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- The abstract should be followed by 3 - 7 key words or short phrases for Indexing purposes. Key words should be separated by semicolons.

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Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those
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(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum 1, 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 comes up to your hands, I hope you notice the changes we have made. There will be more emphasis on review article, and we will start to publish some of the interesting papers that were delivered during the annual meetings. We welcome your comments as well as the scientific activity to be incorporated in the upcoming issues.

The society has been active and we were invited to participate in many conferences such as **Mehala OB/GYN Department Annual meeting, Damietta Governorate Annual meeting as well as the upcoming Ismailia Medical Syndicate meeting**. We are always ready to support any scientific activity all over Egypt.

All over the news, were a health warning about contraceptive pills containing drospirenone, while by no means we should incriminate it till harder proof is found, I would imagine that a body should be present in our specialty to look at this warnings and to contact producers to judge if it should be in the market or not, your feedback on this subject is appreciated.

As always we pray to god to keep Egypt safe and to grant its prosperity.

Chief Editor,
Prof. Mohamed Yehia

Interstitial Ectopic Pregnancies: Laparoscopy Vs. Laparotomy

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Candice Holliday, JD, Research Assistant, Division of Reproductive Endocrinology and Infertility of the Department of Obstetrics & Gynecology at the University of South Alabama, cphollid@umich.edu

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Abstract

As used in the literature, an interstitial ectopic pregnancy can refer to three different situations. A true interstitial ectopic pregnancy occurs in the Fallopian tube's interstitial or intramural segment. When a woman has a single uterine horn, a bicornuate uterus, or a septate uterus, her ectopic pregnancy is a cornual pregnancy. When an ectopic pregnancy occurs in one of the uterine angles, but outside the Fallopian tube, a rare angular pregnancy has occurred.

In the past, an interstitial pregnancy was managed conservatively until over 12 weeks on the premise that the uterine muscle protected against early rupture. Recently, however, evidence contradicts this belief as early rupture is more common than initially thought. With the advances in laparoscopic surgery, laparoscopy is accomplished with great success. That said, if the physician deems it safer to do laparotomy, patient safety is key to management of an interstitial ectopic pregnancy.

Introduction

Management of ectopic pregnancies has progressed in recent years (1-10). In 1% to 2% of all pregnancies, an ectopic pregnancy occurs (7). Of those ectopic pregnancies, 2% to 4% of them are interstitial (11-24). When an embryo implants in the interstitial or intramural portion of the Fallopian tube, an interstitial ectopic pregnancy has occurred (Figs 1.1-1.5).

In order to manage an interstitial ectopic pregnancy, the physician must assess the patient's stability and whether the ectopic pregnancy has ruptured. For patients who must be immediately diagnosed for reasons such as a positive urine pregnancy test, rebound tenderness, and/or hemodynamic instability, diagnostic laparoscopy is preferred (6). If the ectopic pregnancy has ruptured or the patient is otherwise unstable, diagnosis requires laparotomy (15).

Laparotomy

In the 1800s, laparotomy for a diagnosis of ectopic pregnancy was developed (6) (Figs. 1.2, 1.6, 1.7). Over the last 2 decades, and despite the rise of laparoscopic management, laparotomy with cornual resection or cornuostomy has remained a popular surgical management technique in the following circumstances: (1) for hemodynamically unstable patients; (2) for patients where laparoscopy would be complex (obesity, hemoperitoneum, or multiple dense adhesions); and (3) for patients with physicians uncomfortable with laparoscopy (Figs. 1.8-1.9 (A)-(B)) (9).

Statistical Analysis

In the 1970s and 1980s, laparoscopy began to replace laparotomy because, for most patients, laparoscopy was considered more conservative, more safe, and less costly than open surgery (6) (Figs. 1.3-1.5, 1.10-1.13). A wide variety of hemostatic techniques have been used laparoscopically, including tourniquet purse string suture or endoloop (25) or stay sutures (19), intramyometrial injection of diluted pitressin (Figs. 1.3, 1.4, 1.10 - 1.13) (26, 27), electrocauterization, ultrasonic cutting and coagulating surgical device (harmonic scalpel) and fibrin glue. Additionally, many cases of laparoscopic cornuostomy have been undertaken (17, 26, 28-35) (Figs. 1.11-1.13).

