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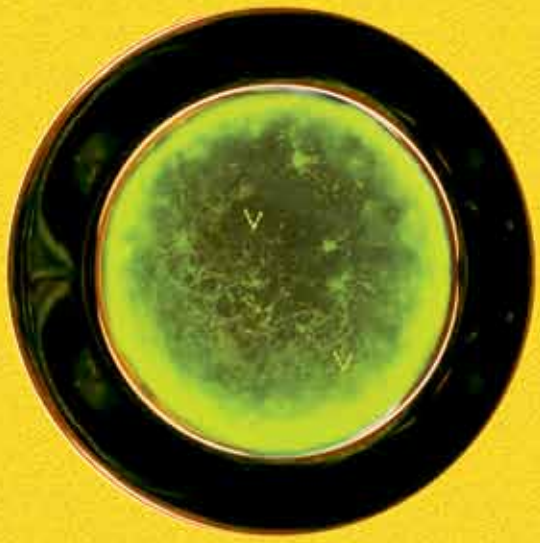

Editor : Mohamed Yahia

THE EGYPTIAN JOURNAL OF FERTILITY AND STERILITY

The Official Journal of the Egyptian Fertility and Sterility Society (EFSS)

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Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those who have made substantial contributions to the study.

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Toppozada MK, Gaafar AA, Shaala SA. In - vivo inhibition of the human non pregnant uterus by prostaglan din E2. Prostaglandins, 1974; 8: 401 - 406.

2- Books:

(a) Personal author: Speroff L, Glass RH, Kase NO. clinical gynecologic endocrinology and infertility. 4th edition, Baltimore, Williams & Wilkins; 1988 : 105

(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum J, Hamberger L. Interaction between prostaglan dins and catecholamines on cervical collagen. In: Toppozada M., Bygdeman M., Hafez ESE, Eds. Prostaglandins and fertility regulation. Advances in reproductive health care. Lancaster, England, MTP Press Ltd., 1985 : 75 - 80.

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CONTENTS

Letter from the editor

Dear colleagues

As this issue reaches you , Egyptians are starting a new era . After the 25th of January , Egypt is no longer the same. As you will notice this issue is three months late, because of the past circumstances and also we changed the printing house. So, we apologise for this delay and hope to catch on with next issues as soon as possible.

We all know the difficulties of scientific research in Egypt. However, we have no excuses not to try to change the face of this country in the shortest time possible . The young doctors in particular who are the hope of the country should try to breach the gap in the shortest time possible . A lot of our egyptian colleagues working in Europe and the States are prepared to help them on different levels . I would like here to mention two of them in particular prof. Peter Rizk and prof. Mostafa abu zeid who expressed their willingness to participate ,not only by coming over to deliver lectures and send scientific papers, but also to train young doctors through hands on training courses on advanced skills , we will announce these courses very soon .

Lastly, we would like to encourage doctors in training preparing their masters thesis to start publishing their work.A special section will be dedicated to them to print their abstract or the summary of their work to get them used to the idea of sending material to be published and revised. We will be waiting for your material as soon as possible.

Last but not least I would like to ask you all to start sending your questions on a new section which will be called ask the expert , the idea is to answer your questions about difficult clinical cases and we will answer them each time by a renowned experts in the field.

May god bless our country with safety , prosperity and peace

Mohamed Yehia

Fibroids and fertility

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ABSTRACT

Uterine fibroids are the most common pelvic tumours in reproductive age women. Although most women with fibroids are fertile, fibroids may interfere with fertility secondary to anatomical distortion and alterations to the uterine environment. Whilst fibroids are associated with infertility in 5-10% of cases, they are estimated to be the sole cause of infertility in 2-3% of cases.

Observational epidemiological studies aimed at elucidating the relationship between fibroids and infertility are inconclusive due to methodological limitations. However, two main pieces of clinical evidence support the opinion that fibroids interfere with fertility. Firstly, in IVF treatment, women with fibroids have an adverse pregnancy outcome. Available evidence suggests that submucosal and intramural fibroids interfere with fertility in decreasing order of importance and subserosal fibroids have no impact on fertility. Secondly, although randomised studies are lacking, myomectomy appears to increase the pregnancy rate in women with submucosal fibroids and possibly in women with intramural fibroids.

Keywords fibroids; myomas; leiomyomas; infertility; IVF; miscarriage; myomectomy; laparoscopy; hysteroscopy; uterine artery embolisation.

INTRODUCTION

Leiomyomas are benign smooth muscle tumours that arise as a result of proliferation of myocytes. They are associated with variable amounts of collagen and fibrous tissue and it is this pathological feature which has led them to being termed as “fibroids”.

Uterine fibroids are the most common pelvic tumours, occurring in 30% of women over the age of 30 years. Their incidence increases with age, and they are more common in certain ethnic populations. The frequency of fibroids reported in literature varies widely due to differences in diagnostic tests used, populations studied and study design. The largest study to date, prospectively followed up 95 061 female nurses in America aged 25-44 years with questionnaires every two years, to determine the incidence of fibroids among premenopausal women by age and race 1. The diagnosis of fibroids was self reported and confirmed for a sample of cases. The crude incidence rate in this study was 12.8 per 1000 woman years. The standardised rates were much higher in black women than in white women, 30.6 and 8.9 per 1000 woman years respectively. Even after adjusting for variables such as body mass index, infertility and contraception, the rates among black women were significantly higher than those amongst white women (RR 3.25; 95% CI 2.71-3.88). Another large American survey included 1364 women aged 35–49 years who were randomly selected from an urban health plan. All recruited women underwent a transvaginal ultrasonography. The cumulative incidence of fibroids at 50 years of age was 70 and >80% for whites and African Americans respectively. The prevalence of fibroids is lower in Europe, although still remarkable from the healthcare point of view. An Italian cohort study documented an incidence of ultrasonographically detectable fibroids of 21% in a series of 341 unselected women residing in an urban zone aged 30–60 years2. A Swedish study recruiting 335 unselected subjects from an urban district and who accepted to undergo a transvaginal ultrasonography showed a prevalence of 3% in women aged 25–32 years and 8% in those aged 33–40 years 3.

Classification of fibroids

Fibroids are traditionally classified according to their anatomical location and are divided into submucous, intramural (the commonest site) or subserous fibroids. Submucous fibroids are those that distort the uterine cavity and are further divided into three subtypes: pedunculated (type 0), sessile with <50% intramural extension of the fibroid (type I) and sessile with ≥50% intramural extension (type II). Intramural fibroids are those which do not distort the uterine cavity and with <50% of the tumour protruding into the serosal surface of the uterus. Fibroids protruding ≥50% out of the serosal surface are considered subserosal, they are further divided into sessile or pedunculated. Fibroids are multiple in two thirds of cases.

Pathogenesis

The underlying pathogenesis and pathophysiology of leiomyomas is highly complex and is far from being completely understood. Cytogenetic examination of leiomyomas reveals that about 40% of them have chromosomal abnormalities 4. These consist of translocations, trisomies, deletions and rearrangements. The rest appear chromosomally normal but exhibit mosaicism within the monoclonal tumour. These karyotype abnormalities have been shown to correlate with fibroid size and site. The mechanisms that link the clinical phenotypes to their underlying genotypes vary. For example, translocations can either up regulate or down regulate a gene and its expressed protein, depending on where the gene sequence is spliced. Trisomies on the other hand generally increase gene expression, through increased gene dosing.
There has also been research looking into the familial inheritance of fibroids. Twin studies have shown a strong susceptibility to fibroid development, with monozygotic twins twice as likely to develop fibroids compared to dizygotic twins5. Familial clustering has been described and there are also several inherited disorders associated with fibroids.

Ovarian steroids oestrogen and progesterone are important in the pathogenesis of fibroids. It has been shown that fibroids have increased levels of oestrogen and progesterone receptors when compared to normal myometrium, and that they also have an increased expression of enzyme P450 that allows the cells to synthesise their own endogenous oestradiol. Oestrogens affect tumour growth by stimulating the proliferation of uterine smooth muscle cells. Progesterone on the other hand, through its production of the bcl-2 protein, increases tumour bulk, by inhibiting programmed cell death 6.

Growth factors increase smooth muscle proliferation and act in a paracrine or autocrine way. They are also responsible for the increase in the extracellular matrix (collagens, proteoglycans and fibronectin) associated with fibroids. The growth factors, transforming growth factor ß (TGFß), heparin binding factor and insulin like growth factor (IGF) have been identified in fibroids 7-9.

Clinical presentation

Despite the epidemiological burden, the majority of women with fibroids remain asymptomatic. Symptoms associated with fibroids include heavy and prolonged periods, pelvic pressure (from large fibroids), pain (resulting from torsion of a pedunculated fibroid or fibroid degeneration), urinary symptoms and constipation resulting from pressure by anterior and posterior fibroids. Whether fibroids cause infertility is the subject of considerable speculation.

Although most women with fibroids are fertile, fibroids may interfere with fertility secondary to anatomical distortion and alterations to the uterine environment. For those women afflicted with fibroids the risks of pregnancy wastage are also increased.

Fibroids and infertility

Whilst fibroids are associated with infertility in 5-10% of cases, they are estimated to be the sole cause of infertility in 2-3% of cases 10. The mechanism by which fibroids have a detrimental effect on fertility remains controversial with various theories being postulated. It has been suggested that the mechanism by which fibroids cause infertility are mechanical in nature. The tumours, if subendometrial or tubal in position, may directly block the passage of spermatozoa. Any tumour that distorts the shape or elongates the endometrial cavity may affect the establishment and maintenance of early pregnancy. Subendometrial tumours are capable of causing endometrial erosion with subsequent inflammation. This state alters the biochemical nature of intrauterine fluid and thus results in hostile environment for the spermatozoa. Alternatively, the subendometrial tumours may disrupt the endometrial blood supply, thus affecting nidation and sustenance of the early embryo. It has also been suggested that the hyperoestrogenic environment associated with fibroids may impair fertility. The effect of fibroids on fertility is dictated largely by the location and size of the fibroid. A decreased risk of fibroids in parous women when compared with nulliparous women has been repeatedly reported. The observation that parity is associated with a reduction in the risk of fibroids could be interpreted in two ways. Parity may be a protective factor or, alternatively, fertility may be partly compromised in women with fibroids. Studies investigating the association between fibroids and history of infertility may be of help in clarifying this issue, but unfortunately evidence on this regard is scarce. Overall, the question therefore remains about causality of the association. Does pregnancy protect from fibroid development or, conversely, do fibroids affect fertility.

Fibroids and IVF treatment outcome

The advent of assisted reproductive techniques (ART) and in particular of in vitro fertilisation (IVF) treatment has offered a useful tool to elucidate the relationship between fibroids and fertility. Results from IVF treatment provide precious information on the impact of uterine fibroids on embryo implantation.

There have been meta-analyses that have aimed to assess the impact of fibroids in IVF cycles. Somigliana et al (2007) published a meta-analysis of studies investigating the influence of fibroids located at different sites in IVF cycles11. Overall, their results showed that myomas negatively affect pregnancy rates. Although based on a small number of studies, submucous fibroids appeared to strongly interfere with the chance of pregnancy: OR (95% CI) for conception and delivery being 0.3 (0.1-0.7) and 0.3 (0.1-0.8) respectively. The impact of intramural fibroids was less dramatic although still statistically significant: OR (95% CI) for conception and delivery being 0.8 (0.6-0.9) and 0.7 (0.5-0.8) respectively. In a follow up study, intramural fibroids were shown to have an adverse effect on live birth rate after three consecutive cycles of IVF treatment 12. In general, these effects appeared to be more relevant when considering the delivery rate compared to the clinical pregnancy rate. Conversely, subserosal fibroids did not seem to affect pregnancy rates.

A recent updated systematic review by Pritts et al (2009)

results were consistent in showing that women with submucous fibroids, compared with infertile women without fibroids, demonstrated a significantly lower clinical pregnancy rate (RR 0.36; 95% CI 0.17-0.73), implantation rate (RR 0.28; 95% CI 0.12-0.64), and ongoing pregnancy/ live birth rate (RR 0.31; 95% CI 0.11-0.85) and a significantly higher spontaneous abortion rate (RR 1.67; 95% CI 1.37-2.05). Women with intramural fibroids also produced significantly lower clinical pregnancy rate, implantation rate and ongoing pregnancy/ live birth rate and a significantly higher spontaneous abortion rate. When women with subserous fibroids were compared with women without fibroids, no difference was observed for any outcome measure.

There is controversy on the impact of intramural fibroids that do not distort the uterine cavity on IVF treatment outcome. The first prospective observational study to report an adverse effect of such fibroids on outcome of IVF was reported by Hart et al (2001)14. However other studies failed to reproduce this significant effect. This was addressed in a recent systematic review Sunkara et al (2010) that looked at 19 observational studies comprising a total of 6087 IVF cycles15. Meta-analysis of these studies showed a significant decrease in live birth (RR 0.79; 95% CI 0.70-0.88) and clinical pregnancy rates (RR 0.85; 95% CI 0.77-0.94) in women with non-cavity distorting intramural fibroids compared to those without fibroids, following IVF treatment. However there is currently lack of evidence from randomised controlled trials whether any intervention in this group of women would improve the outcome of IVF treatment and restore live birth rates to the levels expected in women without fibroids.

Fibroids and Miscarriage

Buttram and Reiter (1981) in their review of published reports from 1957 to 1980 identified a reduction in miscarriage from 41% to 19%, in a cohort of women with symptomatic fibroids who underwent myomectomy10. Women in these studies had symptomatic palpable fibroids which differ to most infertility patients who have asymptomatic fibroids diagnosed on ultrasound examination. Li et al (1999) in a small uncontrolled series of 19 asymptomatic women who conceived with fibroids reported a reduction in miscarriage post myomectomy compared to the pre myomectomy rate (24% vs 60%) 16.

Benson et al (2001) reported a nearly two fold increase in miscarriage rate among 143 women with ultrasonographically identified fibroids in the first trimester, when compared to 715 age matched controls without fibroids (14% vs 7.6%, P<0.5)17. Although the fibroid size was not associated with the spontaneous loss rate, the presence of multiple fibroids was a significant predictor of spontaneous loss and among the 88 patients with only a single fibroid, there was no increased risk of spontaneous miscarriage compared with controls. A meta-analysis of controlled studies of intramural fibroids and IVF outcome which reported on spontaneous miscarriage showed a spontaneous miscarriage rate of 22% in women with intramural fibroids compared with 15.4% in the control group. Data are currently unavailable to evaluate the risk of miscarriage in women with submucosal fibroids. Casini et al (2006) reported miscarriages in five of nine (53%) pregnant women with submucosal fibroids and nine out of 21 women (43%) who underwent prior myomectomy18.

Fertility after myomectomy

Before the advent of less invasive options hysterectomy was the standard treatment for women troubled with fibroid associated symptoms. This option is understandably unacceptable for women wishing to conserve their fertility. Myomectomy which involves the removal of the fibroid with conservation of the uterus is the alternative surgical treatment option for women wishing to conceive. The procedure may be performed abdominally, laparoscopically or hysteroscopically. Several reviews of literature on pregnancy rates following myomectomy have been published. One of the early reviews focussing on studies published between 1933 and 1980 by Buttram and Reiter (1981) reported a 40% pregnancy rate following abdominal myomectomy (480 out of 1202 cases)10. This rate was 54% when patients with other causes of infertility were excluded. Another review by Vercellini et al (1998) confirmed this rate of success following myomectomy19. They reported a post surgical pregnancy rate of 57% across prospective studies. When including women with unexplained infertility, this rate was 61%. The advent of endoscopic surgery did not seem to modify this result. In a review by Donnez and Jadoul (2002) the pregnancy rate among women undergoing hysteroscopic and laparoscopic myomectomy was reported as 45% and 49% respectively20. These findings have further been confirmed by more recent and larger studies.

IVF outcome after myomectomy

Whilst there is a consistent body of literature on the adverse influence of fibroids on pregnancy outcome, the impact of myomectomy has been less extensively investigated. Narayan et al (1994) investigated the effect of myomectomy on a small group of women with submucosal fibroids (n=27)21. They found that the delivery rate was not significantly different in women who underwent myomectomy compared to women without fibroids (37% and 22% respectively, P=0.13). Surrey et al (2005) reported a pregnancy rate of 62% and 68% respectively in women operated for submucous fibroids and controls without fibroids following IVF treatment22. From these studies we can infer that although the overall evidence is scarce, previous myomectomy did not seem to negatively affect the pregnancy rate following IVF treatment.

A comparative study by Bulletti et al (2004) has provided further evidence on the effectiveness of myomectomy prior to IVF treatment23. Women with intramural and/ or subserosal fibroids with at least one lesion >5cm were allocated to myomectomy or no surgery based on their decision. They reported a live birth rate of 25% and 12% respectively in women who did and did not undergo surgery prior to IVF treatment.

Alternative treatments for fibroids

Several non-surgical approaches for the treatment of fibroid associated symptoms have emerged over the last several years with medical therapies as well as radiological interventions being proposed. GnRH agonists, the mainstay of medical therapy for fibroids, work by creating a hypogonadotrophic hypogonadal state and produce a significant reduction in uterine size. Their use in the context of infertility treatment remains questionable since ovulation is generally inhibited during treatment and the fibroids usually resume their pre-treatment dimension within a few months after stopping treatment. Other medical options that may reduce the size of fibroids include the androgenic steroid danazol, the antiprogestagen

mefipristone, the selective oestrogen receptor modulator raloxifene and the aromatase inhibitor fadrozole. Again because of reasons mentioned above their use in the context of infertility treatment remains questionable.

Non medical alternative treatment options for fibroids that have been developed over the recent past include fibroid embolisation, laparoscopic myolysis and MRI guided focused ultrasound. Data regarding pregnancy outcome with these interventions is scanty as most women who wish to conserve fertility have been excluded from these treatments due to safety concerns. Particularly, information on laparoscopic myolysis and MRI-guided focussed ultrasound are absolutely insufficient and the effect of these techniques on pregnancy therefore unknown. Recently, more evidence has been emerging on the effects of fibroid embolisation on pregnancy outcome. In a large survey of 1200 women, Walker and McDowell (2006) recorded 108 women who attempted to become pregnant of whom 31% were successful24. This rate appears to be lower than surgery, but it is difficult to draw definite conclusions as there was no control group. Data regarding pregnancy outcome following uterine artery embolisation tends to support a detrimental effect. An increased risk of miscarriage, preterm delivery, IUGR, abnormal placentation and postpartum haemorrhage has been reported. However, these results are controversial, as studies are underpowered. Based on present evidence fibroid embolisation cannot be recommended in daily clinical practice to women wishing to conserve their fertility.

Recently, uterine leiomyomas have become an attractive target for gene therapy. Gene therapy is the introduction of genetic material into patients' cells to achieve a therapeutic benefit. Gene therapy strategies include: mutation compensation of dysregulated genes; replacement of defective tumour-suppressor genes; inactivation of oncogenes; introduction of suicide genes; immunogenic therapy and anti angiogenesis based approaches. Preclinical studies of gene therapy have shown promising results in uterine leiomyomas and researchers are of the view that this approach is not far from becoming a medical reality.

Given the current evidence, clinicians should pursue a comprehensive and personalised approach taking into account the pros and cons of myomectomy, including the impact of fibroids on fertility, the risks associated with fibroids during pregnancy on one hand and the risks associated with surgery on the other hand.

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• Sunkara SK, Khairy M, El-Toukhy T, Khalaf Y, Coomarasamy A. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and meta-analysis. Hum Repro 2010;25:418-429.

