevated liver enzymes, low platelets) syndrome represents a severe form of preeclampsia/eclampsia characterized by hemolysis, elevated liver enzymes, and low platelets [2] and was described in 1982 by Weinstein [3]. The reported maternal mortality rates from HELLP syndrome range from 1% in the United States [3] to 30% in Turkey [4]. The reported incidence of HELLP syndrome in association with eclampsia ranges from 10.8% to 32.1% [5, 6]. HELLP syndrome, a serious condition in its complete form, is associated with substantial risk for the mother and fetus [7, 8]. Two classifications for the HELLP syndrome are commonly used [9, 10]. The Tennessee System classification is based on the assessment of the following parameters: AST > 70 UI/L, LDH > 600 UI/l, thrombocytes < 100,000/ mm^3. Accordingly, there are two forms: complete (all elements present) and partial HELLP syndrome (one or two elements present). The Mississippi classification relies on the thrombocyte count: class I (< 50,000/mm^3), class II (50,000-100,000/mm^3) and class III (100,000-150,000/mm^3). Martin *et al*., found higher maternal morbidity rates in Class 1 HELLP syndrome [11]. A wide range of complications may arise and the condition represents diagnostic and therapeutic problems; timing and method of delivery are important.

In this study we report the maternal and perinatal outcomes in HELLP syndrome cases at our clinic.

Material and Methods

This retrospective study was performed at Dicle University, School of Medicine, Obstetric and Gynecology Department between January 2004 and December 2008. HELLP syndrome was determined by the presence of all three of the following criteria: hemolysis (characteristic appearance of peripheral blood smear and serum lactate dehydrogenase [LDH] level ≥ 600 U/l or serum total bilirubin level ≥ 1.2 mg/dl), elevated liver enzymes (serum aspartate aminotransferase concentration ≥ 70 U/l), and low platelet count (< 100,000 cells/μl) [10]. Maternal outcomes analyzed included eclampsia, placental abruption, acute renal failure, need for transfusion of blood products, cesarean delivery and maternal death. For each woman, categoric data were collected concerning age, parity, gestational age at diagnosis, mean arterial blood pressure, blood platelet count, peak serum levels of aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH) and adverse maternal outcomes. Reported laboratory results and symptoms, such as headache, visual changes, nausea/vomiting

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and epigastric pain were present on admission. Gestational age was determined according to either last menstrual period or ultrasonography (US) examination.

Results

During the study period 126 women met the strict criteria for HELLP syndrome and there were 21,487 deliveries totally; 0.9% of all deliveries were complicated by HELLP syndrome. Demographic and clinical characteristics of the cases are presented in Table 1. Fifteen cases had severe preeclampsia, four had chronic hypertension and 18 had eclampsia. Gestational ages at diagnosis were ≤ 28, 28 to 32, and > 32 weeks of gestation in 13.5%, 31.7%, and 54.8% of cases, respectively. Nadir blood platelet count < 50,000 cells/μl, peak AST concentration > 150 U/l, and peak LDH concentration > 1400 U/l were present in 25.4%, 62.7%, and 27.7% of the cases, respectively. There was no correlation between gestational age at onset of HELLP syndrome and either nadir platelet count (r = 0.05), peak serum AST concentration (r = 0.06), or peak serum LDH concentration (r = 0.04). Adverse maternal outcomes studied included eclampsia, placental abruption, acute renal failure, the need for transfusion, and death. Acute renal failure was diagnosed in the presence of oliguria or anuria in association with a creatinine clearance of ≤ 20 ml/min or an elevated serum creatinine level of ≥ 2 mg/dl. Fourteen (11.11%) cases had acute renal failure, and seven (50%) were transferred to the nephrology clinic. In 58 (46.03%) cases blood products were transfused. Magnesium sulphate was administered routinely to prevent and control convulsions (a loading dose of 6 g given over 20 min, followed by a maintenance dose of 2 g/h as continuous intravenous solution for at least 24 h postpartum). Corticosteroids (12 mg betametazone intramuscularly every 12 h/2 times) were administered to enhance fetal lung maturity at ≤ 34 weeks' gestation. The cesarean delivery rate was 65.1% and spontaneous vaginal delivery rate was 34.9% (Table 2). Maternal mortality occurred in ten cases (7.93%). Table 3 presents the clinical characteristics of those maternal death cases. The mean birth weight of the cases was 1991.89 ± 957.77 g and 91 (72.22%) fetuses had a fetal weight of < 2500 g. The mean 0-min Apgar score was 4.77 ± 2.28 and the 5-min Apgar score was 7.05 ± 2.03. Sixty-four (55.65%) of these infants had low 5-min Apgar scores and 15 (11.90%) of the fetuses died.

Discussion

Although the term HELLP syndrome was not coined until 1982, its pathological features have been recognized for at least 100 years [12]. However, controversies persist regarding the diagnosis, management, and prognosis of this enigmatic disease. This uncertainty exists partly because the pathophysiological mechanism remains obscure and partly because of disagreement about the criteria used to define this syndrome. Sibai defined standardized strict laboratory criteria for disease diagnosis

Table 1. — *Demographic and clinical characteristics of the cases (n = 126).*

	Mean (SD)	Range
Maternal age (years)	30.32 ± 8,76	18-48
Gestational age (weeks)	32.63 ± 5,87	25-39
Gravidity	4.58	1-16
Parity	3.27	0-13
Blood pressure (systole/diastole) (mm/hg)	144.96/97.61	220-70
Platelets (K/UL)	86.88	142-424
Albumin (g/dl)	2.09	3.5-5.0
Blood urea nitrogen (mg/dl)	39.071	10-45
Creatinin (mg/dl)	0.99	0.6-1.30
Alanin aminotransferase (IU/l)	224.42	0-55
Alanin aminotransferase[1] (IU/l)*	140.22	0-55
Aspartate aminotransferase (IU/l)	379.23	5-40
Aspartate aminotransferase[1] (IU/l)*	215.51	5-40
Total bilirubin (mg/dl)	1.76	0.2-1.2
Lactic dehydrogenase (IU/l)	1418.67	125-243
Lactic dehydrogenase[1] (IU/l)*	875.16	125-243

*: ALT , AST and LDH values after birth.

Table 2. — *Symptoms, complications, and mode of delivery of the cases.*

	n (%)
Headache	107 (84.92%)
Nausea and/or vomiting	99 (78.57%)
Epigastric pain	77 (61.11%)
Visual symptoms	86 (68.25%)
Eclampsia	28 (22.22%)
Placental abruption	26 (20.63%)
Cerebral ischemia-edema	17 (13.49%)
Cerebral hemorrhage	8 (6.34%)
Acute renal failure	14 (11.11%)
Transfusion of blood products	58 (46.03%)
Laporatomy for major bleeding	12
Albumin transfusion	86 (68.25%)
Cesarean delivery	82 (65.1%)
Spontaneous vaginal delivery	44 (34.9%)
Maternal death	10 (7.93%)
Fetal death	15 (11.90%)

Table 3. — *Clinical characteristics of maternal death cases.*

Case	Maternal age (years)	Gestational age (weeks)	Associated pathologies	Delivery route	Day of death	Complication leading to death
1	26	38	Eclampsia	Cesarean	6th	IHH*
2	30	27	Eclampsia	Vaginal delivery	1st	IHH
3	18	33	Eclampsia	Vaginal delivery	1st	Sepsis
4	38	31	Eclampsia	Cesarean	3rd	IHH
5	42	28	Eclampsia	Cesarean	7th	IHH
6	35	26	Stillbirth	Vaginal delivery	1st	Sepsis
7	43	30	Pulmonary embolism	Vaginal delivery	3rd	Sepsis
8	32	31	Fulminant hepatitis	Cesarean	1st	Fulminant hepatitis
9	38	30	Eclampsia	Cesarean	1st	IHH
10	26	37	Eclampsia	Vaginal delivery	4th	IHH

IHH: intrahemispheric hemorrhage.

[10] which we have used in this study to define HELLP syndrome. HELLP syndrome can be diagnosed in pregnant women whose blood pressure elevation was first detected after mid-pregnancy, either with or without proteinuria. Sibai observed that hypertension and proteinuria may be absent or only slight. Even though HELLP syndrome is considered to be a variant or an atypical variant form of severe preeclampsia, its severity is reflected in the laboratory parameters, and not in the usual clinical parameters of blood pressure and proteinuria that typically reflect preeclampsia disease severity [13]. We observed that 7% of women did not have proteinuria in our study. HELLP syndrome is associated with both maternal and neonatal complications. In the literature, there is controversy regarding adverse maternal outcomes in HELLP syndrome. Martin *et al*. [11] reported a significant maternal and perinatal complication rate in patients with platelet count values ≤ 50,000 cells/mm^3, but Haddad *et al*. [15] found that laboratory parameters of HELLP syndrome are not independent risk factors for adverse maternal outcome. Laboratory thresholds that indicate more than 75% risk of serious maternal morbidity are LDH concentration > 1400 U/l, AST > 150 U/l, ALT > 100 U/l and platelet count ≤ 50,000 cells/mm^3. Thirty-two of the women had these values in our study. A decrease in these parameters after delivery is a good prognostic factor; 116 of the women had lower laboratory values after birth in our study. Interestingly, clinical symptoms, such as headache, visual changes, epigastric pain and nausea/vomiting have been suggested to be better predictors of adverse maternal outcome than laboratory parameters [14]. These clinical symptoms were more predictive than laboratory values in our study. The prognosis of the women who had clinical symtoms was worse than the others. HELLP syndrome carries a significant risk to mother and fetus, with approximately 1-3.5% for the mother, with increased proportion of multi-organ failure (MOF) and acute renal failure (ARF). Sixteen of the cases had MOF and 14 had ARF in our study.

Intensive care management of patients with HELLP syndrome producing multiple organ system failure consists of careful monitoring with active and supportive treatment of any complications. Coagulopathy and hemorrhage require aggressive replacement with blood and clotting factors. We treated our cases with coagulopathy and DIC with blood and clotting factors such as red blood cells, platelets, fresh frozen plasma and albumine. Cerebral hemorrhage is a serious complication and has been shown to be a fatal event in 50% to 65% of cases [17]. We had six such cases. We also had eight cases of pulmonary edema. In previous reports, maternal mortality was calculated to be approximately 1%, which was mostly a result of disseminated intravascular coagulopathy and the complications [18]. We only observed ten maternal fatalities in HELLP. Perinatal mortality and morbidity are considerably higher in HELLP syndrome offspring than for the mothers, and are primarily dependent on the gestational age when the condition develops Kim *et al*. reported that newborns with HELLP syndrome have low 5 min Apgar scores [19]. Sixty-four of the cases had lower 5 min Apgar scores in our study. The perinatal mortality rate related to HELLP syndrome is between 7.4% and 34%. Neonates delivered before completing 32 weeks' of gestation have the highest risk of perinatal death [20, 21]. Fifty-seven of the cases delivered before 32 weeks and 15 of the fetuses died.

In conclusion, devastating effects of a hypertensive disorder associated with pregnancy could be prevented by close antenatal follow-up, timely prediction of risk factors and reasonable management strategies. Early detection of high-risk individuals and mild cases by well- trained primary medical personnel and timely referral to advanced tertiary centers will lead to improved perinatal and maternal outcomes in this critical group of patients.

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Evaluation of serum levels of interleukin-10, interleukin-11 and leukemia inhibitory factor in differentiation of eutopic and tubal ectopic pregnancies

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Summary

Purpose of study: To investigate whether serum levels of leukemia inhibitory factor (LIF), interleukin 10 (IL-10) and interleukin 11 (IL-11) are different in reference to the site of implantation. *Methods:* Seventeen patients with laparoscopic diagnoses of tubal ectopic pregnancy (EP) and 19 patients with intrauterine pregnancy delivering healthy term neonates (IUP) were prospectively evaluated for LIF, IL-10 and IL-11 levels. The data were compared by using the Student's t-test, chi-square test, Kruskal-Wallis and the Mann-Whitney U test with Bonferroni's correction ($p < 0.05$) as appropriate. *Results:* A statistically significant difference was observed in serum LIF levels between the EP and IUP groups ($p = 0.002$). Ranges of LIF were 15-300 and 70-1200 ng/ml for the IUP and EP groups, respectively. There were no significant differences between groups in terms of IL-10 and IL-11 levels. *Conclusion:* LIF, but not IL-10 or IL-11, levels may be increased in early tubal ectopic pregnancies when compared to normal intrauterine pregnancies.