Conclusion

Out of the 312 women with PCOS, 122 cases fulfilled the NCEP and the modified ATP III for the diagnosis of MS making the prevalence of MS among PCOS women to be 39.11%. The prevalence of MS increased significantly with age, BMI, IR and free testosterone plasma level (table II). The most dominant component of MS was the increased waist circumference (>88 cm) being present in 77.87% of PCOS women, followed by lowered

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HDL-CL plasma level (75.41%). Hypertriglyceridemia was present in 28.69% and BP elevation was present in only 20.5%. Elevated FPG was detected in only 17.21% of women with PCOS (table III).

Among the studied CV variables, QTc and QTd were significantly higher among the MS PCOS cases compared to non- MS PCOS: significant increase in IVSD, insignificant decrease in EF and increase in cIMT in PCOS cases with MS. On the other hand, the non- MS PCOS cases showed significant prolongation of QTc interval, significant increase in IVSD and significant increase in cIMT (P 0.018) when compared to the healthy control women (table IV). In the present study higher left ventricular mass (LVM), higher left atrium size, and lower LVEF and early to late mitral flow velocity were observed in both groups of PCOS in comparison to control (table IV).

In this manuscript we have addressed the surgical management of interstitial pregnancies. Rupture of an interstitial pregnancy can occur before 12 weeks. Before a rupture occurs, laparoscopic management is the preferred management option. That said, if laparoscopic management is difficult for the clinician, laparotomy is a valid alternative.

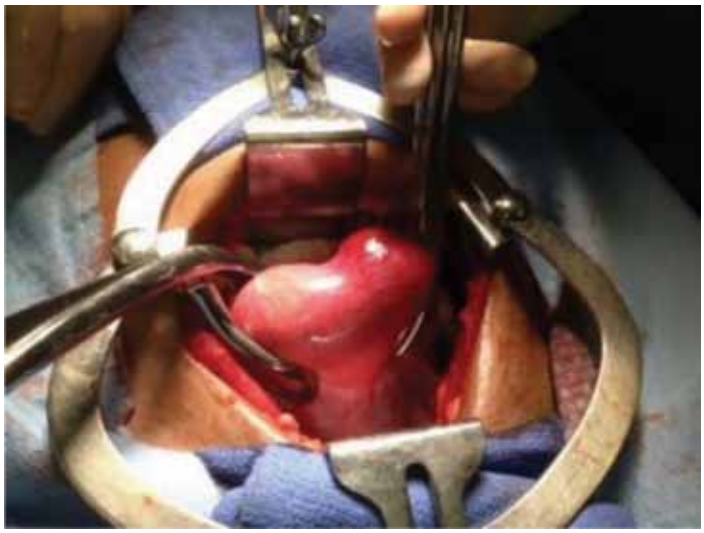


Figure 1.1: Left interstitial ectopic pregnancy. Courtesy of Botros Rizk.

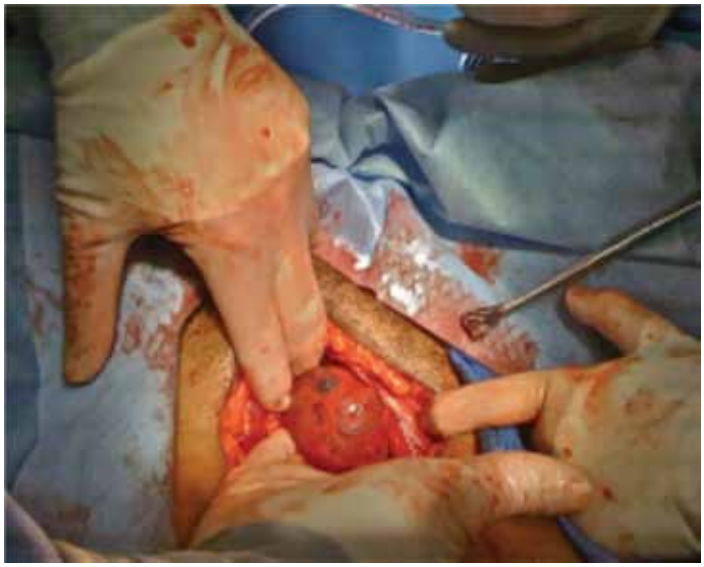


Figure 1.2: Interstitial ectopic pregnancy. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