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Dehydroepiandrosterone supplementation within a gonadotropin-releasing hormone antagonist protocol in patients with poor ovarian response

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ABSTRACT

Objective: The aim of this study was to compare IVF treatment outcomes between pre- and post-treatment cycles among a cohort of women with known decreased ovarian reserve, using dehydroepiandrosterone (DHEA) supplementation.
Materials Methods: A prospective cohort study in a private assisted reproduction centre. Twenty-five women with significantly diminished ovarian reserve had IVF cycle before and after 50 mg/day DHEA for 2 months, with otherwise identical hormonal stimulation.
Results: After treatment with DHEA, patients demonstrated a significant improvement in day 3 FSH (7.1 ± 0.5 vs. 9.9 ± 1.5 mIU/ml) ($p< 0.05$), day 3 E2 (325.46 ± 9.7 vs. 227.37 ± 16.95 pmol/L) ($p< 0.05$), day 3 testosterone (79.84 ±10.93 vs. 54. 64 ±10.31 µg/dl) ($p< 0.05$) , day 3 DHEA-S (99.94 ± 15.4 vs. 62 .54 ± 16.3 µg/dl) ($p < 0.05$), number of follicles demonstrated by ULS on the day of hCG administration[Total (9 ±1.5 vs.6 ± 1.5) ($p=0.01$), ≥ 14 mm (4.5±1.5 vs.3.5 ± 1.5) ($p = 0.01$) and ≥ 17 mm(4.5 ±1.5 vs. 2.5 ±1.5) ($p = 0.01$)], serum E2 level on the day of hCG administration (4760±1524 vs. 3800±1684 pmol/L)($p = 0.001$), number of oocytes retrieved (7.5 ± 0.5 vs. 5.5 ± 0.5) ($p = 0.01$) and number of mature oocytes (Metaphase II) (5.5± 0.5 vs. 3.5± 0.5)($p = 0.01$). Although there were significant differences in fertilization rate (72.6 % vs. 45.8 %) ($p<0.005$), percentage of grade I/II embryos (73.6 % vs. 50.4%) ($p= 0.005$), cumulative embryo score per oocyte retrieved (18.5±1.2 vs. 10.5±1.2) ($p = 0.001$) and cancellation rate (15% vs. 40%) ($p = 0.001$).These were not reflected on biochemical pregnancy, clinical pregnancy rate/cycle or clinical pregnancy rate/embryo transfer.
Conclusions: DHEA supplementation for poor responder patients could have beneficial effects on ovarian follicular function, improvement in hormonal profile and embryological data. However, large prospective randomized placebo controlled trials are waited to demonstrate the improvement in clinical pregnancy rate after DHEA supplementation.
Key Words: DHEA, GnRH antagonist, poor responders, IVF.

INTRODUCTION

Despite considerable advances in assisted reproductive techniques (ART), management of poor responder patients is still a challenge. Although there is lack of uniform definitions, poor response to controlled ovarian hyperstimulation (COH) can be generally defined as unsatisfactory ovarian response in terms of low number of follicles developed, low serum E2 levels, and low number of oocytes retrieved despite adequate ovarian stimulation. However the cutoff points for these parameters that define poor response vary between studies 1, 2.

Poor response to ovarian hyperstimulation is a complication in 5% to 18% of all in vitro fertilization (IVF) cycles. Poor responders have significantly worse IVF outcomes than normal responders, with successful pregnancy rates as low as 2 % to 4 % 3.

Many treatment modalities have been suggested to improve ART outcomes in poor responders. These modalities include : [1] Variations in the type,dose,and timing of gonadotropins,or GnRH analogues(agonists and antagonists), [2] The use of oral contraceptive (OC) pills, clomiphene citrate (CC), aromatase inhibitors, growth hormone/growth hormone releasing hormone (GHRH), corticosteroids, estradiol (E2), testosterone (T), nitric oxide donors, or aspirin as adjuvant therapies. A part from these regimens, an alternative approach suggested for these patients is natural cycle ART 1, 4, 5. Previous studies have found that dehydroepiandrosterone (DHEA) supplementation increases oocyte production and augments ovarian stimulation in individuals who do not respond well to gonadotropin administration 6,7.
DHEA is an endogenous steroid that originates from the zona reicularis of the adrenal cortex and the ovarian theca cells in women. It is produced by the conversion of cholesterol and it is very important in the formation first of T and then E2 in peripheral tissues. Therefore, DHEA is an essential prohormone in ovarian follicular steroidogenesis8, 9.
The concentration of DHEA in women remains high during the reproductive years and progressively decreases with age. Numerous hypotheses have been made on how DHEA

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MATERIALS AND METHODS

This was a prospective cohort study involving poor responder patients undergoing IVF treatment at The Toronto Institute for Reproductive Medicine. Twenty-five women with significantly diminished ovarian reserve had IVF cycle before and after DHEA treatment, with otherwise identical hormonal stimulation from March2008 and March2009.Written informed consent was obtained from all patients and the study was reviewed and approved by our institutional review board.

As it is the policy of our centre to use GnRH antagonist protocol for poor responder patients, poor responders were defined when one or more of the following criteria is present in at least one previous failed ART cycle (using Gn RH antagonist protocol): [1] number of oocytes retrieved less than four, [2] level of E2 <1800 pmol/L on the day of hCG administration, or [3] a prior cancelled stimulation cycle due to poor ovarian response.

All patients had normal liver, kidney and thyroid function and had baseline ultrasound scans on cycle day 3, and blood was drown for serum FSH, LH, E2, progesterone, prolactin , testosterone and DHEA-S. Patients who agreed to participate in the study, began taking micronized DHEA by prescription compounded by a single pharmacy, 50 mg/day (two 25 micronized DHEA) for 2 months. All patients experienced both their pre- and post DHEA treatment IVF cycles at our centre. Monthly repeat baseline ultrasound scan on day 3, and liver, kidney, thyroid and hormonal assay were done. After 2 months of DHEA pretreatment, and while still remaining on this hormone, the subjects had a repeat IVF cycle using the same GnRH antagonist protocol.

Multiple dose GnRH antagonist protocol was given to all patients. Oral contraceptive pills were not used in this protocol. A combination of recombinant FSH, follitropin beta(Puregon, Organon)and hMG (Menopure, Ferring) was started on cycle day 2 and later 0.25 mg of Cetrorelix (Cetrotide, Serono) was administered daily when the leading follicle reached 14 mm in diameter or serum estradiol > 1000pmol/L until the day of hCG injection. An initial gonadotropin dose of 300IU of Puregon and 150 IU of Menopure for the first 5 days, followed by individual adjustments in gonadotropin dose, according to ovarian response was done.
When the average diameter of the leading follicles reached ≥ 18 mm and serum E2 levels were ≥ 2000 pmol/L, 10000 IU of hCG (Preganyl, Organon) was administered, followed 35-36 hours later by an ultrasound-guided transvaginal oocyte aspiration. The same multiple dose GnRH antagonist protocol was used both before and after DHEA treatment.Intracytoplasmic sperm injection (ICSI) procedure was performed 4-6 h after oocyte aspiration for all of the mature oocytes. Oocytes were examined 16-18h after ICSI for pronuclei (PN). Normal fertilization was defined as existence of (2 PN).

The embryos obtained were categorized on day 2 or 3 into 4 categories depending on their morphologic appearance, zonal thickness, cytoplasmic fragmentation and blastomere size .Grade 1[high quality]:embryos with equal blastomeres and no observed cytoplasmic fragmentation; grade II [good quality]: embryos with equal blastomeres and < 20 % fragmentation of the cytoplasm; grade III [fair quality]:embryos with unequal blastomeres and 20-50 % fragmentation of the cytoplasm; grade IV [poor quality]:embryos with unequal blastomeres and > 50 % fragmentation of the cytoplasm.

Cumulative embryo scores were calculated by multiplying the cell number and grade of each embryo, on its day 3 of development, and summing the scores for embryos produced by each patient in each cycle of treatment (Steer et al., 1992). Average cumulative embryo scores were calculated by dividing the cumulative embryo score by the total number of oocytes retrieved.

Embryo transfer was done on day3; one to four embryos were transferred depending on patient's age, embryo quality and the number of embryos available. The luteal phase was supported with 200mg three times per day of natural progesterone vaginally and daily until a pregnancy test was performed. Pregnancy was determined as positive by ß HCG levels after 14days from embryo transfer and confirmed by repeat the test after 48hours.Progesterone treatment was continued up to 12 weeks gestation. Clinical pregnancy was determined by the presence of intrauterine gestational sac 2weeks after pregnancy test. Criteria for cycle cancellation due to poor ovarian response included the presence of fewer than 3 growing follicles on ULS, with E2 level < 750pmol/ L on day7 of stimulation. All IVF cycle parameters including peak E2 level, total number of oocytes retrieved, embryo numbers and average cumulative embryo scores, rate of cycle cancellation, positive pregnancy tests and ongoing clinical pregnancies were analyzed.

STATISTICAL ANALYSIS

SPSS for windows; standard version 10.0.7(SPSS Co., Chicago, IL, USA) was used for the statistical analysis. The student's t-test or Mann-Whitney U test was used to compare the mean values between the endocrine variables, cycle characteristics, embryological data and clinical outcomes between pre and post DHEA IVF cycles. Differences in outcome rates were analyzed using an x^2 or Fisher's exact test. In all statistical analyses, P<0 .05 was considered statistically significant.

RESULTS

Twenty-five women with significantly diminished ovarian reserve had IVF cycle before DHEA treatment, six patients get clinical pregnancy. Five out of them continued their pregnancy and one patient aborted during first trimester. So, only twenty patients had their IVF cycle after DHEA treatment.

There were no significant differences in patient's age, BMI, day 3 LH, progesterone and prolactin. However after treatment with DHEA, patients demonstrated a significant improvement in day 3

FSH (p< 0.05), day 3 E2 (p< 0.05), day 3 testesterone (p< 0.05) and day 3 DHEA-S (p < 0.05) (Table 1).

Cycle characteristics before and after treatments with dehydroepiandrosterone (DHEA) were demonstrated in Table (II).There were no significant differences in duration of gonadotropin stimulation (days), total dose of rFSH(IU), total dose of hMG (IU)or endometrial thickness on the day of hCG administration. However, after treatment with DHEA, there were significant differences in number of follicles demonstrated by ULS on the day of hCG administration [Total (p = 0.01), ≥ 14 mm (p = 0.01) and ≥ 17 mm (p = 0.01)], Serum E2 level on the day of hCG administration (p = 0.001), number of oocytes retrieved (p = 0.01) and number of mature oocytes (Metaphase II) (p = 0.01).

Table (III) demonstrated embryological data and clinical outcomes before and after treatment with dehydroepiandrosterone (DHEA). There were no significant differences in number of embryos transferred or implantation rate. Although there were significant differences in fertilization rate (p<0.005), percentage of grade I/II embryos (p= 0.005), cumulative embryo score per oocyte retrieved (p = 0.001) and cancellation rate (p = 0.001).These were not reflected on biochemical pregnancy, clinical pregnancy rate/cycle or clinical pregnancy rate/embryo transfer.

Table 1: Demographic data and endocrine variables before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Number of patients	25	20	_
Number of cycles	25	20	_
Age (y)	39.6 ± 0.9	40.2 ± 0.9	NS
BMI (kg/m²)	24.73 ± 2.36	24.56 ± 2.43	NS
Day 3 FSH(mIU/ml)	9.9 ± 1.5	7.1 ± 0.5	< 0.05
Day 3 LH(mIU/ml)	5.6 ± 1.7	5.2 ± 1.4	NS
Day 3 E2(pmol/L)	227.37 ± 16.95	325.46 ± 9.7	< 0.05
Day 3 progesterone(nmol/L)	4.65 ± 1.3	4.57± 1.2	NS
Day 3 prolactin (mIU/L)	74±17.5	73±14.8	NS
Day 3 testesterone(μg/dl)	54. 64±10.31	79.84±10.93	< 0.05
Day 3 DHEA-S (μg/dl)	62 .54± 16.3	99.94 ± 15.4	< 0.05

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

Table II: Cycle characteristics before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Duration of gonadotropin stimulation (days)	11.0±1.0	10±1.0	NS
Total dose of rFSH(IU)	2650±1045	2575±1025	NS
Total dose of hMG(IU)	1500±150	1525±100	NS
Number of follicles on the day of hCG administration Total ≥ 14 mm ≥ 17 mm	 6 ± 1.5 3.5±1.5 2.5±1.5	 9 ±1.5 4.5±1.5 4.5±1.5	 0.01 0.01 0.01
Endometrial thickness on the day of hCG administration(mm)	9.3 ± 1.9	9.2 ± 1.7	NS
Serum E2 level on the day of hCG administration(pmol/L)	3800±1684	4760±1524	0.001
Number of oocytes retrieved	5.5 ± 0.5	7.5 ± 0.5	0.01
Number of mature oocytes (Metaphase II)	3.5± 0.5	5.5± 0.5	0.01

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

Table III: Embryological data and clinical outcomes before and after treatment with dehydroepiandrosterone (DHEA)

	Pre - DHEA	Post- DHEA	P value
Fertilization rate (%)	45.8	72.6	<0.005
Number of embryos transferred	2.53±1.2	2.48±1.2	NS
Grade I/II embryos (%)	50.4	73.6	0.005
Implantation rate (%)	7.4	11.8	NS
Cancellation rate (%)	10/25(40%)	3/20 (15 %)	0.001
Cumulative embryo score per oocyte retrieved	10.5±1.2	18.5±1.2	0.001
Biochemical pregnancy (%)	6/25(24%)	6/20(30%)	NS
Clinical pregnancy rate/cycle (%)	4/25(16%)	4/20(20%)	NS
Clinical pregnancy rate/embryo transfer (%)	4/25(16%)	4/20(20%)	NS

Data presented as mean ± SD.P value determined by ANOVA analysis. P<0 .05 was considered statistically significant. NS = not significant.

DISCUSSION

To the best of our knowledge, this is the first trial employing DHEA supplementation for poor responder patients undergoing ovarian stimulation using GnRH antagonist protocol. Lack of a large number of adequately designed prospective randomized trials and inconsistency in inclusion criteria makes it extremely difficult to conclusively demonstrate an advantage of a single protocol for all poor responder patients. However, it is the policy of our centre to use Gn RH antagonist protocol for poor responder patients. This is in policy with several prospective trials which had evaluated GnRH antagonist in comparison with alternative regimens designed for poor responders.

Cheung et al., compared a fixed start (day 6) of multidose GnRH antagonist to a long GnRHa protocol in a prospective randomized trial of 66 poor responders. There were no significant differences in response, although a trend towards higher pregnancy rates/transfer in those receiving Gn RH antagonist (26.38 vs. 17.6%) did not achieve statistical significance. However, extremely high cancellation rates of over 30% were noted in both groups 12.

In the present study, DHEA supplementation for poor responder patients was associated with improvement in hormonal profile in the form of decrease in basal FSH and increase in basal estradiol, testosterone and DHEA-S. These observations were in agreement with Casson et al., 9 and Mamas and Mammas 14 and confirm numerous hypotheses which had been previously suggested on how DHEA promotes fertility.
DHEA and DHEA-S are ubiquitous steroids of primarily adrenocortical reticularis zonal origin. These hormones circulate in high amounts in female reproductive life; however concentrations fall progressively with age 13, leading to speculation that replacement of DHEA and DHEA-S in the elderly may have age-retardant effects 10.

Casson et al., (9) who were the first to report the beneficial effects of DHEA on ovaries with diminished reserve, demonstrated a transient increase in IGF-1 in patients undergoing exogenous gonadotropin ovulation induction after pretreatment for only 8 weeks of DHEA. Such a transient increase in IGF-1 may have been due to either increased production or androgen effect on the liver producing decreased IGF-1 binding hormone 15.

In the present study, we observed also a significant increase in serum E2 level on the day of hCG administration and increase in the number of mature follicles demonstrated by ULS. Furthermore, this was associated with increase in the number of both retrieved and mature oocytes. Our results were in agreement with those of Barad and Gleicher15, although in their study, women received 75mg of DHEA daily (25 mg three times daily) for an average of 17.6≥ 2.13weeks and microdose GnRH agonist protocol was used for ovarian stimulation.

It seems that DHEA supplementation for poor responder patients, through its effect on increasing serum androgen level could produce its effect on ovarian follicular growth. Several theories had been previously suggested to explain the possible mechanism of association between increasing.

androgen level and improvement of follicular growth. Androgens may act as a metabolic precursor 16 or promote steroidogenesis 17, or act as a ligand for androgen receptors 18 or increase insulin-like growth factor (IGF-1) 19 or by other unknown mechanism yet. Haning et al., 20 demonstrated that DHEA is the prehormone for up to 48 % of follicular fluid testosterone, which is the prehormone for E2 during ovulation induction with exogenous gonadotropins.

Moreover, in the present study, DHEA supplementation for 2 months was associated with a significant increase in percentage of grade I/II embryos, cumulative embryo score per oocytes retrieved and decrease in cancellation rate. However these effects could not be reflected on implantation rate, biochemical and clinical pregnancy rates. This was in agreement with Barad et al., 21 regarding embryo quality and score however in contradiction regarding clinical pregnancy rate.

Barad et al., 21 in a case control study, assessed the role of DHEA supplementation on pregnancy rates in 190 women with diminished ovarian reserve. The study group included 89 patients who used supplementation with 75 mg daily of oral micronized DHEA for up to 4 months prior to entry into IVF. The control group composed of 101 couples who received infertility treatment, but did not use DHEA. They demonstrated that cumulative pregnancy rates were significantly higher in the study group (28.4% vs. 11.9 %).
As a result of the possible side effects which could be associated with DHEA including androgenic effects like acne, deepening of voice , facial hair growth, moreover, the long term effects of DHEA supplementation which remain unknown. Furthermore, since DHEA is a precursor of sex steroids. There may be concerns of a possibility of increased risk of estrogen and androgen dependant malignancy. So that is why we decided to use DHEA supplementation for only 2 months and in the minimal dose of 50 mg micronized DHEA per day. As a result, all patients in our study did well with DHEA, with no reported side effects and no impairment in kidney, liver or thyroid functions.

In summary, this study confirms the preliminary studies which reported beneficial effects of DHEA on ovarian follicular function, improvement in hormonal profile and embryological data after DHEA supplementation for poor responder patients. However, large prospective randomized placebo controlled trials are waited to demonstrate the improvement in clinical pregnancy rate after DHEA supplementation. Furthermore, the optimal dosage supplementation of DHEA should be demonstrated to outweigh the benefits of the drug for poor responder patients against the potential side effects.

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Antagonist/letrozole protocol versus microdose flare-up protocol in poor responders: a randomized study

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ABSTRACT

Objective: To compare the efficacy of gonadotropin-releasing hormone (GnRH) antagonist/letrozole (AL) and micro-dose flare-up (MF) protocols on cycle parameters and clinical outcomes in poor responders.

Patients & methods: A randomized prospective study comprised of 150 infertile women undergoing controlled ovarian stimulation (COS) and intracytoplasmic sperm injection (ICSI) and classified as past or potential poor responders based on specific criteria. Participants were prospectively randomized to receive AL protocol (group I, n=75) or MF protocol (group II, n=75). Clinical pregnancy was the primary outcome. Cycle cancellation rate, dose of gonadotropin used, serum E2 levels, number of retrieved oocytes, fertilization rate, and embryo quality were secondary outcomes

Result(s): Patient characteristics were similar between the two protocol groups. There were no significant differences in mean age, number of oocytes, fertilization rates, number of embryos transferred, or embryo score. Peak E2 levels were lower in the AL group, although this difference did not reach statistical significance. Clinical pregnancy per started cycle (33.3% versus 29.3%, P=0.59) and per embryo transfer (36.8% versus 34.4%, p=0.7) were comparable between AL and MF protocols. Trends toward lower cancellation rates were noted among AL group, but these did not reach statistical significance.

Conclusion(s): The treatment outcomes of gonadotropin-releasing hormone antagonist/letrozole protocol and the microdose flare-up protocol seem to be similar in poor ovarian responders undergoing ICSI.

Key words: GnRH antagonist, letrozole, microdose flare up, poor responder, ovaria stimulation.

INTRODUCTION

The success of in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) treatment depends on adequate follicle recruitment. Controlled ovarian stimulation (COS) is gonadotrophin (Gn) induced stimulation of the ovaries for purposes of in vitro fertilisation (IVF) treatment, which specifically aims at inducing ongoing multiple follicle development rather than a single dominant follicle in ovulatory women(1). Protocols for COS are based on this principle. Hence, ovarian response to COS may differ leading to an optimal response or a sub-optimal response, which may be 'poor response' or 'ovarian hyperstimulation syndrome.

Although there is lack of uniform definitions, poor response to COS can be generally defined as unsatisfactory ovarian response in terms of low number of follicles developed, low serum estradiol (E2) levels, and low number of oocytes retrieved despite adequate ovarian stimulation. However, the cutoff points for these parameters that define poor response vary between studies (2, 3).