Key words: Cytokines; Ectopic pregnancy; Fallopian tube; Interleukin 10 (IL-10); Interleukin 11 (IL-11); Leukemia inhibitory factor (LIF).

Introduction

Despite early intervention, ectopic pregnancy (EP) remains a major cause of maternal morbidity and mortality in the first trimester. Transvaginal ultrasound and serial measurements of sensitive β-human chorionic gonadotropin (β-hCG) levels have both assisted in diagnosis and lowered morbidity and mortality. This strategy may not always accurately separate an early normal intrauterine pregnancy (IUP) from an ectopic one because there is no consistent β-hCG pattern that characterizes EP [1]. Therefore, recent research has converged on finding candidate markers to reliably diagnose EP with a single serum measurement, especially to be used in emergency settings.

Although the role of cytokines is well documented in immune reactions like inflammation, their roles in pregnancy are unknown [2]. Their involvement especially in EP has not been clearly defined. Some investigators have published conflicting reports on the association of leukemia inhibitory factor (LIF), a cytokine of the interleukin 6 (IL-6) family, with EP [3-5]. Another IL-6-type cytokine, interleukin 11 (IL-11), has been shown to be involved in regulation of trophoblast invasion [6]. In addition, its serum levels are decreased in women with spontaneous abortion [2].

The aim of the present study was to investigate whether serum levels of cytokines are different in reference to the site of implantation. We have compared serum LIF, IL-11 and interleukin 10 (IL-10) (an anti-inflammatory cytokine which has not been previously studied) levels in IUP and EP groups in an effort to help in predicting the site of implantation and to suggest a possible role in immune-regulatory mechanisms of early normal and ectopic pregnancy.

Materials and Methods

Subjects

In this prospective study, a total of 41 consecutive patients admitted to at our clinic with a diagnosis of either possible EP or early intrauterine pregnancy were enrolled. All women presented with delay of menses, abdominal pain or abnormal bleeding. All women had a gynecologic examination and a transvaginal ultrasound. None of the patients had a history of pelvic inflammatory disease.

Of the 20 patients with a presumptive diagnosis of EP, none had signs of an intrauterine pregnancy, but rather an adnexal mass with free fluid. Only 17 patients with an accurate gestational age who had been diagnosed as having a tubal ectopic pregnancy during laparoscopy were included in the study. One patient was left out due to cervical pregnancy and another two patients were treated by methotrexate only so they had no surgical confirmation of their diagnosis and were left out of the study.

Of the 21 patients who were diagnosed with early IUP, only 19 patients who had an uneventful pregnancy and delivered a healthy term infant were included in the analysis. One woman had a miscarriage and the other had an immature delivery at the 20th gestational week and both were left out of the study.

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Therefore a total of 36 patients were included in the final analysis of the data: the EP group (n = 17) and the IUP group (n = 19). All blood samples, one from each patient, were collected by peripheral venous puncture upon admission. The sera were stored at -80ºC until assays were performed in batches.

The study is accordance with the 1975 Helsinki Declaration on Human Experimentation. It was approved by the local ethics committee and informed consent was given by all participants.

Cytokine assays

The plasma concentration of LIF (in nanograms per milliliter), IL-10 (in nanograms per milliliter) and IL-11 (in picograms per milliliter) were determined by commercially available enzyme linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. LIF/HILDA (Biosource, Belgium) was a solid phase enzyme amplified sensitivity immunoassay performed on a microtiter plate. IL-10 (RnDSystems, USA) and IL-11 (RayBio, USA) were both ELISAs for the quantitative measurement of cytokine in serum.

Statistical analysis

The data on patient characteristics were compared by using the Student's t-test and chi-square test as appropriate. The data on cytokine levels are given as minimum, maximum and the median. They were compared by using Kruskal-Wallis and the Mann-Whitney U test with Bonferroni's correction.

Results were considered significant when p was < 0.05. All statistical analysis was carried out by using SPSSr12 software package.

Results

Age, parity and estimated gestational age of patients in the EP and IUP groups are presented in Table 1. All parameters were similar between the groups. The range of β-hCG levels for both groups was inbetween 297 and 11,106 mIU/ml.

Table 2 gives the median serum markers for IL-10, IL-11 and LIF along with their corresponding minimum and maximum values. IL-11 levels are expressed in pg/ml, while those of IL-10 and LIF are in ng/ml. There were no significant differences between EP and IUP groups in terms of IL-10 and IL-11 levels.

A statistically significant difference was observed in serum LIF levels between the EP and IUP groups (p = 0.002). The median level for LIF in the EP group was calculated to be higher than in the IUP group (120 ng/ml vs 80 ng/ml). The range of LIF levels in IUP was only between 15 and 300 ng/ml, while the measured LIF range spanned from 70 to 1200 ng/ml in the EP group. The scatter diagram of each measured LIF variable is detailed and plotted in Figure 1. A threshold for diagnosis of EP according to LIF levels was not estimated because of the small size of the study group.

Table 1. — *Patient characteristics according to ectopic and normal intrauterine pregnancy groups. Statistically* p *value is considered significant if less than 0.05. NS: not significant; S: significant. †Minimum and maximum parity is given in parenthesis.*

Characteritcs	IUP (Intrauterine pregnancy)	EP (Ectopic pregnancy)	p value
Age (years)	26.8 ± 4.6	28.7 ± 5.9	NS
Parity	0.89 (0-6)†	0.82 (0-4)†	NS
Estimated gestational age (days)	52.7 ± 15.2	44.9 ± 11.0	NS

Table 2. — *IL-10, IL-11 and LIF serum measurements for ectopic and normal intrauterine pregnancy.*

Cytokine and p levels	Group	n	Median	Minimum	Maximum
IL-10 (ng/ml)	EP	17	5	2	38
ns	IUP	19	5	3	14
IL-11(pg/ml)	EP	17	1.8	0.7	85
ns	IUP	19	2.3	0.3	45
LIF (ng/ml)	EP	17	120	70	1200
p = 0.002	IUP	19	80	15	300

ns = not significant.

Discussion

The exact pathophysiologic mechanism underlying the ectopic implantation process is not clear. This lack of knowledge prevents the development of a certain molecular marker (or markers) to differentiate an ectopic pregnancy from a normal intrauterine one with precision. We examined the hypothesis of whether differences in cytokine levels between women with EP and women with IUP during early first trimester may be related to ectopic implantation and immune mechanisms. We also investigated whether the concentration of one of these cytokines may indicate the place of implantation.

LIF has been proven to have a role in the implantation process and ectopic pregnancy [7, 8]. Keltz *et al*. have shown that the LIF messenger ribonucleic acid and tubal secretion of LIF were markedly increased in ectopic pregnancy [9]. Wegner and Mershon evaluated serum LIF as a marker of ectopic pregnancy and interestingly found that LIF levels were lower in ectopic pregnancies when compared to IUP but were not discriminatory enough [5]. Although Kiran *et al*. demonstrated a tendency for increased concentrations of LIF in tubal extracts of ectopic pregnancies, the difference was not statistically significant [10]. Recently increased production and presence of LIF in tubal ectopic pregnancies were also supported by immunohistochemical and Western blot works from Güney *et al*. and Ji *et al*. [4, 11]. In another research, Daponte failed to show a difference in serum levels of LIF from ectopic and abnormal intrauterine pregnancy patients [3]. Our finding of increased LIF levels in blood samples of patients with EP when compared to normal IUP, lends credit to the findings of Keltz, Güney and Ji and colleagues. Although the number of women enrolled in the study is small, it also supports the original hypothesis of Wegner and Mershon's work that serum LIF would be elevated in patients with tubal ectopic implantation [5]. Randomized controlled studies involving a larger number of patients may be warranted to come up with a better answer.

IL-11 has been shown to be important in embryo

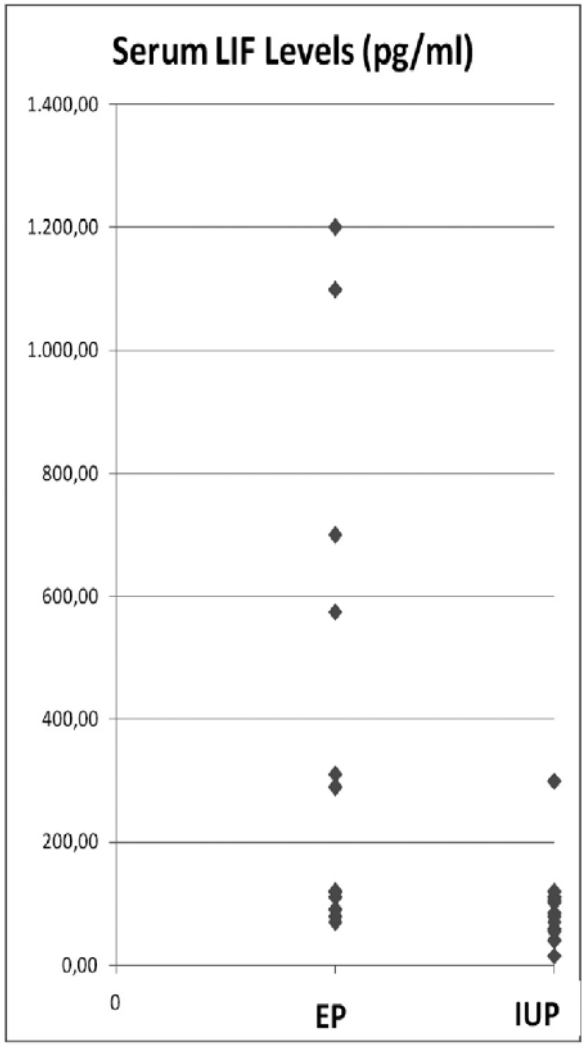

Figure 1. — Scatter diagram of serum LIF levels for ectopic (EP) and intrauterine pregnancies (IUP). No cut-off was calculated due to the small number of cases.

implantation and decidual response in mice and its expression has been shown in eutopic and ectopic human implantation [6, 12]. In addition, IL-11 levels are increased during early normal pregnancy [2]. Although von Rango *et al*. speculated that dysregulated IL-11 expression may be involved in a series of reactions leading to inadequate trophoblast invasion in tubal ectopic pregnancies, their work immunohistochemically showed that in tubal pregnancy staining of IL-11 was similar to the intrauterine situation [6]. Therefore we hypothesized that IL-11 levels should be similar between our study groups, and our results were in concordance with previously published data. IL-11 levels are probably not helpful in distinguishing the site of implantation of a viable pregnancy.

IL-10, the last cytokine we have investigated, has primarily been implicated in limiting and terminating inflammatory responses in the body [13]. Previous research has indicated that IL-10 may be linked with tubal factor infertility due to chlamydial infection and fibrosis [14, 15]. An abnormal implantation of the embryo, e.g. in the tube, unlike the normal one, would evoke an inflammatory response. The hypothesis is that the anti-inflammatory response to EP might lead to increased concentrations of IL-10 in the blood. Our preliminary results showed that this is not the case. The levels of this anti-inflammatory cytokine were similar between women with EP and women with an IUP. Although a conclusion may not be drawn from this data, the anti-inflammation evoked by ectopic and normal intrauterine pregnancy seems to be of similar magnitude and independent of place of implantation.

The major limitation of our investigation was the small number of women enrolled in the research groups, decreasing the power of the study. Another was the absence of serial and time-dependent measurements of the cytokines studied. Whether these concentrations change is not known according to current data.

Conclusion

To summarize, LIF levels seem to be increased in tubal ectopic pregnancies when compared to early normal intrauterine pregnancies. This finding supports the role of LIF in ectopic pregnancy and its possible use in differentiating the site of implantation. Further studies with a greater number of women are needed to establish this role. However, IL-10 and IL-11 levels do not seem to change according to the site of implantation, hence limiting their use in this regard.