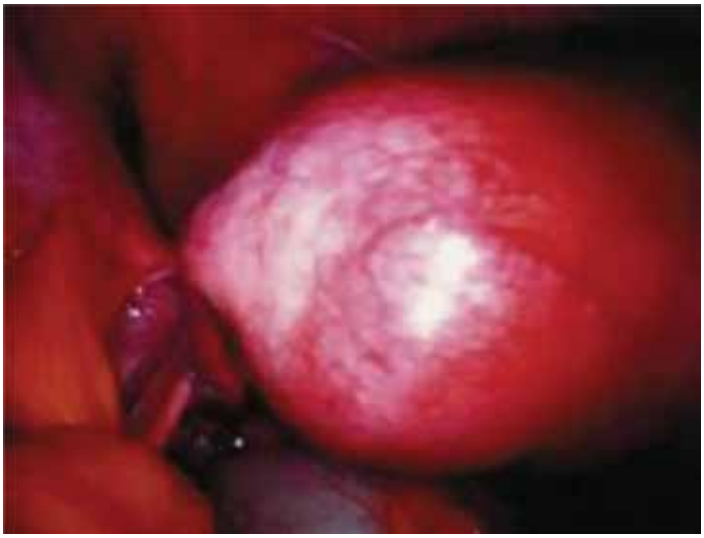


Figure 1.3: Laparoscopic view of interstitial ectopic pregnancy. Reproduced with permission from Rizk B, Abuzeid M, et al. Ectopic pregnancy. In: Rizk B (Ed.). Ultrasonography in Reproductive Medicine and Infertility. Cambridge: Cambridge University Press, 2010. Chapter 31, 264.

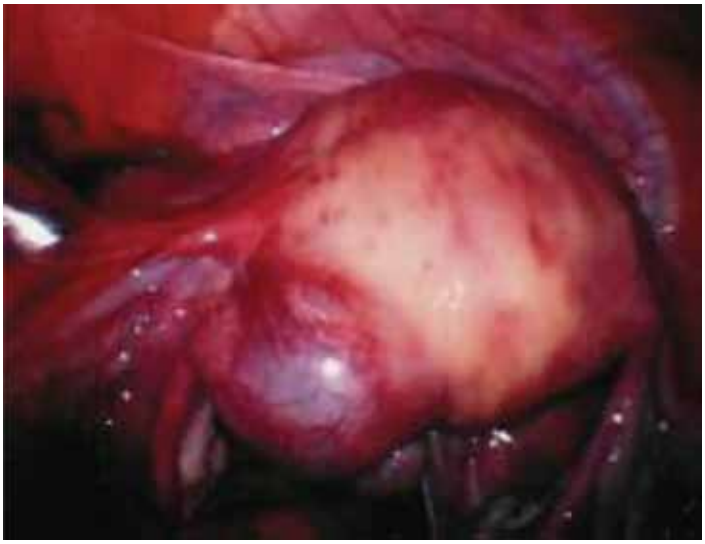


Figure 1.4: Laparoscopic view of interstitial ectopic pregnancy after pitressin injection. Reproduced with permission from Rizk B, Abuzeid M, et al. Ectopic pregnancy. In: Rizk B (Ed.). Ultrasonography in Reproductive Medicine and Infertility. Cambridge: Cambridge University Press, 2010. Chapter 31, 264.