The ideal controlled ovarian stimulation protocol for poor responders has not been clearly defined. A variety of regimens have been employed including the use of increased gonadotropin doses, decreased GnRH agonist (GnRH-a) doses, flare regimes, adjunctive growth hormone, GnRH antagonists, and microdose flare regimes (3). Several studies have supported the use of a microdose GnRH-a flare protocol in this patient group, which demonstrated improved ovarian responses and clinical outcomes (4-6). This approach takes advantage of the initial release of endogenous Gn that is induced by low-dose GnRH-a administration in the early follicular phase in an effort to enhance response to the subsequent administration of exogenous gonadotropins. Although high doses of gonadotropins are typically added, but the effective stimulation largely has been attributed to endogenous gonadotropin release (7).

GnRH antagonists have been administered to poor responders during gonadotropin stimulation with mixed results (8-12). The use of antagonists allows initiation of gonadotropin stimulation in the absence of prior pituitary gonadotropin down-regulation given that these agents are not typically added to the COS protocol until follicular maturation has already been initiated. The aromatase inhibitor letrozole has been employed as a novel approach

to improving gonadotropin response. This agent acts by blocking E2 synthesis with a resulting decrease in negative feedback at the level of the pituitary. The resulting increase in endogenous gonadotropin secretion may enhance the ovarian response to exogenous gonadotropins (13, 14). Moreover, the increased intraovarian androgens, in addition to serving as precursors for ovarian estrogen synthesis, also have been shown to have a fundamental trophic role in primate ovarian follicular development by augmenting FSH receptor expression on granulosa cells (15). The preliminary studies in poor ovarian responders have noted decreased gonadotropin consumption (16) and increased number of oocytes retrieved (17) with the use of letrozole. Therefore, the combination of a GnRHantagonist and letrozole in conjunction with gonadotropin may offer a new alternative to the microdose GnRHa flare protocol for poor responders preparing for IVF.

Several investigators have compared flare and GnRH antagonists in poor responders with conflicting results. Fasoulitis et al. described a trend, though not statistically significant, toward higher implantation and clinical pregnancy rates when using antagonists (11). On the other hand, Demirol and Gurgan 2009, in a randomized study, reported that the microdose flare-up protocol seemed to have a better outcome in poor-responder patients, with a significantly higher mean number of mature oocytes retrieved and higher implantation rate (18).

Actually, these two protocols are popular in terms of treatment of poor responders and efforts to define their efficiency and safety will contribute to the improvement of therapeutic management of poor responder patients. Therefore, the aim of this prospective randomized study is to compare the efficacy of gonadotropin-releasing hormone (GnRH) antagonist/letrozole (AL) and micro-dose flare-up (MF) protocols on cycle parameters and clinical outcomes in poor responders.

PATIENTS & METHODS

The current study was conducted, from September 2007 to September 2010, in private and university IVF units following approval of the institutional review board (IRB) at Zagazig University School of Medicine. The study included 150 patients who were candidates for ICSI and classified as poor responders as described below. All women were ≤ 37 years and underwent pre-cycle ovarian reserve testing, which included an assessment of cycle day 3serum FSH and measurement of antral follicle count (AFC) measuring 2–10 mm during the early follicular phase. Criteria for classification as a poor responder included at least one of the following: day 3 serum FSH level>10mIU/mL, <6 total antral follicles, prior cycle cancellation and prior poor response to COS (peak E2 <500pg/mL and/or <4 oocytes retrieved). An informed consent was obtained from all couples.

Treatment Protocols

A total of 75 patients were assigned to the GnRH AL protocol (group I). On day 3 of the cycle, gonadotropin stimulation was initiated with recombinant FSH(Gonal-F, Serono, Rockland, MA) 300 IU and hMG (menogon, Ferring) 150 IU daily. Letrozole (Femara, Novartis, East Hanover, NJ) 2.5 mg daily was also initiated on day 3 and continued for 5 days. A GnRH antagonist, cetrorelix (Cetrotide, Serono) 0.25 mg SC daily was initiated once the lead follicle reached 14 mm in mean diameter. Serial ultrasound examinations and evaluation of serum E2, LH, and P levels were used to assess follicular maturation. Gonadotropin doses were adjusted after 5 days of stimulation

A total of 75 patients were assigned to the MF protocol (group II). Each patient underwent treatment with 20 ug SC administration of GnRH agonist triptorelin (Decapeptyl;Ferring,), twice daily, from second day of the cycle until the day of hCG administration. The exogenous gonadotropin stimulation started on day 3 of the cycle and consisted of recombinant FSH and hMG (in the doses described above).

Human chorionic gonadotropin (hCG) 10,000 IU IM was administered when at least two follicles achieved a mean diameter of 17 mm and serum E2 levels were ≥ 500 pg/mL. Oocyte aspiration was performed 35hours after hCG administration. ICSI was performed in a standard way. Oocytes were examined 16–18 hours after ICSI for pronuclei (PN). Normal fertilization was defined as existence of two pronuclei (2PN). The embryos obtained were categorized on day 2 or 3 into four categories depending on their morphologic appearance, cytoplasmic fragmentation, and blastomere size (grade I [high quality]: embryos with equal blastomeres and no observed cytoplasmic fragmentation; grade II [good quality]: embryos with equal blastomeres and <20% fragmentation of the cytoplasm; grade III [fair quality]: embryos with unequal blastomers and 20%–50% fragmentation of the cytoplasm; grade IV [poor quality]: embryos with unequal blastomers and >50% fragmentation of the cytoplasm)(19). Depending on patient's age, embryo quality, and the number of embryos available, one to four embryos were transferred 2–3 days after oocyte collection. Cycle cancellation was recommended when fewer than three developing follicles of an appropriate growth pattern were noted.

Luteal phase support

In both groups, daily intramuscular injection of progesterone (Prontogest; Ibsa, Switherland) 100 mg, started from the day of embryo transfer and continued until a negative pregnancy test or a positive fetal heart beat was documented by transvaginal ultrasound. In all groups, serum HCG tests were performed on days 18 and 20 after the administration of HCG. An ultrasound scan was done 3 weeks after a positive pregnancy test to confirm a clinical pregnancy. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (i.e. 18 weeks after fertilization) (20). Ongoing pregnancy was defined as pregnancy developing beyond 20 weeks gestation

Hormone measurements

Serum concentrations of FSH, LH, oestradiol, progesterone and bHCG were determined using Elecsys 2010 (Roche, Germany). For FSH, the analytical sensitivity was <0.1 IU/l with total precision of 2.9%. For LH, the analytical sensitivity was 0.1 IU/l with total precision of 1.6%. For oestradiol, the analytical sensitivity was 5 pg/ml with total precision of 2.3%. For progesterone, the analytical sensitivity was 0.03 ng/ml (conversion factor = 3.18 nmol/l) with total precision of 2.4%. For quantitative bHCG assay, the analytical sensitivity was 0.5 IU/l with total precision of 2.1%.

Outcome measures

Clinical pregnancy was the primary outcome. Cycle cancellation rate, dose of gonadotropin used, serum E2 levels, number of retrieved oocytes, fertilization rate, and embryo quality were secondary outcomes

Randomization

On day 1 of the cycle, included women were randomized into two groups (I and II) using block randomization. Allocation concealment was performed using 150 identical dark-sealed envelopes, prepared by the statistician and kept in the unit's pharmacy. When the woman was eligible and agreed to participate, she was instructed to open the next available envelope to determine the group to which she was assigned. The randomization key was kept with the pharmacy director and not opened until after statistical analysis was performed.

Statistical Analysis

Prior data comparing the CPR between AL and MF was shown to be 37% and 52%, respectively (21). Therefore, 61 women would be required to be able to reject the null hypothesis that the success rates are equal with a probability (power) of 0.8 and Type I error probability of 0.05 using the Chi-square (χ^2) statistic. Since the rate of cancelation during COS was reported to be up to 24% (22), a total of 75 women were included in each arm. Data were statistically described in terms of mean ± standard deviation (SD) and percentages, where appropriate. Comparison of quantitative variables was done using Student t- test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed, except when the expected frequency of events was less than five, in which case the Fisher's exact test was used. Relative risk and 95% confidence intervals (CI) and/ or probability value (p-value) are presented. A *p*-value <0.05 was considered to be statistically significant. All statistical calculations were done using computer programs Excel version 7(Microsoft Corporation, NY, USA) and SPSS version 15 (SPSS, Chicago, IL, USA).

RESULTS

Seventy-five cycles were performed with the AL protocol, and 75 were performed with the MF protocol. The mean age of the patients, mean duration of infertility, basal FSH level and AFC were similar in both groups (table I). Number of previous IVF cycles was comparable (P = 0.66). A trend toward higher cycle cancellation rates that did not reach statistical significance was experienced among patients assigned to MF as opposed to AL (14.6 % vs. 9.3%, P = 0.31). In AL group, a total of seven cycles were cancelled (three cycles owing to impaired response, one to absent oocytes on retrieval, one to absent mature oocytes, one to fertilization failure and one to arrested embryo development) while in MF group a total of eleven cycles were cancelled (four cycles owing to impaired response, cyst was formed in three cases, two cycles duo to absent oocytes on retrieval and two to fertilization failure). These cases were included in the intention-to-treat analyses

The results of COS are displayed in table II. There were no differences in duration or doses of gonadotropins required, numbers of retrieved or mature oocytes. As would be expected, lower peak E2 levels were noted with AL, although this difference did not reach statistical significance. Endometrial thickness and progesterone level on day of hCG were comparable. Fertilization rates, high quality embryos (grade 1 & 2) and number of transferred embryos were also similar between the two groups. There were no statistically significant differences in clinical pregnancy rates (per started cycle and per embryo transfer) between the two groups. Similarly, abortion and ongoing pregnancy rates were also comparable between both groups (table III).

Table I: Baseline characteristics of the AL and MF groups

Group	AL(n = 75)	MF(n = 75)	P value
Age (years)	31.17 ± 3.9	31.85 ± 3.5	0.26
Infertility duration(years)	5.5 ± 1.5	5.14 ± 1.6	0.14
BMI(Kg/m2)	23.13 ± 1.6	23.17 ±1.6	0.88
Basal FSH(IU/L)	7.92 ± 1.4	7.69 ±1.4	0.33
AFC	5.05 ± 1.1	4.88 ±1.1	0.34

Data presented as mean ± SD. P > 0.05 non-significant

Table II: Controlled ovarian stimulation characters of the AL and MF groups.

Group	AL(n = 75)	MF(n = 75)	P value
Stimulation duration	11 ± 1.47	11.2 ± 1.45	0.44
Ampoules number	67.78 ± 10.3	64.9 ± 8.3	0.07
E2 on hCG day	1713.39 ± 308.2	1816.81 ± 359.7	0.07
P on hCG day	1.11 ± 0.33	1.10 ± 0.32	0.9
Endometrial thickness	8.45 ± 1.04	8.41 ± 1.03	0.18
Retrieved oocytes	5.19 ± 1.6	4.86 ± 1.5	0.22
Mature oocytes	4.54 ± 1.3	4.41 ± 1.2	0.55
Fertilization rate(%)	68.33 ± 15.1	66.98 ± 18.1	0.63
Grade I & II embryos	3.86 ± 1.5	3.44 ± 1.1	0.06
Transferred embryos	3.56 ± 1.14	3.32 ± 0.82	0.18

Data presented as mean ± SD unless otherwise specified. P > 0.05 non-significant

Table III: Pregnancy outcome of AL and MF groups

Group	AL(n = 75)	MF(n = 75)	P value	RR(95% CI)
Clinical pregnancy/ started cycle, n (%)	25/75(33.3%)	22/75(29.3%)	0.59	1.2(0.57-2.55)
Clinical pregnancy/ ET, n (%)	25/68(36.8%)	22/64(34.4%)	0.77	1.11(0.51-2.41)
Abortion n (%)	2/25(8%)	2/22(9.1%)	1	0.87(0.08-9.78)
Ongoing pregnancy/ ET, n (%)	23/68(33.8%)	20/64(31.3%)	0.75	1.12(0.51-2.49)

Chi-square test, or Fisher exact test when appropriate. P > 0.05 non-significant

DISCUSSION

Despite improvements in the success of IVF and ICSI in all age groups, the treatment of poor responders remains controversial. One of the difficulties in critically evaluating various COS protocols is the lack of a single universally accepted definition of the poor responder (2, 3). A variety of criteria have been used alone or in combination as inclusion criteria for proposed protocols. The current investigation has not relied on a single criterion but rather on the evidence of prior poor response or presumed poor response based on standard evaluations of ovarian reserve (23). Older women were excluded to avoid conflicting results of mixing women with physiologic age related ovarian insufficiency and non physiologic impaired ovarian response, so as to evaluate a more homogenous population in a truly randomized trial. There is no question that it would be advantageous to predict a challenging group of young women who will respond poorly and tailor an appropriate protocol in advance rather than allowing them to fail to respond to more standard regimens before introducing a more appropriate stimulation regime during a second cycle.

In the current study, a stimulation protocol involving the use of an aromatase inhibitor initiated in the early follicular phase along with gonadotropin stimulation and subsequent GnRH antagonist suppression was compared with a more standard microdose flare GnRH-a regimen. Stimulation parameters, including duration of stimulation and gonadotrophin ampoules were comparable between the two groups. Lower mean peak E2 levels were appreciated in the group receiving the GnRH antagonist, which was presumed to be due to aromatase inhibition induced by letrozole (14). Even so, quantitative results of stimulation were similar between the two protocols as judged by the number of retrieved, mature and fertilized oocytes obtained as well as the number of high quality and transferred embryos (Table 2). There were no statistically significant differences in clinical pregnancy (per started cycle and embryo transfer) and ongoing pregnancy rates, between the two protocols.

Prior randomized and nonrandomized studies have offered varied results. Traditional flare regimes in which higher-dose GnRH-a is administered with minimal delay before initiation of gonadotropin COS are associated with significant increases in follicular-phase serum P and androgen levels, which may exert deleterious effects on follicular development and oocyte quality (24). In an effort to minimize this effect, while maintaining the benefit of stimulating endogenous gonadotropin release, the administration of lower doses of GnRH-a was proposed. Three separate trials employing lower daily doses of GnRH-a, in appropriately selected poor responders, have demonstrated significant benefit over more traditional regimes with regards to improved ongoing pregnancy rates and decreased cancellation rates (4-5,25). Surrey et al. reviewed different regimens used in the poor responders and concluded that a microdose GnRH agonist flare protocol was more uniformly beneficial to the cycle outcome than other regimens (5).

The introduction of GnRH antagonists brought new hope to the treatment of poor responders. Nikolettos et al. showed that patients in the GnRH antagonist group required fewer ampoules of gonadotropins and needed a statistically significantly shorter treatment duration compared with the long GnRHa protocol to achieve the same number of follicles (26). Craft et al. reported a significant reduction in cycle cancellation rates and more oocyte production in a mixed group of patients with poor response or failed cycles during prior standard GnRH-a down-regulation cycles(8). Nevertheless, only an 11.8% live birth rate per completed cycle was achieved. In Cochrane Database Systematic Review 2010, higher number of retrieved oocytes and lower Gn doses were reported upon using antagonist versus long agonist protocol. However, there were no differences in CPR, cancellation rates or miscarriage rates between the two protocols (27).

Although the use of an antagonist eliminates the effect of excessive pituitary gonadotropin suppression induced by a GnRH-a, poor responders may benefit from additional stimulation of endogenous gonadotropin release in the early follicular phase. D'Amato et al. employed a protocol including clomiphene citrate, high dose gonadotropins, and delayed antagonist administration to a group of poor responders and compared results with patients undergoing a long protocol in a prospective nonrandomized trial (28). Although cancellation rates were significantly decreased, no significant differences in pregnancy rates were noted. Once again, low implantation rates (13.5%) were noted. Some possible causes for this phenomenon may be the antiestrogenic effects of clomiphene on the endometrium and potential deleterious effects on oocytes (29). The aromatase inhibitor letrozole acts to increase endogenous gonadotropin release but does not deplete estrogen receptors at the level of the endometrium and could theoretically serve as an ideal alternative to clomiphene citrate in this model (30). Controversy has surrounded the subject of the overall safety of letrozole therapy. A published abstract has suggested a possible increased risk in birth defect rates with letrozole use, which is a source of additional concern (31). However, a larger retrospective trial evaluating 911 newborns who were conceived after administration of this agent to mothers as infertility therapy found no greater overall rates of major or minor malformations than in those who were conceived after administration of clomiphene citrate (32).

Two studies have demonstrated that the addition of letrozole to gonadotropins in poor-responder patients undergoing COS improved follicular response and lessened gonadotropin requirements (14, 33). Garcia-Velasco and colleagues reported the results of an observational pilot trial designed to assess the effects of the addition of letrozole to an antagonist-gonadotropin regimen in a group of poor responders (17). No enhancement of pregnancy rates was noted, but implantation rates were improved. In the current investigation, gonadotropin requirements, oocyte number, and embryo quality as well as pregnancy outcomes were similar between the two treatment groups assigned to either AL or ML protocols. Demirol and Gurgan, in a randomized controlled study upon 90 poor responders, compared the efficacy of the microdose flare-up and multiple-dose antagonist protocols. The two protocols had comparable CPR. However, the MF protocol had significantly higher mean number of mature oocytes retrieved and higher implantation rate (18). Of note, the study included different patient population (older women and those having basal FSH > 15 IU/L) and letrozole was not added to the antagonist group. On the other hand, two recent studies had concluded that microdose flare-up protocol and multiple dose GnRH antagonist protocol seem to have similar efficacy in improving treatment outcomes of poor responder patients (34, 35). Interestingly, in a recent retrospective study upon 1383 poor responders (predicted to have or with a history of poor ovarian response), the MF protocol was used in 1026 cycles and the AL protocol was used in the remaining 357 cycles. The clinical pregnancy and implantation rates were comparable between the two groups (36). So, based on current study findings and others (21, 36), it could be assumed that AL and MF are equally effective protocols for management of poor responders.

In the current study, a trend toward higher cycle cancellation rates that did not reach statistical significance was experienced among patients assigned to MF as opposed to AL (14.6 % vs. 9.3%, P = 0.31). Cancellation due to impaired response, absent oocytes on collection or fertilization failure was comparable between both groups. However, three cases developed cyst formation during COS using the MF protocol while none developed cyst among the AL group. Interestingly, these three cases had AFC ≤ 3 and two of them had been cancelled previously due to impaired response with development of only one follicle during COS. In such group of whom we can call "extreme poor responders" the initial release of endogenous gonadotropins induced by GnRH-a administration in combination with exogenous Gn might have caused these cysts. Actually, none of the published studies reported on cyst formation development and cycle cancelation is generally mentioned broadly without going into much details. So, the current study findings definitely warrant further study in a larger group of these patients.

In conclusion, in the present study, the treatment outcomes of gonadotropin-releasing hormone antagonist/letrozole protocol and the microdose GnRH-a flare-up protocol seem to be similar. Further prospective randomized trials with larger numbers of patients and large meta-analyses with strict inclusion criteria are needed to assess the efficacy of the two protocols in the poor responders.

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Fertility options for muslim people: acceptability & awareness among clients and health service providers

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ABSTRACT

Objective: The available options for infertile Muslim couples are 1: remaining childless permanently 2: Polygynous marriage, 3: Divorce; 4: Fostering an orphan legally, or 5: New reproductive technologies. In nearly 20 nations of the Muslim Middle East and Muslim people in non-Islamic countries, marriage is highly valued and they can not live happily without children. Authors have made an important point regarding deficient counseling concerning awareness, acceptability and offering of these options. To test the above fertility options awareness & acceptability among infertile couples & consultants.
Materials& Methods: 120 Muslim couples & 20 consultants were surveyed over a 6-months period were surveyed for their knowledge of fertility options awareness, acceptability and offering. It was hypothesized that less than 50% of the subjects had an adequate understanding of the available options.
Results: 33% (n= 80) of subjects (for clients) had a score of 5 or more which was considered as having an adequate understanding of the available options, while less than 20 % of doctors (n = 4) offering other options during counseling. .The hypothesis was accepted, giving reason for concern about the effectiveness of consumer education at all levels of fertility management.
Conclusions: In Muslim communities, fertility awareness is generally very poor among clients and consultants. The authors believe that there are many positive implications for promoting patient education about fertility awareness.
Keywords: Fertility options, Muslim, awareness, acceptability.

INTRODUCTION

Infertility as it was described by National Institute for Clinical Excellence NICE 1 is the state of inability to conceive after 2 years of unprotected intercourse .It is more than a physical problem ,it has devastating social, psychological and economic burden 2, which go beyond childlessness, and women bear the major hurt of the burden and made to feel inferior and may be abused or even tortured by the family even when the man is at fault3.