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Analysis of uterine rupture cases in Agri: a five-year experience

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Summary

Introduction: We attempted to establish the frequency of uterine rupture and to address etiological factors, complications, management and maternal and perinatal outcome of complete versus incomplete rupture, with the aim of proposing preventive measures. *Methods:* The clinical records of uterine rupture cases managed at the Obstetrics and Gynecology Department of Agri Maternity and Children's Hospital in Turkey from June 2004 to June 2009 were analyzed retrospectively. *Results:* There were 44 cases of ruptured uterus. Among 24,554 deliveries the total incidence of uterine rupture was 1/558 or 17%. The most common site for the location of rupture was the fundal region (36.36%) followed by the lower segment, isthmic and mixewd types, respectively. *Discussion:* Prevention must necessarily include regular antenatal care and meticulous screening of high-risk patients. Improved organization and access to maternal care, decentralization of obstetric services into peripheral care units in villages to prevent home deliveries and good supervision during labor can reduce the incidence of this preventable obstetric catastrophe.

Key words: Uterine rupture; Hysterectomy; Grandmultiparity.

Introduction

Rupture of a gravid uterus is associated with high maternal and fetal mortality and morbidity. In spite of the recent advances in modern obstetric practice, it remains a life-threatening complication of pregnancy in underdeveloped countries [1]. Agri is situated in the Eastern Anatolian region of Turkey and has a low socio-economic status. The rise in primary cesarean section rates is likely to increase the prevalence of uterine rupture in the developed world as well as in the developing world. Predisposing factors for uterine rupture other than previous cesarean section include obstructed labor, inappropriate induction or augmentation of labor with oxytocic agents, previous uterine trauma, grand multiparity, uterine anomalies, abnormal placentation, fetal anomalies, and no antenatal care [2-4]. Uterine ruptures are commonly classified according to etiology as both traumatic and spontaneous. Spontaneous rupture may be seen in patients with a history of uterine surgery or those with an unscarred uterus. Spontaneous rupture may occur before the onset of labor or during labor. The differentiation between incomplete and complete rupture must be detected [5, 6].

We attempted to establish the frequency of uterine rupture and to address etiological factors, complications, management and maternal and perinatal outcomes of complete versus incomplete rupture, with the aim of proposing preventive measures.

Methods

The clinical records of uterine rupture cases that were managed at the Obstetrics and Gynecology Department of the Agri Maternity and Children's Hospital in Turkey from June 2004 to June 2009 were analyzed retrospectively. Complete uterine rupture was defined as complete separation of the wall of the pregnant uterus. Incomplete uterine rupture was defined as the uterine muscle separated, whereas the uterine cavity was separated from the peritoneal cavity by the visceral peritoneum over the uterus or that of the broad ligament. Information was collected on patient characteristics, including age, socioeconomic status, parity, weeks of gestation, prior cesarean sections and maternal and fetal mortality/morbidity. Low socioeconomic status was defined as yearly income of ≤ $1,000 (US). SPSS 9.05 for Windows was used for statistical analysis.

Results

There were 44 cases of uterine rupture. Among 24,554 deliveries the total incidence of uterine rupture was 1/558 or 17%. Demographic and clinical characteristics are shown in Table 1. Mean maternal age was 27.65 ± 8.22, gravida was 4.24 ± 2.8, and parity was 3.72 ± 2.86. The most common site for the location of rupture was the fundal region (36.36%) followed by the lower segment, isthmic and mixed types, respectively. Fetal death was seen in 19 cases (43.18% of all patients). Maternal death was seen in two (6.8%) cases. Maternal deaths were due to hypovolemic shock. Eleven of the perinatal deaths were intrapartum stillbirths, eight of which were neonatal deaths.

Table 2 details the clinical comparison of complete and incomplete uterine ruptures: there were 29 (65.9%) complete and 15 (34.09%) incomplete. The demographic characteristics of the complete and incomplete uterine rupture patients were similar. Possible etiological factors identified in the cases of uterine rupture were previous cesarean section in 17 (38.6%), prolonged labor in six (13%), use of oxytocics in seven (15%), previous histerescopic surgery in two (4.5%) and fundal pressure in

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Table 1. — *Demographic and clinical characteristics (N: 44).*

Mean maternal age (SD)	27.65 (8.22)
Gravidity (SD)	4.24 (2.8)
Parity (SD)	3.72 (2.86)
No. of previous cesarean sections (%)	17 (38.63)
Gestational age, weeks (SD)	37.12 (4.4)
Low socioeconomic class (%)	39 (88.63)
Location of site of rupture	
Fundal (%)	16 (36.36)
İsthmic (%)	8 (18.18)
Lower segment (%)	12 (27.27)
Mixed (%)	8 (18.18)
Postoperative hospitalization (days, SD)	8.2 (6.6)
No. of referred patients (%)	14 (31.81)
No. of maternal deaths (%)	2 (4.4)
No. of fetal deaths (%)	19 (43.18)
Hysterectomies (%)	18 (40.9)

Table 2. — *Clinical comparison of complete and incomplete uterine ruptures.*

	Complete	Incomplete
Mean maternal age (SD)	31.22 (5.46)	25.61 (6.12)
Number (SD)	29 (65.9)	15 (34.09)
Gravida (SD)	5.08 (3.02)	3.65 (2.11)
Parity (SD)	3.94 (2.4)	3.51(2.22)
Gestational age, weeks (SD)	36.7(3.8)	38.64 (2.6)
Days of postoperative hospitalization (SD)	10.41 (4.6)	6.13 (3.7)
No. of referred patients (%)	9 (64.2)	5 (36.8)
No. of maternal deaths (%)	2 (6.8)	0 (0)
No. of fetal deaths (%)	15 (51.7)	4 (26.6)
Hysterectomies (%)	13 (44.8)	5 (33.3)

eight (18%). No data on the etiological factors were available for four (9%) cases. The mean number of postoperative hospitalization days was 8.2 ± 6.6 (Table 3). Subtotal abdominal hysterectomy and total abdominal hysterectomy were performed in nine (20.45%) cases each and 24 (54.54%) cases had uterine repair. General anesthesia was given in all cases.

Table 3 details the operative procedures.

Table 3. — *Operative procedures (N: 44).*

	Number (%)
Repair alone	19 (43.18)
Repair with tube ligation	5 (11.36)
Int. iliac artery ligation	2 (4.5)
Subtotal hysterectomy	9 (20.45)
Total hysterectomy	9 (20.45)

Discussion

Rupture of the gravid uterus is an important cause of maternal mortality, morbidity and perinatal mortality. The most common risk factor is previous uterine surgery, and most cases of uterine rupture occur in women with a previous cesarean delivery. Although practitioners are more likely to encounter uterine rupture in mutiparous women with a previous cesarean delivery, it is an obstetric complication that must be considered in all women, regardless of parity [7, 8]. Clinicans must be vigilant in accurately identifying primigravidas with a higher risk of uterine rupture, particularly those with previous uterine surgery, including myomectomy and, increasingly, laparoscopic myomectomy [9-11].

Prompt recognition of uterine rupture and an expeditious recourse to laparotomy are critical in influencing perinatal and maternal morbidity. The real incidence of uterine rupture is not known. As some deliveries are supervised at home by traditional birth attendants, we do not know how many patients die because of uterine rupture before reaching the hospital. Not all uterine ruptures present with the typical clinical picture of abdominal pain, hypovolemia, vaginal bleeding, and fetal compromise [12]. Therefore, it is important to maintain a high index of suspicion for uterine rupture in women presenting with some or all of these features, regardless of parity. Vedat *et al*. reported that the incidence was 1:966 in northern Turkey [13]. We found the incidence to be 1:558 or 17%.

Eighteen patients (40.9%) needed a hysterectomy which is much higher than 14% reported from a study in Japan [14], and 5/1,000 deliveries reported from Jordan [15]. The maternal mortality rate was 4.5%, and several other studies from developing countries have reported the same ratios. Maternal deaths occurred in cases where rupture was due to hypovolemic shock.

Prevention must necessarily include regular antenatal care and meticulous screening of high-risk patients. Family-planning advice to reduce grandmultiparity should also be made available. Improved organization and access to maternal care, decentralization of obstetric services into peripheral care units in villages to prevent home deliveries and good supervision during labor can reduce the incidence of this preventable obstetric catastrophe.

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Pregnancy and adnexal torsion: analysis of 20 cases

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Summary

Objective: To study the clinical profile, management and outcome of pregnancy complicated by adnexal torsion. *Methods:* All pregnancy cases complicated by adnexal torsion admitted between January 2001 and January 2009 were analyzed. *Results:* The total number of pregnant cases was 20. Age range of pregnant women with adnexal torsion was 18 to 42 years. Of these cases 70% were seen in the first and second trimester. Seventy percent of cases were operated by the laparotomy route and 30% by laparoscopy. Salpingo-oophorectomy was performed in 70% of cases and detorsion in 30% of cases. Histopathologic examinations revealed five patients (25%) had serous cystadenoma, four patients (20%) mucinous cystadenoma, six patients (20%) dermoid cyst and five patients (25%) hemorrhagic cyst. *Conlusions:* Adnexal torsion as a differential diagnosis of acute abdomen in pregnancy should be considered and we recommend early surgical treatment that will save the adnexa.

Key words:

Introduction

Adnexal torsion is a serious cause of acute lower abdominal pain in women. It is a relatively uncommon condition, with a prevalence of about 2.7% of gynecologic emergencies, but it often constitutes a challenging diagnostic problem in clinical practice [1]. Adnexal torsion may arise in women of any age, but particularly during the reproductive years. Adnexal torsion is rarely observed during pregnancy. Its incidence is approximately 1: 5,000 pregnancies, occurring more frequently in the first trimester after ovarian stimulation for in vitro fertilization (IVF) [2]. The clinical symptoms are nonspecific and could be confused with other acute abdominal conditions, such as acute appendicitis, renal colic, and cholecystitis. Traditionally, abdominal complications during pregnancy have been treated by means of laparotomy. Today, laparoscopy is considered the preferable surgical option until approximately the 16th week of gestation [3]. A prompt diagnosis is essential for conservative, organ-preserving management, because after 36-48 hours of torsion irreversible lesions of the ovary are likely to occur.

Material and Methods

This study was conducted at the Department of Gynecology in Dicle University, Diyarbakir. Twenty pregnant patients were evaluated for adnexal torsion between January 2001 and January 2009.

Results

The total number of pregnant patients with adnexal torsion was 20. Age ranged between 18 and 42 years with a mean of 29.05 (± 6.07) years and mean gravidity was 3.7 (1 to 12). Six (30%) of the patients were in the first trimester, ten (50%) in the second trimester and four (20%) in the third trimester. Mean gestational weeks were 18.5 (10 to 36 weeks). Ten patients (50%) were operated for a diagnosis of acute abdomen, eght patients (40%) were operated for a diagnosis of pelvic mass and two were operated for a diagnosis of intraabdominal hemorrhage. Laparotomy was performed in 17 patients (85%). Three patients (15%) were operated by laparoscopy. Three of 17 patients who underwent laparotomy had cesarean sections performed at the same time.

Salpingo-oophorectomy was performed in 14 cases (70%) and in six cases (30%) cyst extirpation and detorsion were performed. Adnexal masses were torsioned on average three times (1 to 5). Postoperative histopathologic examinations revealed five patients (25%) had serous cystadenoma, four patients (20%) had mucinous cystadenoma, six patients (20%) a dermoid cyst and five patients (25%) a hemorrhagic cyst (Table 1).

Table 1. — *Findings of the adnexal masses*.

Size	< 6 cm	6-12 cm	> 12 cm
	6 (30%)	8 (40%)	6 (30%)
Type	Cystic	Solid	Heterogeneous
	12 (60%)	4 (20%)	4 (20%)
Histopathologic examination	Dermoid	Serous cystadenoma	Mucinous cystadenoma	Hemorrhagic cyst
	6 (30%)	5 (25%)	4 (20%)	5 (25%)

Three (15%) of the patients were delivered by cesarean section. The mean gestational week was 35 (33 to 36 weeks), the mean birth weight of the newborns was 2,700 g (2,300 to 2,900 g), mean APGAR score 7.2 (6 to 9) and all the newborns were healthy.

We began oral niphedipine as tocolytic therapy in six of the 17 patients. Fifteen (88.23%) of the patients had a full-term pregnancy and delivered healthy infants, two (11.77%) of the patients did not have a full-term pregnancy and delivered preterm, and additionally these two preterm infants were healthy.