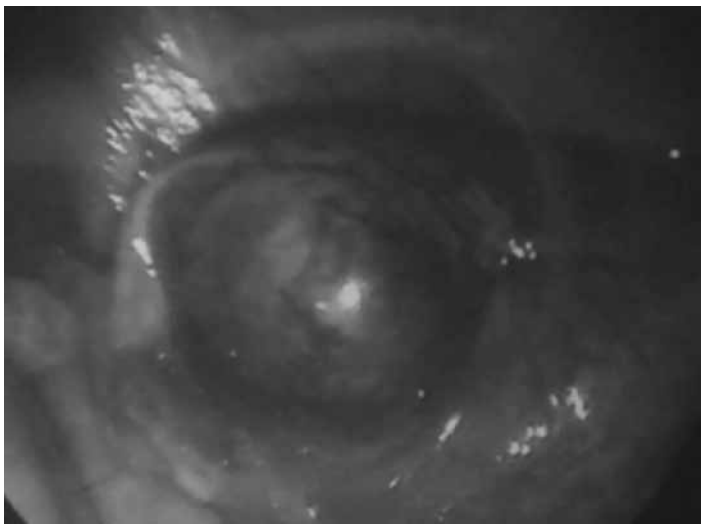


Figure 1.5: Laparoscopic view of interstitial ectopic pregnancy. Reproduced with permission from Rizk B, Abuzeid M, et al. Ectopic pregnancy. In: Rizk B (Ed.). Ultrasonography in Reproductive Medicine and Infertility. Cambridge: Cambridge University Press, 2010. Chapter 31, 264.

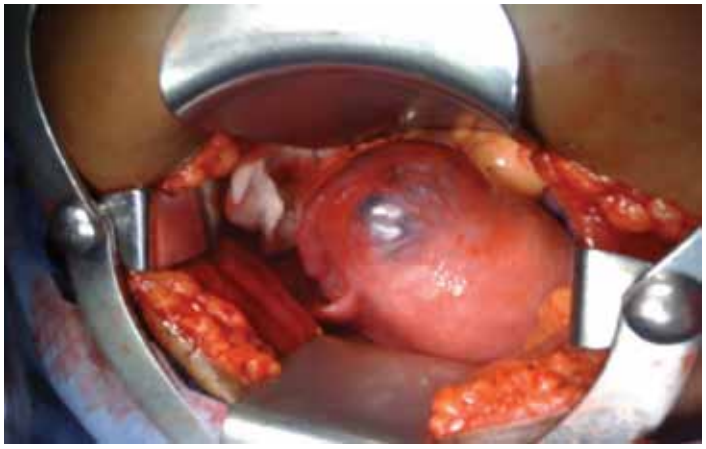


Figure 1.6: Management of interstitial ectopic pregnancy at laparotomy. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