Although infertility is a global problem and estimates of its prevalence are not very uniform and vary from region to region, the available literature figures worldwide up to 8–12% 1 and estimated to be up to 13% in Egypt 4 .In Nigeria where Muslims represent up to 65% of the whole population the prevalence up to 20-30% 5. In 1992 Islam had over 1.250 billion followers and this figure expected to increase to 2.5 billion by the year 2020 With the present rate of population growth 6. Extrapolation to the global population means that 29-44 million of these infertile couples are Muslims because of a relatively high prevalence of infertility among Muslims in developing countries. The rate of tubal occlusion in sub-Saharan Africa with its predominant Muslim population is over three times that in other regions, with the exception of the Eastern Mediterranean.

As Most couples with sub fertility will conceive spontaneously or will be amenable to treatment, so that only 4% remain involuntarily childless 7 and Facing a couple with Intractable infertility, although rare, is extremely distressing not only for the clients but also for the health service providers and alleviation of infertility even by proper counseling therefore becomes a necessity on many levels and it has been declared a public health issue by the World Health Organization (WHO) 8. So help from a counselor may be needed, people may be able to accept their position and see the opportunity to start a new life 9.

United nations declarations of Human Rights in Article 16:1 states that "Men and women of full age, without any limitation due to race, nationality or religion have the right to marry and found a family 10 and the reproductive health insists on the individual's right to reproduce and freedom to decide when and how often 11.

The most basic and desired goal in adulthood is achieving parenthood 10. The situation is the same in Muslim community as the clear message to Muslim spouses is to have as many children as they raise them suitably as the children are a divine gift to the spouses ,and the demand good care for them ,educating and qualifying them to serve society 6.Based on

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understanding of the Islamic teachings and looking to the local moral worlds of infertile Muslims as they attempt to make, in the religiously correct fashion, Muslim community is differ than western world .The former depends upon the presence of a nonbinding but authoritative Islamic religious proclamations called fatwas that have profoundly affected the practice of many aspects in such field. Recently, many fatwas have been issued in Egypt and other Muslim countries 12. In western countries, the available options for a couple with intractable infertility are donation (Egg, sperm, and embryo donation), adoption, surrogacy or accepting a childfree lifestyle. The available options in Muslim world is quite different and they include (1) remaining childless permanently; which is unthinkable in the Muslim community (2) to remain together in a polygynous marriage (if the cause related to the wife), which is rarely viewed as an acceptable option by women themselves; (3) to divorce (if the cause is related to the husband and /or the wife) which appears be traumatic for even both ; (4) fostering an orphan legally, which is rarely viewed as an acceptable option; or (5) to go through New reproductive technologies, including IVF and ICSI, to overcome infertility. So it can be concluded that the acceptable fertility options may vary according to couple's culture, religious, moral beliefs, and the availability of such option.

It is observed that there is little published literature on the level of fertility awareness amongst customers attending fertility clinics in general 13 and especially for special circumstance e.g. Muslim communities. Fertility awareness is generally very poor not only in couples attending a tertiary fertility unit but also among heath service providers. Also, physicians offering the fertility services are different in their acceptability to all options.

As counseling concerning any treatment option for any other medical problems should be the first step it should have the same priority in the management of one of the most sensitive issues for the Muslim families which is infertility. From a patient's perspective, the authors believe that there are many positive implications for promoting customer awareness and their education about the available fertility awareness and acquiring knowledge of different fertility options that are available in their communities can be a very empowering experience, and can ameliorate a potentially solvable problem. From a physician's perspective, offering the options can be met with a valuable rate of acceptance.

It has been our observation for a long time that clients attending the infertility service clinics at our hospital and private sectors have an inadequate understanding of the available fertility options for intractable cases .Until analysis of the results of this study we do not know the root defect whether from deficient clients knowledge or lack of awareness and offering from of the heath service providers to their clients.

Recently there is a trend to increase the patient involvement in health care delivery , evaluate new treatments , as part of audit and also used to evaluate participant opinion of as part of the Continuing Professional Development (CPD) process 14. As the questionnaires can be used in a wide range of situations to gather opinion and collect information and attitude of consumers towards certain modality of treatment, so the present study used this technique to test the above fertility options awareness & acceptability from the infertile couples and offering the package by health care providers.

Materials & Methods

The study questions were does the Muslim people with infertility aware and /or accept all available fertility options? And does their health service providers aware /offering all these options during counseling? The study populations were two groups: the first one included hundred and twenty couples attending three tertiary infertility clinics (infertility unit of Obstetrics & Gynecology department, El-Menia faculty of medicine, El-Menia infertility center for research and treatment, El-Menia university, and private unit managed by the three authors) over a period of 6 months suffering from intractable infertility for infertility investigation and treatment were invited to participate in this study. All the couples had been attempting conception for at least 3 years and had been referred from a specialist .They were assessed initially as having intractable infertility. The second group included twenty consultants offering the service in these three clinics. 16 of them were male while 4 were female doctors. The technique followed in the selection of both groups called "purposive sampling".

Prior to commencement of the study, the research proposal was approved by the ethical committee from the related department. A questionnaire was designed for this purpose (follow chart: 1) .The protocol used in this study was to provide the information in advance to all invited Participants during the initial meeting and then asking them to indicate whether they wish to take part in a survey or not when they come back for the next visit, rather than asking them to fill the questionnaire in one sitting . The interval between the two visits were accompanied with telephone reminder to improve the response rate .Clear contact details of the research coordinator (who is the second author) and main author in case of further enquires from the participants. Stress to all couples that their future health care will not be affected if a subject chooses not to participate in this study.

A separate questionnaire was designed by the authors for each participant (wife, husband & doctor) .The questionnaires were different in the questions offered to suit each participant. Husband and wives were surveyed for their knowledge of fertility options awareness and acceptability that are already available and matched for their believes according to Islamic teachings. The doctors were also surveyed for their awareness and offering the above options for their clients. We used The Arabic language for writing the questionnaire for clients while for the consultants we used English one. The questionnaires were printed in a White paper to avoid tiring of the eyes but marked for each participant category (i.e. red small circle for wives, blue for husband and green for doctors) for easy collections from the prepared boxes. Each questionnaire was labeled with a unique ID number so that easy to identify and also reminders are only sent to non-respondents.

Each questionnaire was composed of 12 questions which were designed to determine the participants' level of knowledge regarding his/her awareness and acceptability of the different available fertility options and their use of this information to enhance their chances of conception. Eight of the questions were multiple choice and four further questions asked subjects to describe details of their concerns and possible causes of why they are not aware or not accepting a particular option. We used Likert scale [15] to ask the participant the extent to he/she agree or disagree about a particular option. Questionnaire for the consultants was composed of also twelve questions but different contents .It covered certain areas as the duration of experience in this field, work load, awareness and offering the above options and why he /she think the reasons behind lack of awareness and lack of offering the available options.

Singing a written consent by each participant. each author was dedicated to one group, the main author for the doctors while the second author for the husbands .As the third author is a female doctor so she dedicated for the wives participating in the study .The questionnaire was completed before or after usual clinical visits anonymously by each subject (wife, husband & doctor) in a separate room and each participant was asked to put it in the prepared boxes that were collected from the three units after

completion of the survey.The questionnaire for the couples was graded into 3 classes:

(A) Level of awareness of each option (given one mark each).
(B) Level of accepting each option (given one mark each).
(C) Level of use such awareness & acceptability to enhance his /her fertility. Answered in open questions.

The questionnaire for the consultants was also graded into 3 classes:

(A) Level of awareness of each option.
(B) Level of offering to the treated couples.
(C) Level of use such awareness on offering during counseling. Answered in open questions.

The questionnaires were initially analyzed for characteristics such as; age of the participants, years of attempting conception, previous visits to the clinic seeking for medical advice about their concerns, perceived cause of infertility, previous counseling about the options available .

These questionnaires were scored by 2 independent clerks. Scores ranged from 0 for subject who had no concept of fertility options awareness, acceptability and/or offering the options to 10 for those who were highly aware, accepting or offering. A cut-off 5 or more or greater was considered as having adequate fertility awareness. The percentage of clients and consultants with fertility awareness & acceptability and offering scores of 5 or greater were statistically analyzed for acceptance of the hypothesis using a chi square test.

All participants were promised to receive feedback if they wish to do so for the results of this study stressing on the anonymity of the information provided separately by each one of them and not all information disseminated. It was hypothesized that less than 50% of the subjects had an adequate understanding of the available options and acceptability.

RESULTS

The response rate in this interviewer- based survey was 78% for the clients (husband & wives), while for the consultants was 82%.The self –complete questionnaire technique which followed in this survey ensured confidentiality and anonymity of all participants .They were allowed to used a separate room to fill it with clarification of vague questions .The Literacy level was sufficient to complete the questionnaire.The mean the mean age ± SD was 27.4 ± 6 for wives while 32.3 ± 4.9 for the husbands.

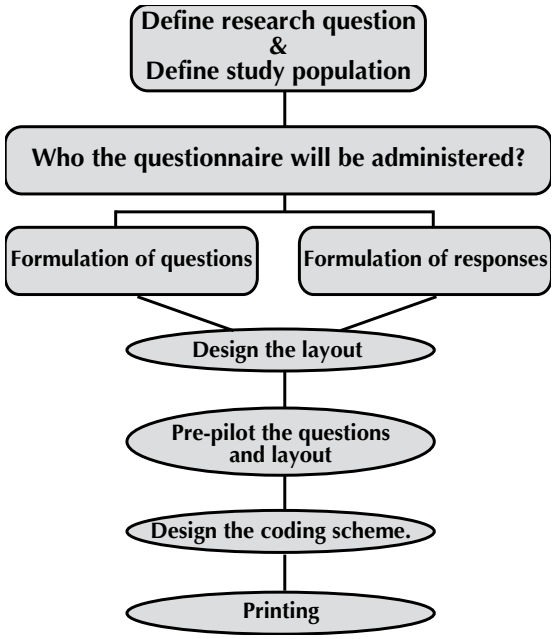
For 43% of couples (both husbands & wives) it was their first visit to a tertiary referral infertility clinic. While 20% of the husbands sought medical advice separately in a tertiary units due to isolated male factors, 75% of wives sought medical advice in a tertiary units for surgical interventions before for improving their fertility options .Seventy one percent had been trying to conceive for 3 years, 13% for 4-5 years, and 16% for greater than 6 years.

Fifty four percent of subjects recorded their understanding of the cause of their infertility as 'unexplained' or did not know at the time of their first visit to the clinic while twenty percent recorded the cause as male factor and twenty six attributed to female factors (figure 1). We found 33% (n= 80) of subjects (for clients) had a score of 5 or more which was considered as having an adequate understanding of the different 5 available options for the Muslim people while 67% not aware with the full options available .

Surprisingly, not all the aware clients accept the options i.e. only 25% (n=60) of the clients of the available fertility options accepting these options as a treatment i.e. 8% although aware but still not accepting the available options. Analysis of consultant's questionnaire revealed that 85% (n=17) of the participating consultants were aware with all options and 15% of them not fully aware .Only three consultants (Less than 20 %) of those who are fully aware (n = 4) offering all the available options during counseling while the others concentrate only to counsel their clients for the available medical options only like surgical interventions or assisted reproductive techniques (figure 3).

The hypothesis that less than 50% of subjects have an adequate understanding of the available fertility options and acceptability accordingly for the clients and offering these options by the service providers was supported (Chi sq. test <0.01).Some reasons were given by the consultants for their lack of offering all options listed in figure (4).

Flow chart (1): Stages of questionnaire design:



Flow chart for the study (2):

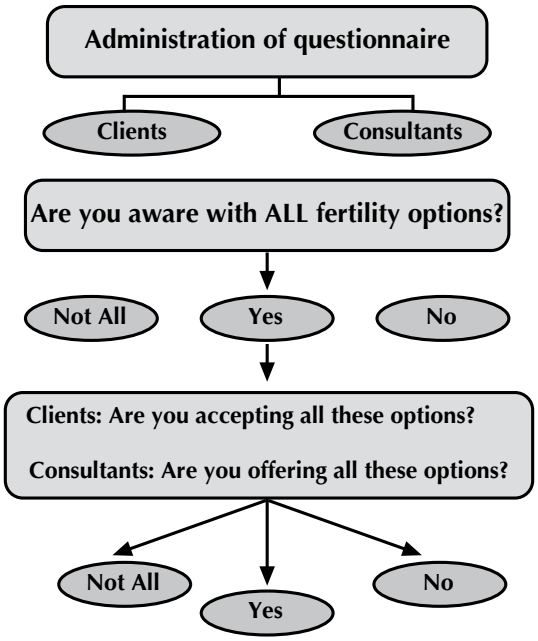


Figure 1: Clients' perception of the cause of infertility.

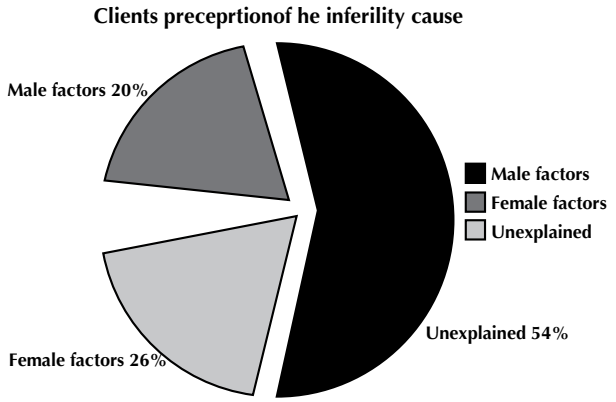


Figure (2): Clients awareness with fertility options.

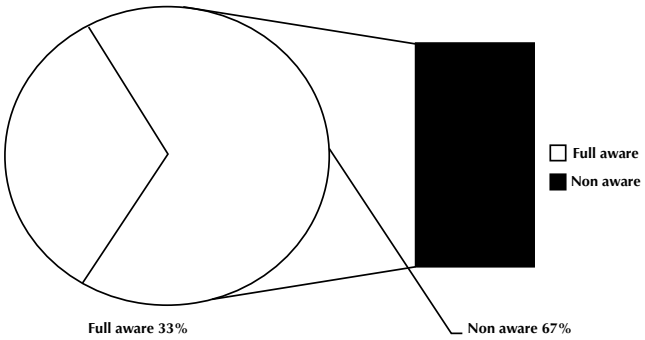


Figure (3): Consultants awareness & offering attitudes.

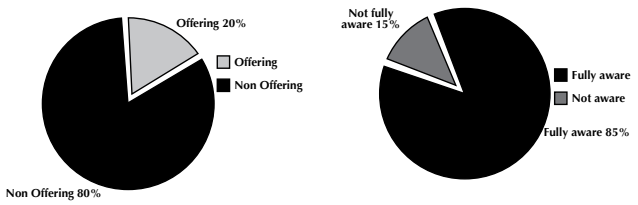
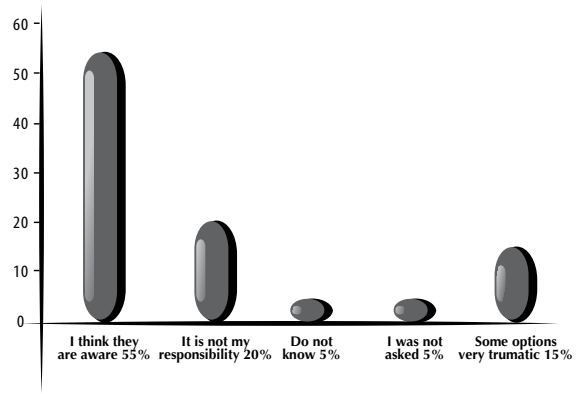


Figure (4) Consultants reasons for non offering.



DISCUSSION

The results obtained from this survey support the hypothesis of the authors that awareness and acceptability among clients and offering by service providers of different available fertility options in Muslim world is very poor giving reason for concern about the effectiveness of consumer education at all levels of fertility management.

The purposive sampling technique that was followed in the selection of the group study is useful because a wide range of possible views can be identified. We used self-complete questionnaire technique which ensured confidentiality and anonymity .Another advantage of this technique is being cheaper to administer. In this study we reported for the first time the area of significant deficiency in the first station in managing infertile couples not only in consumer education but also among service providers in the Islamic world. Of greatest concern is the proportion of couples (67%) and consultants (80%) who were scored as having no awareness or none offering of already available options.

We reported in this survey the majority of participants (71%) had been trying to conceive for 3 years, and the majority also (67%) were non aware with different options until facing the consultants at the tertiary units who also not offering all options (only 20% of them) .Therefore, it is important that during the initial visits with the general practitioners or even the consultants to establish if the couple aware with available fertility options that suits them or not. More importantly, are they accepting these options or not and why?

This study also exposed some misconceptions ,one is the restrictions of fertility options to one or more ,the other big misconception is the lack of acceptance of many consultants to offer other solutions to manage infertility irrespective of client acceptance or not. The majority of the participant in this study were people from higher social groups and non-ethnic minorities (either the couples or the consultants) who are more likely to complete questionnaires than other groups 14 which was clearly reflected in the response rate in all groups and so poor response rates which lead to bias were avoided . Previous studies have shown that people are more likely to respond to questionnaires that cover issues that are relevant to them 16 .As the questionnaire surveys are particularly reliant on the willingness of the participants to take part, considerable efforts were done by the authors to encourage subjects to take part so increasing the response rate e.g. clear explanation of the study proposal to all participants, Stress the anonymity of the survey of the questionnaire filled by each participants, and appointing a female doctor to interview the ladies participated in the study who one of the authors. Other ways used to improve the response rates were reminding the couples before coming to think about the study and using attractive style for the questionnaire. No financial incentives were offered to participants as it's unethical to offer people financial incentives to take part in healthcare surveys 14. Using two settings for introduction and filling the questionnaire intervened by telephone reminder had two advantages; the first was improving the response rate while the second advantage was law cost for the study. The cost of the study has been reduced by other measures like the use of self complete questionnaire and non use of interpreters as there is a single language practiced in the locality.

The number of first time attendees to a tertiary infertility clinic was 43% of couples (both husbands & wives) appear to correlate with the expected high proportion of subjects who either perceived their cause of infertility as unexplained or did not know (54%,figure 1) . While 20% of the husbands sought medical advice separately in a tertiary units due to isolated male factors, 75% of wives sought

medical advice in a tertiary units for surgical interventions before for improving their fertility options which reflect the big burden on females as wrongly believed that the only responsible .

The data obtained showed that 33% only (n=80) of subjects (husbands & wives) achieved the passing score to be aware with the whole list of available options and thought to be fully understand what each option mean while the majority (67%) were not aware at all .Further analysis of these data showed not all subjects who were aware can accept these options .The reasons behind this were many e.g. economic status (as the new reproductive technologies can only be accessed by a few rich couples, who make treatment through expensive private sector services that are increasingly available in many of developing countries where the majority of Muslim people there.), social factors (to accept polygamous marriage or divorce). This leads to aggravation of the problem's magnitude.

Although all consultants were aware the full list of options, only 20% of them offering their clients with these options. Many reasons had been revealed e.g. In 55% of consultants, they sought that the clients have already know these options while in 20% the answer was it is not my job to offer non-medical options e.g. polygamous option.5% of consultants claimed the lack of offering to the clients themselves because they did not ask about the available option or even they can not reveal any cause for non explanation. Due to social circumstances, about 15% of consultants can not offer some options e.g. divorce as it is may be traumatic to even both. One of major concern is the source of medical knowledge is coming from western curriculum rather than respecting the original culture and this is the responsibility of professionalism.

The authors think that there are many positive implications for promoting patient education about awareness of different fertility options in a special situations e.g. Islamic culture not only among customers but also among this service providers. It is very empowering experience to find list of options amid a very frustrating situation of intractable infertility from the patient's perspective. From the doctors' perspective, it enforces the relationship between them & their clients as the latter feel the former responsibility extend beyond medicine. The third merit is the resource and financial perspective; there are occasions where unnecessary or very expensive interventions are carried out instead of simple alternative option e.g. going through three trial of A.R.Ts. is much cheaper than marriage itself from the economic point of view, irrespective of other emotional troubles among both clients .And marriage itself may be encouraged by the other partner to keep the bond stable as much as they can. If both clients and their health service providers have a clear understanding of what each option mean, it would be possible to more accurately select the suitable option in a short time which, of course, will give more meaningful results. Another application of this information is to know when to start and when to take off a particular option.