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Discussion

Adnexal torsion is a rare cause of acute abdominal pain. It accounts for approximately 3% of gynecological emergencies and 10-20% of ovarian torsions occur during pregnancy. Adnexal torsion during pregnancy is a rare condition, more common in the first and early second trimester, and exceptional during the third trimester. In our study, ten (50%) of the cases were in the second trimester, six (30%) in the first trimester and four (20%) in the third trimester. Adnexal torsion is frequently associated with ovarian stimulation treatment for IVF or ovarian masses [4]. The majority of the tumors are functional cysts. In the first trimester of pregnancy these are luteum cysts, which regress spontaneously. This is the reason for delaying excision until the second trimester. Lesions surgically excised in the second trimester include persistent corpus luteum cysts (20%), cystadenomas (24%), dermoids (37%), paraovarian cysts (5%), endometriomas (5%) and leiomyomas (5%) [5]. Malignant tumors are found in 5.9% of the cases [6]. Cystadenomas (45%), dermoid (30%) and hemorrhagic cysts (25%) were detected in our study but endometriomas, leiomyomas and malignant tumors were not detected.

Hasiakos *et al*. reported four cases with adnexal torsion during pregancy and all of their cases were in the first trimester and operated by laparoscopy [7]. Fourteen of our cases were in the second and third trimester, thus we performed laparotomy in 17 cases and laparoscopy in three.

The preoperative diagnosis is difficult, especially in pregnant women. Torsion of the ovarian pedicle results in circulatory stasis that is initially venous, but becomes arterial as the torsion and the resultant edema progress. When complete torsion with hemorrhagic necrosis is suspected, immediate surgery is necessary. If there is a delay in the diagnosis and the torsion persists for more than 36-48 hours the lesions of the ovary are irreversible and a conservative, organ-preserving approach is not possible. However, it has been described that viable ovarian tissue can be detected even after 48 hours of torsion [8]. We performed salpingo-oophorectomy in 14 cases and cyst extirpation and detorsion in six cases.

Conclusion

Adnexal torsion is a rare event during pregnancy which requires a differential diagnosis from other diseases presenting with abdominal pain. It necessitates prompt surgical intervention because any delay leads to irreversible ovarian necrosis, so that salpingo-oophorectomy is ultimately necessary.

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Case Reports

Intrauterine fetal demise due to streptococcal toxic shock syndrome: a case report

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Summary

Background: Toxic shock syndrome caused by group A streptococci (GAS) is rare around the time of delivery, but it may predispose pregnant women to a life-threatening condition. *Case:* A 32-year-old primigravida at 21 weeks of gestation was taken to our hospital with acute severe abdominal pain following fever. On admission the fetus was found to be dead, and intrauterine fetal demise due to placental abruption was suspected. An emergency cesarean section found no sign of placental abruption. Soon after the surgery, the patient went into shock but was successfully treated with intensive care. Although repeated blood cultures failed to detect microorganisms, the patient was positive for streptococcal pyrogenic toxin A, which is a superantigen of GAS. *Conclusion:* Once GAS infection is suspected, regardless of negative blood cultures, supportive care in the intensive care unit is mandatory.

Key words: Pregnancy; Streptococcus pyogenes; Superantigen; Toxic shock syndrome.

Introduction

Despite recent advances in the use of antibiotics and support therapies, pregnant women and their fetuses are susceptible to serious and life-threatening infections.

Group A streptococci (GAS), such as *Streptococcus pyogenes,* cause uncomplicated pharyngitis, scarlet fever, impetigo, and acute rheumatic fever, and sometimes lead to severe manifestations, e.g., sepsis, necrotizing fasciitis, and toxic shock syndrome. In the field of obstetrics, infections with GAS have traditionally represented the most common cause of puerperal sepsis, although the incidence has decreased in recent years. Recently, the numbers of maternal and fetal deaths due to severe GAS infection before, during or shortly after delivery have been reported [1, 2]. We report on a case of intrauterine fetal demise at 21 weeks of gestation associated with streptococcal toxic shock syndrome.

Case Report

A 32-year-old Japanese primigravida developed a fever and presented to her primary obstetrician at 20 weeks of gestation. Her previous medical history was unremarkable, and the current pregnancy was otherwise uncomplicated. She was diagnosed as having influenza B and was given oseltamivir phosphate, which rapidly alleviated her fever. One week later, she became febrile again. Although she was administered an oral cephalosporin empirically, her body temperature was > 39°C the following day. She was hospitalized immediately, and a carbapenem was administered intravenously. The following morning, the patient complained of lower abdominal pain that rapidly increased in intensity, and she was taken to our hospital. On admission, the patient was conscious but appeared to be in anguish. Her body temperature was 37.8°C, blood pressure was 127/66 mmHg, and pulse rate was 133 bpm. Her abdomen was hard, and the uterus felt hypertonic with mild tenderness. Her cervix was 2.0 cm in length and dilated 1 cm with mild vaginal discharge. Ultrasonography revealed that the fetus was dead and that the placenta had thickened. The patient had the following clinicopathologic characteristics: white blood cell count, 18,500/mm^3, with 87.5% neutrophils; hemoglobin, 11.4 g/dl; platelet count, 17.6 × 10^4/mm^3; and C-reactive protein, 8.0 mg/dl. Activated partial thromboplastin time was prolonged to 39.1 seconds (control, 35.6 seconds), and fibrin/fibrinogen degradation products (FDP) and D-dimer were elevated to 200.7 and 57.8 g/ml, respectively. Placental abruption with intrauterine fetal demise and disseminated intravascular coaglopathy (DIC) was considered. Three hours after admission, an emergency cesarean section was performed, and a stillborn fetus weighing 456 g was delivered. Laparotomy produced no evidence of placental abruption, purulent myometritis or neighboring inflammation. Uterine contraction during surgery was fair, and blood loss was estimated to be 900 ml.

Soon after returning to the obstetric ward, the patient complained of dyspnea. Her blood pressure was 90/40 mmHg, and this was not increased by rapid fluid replacement. Laboratory data suggested a progression of DIC, with hemoglobin level of 6.2 g/dl, platelet count of 7.7 × 10^4/mm^3, FDP of 776.5 µg/ml, and D-dimer of 268.2 g/ml. The patient was admitted to the intensive care unit (ICU), and was treated for DIC with red blood cell and platelet transfusions, danaparoid sodium, and antithrombin. As septic shock or toxic shock syndrome was strongly suspected, meropenem was initiated intravenously at a dosage of 3 g/day. On the first day in the ICU, fluid replacement was administered up to 6,400 ml, to maintain blood pressure, but the patient did not show any response to diuretic agents. One day later, hydrocortisone was administered, and her condition gradually improved along with diuresis. In parallel with the supportive therapies, blood, vaginal, and urine cultures, and serological analyses were repeatedly performed during her hospitalization in an attempt to detect microorganisms. However, no infecting microorganisms were detected before her return to the obstetric ward day 7 and subsequent discharge on day 16 postsurgery.

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After the patient was discharged from the hospital, samples of her sera that had been stored at -20°C were evaluated for the presence of staphylococcal enterotoxins A, B, and C (SEA, SEB, and SEC), toxic shock syndrome toxin-1 (TSST-1), and streptococcal pyrogenic toxin A (SPEA), using enzyme-linked immunosorbent assays (ELISA) [3] (Table 1). On the day of her operation, the patient had a serum SPEA level of 19.2 pg/ml, which was higher than the cut-off value. On postoperative day 13, the level of SPEA was still high, while at two months post-surgery, it was below the level of detection. These findings indicate that the patient suffered from streptococcal toxic shock syndrome, which resulted in the death of the fetus.

Table 1. — *Changes in serum concentrations of superantigens over time*.

	Postoperative			Cutoff value (pg/ml)	
	Day 0	Day 13	2 months	positive	false-positive
SEA	2.66	N/D	0.24	8	6
SEB	0.77	N/D	0.00	50	30
SEC	53.0	N/D	N/D	70	50
TSST-1	0.00	0.27	0.00	25	15
SPEA	19.2	10.1	0.00	10	8

SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEC, staphylococcal enterotoxin C; TSST-1, toxic shock syndrome toxin-1; SPEA, streptococcal pyrogenic toxin A; N/D, not determined.

Discussion

According to the definition of streptococcal toxic shock syndrome proposed by the Centers for Disease Control and Prevention of the United States [4], this patient could not be regarded as even a probable case, since GAS were not isolated. However, we believe that this patient had streptococcal toxic shock syndrome, based on several lines of evidence.

This case shared several clinical features with a definite case documented in our hospital in 1996 [5], in which abnormal uterine contraction, highly elevated FDP, and fetal heart rate abnormalities, which are common signs of placental abruption, were noted. It is noteworthy that the onset of streptococcal toxic shock syndrome is sometimes followed by a flu-like prodrome [1, 5]. Our patient was treated for influenza one week before the onset of the disease. Recent studies have shown that influenza virus infection can promote secondary bacterial infections, and that superinfection with influenza virus can cause a lethal GAS infection. Okamoto *et al*. reported that influenza virus infection enhanced adhesion and internalization of GAS and caused incremental increase of proinflammatory cytokines, interleukin-6 and tumor necrosis factor , in the alveolar epithelial cells in mice [6, 7]. Indeed, Harre et al. reported a fatal case of GAS myopericarditis following by influenza A infection [8]. Given the current concern about a global pandemic of influenza, physicians should consider the possibility that superinfections with influenza viruses and GAS may result in lethal, invasive GAS infections.

Although blood culturing is the most important diagnostic procedure for identifying microorganisms, culture-based microbiological identification procedures are comparatively slow and have limited sensitivities. An additional complication is that the blood cultures may be negative because the patient has already received antibiotics. Since severe GAS infections are associated with early onset and rapid progression [9], rapid and convenient detection systems for GAS are really needed. In the present case, we used ELISAs to test for five bacterial superantigens, SEA, SEB, SEC, TSST-1, and SPEA, which are responsible for staphylococcal or streptococcal toxic shock syndromes. GAS produces several superantigenic toxins, including SPEA, streptococcal pyrogenic toxin C, and streptococcal superantigen, and the release of these toxins causes streptococcal toxic shock syndrome. Among these toxins, SPEA is the most pyrogenic, and it can directly stimulate T cells to release massive amounts of proinflammatory cytokines [10]. Tanaka et al. reported a definite case of streptococcal toxic shock syndrome, in which the serum level of SPEA was remarkably elevated [11]. In the present case, serum SPEA was positive, although we failed to detect GAS in repeated blood cultures. We believe that the detection of superantigens is a helpful diagnostic tool for GAS infections.

Once streptococcal toxic shock syndrome is suspected, intensive therapy should be initiated immediately. However, a successful therapy for invasive GAS infection has not been established. The CDC recommends high-dosage parenteral penicillin and clindamycin for toxic shock syndrome. Clindamycin is considered to be effective in the reduction of toxin production through the inhibition of protein synthesis. Intravenous administration of human immunoglobulin G may also be useful in reducing mortality [1, 11]. In addition, the administration of a glucocorticoid may reduce the toxicity of T-cell activation and the specific responses to superantigens [12]. In the present case, the condition of the patient seemed to improve after hydrocortisone was administered.

In summary, severe GAS infection should be considered when a pregnant woman presents mimicking placental abruption following influenza infection. Once GAS infection is suspected, regardless of negative blood cultures, supportive care in an intensive care unit is mandatory.

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A novel highly effective therapy for severe vasomotor symptoms in an estrogen deficient woman – case report

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Summary

Purpose: To describe a novel highly effective therapy for vasomotor symptoms associated with diminished oocyte reserve. *Methods:* A 58-year-old estrogen deficient woman with severe vasomotor symptoms was treated with 20 mg per day of dextroamphetamine sulfate. *Results:* A marked immediate improvement was noted. *Conclusions:* An acquired disorder of the sympathetic nervous system may be the etiologic factor for vasomotor symptoms in women with diminished egg reserve and treatment with sympathomimetic amines seems highly effective. This case will hopefully stimulate a larger series to determine its efficacy in a larger population.

Key words: Vasomotor symptoms; Estrogen deficiency; Sympathomimetic amines; Orthostatic edema.