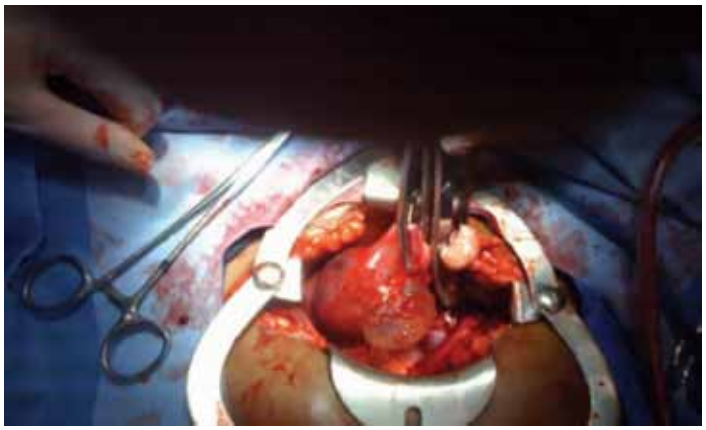
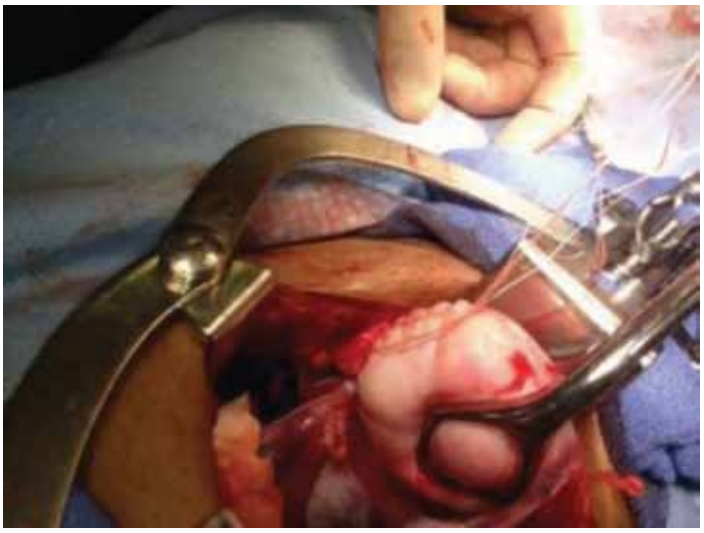
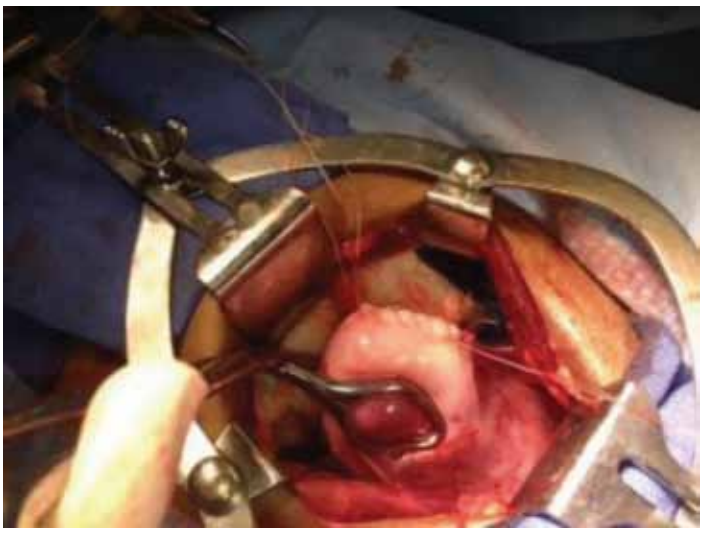


Figure 1.7: Management of interstitial ectopic pregnancy at laparotomy. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

Figure 1.8: Cornual resection of left interstitial pregnancy and stay sutures in place. Courtesy of Botros Rizk.





Figures 1.9 (A) and (B): Suturing of uterine cornu after removal of left interstitial ectopic pregnancy. Courtesy of Botros Rizk.

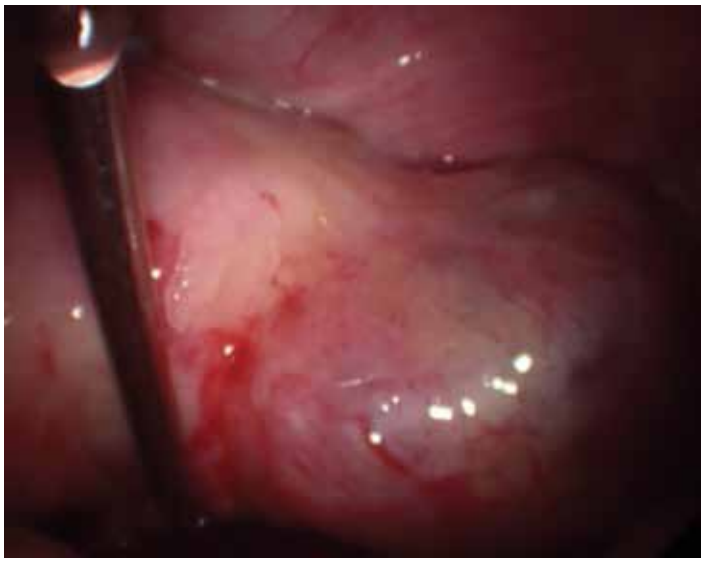


Figure 1.10: Laparoscopic view of right interstitial ectopic pregnancy after pitressin injection. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