Other researchers 17 have demonstrated that it is possible to successfully instruct 70% of regularly menstruating infertility patients in aspects of fertility awareness. So, incorporation of trained person e.g. nurse, social worker or even Sheikh or Imam (a person who teach religion and the people trust) into the tertiary referral units would be a logical step to rectify this highlighted lack of knowledge among clients. Running courses for the consultants also very empowering to highlight the deficient areas of counseling. The authors do not know the extent to which these results applies to other Muslim people in other communities as they conducted this survey among Sonia group in one of the Muslim countries, although we are very confident that will benefit similar category after application of national law. It is for communities to judge for themselves how valid the results for a given population.

There is little published literature on the level of fertility awareness amongst patients attending fertility clinics especially for special circumstance e.g. Muslim communities. Awareness with the available Fertility options awareness for Muslim people is generally very poor among couples attending tertiary units .Offering these options by heath service providers is also very poor. Also, physicians offering the fertility services are different in their acceptability to all options.

The authors believe that there are many positive implications for promoting patient education about the available fertility options for Muslim people. From a patient's perspective, acquiring knowledge of different fertility options can be a very empowering experience, and can ameliorate a potentially solvable problem. From a physician's perspective, offering the options can be met with a valuable rate of acceptance.

There is urgent need to develop culturally sensitive guidelines respecting the moral and religious of the target population augmented with political willingness to relief the burden of infertility by offering more options from the counseling physicians to the clients. As majority of Muslim people living in developing countries, so tackling of infertility should focus on a stepwise approach beginning with the establishment of preventive services in all of these countries, through integrated infertility treatment at the primary and secondary care levels and ending up with well organized, referral units which should also develop and offer affordable assisted reproductive technology.

Support from friends, and family is vital for couples coming to terms with infertility and accepting a child-free lifestyle. Attendance of non-medical person e.g. Sheikh to the counseling sessions helping the doctors in offering all available options are very encouraging to improve the pregnancy rate through going through the assisted techniques. The authors also encourage the use of Information leaflets that include these options either in the Muslim countries or even for the Muslim minorities in non- Muslim countries. Finally, the role of media can not be neglected to overcome this poor awareness that will consequently reflected upon patient satisfaction.

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Predictive value of sonohysterography in the assessment of uterine cavity in patients with uterine defects: a comparative study

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ABSTRACT

Objective: To compare between hysterosalpingorame, transvaginal Ultrasound, sonohysterography and hysteroscopy for assessment of uterine factor in sub fertile women.
Patients and methods: in a prospective comparative study a hundred of age-matched women with a history of sub fertility (primary or secondary) were recruited. For all women hysterosalpingorame, transvaginal ultrasound, sonohysterograghy and hysteroscopy were done.
Results: Of the study group hysteroscopy diagnosed 70% as normal findings and 30% had uterine abnormalities that include endometrial polyps (9%), uterine anomaly (11%) and submucous myoma (10%) . while sonohysterograghy revealed 27 uterine abnormalities with an overall sensitivity, specificity and negative predictive value of 90%, 100%, and 95.8% respectively. Hysterosalpingography diagnosed 21 uterine abnormalities with an overall sensitivity, specificity and negative predictive value of 70%, 100% & 88.6% respectively. While transvaginal ultrasound examination revealed 89 cases of normal findings, and diagnosed 11 uterine abnormalities with an overall sensitivity, specificity and negative predictive value of 36.6%,100%,95.8% respectively.
Conclusion: Sonohysterograghy is an easier, less expensive, safer and better tolerated alternative to diagnostic hysteroscopy for patients with uterine filling defects noted on HSG. It is highly sensitive and specific as high as the hysteroscopy. It can be used as a preliminary test for screening the patients who are candidate for operative hysteroscopy as normal sonohysterographic examination will not need further evaluation by office hysteroscopy.
Key words: Sub fertility, uterine factor, sonohysterography, hysteroscopy.

INTRODUCTION

Subfertility, defined as the inability to conceive despite regular unprotected sexual intercourse over 1–2 years, varies significantly in prevalence in different parts of the world. Causes including male factor, female (e.g. ovulatory disorder, tubal factor, adhesion or endometriosis, abnormalities in cervical mucus sperm interaction (cervical factor), Uterine abnormalities (uterine factor) or even unexplained factors 1.

Special investigative techniques for evaluating the uterus, tubes and pelvis have become increasingly popular among gynecologists. Initial evaluation of the infertile couples involves at first evaluation of the male factor which can be excluded by normal semen analysis while evaluation of the female factors involving assessment of ovulatory function ,evaluation of the normal pelvic structural anatomy and detection of tubal patency with finding out any structural abnormalities of the reproductive tract either congenital abnormalities as unicornuate uterus, bicornuate uterus , uterus didelphyse or septate uterus or acquired abnormalities as intra uterine adhesion or uterine fibroids 2.

There are various methods for evaluating the uterine cavity. Hysterosalpingography (HSG) is a widely used diagnostic tool. The overall risk of infection with HSG was reported to be <1%, but in a high-risk population infection can occur in 3% of cases. At present, ultrasonography is a basic diagnostic tool in the field of sub fertility. Sonohysterography (SHG), in which the uterine cavity is scanned while it is infused with sterile saline, is a new diagnostic tool for the detection of intracavitary abnormalities 3. Hysteroscopy is the 'gold standard' in the diagnosis of intrauterine pathologies 4. In unexplained sub fertility, hysteroscopy may be performed simultaneously with laparoscopy to evaluate the uterine cavity and cervix 5.

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PATIENTS AND METHODS

This is a prospective comparative study performed at Suzan Mubarak Neonatal & Maternity Hospital, Department of Obstetrics and Gynecology, Menia University, Faculty of Medicine after approval of the ethical committee of the department from December 2006 up to December 2007 in which 100 women with primary or secondary sub fertility were recruited. Written consent was taken from all women after full explanation of the study procedure. This study was pre-approved by the ethics research committee at Suzan Mubarak neonatal & maternity University Hospital.

The inclusion criteria included all women in the reproductive period with primary or secondary subfertility and regular marital life ,normal semen analysis, normal ovulatory cycles evidenced by follicular monitoring or normal level of mid luteal serum progesterone. All women with Irregular marital life, husband with abnormal semen analysis, ovulatory dysfunction, or PID were excluded from the study. All women were subjected to detailed history taking; thorough general examination was done involving, abdominal and pelvic examination. For all women HSG, transvaginal sonohysterograghy (TVS) and Hysteroscopy were done.

HSG was done immediately in the first half of menstrual cycle In the Radiology Department of Menia University Hospital .All abnormalities were fully reported. TVS was performed by a single operator using a transvaginal probe with a 7.5 MHz transducer (MEDISON BW-128) after complete emptying of the urinary bladder. The uterus was imaged in three major scanning planes with transvaginal sonography. These include views in its long axis, an oblique semicoronal or semiaxial plane, and a short axis view. After taking measurements of endometrial thickness and length of the uterus and cervical canal, specific observations were made to ascertain the normality of the endometrium, myometrium and endometrial-myometrial interface. Any deviation in the uterine cavity shape, outline and echogenicity of the endometrium was imaged and recorded. Similar observations were made for the cervix and endocervical canal. Afterwards, SHG was performed with the patient in the dorsal lithotomy position. Under general anesthesia a forward oblique 30c, 4 mm hysteroscopy (Karle Storz Hysteroscopy) was performed.

RESULTS

Table 1 shows patient's characteristic. The age of the studied group, one hundred patients ranged between 21 and 40 years old, with mean of 29.1±6.4 years. The parity ranged from 0 to 4, with mean of 2.2±1.4. The study included 55 women (55%) with primary subfertility, and 45 women (45%) with secondary subfertility. Table 2 shows the hysteroscopic findings of the studied patients. 70% had normal findings, and 30% had uterine abnormalities that include endometrial polyps (9%), uterine anomaly (11%) and submucous myoma (10%).They are distributed according to the type of subfertility. There was a significant increase in uterine abnormalities with secondary infertility when compared to primary infertility (53.4% vs. 11%). Table 3 shows distribution of the intrauterine pathologies according to the diagnostic methods and their comparison with hysteroscopic findings. Transvaginal ultrasonography alone was able to detect eleven intrauterine lesions (36.6%). HSG was able to detect more of these lesions (70%). On the other hand, except for three endometrial polyps, all intrauterine pathologies (90%) were detected by sonohysterography. Table 4 shows true and false positive and negative diagnostic values obtained by different methods for diagnosis of uterine abnormalities. There were no false positive results obtained by any method on diagnosis of any uterine abnormality. Table 5 shows shows statistical analysis of the methods with respect to their diagnostic capabilities of endometrial polyps (the gold standard is hysteroscopy). Sonohysterography had the highest sensitivity (66.7%) and had specificity of 100%, positive predictive value of 100% and negative predictive value of 91.3%. Table 6 shows statistical analysis of the methods with respect to their diagnostic capabilities of uterine anomaly (the gold standard is hysteroscopy). Sonohysterography had the highest sensitivity (100%) and had specificity of 100%, positive predictive value of 100% and negative predictive value of 100%. Table 7 shows statistical analysis of the methods with respect to their diagnostic capabilities of submucous myoma (the gold standard is hysteroscopy). Sonohysterography had the highest sensitivity (100%) and had specificity of 100%, positive predictive value of 100% and negative predictive value of 100%.

Table 1: Patient's characteristics & type of subfertility.

Patient's characteristics	Range or No. (N=100)	Mean ±SD or %
Age	21-40	29.1±6.4
Parity	0-4	2.2±1.4
Type of subfertility		
Primary	55	55%
Secondary	45	45%

Table 2: Hysteroscopic findings according to the type of subfertility

Hysteroscopic findings	Primary subfertility (N=55)	Secondary subfertility (N=45)	P-value
Normal	49 (89%)	21 (46.6%)	0.0001**
Abnormal	6 (11%)	24 (53.4%)	0.0001**
Endometrial polyp	2 (3.6%)	7 (15.5%)	0.04*
Uterine anomaly	2 (3.6%)	9 (20%)	0.01*
Submucous myoma	2 (3.6%)	8 (17.7%)	0.02*

Table 3 Distribution of the intrauterine pathologies according to the diagnostic methods and their comparison with Hysteroscopic findings.

Method	Endometrial polyp	Uterine anomaly	Submucous myoma	Total (%)
TVS	3	5	3	11 (36.6%)
HSG	3	10	8	21 (70%)
SHG	6	11	10	27 (90%)
Hysteroscopy	9	11	10	30 (100%)

Table 4 True and false positive and negative diagnostic values obtained by different methods for diagnosis of uterine abnormalities (the gold standard is hysteroscopy.

Uterine abnormality	No.	Method	True positive	False positive	True negative	False negative
All abnormalities	30	TVS	11	0	70	19
		HSG	21	0	70	9
		SHG	27	0	70	3
Endometrial polyp	9	TVS	3	0	21	6
		HSG	3	0	21	6
		SHG	6	0	21	3
Uterine anomaly	11	TVS	5	0	19	6
		HSG	10	0	19	1
		SHG	11	0	19	0
Submucous myoma	10	TVS	3	0	20	7
		HSG	8	0	20	2
		SHG	10	0	20	0

Table 5 Statistical analysis of the methods with respect to their diagnostic capabilities of endometrial polyps (the gold standard is hysteroscopy).

Method	Sensitivity	Specificity	Positive predictive value	Negative predictive value
TVS	33.3%	100%	100%	77.7%
HSG	33.3%	100%	100%	77.7%
SHG	66.7%	100%	100%	91.3%

Table 6 Statistical analysis of the methods with respect to their diagnostic capabilities of uterine anomaly (the gold standard is hysteroscopy).

Method	Sensitivity	Specificity	Positive predictive value	Negative predictive value
TVS	45.4%	100%	100%	76%
HSG	90.9%	100%	100%	95%
SHG	100%	100%	100%	100%

Table 7 Statistical analysis of the methods with respect to their diagnostic capabilities of submucous myoma (the gold standard is hysteroscopy).

Method	Sensitivity	Specificity	Positive predictive value	Negative predictive value
TVS	30%	100%	100%	74%
HSG	80%	100%	100%	90.9%
SHG	100%	100%	100%	100%

DISCUSSION

Uterine cavity abnormalities can be the cause of subfertility in 10-15% of women; abnormal uterine findings occur in approximately 50% of infertile women 1. This high prevalence of uterine abnormalities, make inspection of the uterine cavity is a routine work in the evaluation of infertile women 2.

Transvaginal ultrasonography (TVS) is safe, noninvasive, and readily available tool in the office setting. Findings on TVS that have been used to describe uterine findings e.g. myometrial involvement, endometrial thickness, heterogeneity, cavitary lesions, and determine which patients should undergo further investigation. Despite the increased use of high-resolution abdominal and TVS, they have limited usefulness in evaluating the exact location of endometrial cavity pathology 6. Moreover, the usefulness of endometrial thickness is predicated on visualization of a clear longitudinal stripe of endometrium. This may be challenging in cases of endometrial distortion resulting from uterine fibroids or abnormal uterine angulation 7.

Hysterosalpingography (HSG) is the most commonly used technique in the evaluation of subfertility. It is recommended for the study of the uterine cavity in the diagnosis of and treatment planning for other gynecologic problems such as intrauterine adhesions and congenital anomalies. Endometrial lesions are shown as filling defects or uterine wall irregularities. HSG also enables visualization of the general configuration of the cavity. Disadvantages of HSG include pelvic exposure to radiation, use of iodinated contrast medium, and patient discomfort 7.

It is associated with considerable false-positive findings for both tubal patency and uterine cavity abnormalities 8. Furthermore, HSG often cannot differentiate myomas from polyps or various mullerian anomalies 9. The sonohysterography (SHG) was provided to describe the instillation of saline into the uterine cavity during ultrasound. Saline infusion sonography (SIS) has also been used to describe this procedure. This procedure has some major advantages over HSG, including simplicity, cost, minimal invasiveness, absence of ionizing radiation, and a high level of diagnostic accuracy 3 in detection of possible intracavitary defects, including polyps, submucosal leiomyoma(s), and endometrial carcinoma 10.

Hysteroscopy has increasingly been gaining acceptance and is today a necessary tool in the investigation of female subfertility. It permits direct visualization of the cervical canal and the uterine cavity, enabling observation of the shape and vascular pattern of any abnormality. It also permits direct biopsy of lesions. Hysteroscopy is an office procedure performed without local or general anesthetic and causes minimal discomfort to the patient 7 .

Hysteroscopy has traditionally been performed as an adjunct tool to evaluate abnormalities suspected as a result of HSG evaluation. Recent studies have shown increased benefit from combining diagnostic hysteroscopy and HSG in the evaluation of female subfertility 11. The accuracy of hysteroscopy, SHG, HSG and transvaginal ultrasound in the diagnosis of uterine cavity abnormalities in infertile women has been compared in various studies 7, 9, 12, 14, 16, 17, 18, 19. In our study we evaluated the diagnostic accuracy of transvaginal ultrasound, HSG, SHG and hysteroscopy in uterine cavity diseases in infertile women, with hysteroscopy considered the gold standard.

In the present study, TVS alone was able to detect eleven intrauterine lesions (36.6%) while HSG was able to detect more of these lesions (70%). On the other hand, SHG was able to detect 90% of intrauterine pathologies. SHG had the highest sensitivity and negative predictive values for diagnosis of all uterine abnormalities 90% and 95.8%, respectively) than HSG (70% and 88.6%), and TVS (36.6% and 78.6%, respectively).

In agreements with our results ,a previous study by Alatas et al. ,in which TVS, SHG, HSG and finally hysteroscopy were performed in 37 patients with primary and 25 patients with secondary subfertility. Suspected uterine anomalies were also confirmed by laparoscopy. TVS was able to detect 36.3 % while HSG was able to detect 72.7% of uterine pathologies. SHG was able to detect all the anomalies except for a single endometrial polyp (90.3%). However, there was no significant difference between the diagnostic capabilities of these methods 12.

In another study in which the researchers assessed the value of SHG in evaluating both the uterine cavity and tubal patency in 84 infertile women and to compare its results with HSG, diagnostic hysteroscopy and laparoscopic chromo-pertubation. As regards the appearance of the endometrial cavity, the results of SHG agreed with hysteroscopy in 72.2% while HSG agreed with hysteroscopy in 75.6% of cases. They concluded that SHG is similar to HSG as regards the appearance of the endometrial cavity but it is inferior to it for evaluating tubal factor 13.

Gronlund et al assessed the diagnostic value of SHG in the evaluation of Sixty-six women (41 with metrorrhagia, 20 with subfertility and five with habitual abortion) .Hysteroscopy was taken as the standard. The overall sensitivity and specificity for SHG was 90.9% and 100%, respectively. The positive and negative predictive values were 100% and 90%, respectively. When examining the metrorrhagia and subfertility groups separately the sensitivity and specificity and predictive values were found to be 88.5%, 100%, 100% and 76.9% (metrorrhagia) and 100% for all parameters in cases of subfertility. No complications were recorded during the procedures. Therefore, those authors concluded that SHG is a simple, fast, well tolerated and accurate method to evaluate the uterine cavity in patients with metrorrhagia or subfertility 14 .

In a prospective study for the evaluation of uterine and tubal pathologies evaluating the diagnostic accuracy of HSG and sonohysterosalpingography in detecting tubal and uterine abnormalities. With surgical findings as the gold standard, sonohysterosalpingography had a sensitivity of 78.2%, a specificity of 93.1%, a positive predictive value of 82.7%, and a negative predictive value of 91%.

For total tubal and uterine pathologies, the findings for the same parameters using HSG were 76.3%, 81.8%, 90.9%, and 59.2%, respectively 15.

HSG versus hysteroscopy in the detection of intrauterine abnormality in Seventy-eight infertile women showed a sensitivity of 81.2% compared with that of hysteroscopy and a specificity of 80.4%, with a positive predictive value of 63.4% and a negative predictive value of 83.7% 7.

Ragni et al. evaluated the accuracy of TVS, SHG compared to hysteroscopy in the diagnosis of intrauterine pathology in a population of infertile patients. The TVS sensitivity and specificity compared with hysteroscopy were 91 and 83% respectively. Using TVS, a 9.2% false positive rate (9 cases) and a 5.1% false negative rate (5 cases) were detected compared to hysteroscopy. The TVS PPV and NPV were 85.4 and 90% respectively. SHG yielded better results: sensitivity and specificity when compared to hysteroscopy were 98 and 94% respectively. The SHG PPV and NPV were 95 and 98% respectively 17.

In the detection of intrauterine pathologies in infertile women Bartkowiak et al. evaluated the diagnostic accuracy of saline infusion SHG .The findings were compared to the results of two widely used procedures: TVS and hysteroscopy. Intrauterine pathologies were diagnosed in 25% of patients. TVS detected 6 (37.5%) and SHG revealed 11 (87.5%) of 13 intrauterine pathologies finally visualized at diagnostic hysteroscopy 18.

In the present study, SHG had sensitivity, specificity and predictive values of 100% in the evaluation of intrauterine anomalies and submucous myomas when compared to hysteroscopy. We observed endometrial polyps and submucous myoma as protrusions into the saline-filled intrauterine cavity under sonohysterographic imaging. An endometrial polyp was detected as a sessile, homogeneous echogenicity without distortion of the endometrial–myometrial junction.

Because a submucous myoma originates from the myometrium, the integrity of the uterine wall and the relationship of the lesion to the endometrial floor (sessile or pedunculate) were easily determined. These details were very useful during hysteroscopic surgical management of the lesions. In mullerian anomalies, SHG has the advantage of evaluating both the interior and exterior surfaces of the uterus at the same time. In this way, it is easier to distinguish between septate and bicornuate uteri. In cases of septate uteri, the thickness of the septum and its relationship to fundal myometrium can be measured. These details are useful while performing hysteroscopic metroplasty.

Regarding these findings concerning the diagnostic value of SHG over the other methods (TVS and HSG) in diagnosing of endometrial polyps, uterine anomalies and submucous myoma, similar results to the present study were obtained by 12, 17,18, 20, 21.

Kamel et al. compared the diagnostic accuracy of TVS and vaginal SHG in detecting endometrial polyps. TVS resulted in false positive and false negative rates of 25% and 36.2% respectively while For SHG were 5.4% and 8% respectively. Combining both techniques further improved such rates to 2.9% and 2.8% respectively but not significantly. The sensitivity and specificity were 64.5%, 75.5% for TVS, and 93.1%, 93.9% for SHG. In the present study, the sensitivity and specificity for endometrial polyps were 66.7% and 100% with SHG and 33.3% and 100% with HSG and TVS 21.