Introduction

A simple highly effective therapy for vasomotor symptoms associated with the climacteric period, when there is a decline in ovarian oocyte reserve, is estrogen replacement. However, in some women estrogen treatment is not an option due to side-effects, medical contraindications, e.g., history of breast cancer, migraine headaches, thrombosis, or personal fears related to recent publicity concerning breast cancer and estrogen.

Alternative therapies in lieu of estrogen, e.g., clonidine or venlofaxine, have had limited success in treating hot flashes, flushes and sweats. There was a case reported where a very effective control of vasomotor symptoms was provided by treating a normal estrogenic female with vasomotor symptoms with dextroamphetamine syndrome [1]. This was attributed to her having a condition involving a defect in the sympathetic nervous system leading to the inability to compensate at the precapillary sphincter level to inhibit leakage of fluid from intravascular to extravascular spaces related to the increase in hydrostatic pressure that occurs with standing [2-5]. The most effective treatment for this idiopathic edema condition is sympathomimetic amines, especially dextroamphetamine sulfate [2-7].

In the young lady whose vasomotor symptoms responded to sympathomimetic amines, the theory was that her symptoms were related to cerebral edema putting pressure on the temperature regulation center of the brain. Treatment with sympathomimetic amines by reducing generalized edema also reduced cerebral edema, thus obviating the vasomotor symptoms. Thus it was thought that the use of sympathomimetic amines may improve vasomotor symptoms only in the specific circumstance of idiopathic edema where the edema is compressing the temperature regulation center which would be a relatively rare event.

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However, the possibility exists that vasomotor symptoms in women of advanced age could be related to a defect in the sympathetic nervous system correctable by treatment with sympathomimetic amines. A test case of a 58-year-old woman with estrogen deficiency and very severe vasomotor symptoms who refused estrogen therapy and accepted therapy with dextroamphetamine sulfate sustained release capsules (10 mg) upon awakening and at noon is reported.

Case Report

A 58-year-old woman whose last menstrual period was at age 52, complained of severe hot flashes, flushes and sweats that had begun eight years before (when she was still menstruating), but that had now reached an unbearable point. She stated that for the past year she felt extremely warm and would break out in a sweat every half hour. These episodes would last 5-10 min. She also complained of waking up drenched at least four to five times per night and would need to throw off her covers.

Despite these severe vasomotor symptoms and the probability of great relief from using estrogen, because of the media publicity and her previous physician's advise, she did not want to take estrogen. In fact even though she was experiencing dysparunia at the introitus, she did not even want vaginal estrogen cream.

She was advised that in our experience the alternative medications, e.g., clonidine or venlofaxine, have had marginal success with potential side-effects. However, since her other main complaint was the inability to lose weight despite dieting, we suggested that she consider dextroamphetamine sulfate. This would help the weight problem, if it was related to fluid retention, and potentially could help the vasomotor symptoms (though our experience was only in one patient) [1].

Since she did have an abnormal water load test, excreting 63 ounces of urine in four hours despite only ingesting 48 ounces while supine, but only excreting 35 ounces erect, she agreed to try.

Within two days of taking the sympathomimetic amine her hot flashes and flushes diminished to only one per day of a

shorter duration and much less intensity. She no longer reported any night sweats during the two months of treatment.

Interestingly, she had stated in the beginning that she was trying to lose about 15 pounds, but was unable to do so. She weighed 164.5 pounds before treatment and went down to 150.5 pounds after two months of treatment. Her pretreatment heart rate was 68 bpm and was 80 bpm after dextroamphetamine sulfate therapy. Her pretreatment blood pressure was 116/72 and was 108/78 after two months of dextroamphetamine sulfate. She stated that she experienced no side-effects. Her abnormal free water clearance was not related to hypothyroidism since prior to therapy her thyroid hormone levels were normal.

Discussion

The beneficial effect of the sympathomimetic amine treatment was too quick to be explained by a relief in pressure on the temperature regulation center of the brain by reduction of edema. This favors that one etiology for vasomotor symptoms may be an acquired disorder in the sympathetic nervous system.

Thus, it seems likely that sympathomimetic amine therapy could be beneficial for a wider population. When the first case was published showing benefit in a young woman with normal estrogen, it was considered that the condition is rare and that sympathomimetic amine therapy may benefit a very limited population.

An abnormality in orthostatic fluid retention is common [5, 7]. The possibility exists that since this disorder is usually associated with an abnormal free water clearance, possibly the benefit will only be found in women with abnormal water load tests. Based on this case, if it was subsequently found in larger studies that dextroamphetamine sulfate is very effective for reducing vasomotor symptoms associated with diminished egg reserve, the abnormal orthostatic water retention may be more of a method to identify women with a defect in the sympathetic nervous system who may respond to therapy rather than be the actual cause of the symptoms. Of course it would be interesting to determine if sympathomimetic amines are effective even in those women who do not appear to have orthostatic water retention.

We have used dextroamphetamine sulfate in some women for over 30 years. It is well tolerated with minimal side-effects. There is no dependence or any withdrawal symptoms if dosages are kept to 30 mg or less.

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Anencephalic conjoined twins with mirror-image cleft lip and palate

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Summary

This is a case presentation of a conjoined twin (cephalothoracopagus) pregnancy with anencaphaly and mirror-image cleft lip and palate, affecting the left side of one twin and the right side of the other twin. The pregnancy was terminated at 26 weeks. The case is discussed with information in the literature.

Key words: Conjoined twin; Anencephaly; Cleft lip; Cleft palate.

Introduction

Conjoined twins are a rare complication of monochorionic twinning. The incidence is somewhere between one in 50,000 and 100,000 births (or 1 in 500-600 twin births) [1]. The most famous conjoined twins were Chang and Eng Bunker, born in Siam (now Thailand) in 1811 and joined by a small bridge of union at the umbilicus (omphalopagus). These twins gave rise to the popular term 'Siamese twins' [2]. Classification of conjoined twins is typically based on the fused anatomic region followed by the suffix, 'pagus', to indicate fastened. A simplified classification system for the eight classic types of conjoined twins was proposed by Spencer [3]. Conjoined twinning and cleft lip and palate have been previously reported in the literature [4-7]. Cleft lip and palate in cephalothoracopagus twins has also been reported [8, 9]. Mirror-image cleft lip and palate in thoracopagus twins have been described [10-15].

In this paper, we report a case of cephalothoracopagus conjoined twins with anencephaly and mirror-image cleft lip and palate who were born in Turkey. To our knowledge, this is the first report of such a case in the world.

Case Report

A 32-year-old woman, gravida 4, para 2, at 26 weeks of gestation was referred to our Perinatology unit for pregnancy termination with a presumptive diagnosis of anencephaly in the fetus. Her past medical history was unremarkable. She did not have any systemic disease, tobacco or alcohol use. Familial history for any kind of anomaly was negative. Obstetric history revealed that she had two healthy children and a term intrauterine unexplained death. She received no prenatal care until the 26th week of gestation. She had no drug usage during pregnancy. The ultrasound examination performed in another hospital revealed anencephalic fetus; thus the patient was referred to our center with a presumptive diagnosis of anencephaly for pregnancy termination. A diagnosis of conjoined twins had been missed by using 2-D sonography. Medical termination of the pregnancy was performed by using misoprostol. A set of conjoined female twins with craniothoracopagus anomaly with a total weight of 1220 grams was evacuated. Anatomical features included development of two faces with mirror-image configuration cleft lip and palate, two brain stems, two vertebral columns, four lower limbs, four upper limbs, two female genitalia, and a single shared anencephalic head (Figure 1, 2). The family refused further investigation, including autopsy, due to their strict religious beliefs.

Discussion

Conjoined twinning is a rare congenital condition that has an incidence of one in 50,000 births [1]. The embryologic origin of conjoined twinning is still debated. Two contradicting theories exist – the fusion and the fission theories [16]. According to the fission theory, which has been the generally accepted one, during the normal course of monozygotic twinning, division by an unknown stimulus occurs at around 13 days post-fertilization when the embryo is too large to separate fully and remains united at one pole or the other, or at a point between the poles [17]. The fusion theory suggests that the inner cell mass divides fully, but the two monozygotic embryos stay close enough to share the amnion alone or both the amnion and yolk sac. Then, they might come in contact with one another and become reunited (fused) resulting in either ventrally or dorsally conjoined twins [16].

Conjoined twins are typically classified by the point at which their bodies are joined. The most common types of conjoined twins are thoraco-omphalopagus, thoracopagus, omphalopagus and craniopagus. Other less common types of conjoined twins include cephalopagus, synecephalus and cephalothoracopagus. In cephalothoracopagus, bodies are fused at the head and thorax. In this type of twins, there are two faces facing in opposite directions, or sometimes a single face and an enlarged skull. Our case had two faces, two brain stems, two vertebral columns, four lower limbs, four upper limbs, two female genitalia, and a single shared head. Congenital malformations usually occur in conjoined twins. The malformations may or may not be associated with the site(s) of fusion. Most commonly these malformations include

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Fig. 1

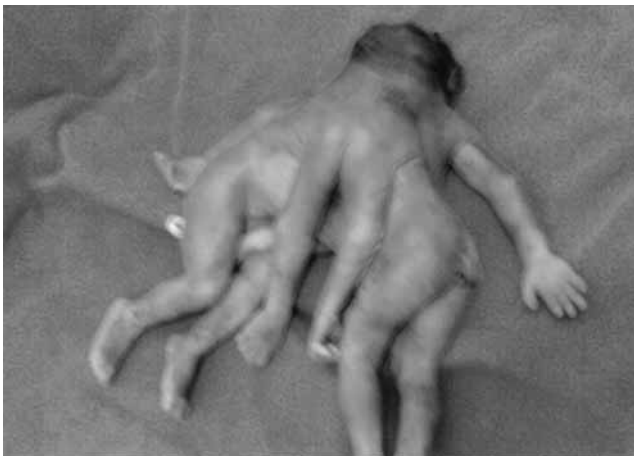

Figure 1. — Conjoined twin with cephalothoracopagus.

Fig. 2

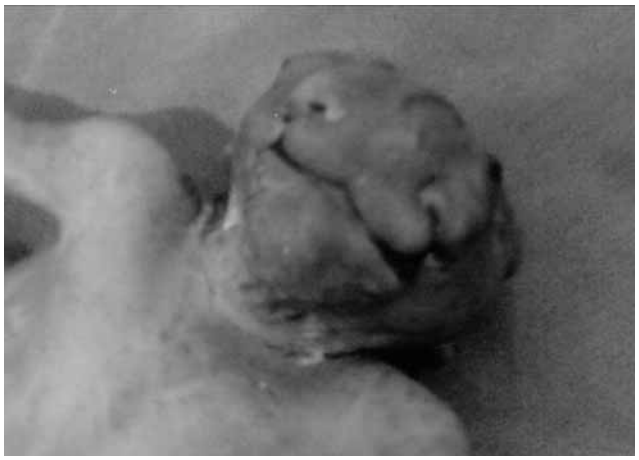

Figure 2. — Conjoined twins showing two faces with mirror-image configuration cleft lip and palate.

neural tube defects and orofacial clefts [18]. Our case had two faces with mirror-image configuration cleft lip and palate and a single shared anencephalic head. Anencephaly and orofacial clefts are multifactorial in origin, arising from both genetic and environmental factors.

The present case as well as other reports on mirror-image clefts in conjoined twins [10-15] supports the fission theory. Mirror-image clefts in conjoined twins have also been suggested to occur from some environmental factors such as poor blood supply [15]. Anencephaly in the case of laterally fused heads was thought to be associated with mechanical difficulty in closing the rostral neuropore [9].

Besides the etiologic factors, diagnosis and route of delivery are important issues in conjoined twins. In our case, the patient was referred with a diagnosis of anencephaly, and the true diagnosis had been missed antenatally. Recently, several case reports have addressed the role of 3-D sonography in the diagnosis [19, 20]. Vaginal delivery was established uneventfully. Multidisciplinary prenatal assessment of conjoined twins is essential to appropriately counsel parents, to manage the pregnancy, and to create an appropriate delivery plan.

To our knowledge, such a case has not been reported to date. We presented this case due to its rarity. This case report supports the importance of fission theory. Further case reports are needed to understand the pathophysiology of this condition.