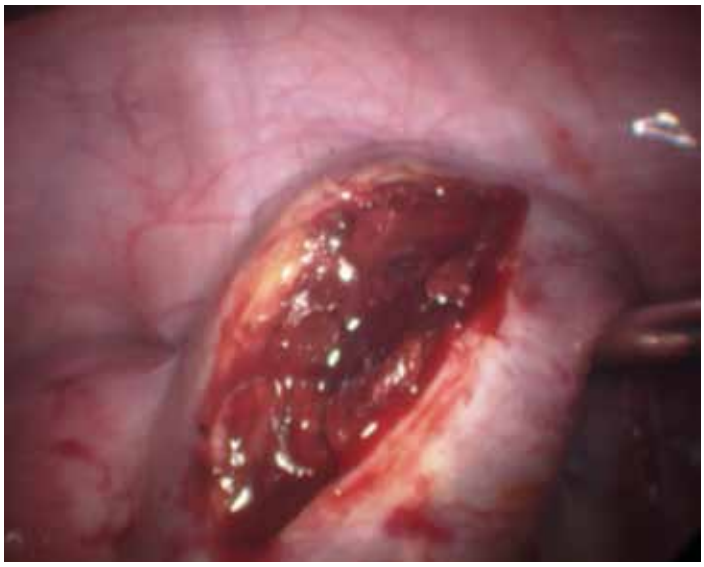


Figure 1.11: Laparoscopic view of cornuostomy incision in right interstitial ectopic pregnancy. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

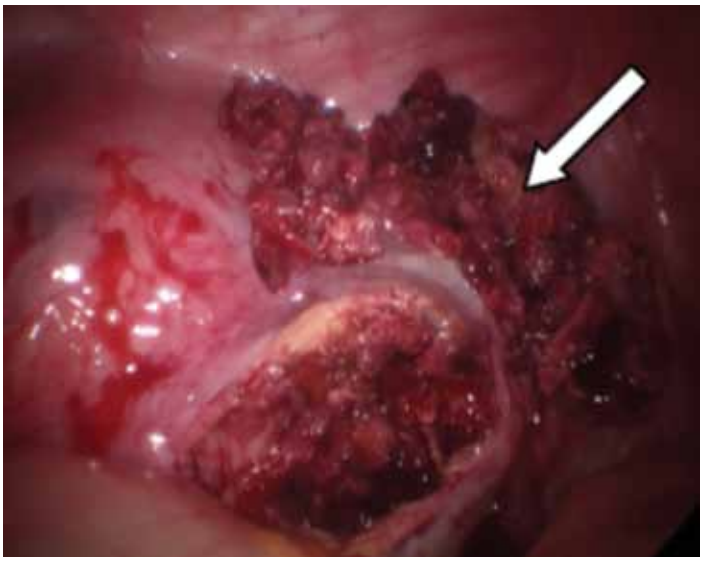


Figure 1.12: Laparoscopic view of cornuostomy in right interstitial ectopic pregnancy after trimming the edges of the incision. Products of conception seen on anterior surface of the right broad ligament (arrow). Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