The advantage of SHG for the diagnosis and location of submucous myomas and focal endometrial lesions was confirmed by Becker et al. who assessed whether SHG provides added diagnostic value over TVS in patients with suspected or known myomas by comparing diagnostic confidence, interobserver agreement, accuracy, and change in diagnoses when 2 independent observers interpreted TVS alone and later interpreted TVS and SHG together. The added information provided by SHG resulted in improved diagnostic confidence for most parameters. Sensitivity values for submucous myomas and focal endometrial lesions were 100% and 90% for TVS and SHG together and 100% and 70% for transvaginal sonography alone 22.

Nass Duce et al. evaluated the diagnostic value of SHG in the evaluation of submucosal fibroids and endometrial polyps. The findings were then compared with histopathological results. The sonohysterographic diagnosis was fibroid in seven patients, endometrial polyp in 23 patients and simple hyperplasia in two patients. Histopathological findings confirmed diagnosis in all except three patients with endometrial polyps, who had normal secretory endometrium 23.

In the study by Bartkowiak et al., transvaginal ultrasound failed to visualize three submucous myomas, one endometrial polyp and two cases of septate uteri. One submucous myoma and one endometrial polyp were not identified with SHG [18]. Therefore, the most important advantage of SHG over HSG, TVS and hysteroscopy is the ability to visualize both the uterine cavity and the myometrium by a single technique. SHG can ameliorate the surgical approach to myomas defining the intramural and submucous extension of a myoma 17. Furthermore, Alatas et al. and Goldstein et al. confirmed that SHG could also better define a congenital abnormality, suggesting the diagnosis of the type of alteration (e.g., differentiating a septate uterus from a bicornuate uterus), and it also allows for the simultaneous detection of ovarian abnormalities 3, 12, 24, 25.

An expected complication of SHG is the possibility of intracavitary infection. To avoid such complications in the present study, patients with mucopurulant discharge were excluded from the study group. The patients who remained in the study group did not receive prophylactic antibiotics because al procedures were performed under totally aseptic conditions. None of the patients developed an infectious complication. The risk of a postprocedural infection should be accepted as similar to that involved in traditional intrauterine manipulations, i.e. HSG. In addition, the procedure was painless and well tolerated in every case.

Overall, the present study was in agreement with other studies in the literature which clarified that SHG was a test that could be easily learnt by expert transvaginal ultrasonographists. SHG was performed in a very short time and no adverse events were experienced. Moreover, patient's discomfort is practically absent, compared to hysteroscopy. We therefore propose that a patient showing a normal uterine cavity at SHG might not require any further evaluation, avoiding an unnecessary diagnostic hysteroscopy. Hysteroscopy could be performed in doubtful diagnosis cases or when a biopsy and a histological evaluation are needed.

Therefore, in experienced hands, SHG is an easy, safe, and well-tolerated alternative to diagnostic hysteroscopy in the initial evaluation of uterine cavity infertile women. We recommend the routine use of SHG in the diagnosis of uterine cavity of infertile patients, reserving hysteroscopy only in selected cases.

From this study the authors concluded that sonohysterograghy is an easier, less expensive, safer and better tolerated alternative to diagnostic hysteroscopy for patients with uterine filling defects. It is highly sensitive and specific as high as the hysteroscopy. It can be used as a preliminary test for screening the patients who are candidate for operative hysteroscopy as normal sonohysterographic examination will not need further evaluation by office hysteroscopy. However, SHG can't completely substitute HSG as HSG provide a good visualization of the tubes and its patency; it can suspect presence or absence of pelvic adhesions which may be taken as an important cause of sub fertility.

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Prediction of preterm birth using a modified classification of gram- stained vaginal smears

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ABSTRACT

Objective: The aim of this study was to identify women at risk of spontaneous preterm birth through a 4-category Gram-stained vaginal smear method and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery.
Patients & Methods: This was a prospective study correlating first trimester Gram-stained vaginal smears with spontaneous preterm birth. Smears were categorized as normal, bacterial vaginosis-like, grade I-like (atypical gram-positive rods) or purulent grade I (lactobacilli-dominated smears showing heavy leukorrhea of unknown cause).
Results: Abnormal Gram stain vaginal smears were associated with more than 5 fold increase in the odds of having a preterm birth when applying the modified 4 category scoring system. The presence of bacterial vaginosis (BV) like, Grade I-like and Grade I-PNL were associated with a significant increase in the odds of preterm labor (4, 7.8 and 6.1, respectively).
Conclusion: Pregnant women with abnormal Gram-stained vaginal smear have a higher risk of preterm labor. Also, the modified 4-category Gram-stained vaginal smear classification is a useful tool in predicting preterm labor.
Key words: preterm birth; bacterial vaginosis; Gram stain; vaginal smear

INTRODUCTION

Preterm birth is defined as delivery of an infant before 37 weeks' gestation. Preterm birth is a serious problem occurring in 11% of all pregnancies. (1) It remains a leading cause of neonatal mortality and morbidity. (2) Bacterial vaginosis (BV) is a vaginal condition that can produce vaginal discharge and results from an overgrowth of certain types of bacteria that naturally exists in the vagina. BV, as well as other infections, had been found to be associated with an increased risk of preterm delivery in many studies. (3-5) BV had been shown to exist in almost 20% of pregnant women, however, most cases remain asymptomatic, and not all women with the condition will deliver prematurely. (6)

Various tests as Amsel's criteria, gram staining, oligonucleotide probes and culture of organisms of bacterial vaginosis can be used for the diagnosis. (7) The Nugent scoring system of a vaginal Gram stain is the most commonly used classification of vaginal microflora status. (8) Other classifications as that by Ison and Hay had also been validated and gained popularity. (9) However, these scoring systems may not reflect the diversity and complexity of the vaginal microflora. (10, 11) Meanwhile, a number of studies had found that other nonbacterial cell types may be involved in the vaginal inflammatory response that could trigger preterm labor. (12, 13)

In 2007, Verstraelen suggested a modified 4-category Gram-stained vaginal smear classification to identify women at risk of spontaneous preterm delivery. (11) The aim of this study was to identify women at risk of spontaneous preterm birth through the modified 4-category Gram-stained vaginal smear method, proposed by Verstraelen, and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery.

MATERIALS AND METHODS

This prospective study was conducted at the outpatient obstetric clinic in Mansoura University Hospital during the period from May 2008 to December 2010. It included 264 pregnant women, 5 cases were excluded due to spontaneous abortion, so the total number in this study was 259 pregnant women. A written informed consent was taken from all pregnant women in this study. Gestational age was calculated from the first day of the last menstrual period and confirmed by early ultrasound examination.

The exclusion criteria included: previous preterm labor, documented infection, multiple pregnancy, diabetic patients and women who received antibiotic or antifungal (local or systemic) treatment during pregnancy before sampling.

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Each pregnant woman was subjected to Vaginal sample during her first trimester by inserting a sterile cotton-tipped wooden swab into the vagina for the purpose of vaginal microflora status assessment .The swab was rolled round through 360 degrees against the vaginal wall at the vault and carefully withdrawn to prevent contamination. Swabs were then smeared on a plain glass slide and air-dried at room temperature and heat fixed. Then, slides were Gram stained and examined under oil immersion lens at X100 magnification.

Accordingly, Gram-stained vaginal smears were categorized as normal, bacterial vaginosis-like; grade I-like (atypical gram-positive rods) or purulent grade I (lactobacilli-dominated smears showing heavy leukorrhea of unknown cause).

We found that Grade I-like smears resemble grade I smears at first glance; however, they show predominantly atypical gram-positive rods, in particular curved or irregularly shaped (bifido- or corynebacterioform) gram-positive rods, that cannot be categorized as Lactobacillus (or as Bacteroides , Gardnerella or Mobiluncus) cell types and, therefore, these smears could not be assigned an Ison-Hay score.

The purulent grade I smears (grade I-PMN) were found to have heavy leukorrhea with neutrophils (PMN) in the presence of normal numbers of Lactobacillus spp. with absence of pathogens such as Candida spp. Accordingly, these smears would be considered normal according to Nugent_ or Ison and Hay. They show large numbers of neutrophils and hence an inflammatory response of unknown cause.

Following blinded allocation of each vaginal smear to 1 of the 4 categories, a comparison was made between patients with an 'abnormal Gram stain' (BV-like, grade I-like or grade I-PMN,) and those with a 'normal Gram stain' (grade I). Other clinical data including maternal age, weight and height, obstetric history mode of conception and any special habit, as smoking, were collected in a routine manner. Also, basic investigations during antenatal care such as complete blood picture, Rh factor, blood glucose level and urine analysis were done to all women in this study.

Spontaneous preterm birth is defined as birth before 37 completed weeks of gestation (delivery between 25 and 36 weeks + 6 days) following spontaneous onset of labor or rupture of membranes. The obstetric outcome was assessed using the mean birth weight (low birth weight was defined as <2500 g) and gestational age at delivery. Outcome was recorded as early miscarriage (EM) if was ≤13 weeks or late miscarriage (LM) if was between 14 and 24 weeks.

Statistical analysis

Continuous variables were compared with the independent samples test. Categorical variables were compared with ordinary chi-square tests. For any reported measure, statistical significance was accepted, as the 2-tailed probability level was < 0.05. All statistical analyses were performed using the statistical software package SPSS v16.0 (SPSS, Inc, Chicago, IL).

We calculated the sensitivity, specificity, positive and negative predictive values, likelihood ratio for a positive and a negative test result and odds ratios. Calculations were performed using Meta Disc software (version 1.4). Ninety-five percent confidence intervals are also reported. Positive likelihood ratios above 10 and negative likelihood ratio below 0.1 have been noted as providing evidence of high diagnostic accuracy, whereas those above 5 and below 0.2 give evidence of moderate diagnostic accuracy.

RESULTS

From the 259 women, one vaginal smear was obtained during the first trimester. Basic characteristics of the study population are shown in Table 1. None of the participants reported smoking.

Table1: Basic clinical characteristics of the study population (n=259)

	Without spontaneous preterm birth (N = 236)	Without spontaneous preterm birth (N = 23)	P value
Maternal age mean (years) ± SD	27.1 ± 5.0	25.3 ± 4.1	0.10
Body mass index mean (kg/m2) ± SD	29.4±2	30.1±2.4	0.11
Parity mean ± SD	2.5±2.1	2.1±1.9	0.38
Infertility treatment % (n)	4%(10)	8%(2)	0.07
+ ve Gram stain	30%(72)	70%(16)	<0.001

From the 259 women, 66 % presented with grade I microflora on each occasion (171/259), 18.5% of the women (48/259) had BV-like microflora, 6% grade I-like microflora (16/259), while 9.2%(24/259) showed heavy vaginal leukocytosis (grade I-PMN). Seven women from the group of women with lactobacilli-dominated microflora in the absence of leukorrhea had preterm birth (7/171). Preterm birth was observed in 7 ladies with BV-like microflora (7/48), 4 ladies of those with grade I-like microflora (4/16) and 5 from women who showed an episode characterized by a purulent grade I stain (5/ 24).

The sensitivity, specificity, +ve likelihood ratio (+LR), –ve likelihood ratio (-LR) and odds ratio (OR) for the classification compared to women with lactobacilli-dominated microflora (normal Gram stain) are shown in table 2.

Table 2 shows a remarkable increase in the sensitivity of the 4-category Gram stain scoring method for the prediction of preterm birth from 50%, when only accounting for BV-like microflora, and up to 69% when taking into account the two other categories grade I-like and grad I-PNL. Meanwhile, the overall presence of an abnormal Gram stain was associated with more than 5 fold increase in the odds of having a preterm birth compared to women with lactobacilli-dominated microflora in the absence of leukorrhea (table 2).

The presence of Grade I-like and Grade I-PNL were associated with a significant increase in the odds of preterm labor (7.8 and 6.1, respectively). The +LR and the –LR for the abnormal Gram stains were 2.5 and 0.62, respectively.

Table2: Diagnostic accuracy of the modified classification of Gram-stained vaginal smears in relation to the occurrence of preterm delivery.

Gram stain category	Sensitivity (95%CI)	Specificity (95%CI)	+LR (95%CI)	- LR (95%CI)	OR (95%CI)
BV-like	0.50 (0.23-0.77)	0.80 (0.73-0.85)	2.50 (1.38- 4.51)	0.62 (0.36-1.06)	4.00 (1.32-12.04)
Grade I-like	0.36 (0.10-0.69)	0.93 (0.88-0.94)	5.33 (2.05-3.84)	0.68 (0.43-1.06)	7.81 (2.00-30.46)
Grade I-PMN	0.41 (0.15-0.72)	0.89 (0.84-0.93)	4.01 (1.81 -8.87)	0.65 (0.40-1.05)	6.16 (1.78-21.34)
Abnormal Gram stain	0.69 (0.47-0.86)	0.69 (0.63-0.75)	2.280 (1.63 -3.17)	0.43 (0.23-0.81)	5.20 (2.05-13.20)

- Positive likelihood ratio (+LR)
- Negative likelihood ratio (-LR)
- Odds ratio (OR)

DISCUSSION

For decades, the important objective of reducing the preterm birth (PTB) rate has presented a challenge. In the past two decades, BV has been consistently associated with adverse outcomes of pregnancy. (14) Specifically, BV has been shown to be associated with a two-fold increase in risk for preterm delivery. (5)

Bacterial endotoxins cause activation of decidual cells, macrophages, lukocytes and monocytes which in turn cause the secretion of cytokines, chemokines and phospholipase A2 that activate cascades leading to labor (ILI, 6 and 8, TNFa). Inflamation, prostaglandin release and preterm activation of fetal hypothalamopituatry-adrenal (HPA) endocrine cascade occurs as a result. The cumulative effect of this results in preterm labor. (15)

The aim of this study was to identify women at risk of spontaneous preterm birth through a 4-category Gram-stained vaginal smear method and to examine the diagnostic accuracy of this classification in relation to the occurrence of preterm delivery. Our findings had shown that extending the spectrum of vaginal Gram stain diagnosis may identify a much higher proportion of women at risk of spontaneous preterm birth than has been reported.

Our results also agree with the findings from many studies. (3) (4) (16) On the other hand, the results of our study are in disagreement with the findings from a recent study who denied the association between preterm labor and bacterial vaginosis. (17)

It has been suggested that 'abnormal vaginal microflora' probably encompasses a wide range of changes in microflora (not confined to bacterial vaginosis) though few investigators seem to have addressed this issue. (10) (12) In our study we found that abnormal Gram stain vaginal smears were associated with more than 5 fold increase in the odds of having a preterm birth when applying the modified 4 category scoring system. The group of patients with grad I- like microbacilli and those with grad 1- PNL had shown a significant increase in the odds of preterm labor. Our results are consistent with the findings by Verstraelen and his colleagues. (11)

On the other hand, the +LR and the –LR, of an abnormal gram smear, indicate the low accuracy of the test in the prediction of preterm labor. This discrepancy between the significant increase of odds ratio and the low performance of the test could be due to the small sample size in our study as the likelihood ratio takes into account the prevalence of the condition in the population. This is consistent with current evidence where screening for bacterial vaginosis is only offered to high risk group where it is useless in the general low risk population. (18) The population in our study was a low risk population.

Although, in this study, each pregnant woman was subjected to Vaginal sample during her first trimester only our results were comparable to that of Verstraelen et al, 2007 study who collected vaginal samples during first and second trimester from all pregnant women. (11)

Our study had a restrictive inclusion criterion that was designed to test our aim. This may be considered as a drawback as other independent important risk factors had not been tested especially a past history of preterm labor. A larger study with more flexible inclusion criteria may provide a better platform to examine predisposing factors for preterm labor.

To sum up, pregnant women with abnormal Gram-stained vaginal smear may have a greater risk of preterm labor than pregnant women with normal Gram-stained vaginal smear. The modified 4-category Gram-stained vaginal smear classification is a useful tool in predicting preterm labor.

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Ovarian reserve in infertile women with chronic pelvic inflammatory disease

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ABSTRACT

Objective: To assess ovarian reserve among infertile women with chronic pelvicinflammatory disease (PID).
Materials and Methods: A prospective study comprised of 35 women (study group A) with clinically and laparoscopically diagnosed PID and 15 cases as control. All cases were assayed for day 3 serum FSH, E2 and inhibin B.
Results: In group A, day 3 FSH & E2 were significantly higher than control (11.2±6 mIU/m1 and 68.5±21 pg/ml versus 5.3±3 mIU/ml and 41.2±16 pg/ml, $P < 0.05$). While serum inhibin B was significantly reduced in group A (40±19 pg/ml) compared to the control (60±10 pg/ml). Serum inhibin B was negatively correlated with serum FSH in patients with PID.
Conclusion: Ovarian reserve appears to be relatively diminished in women with PID. This observation denotes progressive loss of ovarian reserve in cases of PID due to poor follicular development.
Key words: Ovarian reserve, Infertility, Chronic pelvic inflammatory disease .

INTRODUCTION

Ovarian reserve means the presence of sufficient number of follicles available for recruitment and development that will yield a cohort of eggs capable to lead successfully a conception cycle 1. Screening of ovarian reserve has been studied by many fertility centers to evaluate women reproductive potentials, both in the general fertility population 2, and for couples undergoing in vitro fertilization "IVF" 3.

PID is a common benign gynaecological disease that affects about 30% of infertile women based on clinical and laparoscopic evaluation. The association between infertility and PID is well established 4. The development of in vitro fertilization- embryo transfer (IVF-ET) has provided a new therapeutic approach for infertility. However, the results of IVF for patients with chronic PID are controversial. Several investigators had reported that the outcome of IVF was poorer for patients with PID if associated with poor ovarian reserve 5, 6.

Association between PID and poor follicular development has been proposed, resulting in abnormal steroid hormone production. So, ovarian reserve assessment is essential to identify the poor responder before initiation of controlled ovarian hyperstimulation or assisted reproductive programs, to lower the risk of cancellation and improve the pregnancy rates 7. The aim of the present study was to determine ovarian reserve in infertile women with PID and compare that to normal fertile women as a control.

MATERIAL & METHODS

This study was carried out in Departments of Obstetrics & Gynecology and Clinical Pathology, Faculty of Medicine, Mansoura University from the period of May 2007 to October 2009. Thirty five 35 patients with clinically and laparoscopically diagnosed PID (pelvic adhesion, pre-tubal adhesion, hydrosalpinx and pyosalpinx, in cases of exacerbation, edema, hyperemia and dilated blood vessels of the pelvic structures) 4 as study group. Fifteen women with normal reproductive outcome were as a control group, ultra-sonographic assessment was done to exclude any gross pelvic lesions. An informed consent was taken from all cases in the study.

At day 3 of menstruation, 3 blood samples were withdrawn from both patients and controls (2 ml) each at 10 minutes interval to avoid fluctuation into plain tubes. An equal volumes of the separated sera were pooled and kept frozen (-20°C) till analysis of serum FSH by chemiluminscence immunoassay using (immulite analyzer Dpc Los Angles) according to Babson 8, serum estradiol (E2) by electrochemiluminscence immunoassaay using (Roche Elecsys 1010 immunoassay analyzer) according to method of Jonsen et al. 9 and serum inhibin B was assayed by enzyme immunoassay Kit Biosource-Belgium) according to Groom et al. 10.

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RESULTS

Table (I) show clinical data of study and control groups as regard age, BMI (body mass index) and cycle length. Table (II) show statistical data of hormonal assay comparison between study group A and control group. Table (III) for correlation coefficient between inhibin B and FSH & E2 in PID patients and control group confirmed the previous findings.

Table I : Clinical data of study group (35 cases) and control group (15 cases)

Parameter	Group		FSH	E2
Inhibin B	Control Group	r	- 0.1	0.09
		p	> 0.05	> 0.05
Inhibin B	Study Group	r	- 0.3 *	0.1
		p	< 0.05	> 0.05

95% CI = Confidence interval. P value: Not significant

Table II : Comparison of hormonal assay between study group and control group

Parameter	Study group (No= 35) M±SD	Control group (No=15) M±SD	95% CI	P value
Age (y)	30 ± 2.6	31 ± 3.2	27.6 - 31.8	0.12
Body mass index BMI (Kg/m2)	21.9 ± 3.1	20.12 ± 4.2	18.3 - 22.6	0.09
Cycle length (d)	29±4	28 ± 5	25.2 - 30.1	0.13

Table III : Correlation coefficient between inhibin B and FSH & E2 in PID patients and control group

Parameter	Study group (No= 35) M±SD	Control group (No=15) M±SD	P value
Day 3 FSH (m111/m1)	11.2 ± 6	5.3 ± 3	0.05
Day 3 Es (pg/ml)	68.5 ± 21	41.2 ± 16	0.05
Day 3 Inhibin B (pg/ml)	40 ±18	60 ± 10	0.05

* Inhibin B is negatively correlated with FSH level in study group

DISCUSSION

Test for ovarian reserve is an important first step for many patients who are seeking for infertility treatment. Identification of patient with diminished ovarian reserve may identify the couple with decreased chance for getting pregnancy 11. To our knowledge and computer search, no information had been reported about assessment of ovarian reserve in patients with chronic PID.