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Ovarian torsion; early diagnosis by MRI to prevent irreversible damage

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Summary

Background: Early diagnosis of ovarian torsion can help prevent irreversible damage to the adnexal structures in women desiring to maintain fertility. *Case:* The patient was transferred by ambulance for a six-hour history of severe lower abdominal pain. Magnetic resonance imaging (MRI) revealed bilateral enlarged ovaries measuring 5 x 6 cm (right) and 4 x 5 cm (left) with a right twisted and thickened peduncle. Ultrasonography failed to detect the peduncle changes. At surgery, the right adnexa was twisted 180° in a clockwise direction with no findings suggestive of gangrenous change, hemorrhagic infarction or ischemic change. Detorsion of the twisted ovary was performed. *Conclusion:* Detection of tube torsion at MRI may be useful in the preoperative evaluation for surgical detorsion of twisted adnexa encountered in enlarged ovaries.

Key words: Ovarian torsion; Polycystic ovary; Detorsion of twisted adnexa; Ovarian drilling; MRI.

Introduction

Adnexal torsion is an uncommon but serious cause of lower abdominal pain; it is the fifth most common gynecologic emergency, with a reported incidence of 3% in one series of acute gynecologic complaints [1-3]. It commonly accompanies an ipsilateral ovarian tumor or cyst but can also occur in normal ovaries, usually in children [1-6]. If the adnexal torsion is complete and goes undiagnosed and untreated, hemorrhagic infarction may occur in the involved ovary and may lead to peritonitis and death [5]. Early diagnosis can help prevent irreversible damage to the adnexal structures and may thus allow conservative, ovary-sparing treatment in women desiring to maintain fertility. However, adnexal torsion occasionally presents a diagnostic dilemma, largely because of the related but nonspecific clinical, laboratory, and imaging findings. This report describes the accuracy of magnetic resonance imaging (MRI) in the preoperative evaluation for surgical detorsion of a twisted adnexa encountered in enlarged polycystic ovaries in comparison with ultrasound (US) findings.

Case Report

Our patient was a 22-year-old Japanese nuligravida known to have polycystic syndrome (PCOS). PCOS was diagnosed a few weeks before when she had been admitted to hospital because of irregular menstrual cycles. The diagnosis was verified by ultrasonographic morphology and endocrine analysis. She was transferred by ambulance due to a six-hour history of lower abdominal pain. She was pale, sweating, and could not lie quietly and moved about, seeking a comfortable position. There was a board-like rigidity with rebound tenderness in the right lower abdomen. MRI revealed bilateral enlarged ovaries measuring 5 x 6 cm (right) and 4 x 5 cm (left) with a right twisted and thickened peduncle (Figure 1). US was consistent with the MRI findings of PCOS, but failed to detect the stalk conditions. A negative pregnancy test ruled out the presence of ectopic pregnancy. All emergency routine laboratory tests were within normal range. A preliminary diagnosis of a torsed right enlarged ovary was made. Consideration of ovarian salvage led us to prompt exploratory laparotomy. At surgery, the ovaries were polycystically enlarged like a bulging mass measuring 5 x 6 x 6 cm (right) and 4 x 4 x 3 (left). The right adnexa was twisted 180° in a clockwise direction with no findings suggestive of gangrenous change, hemorrhagic infarction or ischemic change. The uterus grossly appeared normal. No other findings which caused her severe abdominal pain were detected in the abdominal cavity. Detorsion of the twisted ovary and drilling of the bilateral ovaries were performed. The patient was discharged on the fifth postoperative day after an uneventful recovery.

Discussion

Torsion should be suspected in patients with an enlarged ovary who have abdominal or pelvic pain. The enlarged ovary apparently has a polarity that allows it to twist along the pedicle; polycystic ovary is rare as a source of the twisted ovarian enlargement in most series [1, 2, 4, 5]. If the trend toward increased ovarian salvage rates continues, then early diagnosis of ovarian tortion is warranted. The common MRI findings in adnexal torsion include tube thickening, smooth wall thickening of the twisted ovarian cystic mass, ascites and uterine deviation to the twisted side [6], although it is unclear if these findings are sufficient to determine whether there is a chance of ovarian preservation. Recent studies have proposed an aggressive approach to ovarian salvage. In a retrospective study by Houry and Abbott [3], detorsion was possible in eight of 87 cases (9%) when the patients had surgery within 24 hours. Cohen *et al*. [7] were able to preserve twisted ischemic adnexa encountered at laparoscopy in 58 women with bluish-black adnexa with minimal post-

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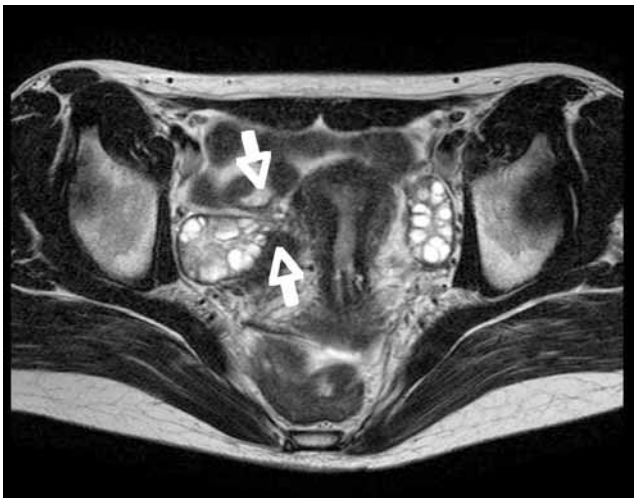
Fig. 1a



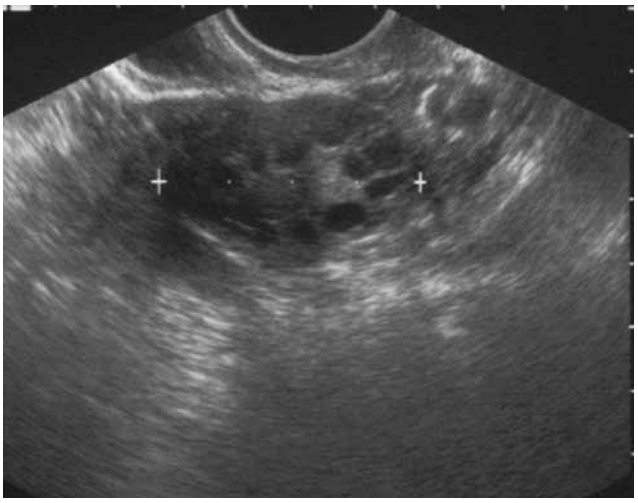


Fig. 1b

Figure 1. — Torsion of right enlarged polycystic ovary in a 22-year-old woman with a 6-hour history of lower abdominal pain. a) Axial abdominal MRI (T2-weighed) shows bilateral adnexal enlargement with torsed and thickened right peduncle (arrowhead). b) Ultrasonography revealed PCOS findings, but not tube torsion.

operative morbidity. Follicular activity was later shown in the ovaries in 54 of the 58 cases. In our study, detection of tube torsion at MRI was useful in the possible diagnosis of a twisted polycystic ovary. We were inexperienced in laparoscopy, but the detorsion may best be done by laparoscopy. Early diagnosis and prompt detorsion within 24 hours could help prevent irreversible structural damage and allow conservative, ovary-sparing treatment.

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Idiopathic edema, a condition associated with pelvic pain and other symptoms in women, as a remedial cause of chronic cold induced urticaria

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Summary

Purpose: To determine if the treatment of cold induced urticaria refractory to conventional antihistamine-type therapy would respond to treatment with sympathomimetic amines. *Methods:* Dextroamphetamine sulfate (15 mg) extended release capsules were prescribed to be taken daily in the morning. *Results:* The cold-induced urticaria completely disappeared and antihistamine therapy was discontinued. *Conclusions:* Treatment of chronic refractory cold-induced urticaria effectively responds to treatment with sympathomimetic amines similar to other cases of chronic refractory urticaria that are not merely cold induced. Manifestation of idiopathic orthostatic edema, a condition predominantly of women, should always be considered in the differential diagnosis of baffling medical conditions.

Key words:

Introduction

There is a condition involving a defect in the sympathetic nervous system, especially prevalent in women, that is an etiologic factor in a variety of disorders which are refractory to conventional therapy [1]. These various treatment-resistant syndromes respond quickly and effectively to treatment with sympathomimetic amines [1].

Dextroamphetamine sulfate has proven effective for chronic urticaria in women who were not responding to conventional therapy including antihistamines and glucocorticoids [2, 3].

The case described here reports a different type of urticaria, i.e., cold-induced urticaria that was resistant to conventional therapy but responded to treatment with sympathomimetic amines.

Case Report

A 16-year-old female presented with a history of cold urticaria. The problem started one year before her presentation when she developed erythematous papules when she iced her ankles at La Crosse practice. This continued throughout La Crosse season only when she iced her ankles in the spot where the ice touched her skin. However several weeks later when she went swimming she developed hives from her feet to her neck shortly after her body plunged into the somewhat cool water. From that time on whenever she was exposed to cold weather, or cool water, she would break out in hives.

She stated that her symptoms were severe and did not respond to the use of benedryl, loratadine and hydralazine. The laboratory evaluation by her dermatologist showed normal liver and renal function as well as normal serum electrolytes and glucose level. Her complete blood count showed a normal hemogolobin, hematocrit and platelet count and the white blood cell count was normal at 6.9 x 10^3. Her percentage of eosinophils was normal at 3%. The sedimentation rate was normal at 14 (nl ≤ 20 mm/h), the antinuclear antibody screen was negative, the Epstein Barr (EB) virus antibody panel showed EB virus UCA IgM to be 0.00 but EB virus UCA IgG antibody was positive at 5.27 (EIA value positive = ≥ 1.10), and EBV nuclear antigen was > 5.00 (nl > 1.10). Fasting serum glucose was 78 ng/dl (nl 65-99 ng/dl).

Further immune studies showed complement component C3C at 122 ng/dl (nl 90-180), complement C4C at 26 ng/dl (nl 16-47), serum cryoglobulin was negative (normal), the eosinophil count normal at 207 cells/ul (nl = 15-500), and the serum tryptase (a marker for mastocytosis and mast cell degranulation) was 6 ng/nl (nl = 2-10). The immunoglobulin E was slightly increased at 118 ku/l (nl ≤ 118).

Endocrine studies showed the total thyroxin level to be 9.2 mcg/dl (nl - 4.5-12.5 mcg/dl). Thyroid peroxidase antibodies were < 10 IU/nl (nl = < 35 IU/nl). The serum thyroid stimulating hormone (TSH) level was increased to 6.12 mIu/ml. Based on the TSH level the patient was started on L-thyroxin (50 mcg) but did not show improvement in her urticaria after two months of therapy.

She was evaluated for idiopathic orthostatic cyclic edema, a condition sometimes associated with chronic urticaria but she did not have any of the classic symptoms of nocturia, facial and finger edema in the morning, edema of the feet and legs in the evening, unexplained weight gain, abdominal distention or decreased urination while standing.

At the time of the initial visit she had been taking cetirizine, loratadine, montelukast and ranitidine. A water load test was performed where she drank 1500 ml of water over a half hour period on two consecutive days. The first day she measured her urinary output in the supine position over a four-hour period and the next day followed the same instructions but was standing for four hours. The urinary output was 1650 ml supine vs 825 ml standing (normal should be > 75% of ingested water load in either position) [4].

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She responded very well to dextroamphetamine sulfate XR and was eventually able to discontinue all other therapy. She has remained symptom free for 16 months.

Discussion

The fact that this teenager failed her water load test suggests that her cold urticaria was another manifestation of a condition known as idiopathic orthostatic cyclic edema [5, 6]. This condition is caused by an increase in vascular permeability in the erect position because of a flaw in the sympathetic nervous system. The increase in hydrostatic pressure that occurs with standing would generally result in fluid diffusing from the intravascular to extravascular spaces were it not for a compensatory closure of pre-capillary sphincters through the sympathetic nervous system [6].

Dextroamphetamine sulfate, a sympathomimetic amine, has proven very effective in correcting this defect [4-6]. Whether related to the swelling of interstitial tissues per se or absorption of toxins into tissues related to the increase in vascular permeability the condition has been associated with interstitial cystitis and pelvic pain unresponsive to conventional therapy but yet significantly improves with dextroamphetamine sulfate therapy [7, 8]. Other pain syndromes in women that had been refractory to conventional therapy but respond to dextroamphetamine sulfate therapy include chronic esophageal pain, gastroparesis, arthritis, fibromyalgia, and headaches [1, 9-11].

The previous cases of chronic urticaria that were unresponsive to conventional therapy but responded quickly and efficiently to sympathomimetic amine therapy were hypothesized to be related to the defect in vascular permeability resulting in a release of histamines [2, 3]. When presented with the case of hives only induced by cold it was not clear that the mechanism would be the same as the unusual urticarial condition previously described [2, 3]. This case shows that cold-induced urticaria not responding to antihistamine therapy may show considerable improvement with sympathomimetic amine therapy. Though glucocorticosteroid therapy was not tried, even if it would also prove to be effective, the treatment would be long term and thus a lot less potential complications would be expected from long-term amphetamine therapy vs corticosteroid treatment.

Idiopathic edema is also a cause of inability to lose weight despite dieting and responds well to amphetamine therapy [4]. This young lady did not complain about her weight, maintained the same dietary habits, but a consequence of therapy (in this case beneficial) was weight loss.

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Huge endometriosis presenting like an ovarian tumor: CT appearance

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Summary

A 32-year-old female with a clinical history of abdominal swelling underwent CT of the abdomen. A huge biloculated cystic mass with a mural nodule in the abdominal and pelvic region was seen. The lesion showed slightly homogeneous enhancement. The imaging findings suggested an ovarian tumor. Histopathological evaluation after surgical resection revealed that the lesion was a bilateral ovarian endometriosis.

Key words: Endometriosis; Computed tomography; Ovarian diseases.

Introduction

Endometriosis is an extrauterine growth of the endometrial tissue. The ovaries, cul-de-sac, posterior broad ligament and uterosacral ligament are the most common sites affected [1]. The gastrointestinal system, abdominal wall, liver and chest can also be involved [2-7]. Reported computed tomography (CT) findings of endometriosis include mostly well-defined cystic masses several centimeters in size [1, 7, 8]. In this case report, we present a case of rare giant bilateral ovarian endometriosis with a mural nodule which was located in the pelvic and abdominal region extending to the liver and gall bladder, suggestive of an ovarian tumor.

Case Report

A 32-year-old female with a 6-week clinical history of abdominal swelling underwent CT of the abdomen. There was no history of pelvic surgery, dysmenorrhea or pelvic inflammatory disease. Her hemoglobin level was 9.16 g/dl, hematocrit 27.8% and sedimentation rate 78 mm/hour. CA 125 was 57.2 u/ml, CA 15-3 29.33 u/ml, CA 19-9 52.11 u/ml, and CEA 2.2 ng/ml. Her other laboratory findings were unremarkable.

A CT scan using 8-mm slices was performed with a spiral CT unit (Somatom Balance; Siemens, Erlangen, Germany). CT of the abdomen and pelvis following intravenous contrast application revealed a slightly homogeneously enhancing biloculated cystic mass with thin walls. The mass, across the midline, measured 20 x 20 x 18 cm within the abdominal and pelvic region with extension to the inferior aspect of the liver and gall bladder and superior aspect of the left and right ovary (Figure 1a, b). The mural nodule was observed at the anterior wall of the right cystic mass (Figure 1c). There was neither ascites nor lymphadenopathy. The CT features of the lesion were mostly consistent with tumor of the ovary. The uterus was normal.

At surgery, a soft cystic lesion originating from the right ovary about 20 cm in diameter and a cystic lesion originating from the left ovary about 10 cm in diameter were determined. Cystic lesions were attached to each other in the posterior level of the uterus. Right salpingo-oophorectomy with endometrioma excision and left endometrioma excision were done. Histologic examination of both the cystic masses showed areas of endometrial surface epithelium and endometrial stroma together with common hemorrhagic areas at the cyst wall (Figure 1d) and the diagnosis was bilateral ovarian endometriosis.

Discussion

Endometriosis is an ectopic collection of endometrial tissue. Symptoms regarding endometriosis are mostly pelvic pain and infertility. It has been reported that ectopic endometrium often behaves unpredictably, which makes the diagnosis difficult. Treatment for endometriosis can be medical or surgical depending on the severity of symptoms [1].

Endometriosis has no pathognomonic finding at ultrasonography, CT or magnetic resonance imaging (MRI). The main role of CT and MRI is to determine the extent of the lesions [3, 4]. Endometriomas can be unilocular or multilocular with thin or thick septations. Reported CT findings of endometriosis include mostly well-defined cystic masses that are several centimeters in size. They rarely exceed 15 cm in diameter [1, 9]. Malignancy should be ruled out in large lesions with wall nodularity [1]. In our case, endometriosis had developed as a slowly enlarging mass and it exceeded 20 cm in diameter. The lesion extended to the inferior aspect of the liver and gall bladder in the superior region and the uterus and left ovary in the inferior region.

Malignant transformation, which is a fatal complication of endometriosis, is rarely reported [1, 5]. Endometrioid carcinoma is the most common neoplasm arising from endometriosis [1]. The most important finding for a diagnosis of malignant changes in endometriosis is a contrast-enhanced mural nodule at the wall of the lesion [8].

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Fig. 1a

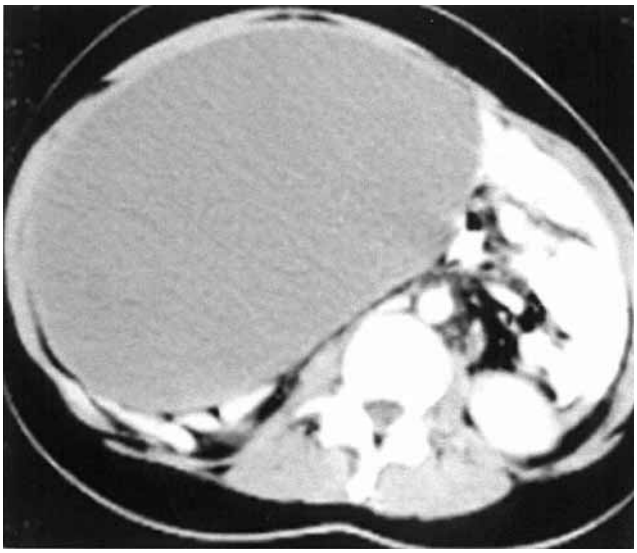

Fig. 1b

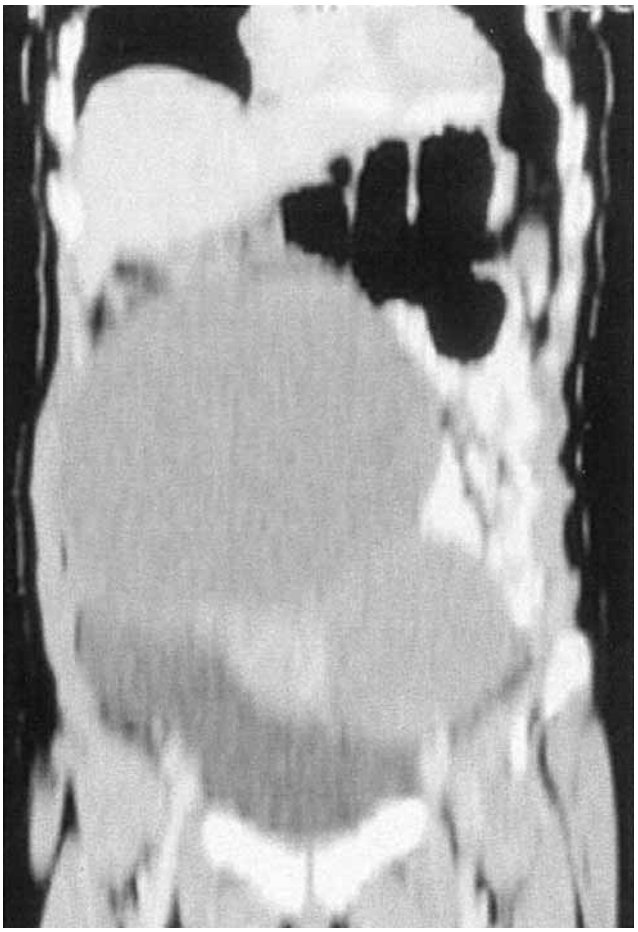

Fig. 1c

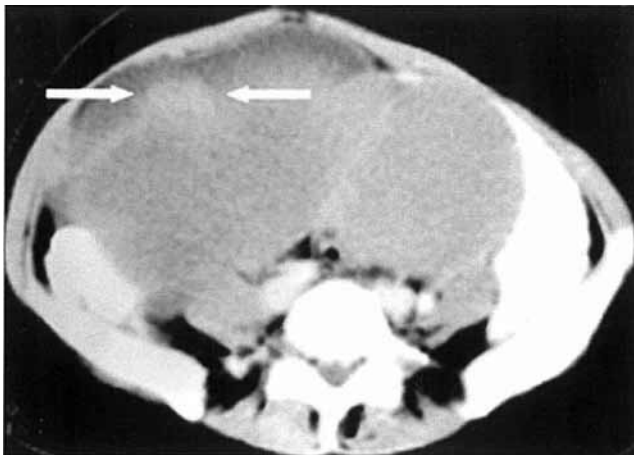

Fig. 1d

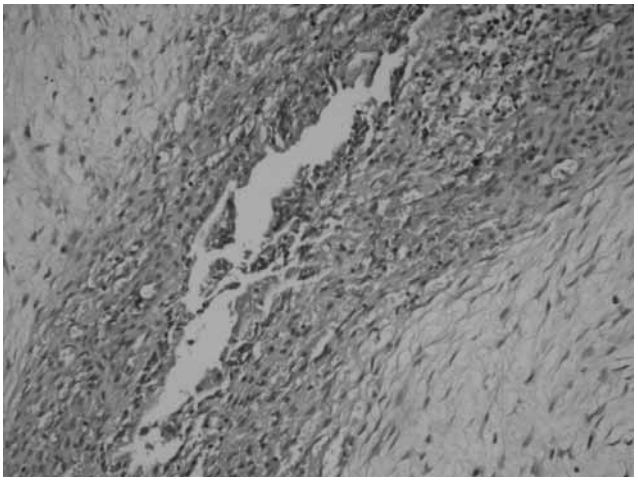

Figure 1. — Axial CT image showing a giant thin-walled cystic mass (a). Coronal reformatted image showing a bilocular cystic lesion in the abdominal and pelvic region (b). Axial CT image showing a bilocular cystic mass with mural nodule (arrows) (c). Histological section of the lesion showing surface epithelium in the stroma of the endometrium (H&E, × 200) (d).

However, decidual changes such as hypertrophy of the stromal cells of ectopic endometrial tissue can form as mural nodule and mimic malignant tranformation or solid component of the ovarian tumor [8]. The differentiation of a malignant mural nodule and decidualized endometrial changes are not possible by any imaging modality [8]. In our case, there was the mural nodule at the lesion wall, however the histopathological evaluation determined the absence of malignancy.

The most common giant cystic masses of the abdominopelvic region in the reproductive age group are epithelial tumors originating from the ovary, such as serous and mucinous tumors. Thin regular walls or septa, absence of invasion, homogeneous CT attenuation or MRI signal intensity, and a diameter less than 4 cm are common features of benign ovarian cystadenomas [10]. The internal solid components are usually absent in benign cystadenomas, but if present, they are mostly

small. Mucinous cystadenoma tends to be larger than serous cystadenoma during the diagnosis. Thick-irregular walls and septa and a large solid component are suggestive features of malignant epithelial ovarian tumors [10]. Hemorrhagic cysts should be also included in the differential diagnosis. Hemorrhagic cysts may have a complex appearance due to fibrin strands and resolve in a few weeks on follow-up examination [1].

In summary, the CT findings of a rare giant endometriosis that developed as a slowly enlarging mass have been described. Endometriosis should be considered in the differential diagnosis of giant cystic masses with a mural nodule located in the abdominopelvic region.