In our work, study and control groups have the same mean age and body mass index. Age has been found to be a risk factor for low ovarian response due to aging ovary progressively loses its follicular pool and pituitary reacts by increasing F.S.H. secretion which in turn leads to an increase in basal E2 resulting in diminished ovarian reserve 12, also, BMI (25-28 Kg/m2) had significant higher rate of anovulation than women with normal BMI 17. So no effect for either age or BMI on the fertility in the present study (Table 1).

Also, in the present study relatively diminished ovarian reserve was observed in PID patients (F.S.H. 11.2±6 mIU/ml, E2 68.5±21 pg/ml) as compared to control group (F.S.H. 5.3±3 mIU/ml, E2 41.2±16 pg/ml). Serum FSH is an indirect indicator of ovarian reserve and its elevation reflects a decrease in the negative feedback of the ovary on the pituitary gonadotropin secretion 13. Basal serum FSH had been reported to be the best marker for assessing ovarian reserve and predicting the response to superovulation with a good correlation to pregnancy rate 14. Measurement of basal E2 in addition to FHS might improve the ability to predict fertility potential. Buyalos et al. reported that day 3 E2 level less than 80pg/m1 with normal FSH level in women of 38-42 years gives a good prognosis of successful treatment 15.

Inhibin B is one of the ovarian peptide (a heterodimeric glycoprotein) released by gralnulosa cells of the follicle early in the menstrual cycle having an inhibitory effect on the pituitary FSH release 8. So, it is considered as a direct marker of ovarian function. Low inhibin level was associated with poor response to superovulation (16) Measurement of basal inhibin B level serves as an attractive indicator of ovarian function as it probably precedes the increase in FSH precipitating its release 8.

Serum inhibin B was significantly reduced in group B PID (40 ± 18 pg/m1) compared to control (60 ± 10 pg/ml) but did not differ in group A compared to control. Inhibin B was negatively correlated with FSH level in patient with advanced PID in group B (Table 3). Defective follicular growth in PID results in reduction of inhibin B and defective steroidogenic activity. As the natural cycles in women with PID have been shown to have longer follicular phase 8. Diminished ovarian reserve in PID may appear quite logic. This may be attributed to the local destructive process that may be associated with chronic PID and/or fibrosis associated with inactive old lesions. Added to this explanation PID associated with chemical and cellular local ovarian and pelvic milieu related to increased number of prostaglandins, activated macrophages and oxygen free radicals resulting in decreased inhibin B levels leading to increasing FSH which in turn increasing basal E2 17,18,19.

In conclusion, women with chronic PID have relatively diminished ovarian reserve. So, evaluation of patients with PID for ovarian reserve via serum inhibin B, FSH and E2 is essential before initiation of infertility treatment.

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Factor v leiden mutation (fvlm) compared to placental histopathology in recurrent unexplained second and third trimester fetal loss

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ABSTRACT

Objective: This study was performed to detect FVL mutation in women with unexplained second and third trimester fetal loss and to compare the presence of FVL mutation with placental histopathology in these women.
Patients & Methods: The present study included two groups: group (I) represented sixty pregnant patients with history of recurrent unexplained fetal loss. The group is subdivided ***into group (Ia):*** 30 patients in their 2nd trimester and group (Ib): 30 patients in their 3third trimester. Group (II): 20 healthy multiparous patients with no previous history of fetal loss as a control group. Ultrasound scanning of the placenta and blood sampling for detection of FVLm for all women were done during pregnancy. Histopathologic examination of the placenta was performed after the pregnancy has ended.
Results: There was no significant difference in the presence of FVL mutation between patients with recurrent unexplained fetal losses (5/60 = 8.3%) and the control group. Normal factor V genotype was detected in 55/60 of the patient with fetal loss (28/30) of the 2nd trimester abortion and 27/30 of the 3rd trimester fetal death; these results were not statistically significant. Ultrasonography of the placenta reflected pathological findings (calcification and degeneration) in second trimester abortion and third trimester intrauterine fetal death which was highly statistically significant than the control group ($P<0.01$). Histopathological examination of the placenta (infarction and thrombosis) was statistically of higher significance in cases with history of fetal loss than the control group ($P< 0.01$).
Conclusion: Screening for FVL mutation is not recommended as routine screening test in cases with recurrent fetal loss although still highly indicated in cases with past history of thrombosis.
Key words: Thrombophilia, Recurrent pregnancy loss, Facto V Leiden mutation.

INTRODUCTION

Recurrent fetal loss is a significant public health problem with two or more losses affecting up to 3-5% women. Identified causes of recurrent fetal loss include chromosomal abnormalities, anatomic alterations of the uterus, autoimmune and endocrinal abnormalities. However, a significant fraction of poor pregnancy outcomes remains unexplained by these factors and many researches have focused on identifying further risk factors. Given that a successful pregnancy outcome is highly dependent on the establishment and maintenance of an adequate placental circulation1 (Martinelli et al., 2000).
It is possible that abnormalities of placental vasculature leading to inadequate feto-maternal circulation may be responsible for at least some poor pregnancy outcomes. This has led to an interest in the thrombophilia as risk factors for fetal loss. Factor V Leiden mutation is the most common form of inherited thrombophilia. A point mutation in the factor V gene at nucleotide position 506 resulting in an arginine to glutamine substitution, reduces the sensitivity of the factor V protein to inactivation by activated protein C (activated protein C resistance) resulting in pro-coagulant state and an inserted risk of thrombosis 2(Tracy and John, 2004).
Factor V Leiden mutation is present in 4–10% of people of Caucasian origin. The mutation induces a hypercoagulable state which increases the risk of venous thrombosis 7-fold among heterozygous carriers and 80-fold among homozygous carriers compared to non carriers. It has been suggested that factor V Leiden mutation may be associated with negative outcomes of reproduction such as recurrent abortion, preeclampsia, prematurity and small for gestational-age neonates3 (Pauer et al., 2003).
The aim of this study is to detect the prevalence of factor V Leiden mutation in pregnant women with history of unexplained recurrent 2nd and 3rd trimester fetal loss and comparing it with placental histopathology in these women.

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MATERIAL & METHODS

This study was conducted in the Obstetrics Department in Al Zahraa University Hospital between December (2006) and November (2009). Eighty pregnant women were included in this study and divided into two groups. Group (1) (Study group) included 60 pregnant women with history of recurrent two or more unexplained fetal loss. This group was further subdivided into; group (Ia) of 30 patients presented with second trimester abortions and group (Ib) of 30 patients in third trimester of pregnancy with intrauterine fetal deaths. Group (II) (control group) consisted of 20 healthy women with uncomplicated pregnancies and with no history of fetal loss, both groups were matched for age, parity and blood group.

All cases had no previous history of thrombosis and were subjected to detailed history and clinical examination. Also, they had routine investigations as blood group and Rh, CBC, urine analysis and culture and sensitivity, random blood sugar, HBs Ag screening. Pelvic U/S was done then 2ml of blood was withdrawn in sterile EDTA containing tubes and stored at 4 degrees for detection of factor V gene by PCR. Postpartum placental sample was taken from feto-maternal side for histopathology examination (taken in natural buffered formalin and stored at room temperature).

Exclusion criteria included patients age above 40 years, systemic lupus disease, RH negative patients, elevated TORCH antibodies, medical disease with pregnancy e.g. diabetes mellitus, hypertension, renal disease, fetal malformations, positive APS or cases with hypothyroidism.

Material assay:

Two ml of venous blood withdrawn from each patient and control by venepuncture under complete sterile conditions, and put the sample on sterile EDTA continuing tubes, collected and stored in 4ºC until the factor V assay. Determination of factor V Leiden was done by genomic DNA extraction followed by PCR amplification. Placental biopsies taken from fetomaternal surface were formalin fixed paraffin embedded and five micron section were prepared for routine hematoxylin and eosin stain. Sections were examined microscopically for infarctions and vascular thrombosis. Data was statistically analyzed using SPSS (statistical package for social science) program version 13 for windows and Epi info program version for all the analysis.

RESULTS

Eighty pregnant women were included in this study and divided into two groups. Table (1) shows that both groups were matched as regard maternal age blood groups and marital periods. Table (2) for the relation between FVL (wild and positive) and placental findings by US and histopathology in the study group shows that all women that were positive FVL showed placental pathological changes. There was highly significant difference between FVL and placental pathology detected by US and histoapthological examination ($P < 0.01$). There was no statistically significant difference in the presence of FVLm in the studied groups ($P < 0.05$). Table (3) for Logistic regression analysis of predictors of FVL in aborted cases shows that Parity, placental histopathology, maternal age, marital period, number of recurrent fetal loss, gestational age are dependent predictors of FVL positive.

Table III : Correlation coefficient between inhibin B and FSH & E2 in PID patients and control group

Studied variables	Group I (Cases) GIA (no. 30)		Group I (Cases) GIB (no. 30)		(Group II) Controls (n. 20)	
≺ Age (years)						
Range	22 - 39		22 - 40		22 - 32	
Mean	29.7		31.07		27.85	
± SD	±3.9		±5.8		± 2.89	
≺ Parity	No	%	No	%		
- P0	5	16.7	0	0.0	0	0.0
- P1	15	50.0	0	0.0	0	0.0
- P2	10	33.3	10	33.3	7	35.0
- ≥ P3	0	0.0	20	66.7	13	65.0
Gestational age						
Range	13 – 20		25-36		18-36	
Mean	16.5		28.9		28.7	
± SD	± 1.91		± 2.56		± 5.5	
≺ No of abortion						
Range	3 – 8		---		---	
Mean	4.2					
± SD	± 1.27					
≺ IUFD						
Mean± SD	---		3.87± 0.89		---	
≺ Marital period (years)						
Range	5 – 19		3 – 18		4 – 15	
Mean	9.73		9.03		8.75	
± SD	±3.43		±4.29		± 3.11	
≺ Blood group						
A	13	43.3	13	43.3	10	50.0
B	6	20.0	6	20.0	5	25.0
AB	5	16.7	6	20.0	5	25.0
O	6	20.0	5	16.7	0	0.0

Table (2) Relation between FVL (wild and positive) and placental findings by US and histopathology in the study group

Studied variables	FVL Wild (no. 55) No.	%	Positive (no. 5) No.	%	X^2	P-value
Placenta in US						
Normal placenta	0	0	0.0	0.0	16.8	<0.05*
Placental calcification	9	16.4	0.0	0.0		
Placental degeneration	46	83.6	5	100		
Placental histopathology						
No infarction	8	14.5	0	0.0	15.46	<0.01**
Focal infarction	5	9.2	0	0.0		
Severe infarction	2	3.6	2	40.0		
Wide infarction	39	70.9	2	40.0		
Thrombosis	1	1.8	1	20.0		

Table (3): Logistic regression analysis of predictors of FVL in aborted cases

	Positive FVL R square = 0.04 β	SE	Odds ratio (95% CI)
Constant	-10.17		
No parity	-1.4	1.17	0.25 (0.02-2.43)
Placental histopathology	0.45	0.62	1.57 (0.47-5.29)
Maternal age	0.1	0.2	1.11 (0.75-1.64)
Marital period	0.18	0.21	1.19(0.79-1.79)
Recurrent fetal loss	0.21	0.39	1.23 (0.57-2.69)
Gestational age	0.04	0.08	1.05 (0.89-1.23)

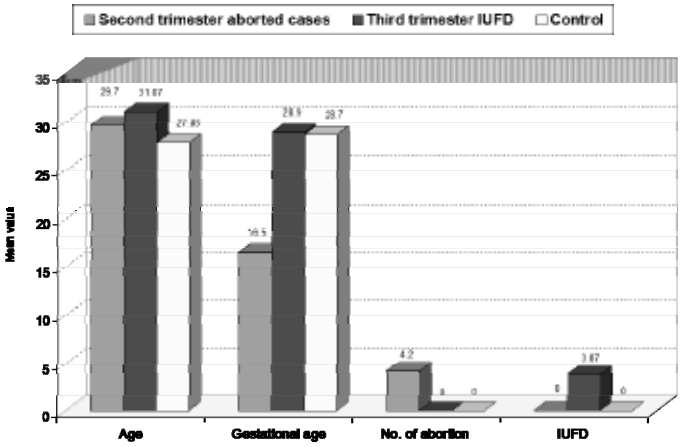


Figure (1): Shows the distribution of age, gestational age, no of abortion and IUFD distribution in studied groups.

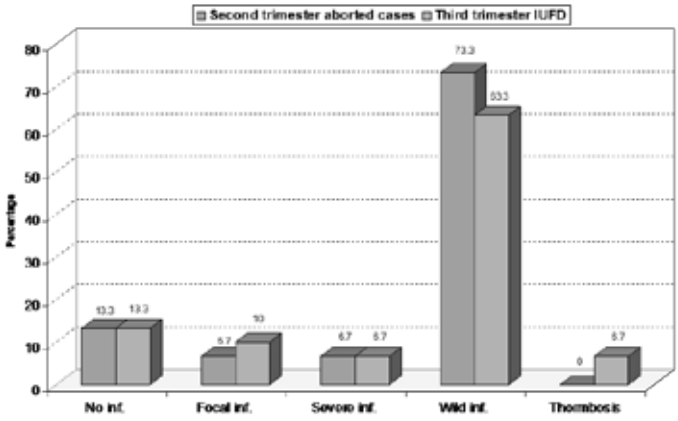


Figure (2): Shows the placental histopathology in the studied groups.

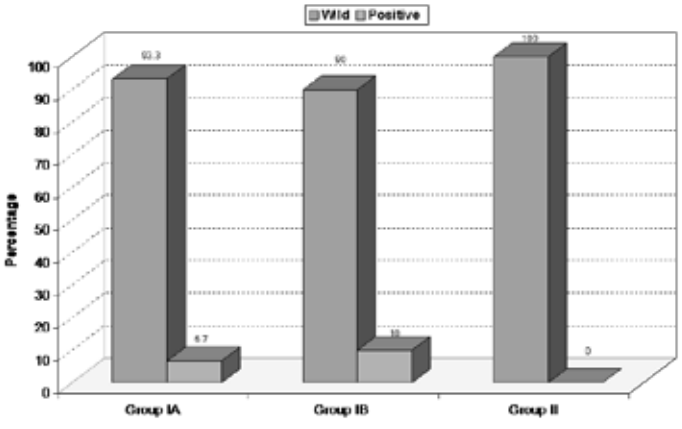


Figure (3): Shows the distribution of FVL mutation in the studied groups.

Figure (4): Chorionic tissues showing viable chorionic villi (on the right) while the left side shows ischemic necrosis due to infarction (H&Ex100) in group IB.

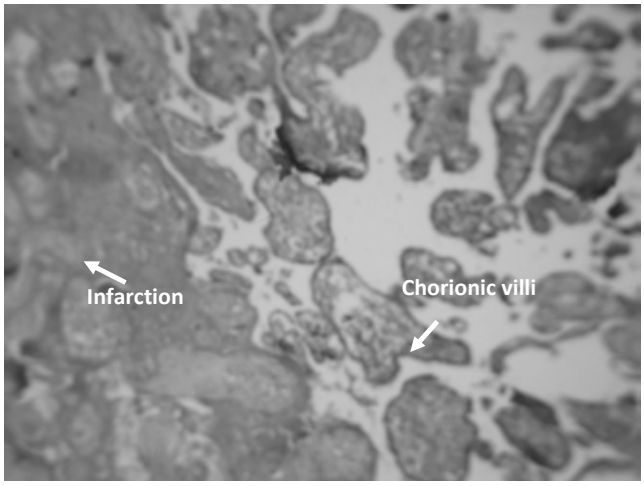


Figure (5): Infarcted placental tissue with evidence of intravascular thrombosis formation (H&E x 100) in case of group IB.

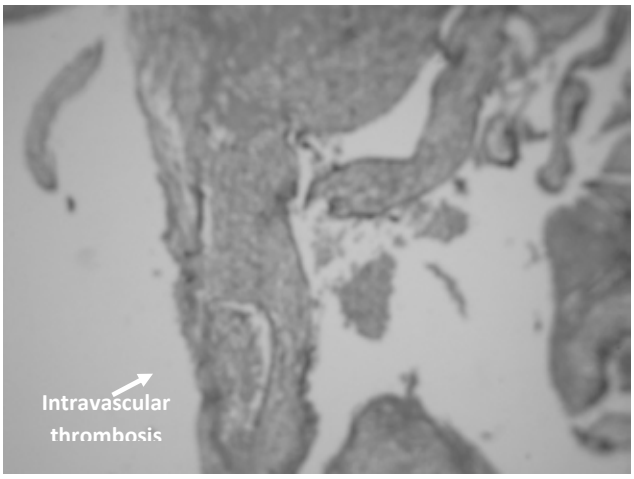


Figure (6): A normal fundal anterior placenta in a 26 weeks pregnancy.

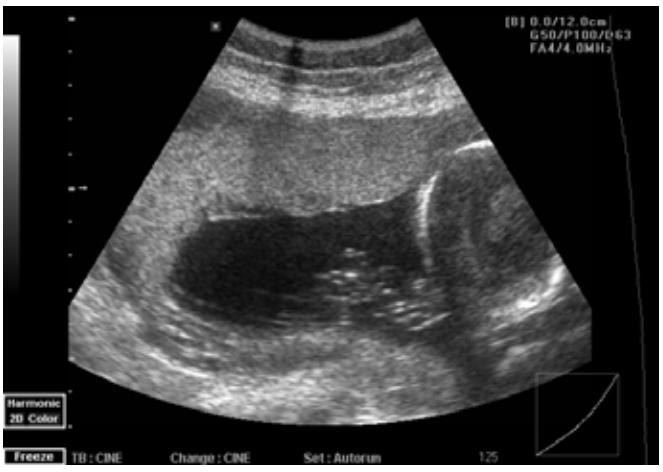


Figure (7): An anterior placenta of a 27 weeks pregnancy showing multiple placental cysts surrounded by calcificaltion. Doppler study of umbilical vessels showed compromized utreoplacental circulation.