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Case report: sacral parasitic twins

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Summary

Introduction: Sacral parasitic twins originate from one fertilized ovum and they have one placenta and the same sex. *Case Report:* A 23-year-old woman was referred to our clinic. Examination by touch revealed a mass that was in the sacral region but the borders could not be fully examined. The solid mass, which was conjoined to the sacrum, had a soft texture. The infant's appearance was macroscopically normal. When the mass was examined by palpation, there were structures which felt like extremities. The mass was 20 x 11 x 9 cm in size. *Conclusion:* The differential diagnosis should include sacrococcygeal teratoma. In our case the differential diagnosis was done by histopathologic findings. This case, which involved a tumoral formation at the sacral region in the antenatal period, was detected during delivery. A sacral parasite is a rarely seen phenomenon and as such the diagnostic information of this case could be useful.

Key words: Parasitic twin; Teratoma; Ultrasonography.

Introduction

Sacral parasitic twins originate from one fertilized ovum and they have one placenta and the same sex. The incidence of this condition has been documented as being 1/40,000-1/200,000 live births [1]. Females are affected more frequently than males by a ratio of three to one. Compared to the USA it is seen in India and Africa more frequently [2]. After the 13th day following fertilization, twins should start to split. It is assumed that the most important factor that underlies formation of conjoined twins is the failing of this complete separation. Conjoined twins are classified according to their conjoined body parts [1, 2].

Parasitic twins (heteropagus) are asymmetric conjoined twins. The parasitic twin is completely dependent on its twin and is smaller and less likely to the organism. It is difficult to deliver such patients. A review of the literature reveals 28 cases reported thus far. We present this rare occurence.

Case Report

A 23-year-old woman was diagnosed as having a "twin pregnancy with pain" in another center and was referred to our clinic. Her gravidity was three, parity was two and number of live children was two. Antenatal care or ultrasonography was not performed. The patient was referred to our clinic urgently because labor had started. There was one fetal cardiac activity. At the patient's vaginal examination the cervix was completely effaced and dilatated. The head descent by station was +2, and heart beat sounds were positive. After the head, the anterior and posterior shoulders were delivered. At the umbilicus level the fetus could not be delivered so controlled fraction was applied. The McRoberts and Rubin's maneuvers were attempted but failed. Examination by touch revealed a mass that was in the sacral region but the borders could not be fully examined. The heart rate of the fetus was descelerated. Surgery was initiated immediately. The Pfannenstiel incision was performed to access the abdomen and a lower uterine incision was made. A 4,200 g, 50 cm in length female fetus was delivered by cesarean section and the Apgar score of the infant was 0. Postoperative resuscitation was carried out with no results. Atony, bleeding or other maternal complications were not seen.

The solid mass which was conjoined at the sacrum had a soft texture (Figure 1). The infant's appearance was macroscopically normal. When the mass was examined by palpation, there were structures which felt like extremities. The autopsy revealed that the fetuses anus opening was displaced to the left because of the sacral mass. A mass 20 x 11 x 9 cm in size which had continuity with the fetal skin in the sacrococcygeal region was documented. The outer surface of the mass was smooth and had a partly lobulated contour. At the surface of the mass, organoid-like tissues as bone, cartilage and fatty tissue (extremity parts) were detected (Figure 2).

Discussion

Prenatal ultrasonography (US), echocardiography, and 3-dimensional magnetic resonance imaging (MRI) which generally gives detailed information about conjoined twins, also helps in deciding whether the pregnancy should continue or be discontinued [1, 2]. Gestations that cannot be separated and have common important anomalies have to be ended. Conjoined twins can be recognized even at the 23rd gestational week by US. The presented case was not properly followed and antenatal diagnosis was not performed, thus appropriate treatment was not carried out [3, 4].

Systematic classification of conjoined twins was first established by Schwalbe *et al*. in 1905 [5]. The differential diagnosis should include sacrococcygeal teratoma. Teratoma is usually accompanied by malignancy. In our case the differential diagnosis was done by histopathologic findings. For this reason teratoma is considered as

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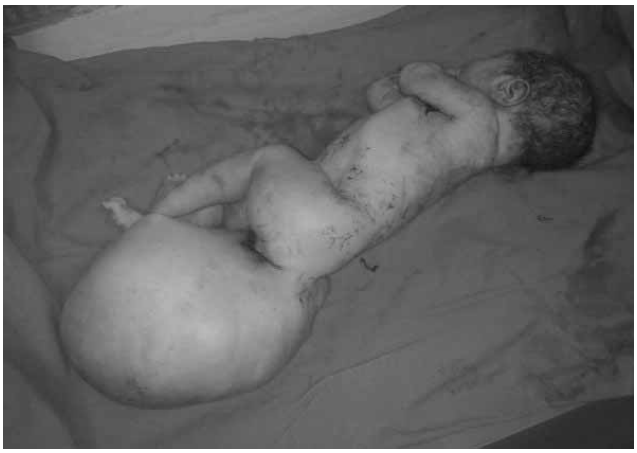
Fig. 1



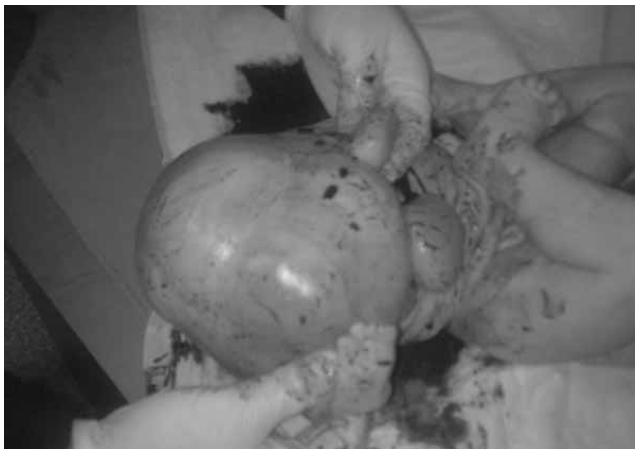


Fig. 2

Figure 1. — A 20 x 11 x 9 cm mass which has continuity with fetal skin in the sacrococcygeal region.
Figure 2. — At the surface of the mass organoid-like tissues as bone, cartilage and fatty tissue (extremity parts) were detected.

a different concept from a parasitic twin. Teratoma consists of internal organs which are at different developing stages [6-9]. In conjoined twins DNA typing and karyotype analysis studies have been done. Spencer *et al*. reported that parasitic twins were always monozygotic [8]. We do not have the facilities for these analyses, thus they are not performed. For a female who has a parasitic mass and the surface tissue of the mass looks like scrotal tissue, benefits of DNA typing could not be underestimated.

Spencer *et al*. reported 20 parasitic cases, 18 of which were localized and two with extensive conjunctions. All of these cases were medial to the dorsal midline [6, 8]. A literature review revealed eight more cases [3, 7, 9, 10]. When all reported cases were reviewed it was found that 18 of the cases were females and five were males, including our case; six of the case sexes could not be detected [6]. Anatomy and morphology of the reported parasites were related to the affected site. Upper extremities and related bones are seen in parasitic twins which are localized at the cervical and upper thoracic region. Lower extremities and bones are seen in parasitic twins which are localized at the lumbar and dorsal region [10]. In a parasitic mass, existence of polymorphic tissue belonging to a multi-organ system supports the theory established by Spencer; this theory suggests that these lesions are aborted or parasitic twins [8].

There are studies reporting that benign teratoma and lipomatosis tissues were detected in a parasitic mass [10]. This condition shows that there is a thin line between duplication aborted by teratoma, fetal inclusion, many types of parasitic twins and conjoined twins. When all reported cases were reviewed it was noted that cesarean section was performed in all cases because of dystocia.We attempted a vaginal delivery at first but had to resort to cesarean section.

This case involved a tumoral formation at the sacral region in the antenatal period which was detected during delivery. After histopathologic examination it was revealed that the mass was a sacral parasite (pigopagus parasiticus) and definitely different from teratoma. This case is presented to share diagnostic information as sacral parasite is a rarely seen phenomenon.

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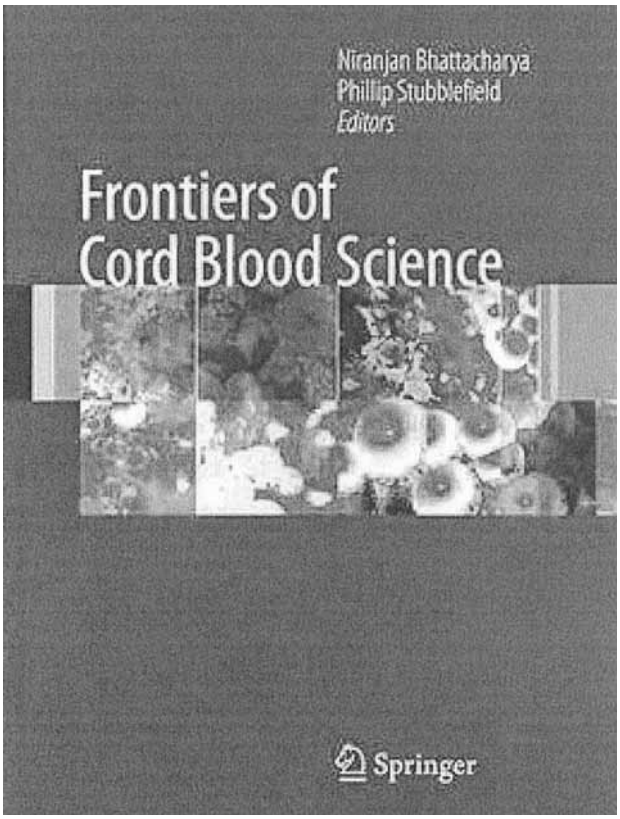
Book Review

FRONTIERS OF CORD BLOOD SCIENCE

Publisher: Springer-Verlag, London 2009.
The book is edited by Niranjan Bhattacharya, Senior Consultant and Advisor at Advanced Medical Research Institute and Phillip Stubblefield, Emeritus Professor, Department of Obstetrics and Gynaecology of Boston University.



The contens of this book are extremely important for developing the progresses that have been made in the last 25 years in the field of transplantation of umbilical cord blood stem cells.

Stem cells have been used to treat a variety of different pathologies and the results have always been encouraging.

This text is responsible for highlighting the importance of these procedures, inducing researchers to setup large-scale collections, blood banks and distribution.

The contents explore all fields of application, which have increased in the last 25 years: from Fanconi's anaemia to cell and gene therapies, from oncology to neurology and from transplants to bioengineering.

The first section illustrates the production and operating characteristics, and basic scientific studies of non-haematopoietic stem cells and of stem cell progenitors deriving from human blood of the umbilical cord.

All the topics are expertly handled by each author and, above all, are extremely clear and exhaustive even for people who are not familiar with this branch of medicine.

The extensiveness of the topics covered makes it a useful guide for specialists in this sector.

The second and third sections illustrate the different fields of use, and also report personal experiences that demonstrate the in-depth research that went into the topic.

The problems connected to banking and bioethics deriving from the use of these stem cells are very clearly outlined in the fourth and sixth sections.

Lastly – but not less important – is the fifth section dedicated to bioengineering, where the stem cells are used to improve the bio-friendliness of a mechanical prosthesis or implant.

The full potential of these cells has yet to be explored in a definitive and complete manner.

The contents of this text shall be responsible for spreading the knowledge acquired to date and, therefore, for inducing researchers to explore all possible fields of future applications, thus amplifying the therapeutic possibilities for illnesses that are still incurable.

We consider this text to be of high scientific importance and are certain it will lead to further progress in clinical research and application.

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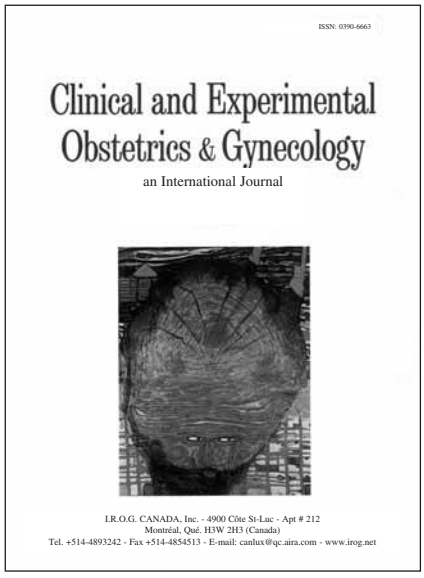

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