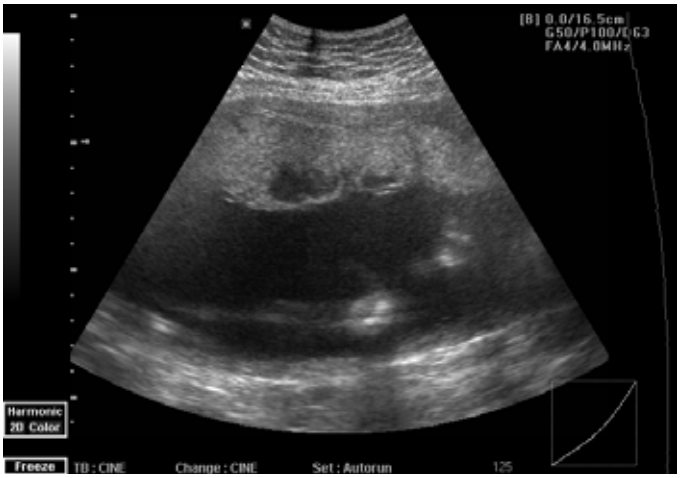


Figure (8): A third trimester fundal placenta showing calcifications and fibrin deposition early in 31 weeks gestation. The fetus who was growth restricted was delivered at 34 weeks gestation

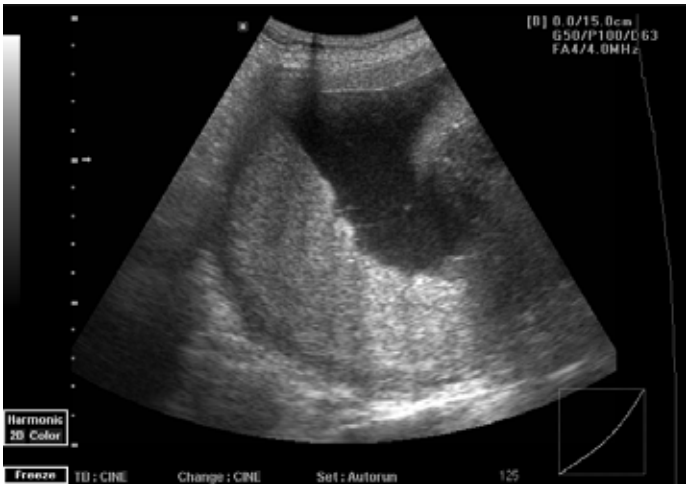
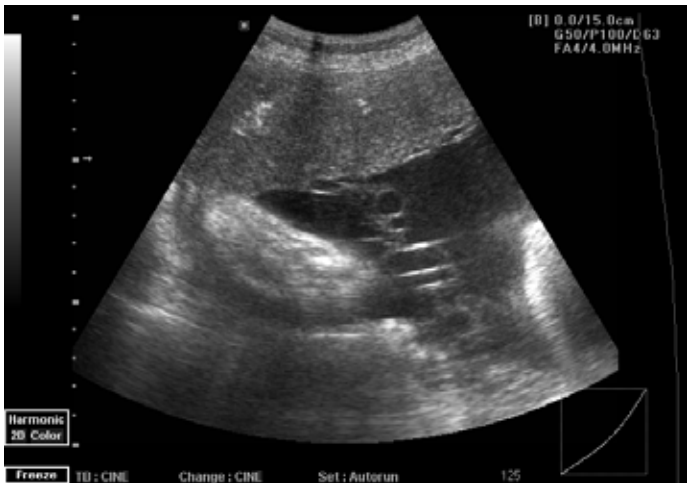


Figure (9): A fundal anterior placenta in 33 weeks pregnancy showing calcification and exaggerated coiling of the umbilical cord.



DISCUSSION

The current study was done to detect FVL mutation in women with unexplained second and third trimester fetal loss and to compare the presence of FVL mutation with placental histopathology in these women.

There were highly statistical significant differences between control group and second trimester cases and also the third trimester cases regarding US findings of placental pathology (p- value < 0.01). As the control cases showed no placental abnormalities. However there was no significant change in the placental pathology, detected by ultrasound between cases of second trimester abortion and intrauterine fetal death (P> 0.05). Suggesting that the importance of ultrasound in reflecting placental pathology in cases of second trimester abortion and third trimester intrauterine fetal death.

As regard placental histopathology the present study showed highly statistical significant differences between control and both second trimester cases and third trimester fetal death groups (p- value < 0.01). On the other hand the present study showed that there was high incidence of placental infarcts and thrombosis in both women with and without thrombophilia. These findings were in agreement of the results of 4(Mousa and Alfirevic, 2000).

On the contrary, Many et al., 2001, have compared the placental findings in women with severe pregnancy complications with and without thrombophilias5. The number of women with villous and multiple infracts was significantly higher in women with thrombophilias. The number of placentas with fibrinoid necrosis of the decidual vessels was also significantly higher in women with thrombophilias. The results of the present study shows that there was no significant difference in the presence of FVL mutation between patients with recurrent unexplained fetal losses (5/60=8.3%) shown as 6.7% of the 2nd trimester abortion cases (2/30) and 10% of 3rd trimester fetal loss (3/30) and the control group. These results supported that FVL mutation may play a role in only some cases of unexplained recurrent fetal loss.

Grandone et al., 1997, looked at 43 Italian women-with 2 or more unexplained losses. They found that the mutation was found in 7 of 43 patients (16.28%) and in 5 of 118 controls (4.24%). Among the 16 women with stillbirth, 5 (31.25%) tested positive for the mutation. Two of the 27 women who suffered first-trimester losses (7.41%) had the mutation. They concluded that the frequency of the factor V Leiden mutation is significantly higher among women with recurrent pregnancy loss and that the mutation is particu¬larly associated with late events6.

Meinardi et al., 1999, evaluated fetal wastage in 228 women with the factor V Leiden mutation and in 121 non affected controls. Approximately 32% of carriers and 22% of non-carriers experienced fetal loss. Carriers had a 29.4% rate of miscarriage, and non-carriers had a 17.4% rate. Stillbirth rates were similar for both groups, 5.7 % and 5.0% respectively, Carriers had a 10.1% risk for recurrence, and non-carriers had a 4.1% risk. These investigators concluded that stillbirth and miscarriage are increased in women with the factor V Leiden mutation7.

Although the above investigators found an association between the factor V Leiden mutation and recurrent preg¬nancy loss, other studies have suggested that there is no such association. Rai et al., 2001, found that the prevalence of FVL was not high than in controls for both first and second trimester abortions8. This discrepancy may stem for the different study design. Carp et al., 2002, reported that thrombophilia was not found to be associated with recurrent pregnancy loss9. Mukhopadhyay et al., 2009, study did not come up with a significant association between FVL and RPL among a north Indian patients, but a trend toward probable association between FVL and women with more than two habitual recurrent abortion was found in our study population10. Another two studies on Indian population did not find any association

between FVL and RPL cases Biswas et al., 2008. Similarly, some other studies conducted on different world populations suggested that FVL is not a predisposing factor for RPL (Aksoy and Karabulut, 2005)11, 12.

We concluded that FVL mutation may play a role in only some cases of recurrent unexplained fetal loss in the second and third trimester of pregnancy. Neverthless, study of FVLm is not recommended in all cases of recurrent unexplained fetal loss. But in those cases with inherited thrombophilias, although playing a small contributary role in recurrent abortions, it may be the straw that breaks the camel's back regarding the thrombotic risk faced by these patients during pregnancy and more important after delivery . Therefore, it remains highly indicated to be searched for in these cases. Ultrasonographic study of the placenta is essential for detection of the placental calcification and thrombosis in cases of unexplained recurrent fetal loss This must be confirmed by postpartum histopathological examination of the placenta.

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NEWS

This section has been prepared by Dr. Mahmoud Shawer, FRCOG and Professor Ahmed Badawy to present some of the latest news in the specialty

Maternal age & pregnancy outcomes

Maternal age does matter in terms of outcomes to both mother and fetus. "Advanced maternal age" is preferred to the term older mothers and for comparative purposes 40 to 44 years is deemed advanced maternal age and 45 years and older as very advanced maternal age. Studies from Norway give insight into the overall outcomes of maternal age and the effect of length of gestation. (Yogev et al and Haavaldsen et al AJOG 2010;203:558e1-7 and 545e1-8). Firstly, the incidence of gestational diabetes and hypertensive complications was higher in those 45 years or older compared with the controls; 17 vs 6% and 20 vs 5% respectively. The rates of caesarean delivery, placenta praevia, postpartum haemorrhage and adverse neonatal outcomes were also significantly raised. Secondly, in women 40 years or older the length of gestation did matter with the risk of fetal death being 3 times that of controls at 38 to 40 weeks and by 42 or more weeks this had risen to 5 times. The authors point out these risks has been attenuated by more assertive obstetric intervention in recent years.

Ovarian cancer research

The origins of ovarian cancer are a long way from being unravelled but associations exist with family history, breast cancer, multiple ovulations, endometriosis and specifically with BRCA 1 & 2 mutations and possibly with another genetic marker. Serous carcinomas make up the majority of ovarian cancers but clear-cell carcinomas, although less common, are the second most lethal form of the disease. Clear-cell carcinomas are often linked to endometriosis and may arise when there is a lack of the tumour-suppressor gene called ARID1A. Wiegard et al (NEJM 2010; 363:1532-43) looked for ARID1A mutations in the histological specimens of clear-cell, endometrial and serous carcinomas and found incidences of a half, a third and zero in the tissues examined. Some disruption of the tumour-suppression mechanism was also found in local endometriotic tissue which induced the authors to speculate that ARID1A mutations could be linked to an early event of the transformation of endometriosis to cancer. This is a long way from being proved but indicates the trail which researchers are following.

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Proton pump inhibitors in pregnancy

Proton-pump inhibitors (PPIs) are the most efficacious drugs for the treatment of gastro-oesophageal reflux. The effect of progesterone is relaxation of the cardiac sphincter, even in the first trimester, with resultant reflux and often the consequent use of PPIs. These drugs are available without prescription in many countries so the chances are high that many developing fetuses will be exposed to omeprazole and other PPIs. To investigate the safety of PPIs in pregnancy, Pasternak and Hviid (NEJM 2010; 363;211-23) looked at the frequency of major birth defects in pregnancies where the mother had taken PPIs and compared the outcomes to the overall incidence of congenital anomalies in Denmark. They gathered data over more than a decade from their Medical Birth Register, prescriptions and information from individuals and found there was no significant association between PPI use in early pregnancy and the frequency of birth defects. Given the duration of the survey and the quality of the data capture, it seems safe to assume that PPIs are not teratogenic.

Apgar scores and cerebral palsy

Sixty years ago Virginia Apgar created her scoring system that measures a baby's vitality at birth. She did this partly to focus on the neonate but also to objectively measure the infant's cardio-respiratory and neurological status. It has become accepted worldwide and is the only general clinical assessment record of entire populations, thus providing data across time and countries. A persistently low Apgar score beyond the first minute of life is an indicator of nervous system depression and can be used for identifying at-risk infants for attention and follow-up. Most babies with low Apgar scores recover quickly but a small number have persistent or worsening signs (Paneth BMJ 2010;340:c5175). Low Apgar scores do not mean asphyxia has occurred at birth or in labour nor do they correlate well with later neurological or cognitive outcomes but a low score at 5 minutes indicates an increased risk of disability. A study from Norway on over 500 000 babies indicates that low scores are strongly associated with cerebral palsy in children of normal birth weight and modestly in children of low birth weight (Lie et al BMJ 2010;341:c4990). They showed that at term 0.1% of babies with Apgars of 10 had cerebral palsy but 10% of those with Apgars of 3 or less were later diagnosed with cerebral palsy. The association of CP with low birth weight infants was 4% with high Apgars and 17% with low Apgars. All forms of cerebral palsy correlated with low scores but the most pronounced was quadriplegia. In conclusion the authors reiterate that 90% of children with low Apgar scores did not develop cerebral palsy.

Analgesics in pregnancy

There appears to be an association between taking analgesics in pregnancy and male sub-fertility. There certainly have been concerns about aspects of male fertility in recent decades such as a higher proportion of male factor problems, lower sperm counts and rising numbers of cases of cryptorchidism. Could there be a link to the use of analgesics in pregnancy at the time of male organogenesis which is around 14-16 week gestation? Kristensen et al from Denmark (Hum Reprod (2010;doi:10.1093/humrep/deq323) draw attention to connections between the potent antiprostaglandin properties of paracetamol, aspirin and ibuprofen with the inhibition of normal testicular formation and androgen function. These analgesics are taken by probably half of all pregnant women at some stage of gestation but are often not considered medication and are widely under-reported. The researchers showed strong links between analgesia intake in the early midtrimester and the incidence of cryptorchidism. There was a rising incidence with dose and the ingestion of more than one analgesic medication. Their data are robust and the authors back up the theory with animal models showing the association of normal translocation of the testes with androgen production. Failure of proper testicular migration is the best described risk factor for poor quality semen counts and testicular germ cell cancer so it is prudent to reinforce the avoidance of any prostaglandin synthetase inhibitors at any stage of pregnancy and particularly at the time of critical organ development.

Cu-T as EC

Intra-uterine contraceptive devices (IUCDs) are becoming more acceptable as a means of long-term reversible contraception. After decades of misplaced association with increased risks of pelvic inflammatory disease IUCDs are finding a wider place in the contraception spectrum in nulliparous or multiparous women and as an effective form of emergency contraception (EC). A good percentage of Egyptian wives have their husbands working away, inside Egypt or abroad, and drop in unexpectedly. Supporting the notion of a safe alternative to hormonal EC, a report from China by Wu et al (BJOG 2010; 117:1205-10) adds evidence to the growing bank of data concerning IUCDs. The researchers fitted women seeking post-coital contraception with a copper releasing IUCD within 5 days of intercourse – and followed them up for a year. Of the nearly 2000 patients 98.5% had the device fitted without a problem and none conceived in the first cycle while the post-insertion continuation rate was 94% over 12 months with a pregnancy rate of 0.23 pregnancies per 100 women. These are extraordinarily positive outcomes. Most of the women were multiparous, in their 20s and two thirds had previously had a termination of pregnancy which reflects China's strict family planning policy. Perhaps these data will encourage other agencies to promote copper containing IUCDs as a safe and effective form of contraception – which they are.

Caesarean technique

As caesarean section rates raise the need for safe techniques gains importance. Proper investigation of points of contention in the operation itself are surprisingly lacking so the Caesar Study Group mainly from the UK is a welcome contribution to our knowledge (BJOG 2010:117:1366-76). In over 3000 women the Study Group looked at 3 steps in the procedure, namely:

- Single versus double layer closure of the uterus
- Closure versus non-closure of the pelvic peritoneum
- Liberal versus restricted use of subrectus sheath drains

None of the alternatives made any difference to the short-term morbidity experienced so there is no objective evidence to promote the use of one or other of the techniques. Infectious morbidity which was loosely defined as a maternal febrile illness postnatally, endometritis or wound infection requiring antibiotic treatment, ran at 17% across the board. Long-term outcomes especially involving the uterine scar integrity in a subsequent labour await clarification but detailed points in surgery seem irrelevant to immediate post-operative infections morbidity.

Aspirin & colon cancer

Aspirin is taken by women and men for protection against cardiovascular pathology recurrence and it appears to also protective against colon cancer. It is known that high doses (500 mg per day) help to prevent colorectal malignancies but lower doses (80 – 350 mg per day) may also be effective. Rothwell et al (Lancet 2010;doi:10.1016/30140-6736(10)1543-7) followed up patients taking low-dose aspirin for 20 years and found they had an incidence hazard ratio of 0.76 for colon cancer but no reduction in rectal cancers. Proximal colon cancers were reduced most markedly, as were overall mortality rates. Another way to protect against colorectal cancer is by lifestyle modification. Since it is a disease of westernised countries men and women can reduce their risk by quitting smoking, increasing physical activity, retaining a healthy body mass index, eating sensibly and taking alcohol in moderation. Eating sensibly means restricting total calorie intake, more than 600g of fruit and vegetables per day, less than 500g of red or processed meat per week, greater than 3g dietary fibre per MJ of dietary energy and less than 30% of the total energy from fat. For us, busy gynaecologists, coming home late in the evening; do not eat everything in the kitchen. Kirkegaard et al from Denmark (BMJ 2010; 341:c5504) worked out the statistical advantages of singly or collectively "obeying the rules" in a large middle-aged population group over 10 years. They found that those observing the recommendations reduced their risk of colon cancer by a quarter and each component adhered-to offered an advantage.

Obese genotype

It seems there are genetic markers that predispose towards obesity. There are at least 12 genetic loci that are associated with weight gain and these can be determined by genomic coding. Each allele is linked to a small proportion of raised Body Mass Index and have been dubbed bad, fat or harmful genotypes (Li et al PLoS Med 2010; 7:e1000332). The bad news is that in 2005 about 400 million adults world-wide were obese and this figure is expected to increase to 700 million in 2015. Perhaps one fifteenth of these millions are in our country. The good news is that exercise has a significant effect on the gene expression and even relatively small amounts of physical activity can attenuate the genes' effects. Half an hour of pulse-raising exercise 5 times a week is ideal but far less makes a difference so diet plus exercise remain the cornerstones of a healthy lifestyle.

Weight gain & birth weight

There is a remarkable consistency between weight gain in pregnancy and birth weight excluding weight gain due to oedema. Looking at mother's weight gain in one pregnancy and the baby's weight then comparing it with her weight gain in the next pregnancy and that baby's weight, gives an accurate idea of the relationship between weight gain and birth weight. Ludwig & Currie (Lancet 2010; 376:984-90) studied over a million pregnancies in the US and confirmed the close correlation between weight gain and birth weight. Taking 10kg as their baseline, they found that women gaining more than 20kg had babies with an odds ratio of 2 of weighing more than 4 kg. They conclude that controlling weight gain in pregnancy will reduce antenatal and intrapartum problems and probably lead to healthier infants, children and adults. The problem is that there is little evidence that interventions during pregnancy are any good at controlling weight gain or reducing the incidence of macrosomia (Dodd et al BJOG 2010;117:1316-26 and Ronnberg & Nilson BJOG 2010;117:1327-34). Will it take social change to stem the tide of overweight and obese women in Egyptian private or even governmental antenatal clinics? If so, solutions seem a long way off.

Stillbirth recurrence

If a woman has a stillbirth in her first pregnancy, it is unclear as to her chances of a live-birth next time. Fortunately stillbirths remain rare occurrences and the existing literature suggests there is no increased risks the next time around so those experiencing an unexplained stillbirth beyond 24 weeks gestation have been reassured. A cause of death can be ascertained in about half of stillbirths and obviously steps will be taken to guard against a recurrence of the precipitating factors the next time round. In a large Scottish study over 20 years Bhattacharya et al (BJOG 2010; 117:1243-7) found that the risk of subsequent stillbirth was nearly double in those women whose initial pregnancy ended tragically compared with other women's successful outcomes. Associated factors were placental abruption, preterm delivery and low birth weight. Clinically little can be done apart from standard advice, especially about smoking and alcohol consumption and close monitoring. Attention to parental anxiety is essential but stillbirth remains a rare event so statistical reassurance is still warranted despite the increased risk according to the latest evidence

Miscarriage & subsequent pregnancy

It is not known how long a woman should wait after a miscarriage to have the best chance of achieving a subsequent optimal pregnancy outcome. It is calculated that after a term pregnancy delaying the next conception for 18 months but not longer than 2 years gives the best statistical odds but miscarriages may be different. Love et al (BMJ 2010;341:c3967) studied a large cohort of Scottish women who had a miscarriage and then another pregnancy and found that, compared to waiting 6 months, those conceiving sooner had better outcomes in terms of fewer repeat miscarriages, terminations, ectopics and stillbirths. Those waiting 2 years had a greater risk of adverse outcomes, so it seems there is little scientific evidence for the need to delay "trying again" but the psychological factors are difficult to quantify. Certainly contraception should be part of every miscarriage debriefing but the woman's wishes would seem more important than statistical advice. The psychological effects of a miscarriage for a woman can be profound but less is known about its effect on the father and the in-laws. Although their role is usually seen as supportive, they too can undergo stresses after an early pregnancy loss – especially if the pregnancy was really needed. Kong et al (BJOG 2010;117 1211-9) studied couples experiencing a miscarriage and found that although the endurance of distress in men was shorter than in women, he was at risk of psychological symptoms, mainly grief and this could affect the couple's relationship. Another example of where the helper may need help.

Intimate partner violence in Egypt

Intimate partner violence is synonymous with domestic violence. It is the leading contributor to mental health problems among women of reproductive age, mainly resulting in depression which is present in half of abused women. In Egypt and other Islamic countries the problem is hidden under a heavy cover of social pressures and fear for children future. Intimate partner violence exists in all communities, in all cultures and ranges from physical violence to sexual and verbal abuse as well as controlling or isolating behaviours. Husbands, partners or male family members are the main culprits and there are strong associations with alcohol, drug abuse and religious fanaticism. Women are often made to feel ashamed or "deserving" of violence against them and put up with unhealthy situations because of family or social pressures. The longer a woman stays in an abusive relationship the greater her physical and mental health risks. Common symptoms of emotional abuse are depression, sleep disturbances, sexual dysfunction, eating disorders or suicidal thoughts. Many women who develop these symptoms are afraid of seeking assistance as they feel they may be labelled or stigmatised as mentally disturbed and be deprived of their rights especially regarding their children. While women are encouraged to confront their situation and seek help, the actual assistance given may be temporary or inadequate resulting in a backlash of increased violence. Few publications show significant levels of improvement from short-term interventions but at the very least they highlight the very existing nature of the problem (Taft & Hegarty JAMA 2010; 304:577-9). If a doctor discovers a victim of domestic violence in Egypt , then what? No statistics, no system for management, and no social or police help. Western communities have their own social assistance but poor Egyptians suffer in silence. More research to define effective interventions is needed and clinicians do need to enquire about abuse. Asking about substance abuse either personally or by her partner may be a better way of opening the door to revelations than by direct questions about abuse. It should also be remembered that pregnancy is a particularly vulnerable time for women so extra care and diligent enquiries are appropriate at this time.

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