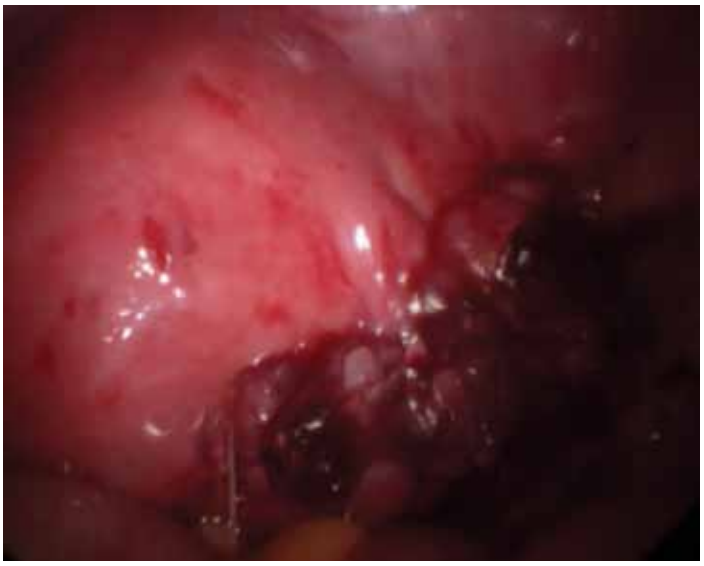


Figure 1.13: Laparoscopic view of cornuostomy in right interstitial ectopic pregnancy after repair with a few interrupted figure of 8 sutures with 0-Vicryl sutures. Reproduced with permission from Rizk B, Owens A, Abuzeid M. Ectopic pregnancy ultrasonographic diagnosis and management. In: Rizk B, Puscheck E (Eds). Ultrasonography in Gynecology. Cambridge: Cambridge University Press, in press for 2012. Ch 9.

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Dimethylsulfoxide vs. 1, 2 Propandiol as Cryoprotective Additives during Vitrification in Cryopreservation of Human Embryos

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2 Obstetrics and Gynecology Department; Faculty of Medicine; Suez Canal University

Abstract

Objective: Assessment of the survival rate of human embryos after vitrification/thaw process using two different freezing (vitrification) media with two different solvents; Dimethylsulfoxide (DMSO) based medium and 1,2Propandiol (PROH) based medium. These two solvents act as cryoprotective additives (CPA) that provide a protective effect for human embryos vitrification.

Subject & Methods: Day2 or day3 divided human embryos were exposed to DMSO or PROH based media before plunging into Liquid Nitrogen (LN2) storage. Later on, during replacement cycles, the embryos were thawed and their survival rate was evaluated.

Results: Two hundreds and twelve embryos in vitrification process were into two groups. Group A (114 embryos) were treated with sequential vitrification medium based on DMSO and Group B (98 embryos) were treated with PROH based medium. Thawed embryos were transferred to physiological media designed for cleavage stage embryos and kept in the CO2 incubator for 2 to 8 hours. After vitrification/thawing procedure, the survival rates of group A (DMSO) and group B (PROH) were 85 survived embryos of 114 (74.6%) and 52 survived embryos of 98 (52%) respectively (P value<0.05).

Conclusion: DMSO based medium used for vitrification/thaw process of day2 or day 3 divided embryos demonstrated a significant higher embryo survival rate than that of PROH based vitrification medium.

Keywords: Embryo cryopreservation; Dimethylsulfoxide; DMSO; 1, 2 Propandiol; PROH; Vitrification.

Introduction

The use of controlled ovarian stimulation protocols during ART programs led to the production of large numbers of human oocytes and consequently embryos. In a routine IVF practice, 60 % of stimulated IVF cycles may yield surplus embryos suitable for storage by freezing (Cryopreservation) [1]. Cryopreservation now plays a pivotal role in clinical assisted reproduction and has a profound impact on treatment strategy [2]. The first successful pregnancy after transfer of a frozen-thawed embryo was achieved in 1983 by [3] using a slow freezing protocol with dimethylsulfoxide (DMSO). Since then, thousands of other babies have been born as a result of the transfer of frozen embryos.

Cryopreservation is the technique of freezing cells and tissues at very low temperatures (-196°C) at which the biological material remains genetically stable and metabolically inert, while minimizing ice crystal formation. In general, when a tissue is subjected to low temperatures, ice crystals will eventually form. These crystals may disrupt the cell membrane leading to the death of the cell. The goal of cryopreservation is to replace some of the water with other compounds that will not form large crystals when frozen. These compounds are known as the cryoprotective additives (CPA) [4]. The most common methods of human embryos cryopreservation are the Slow Freezing and the Vitrification:

1.Slow Freezing

Slow-freezing protocol means a slow, controlled rate of cooling, after exposing the embryos stepwise to low concentrations of CPA. During slow-cooling, cells dehydrate, shrink and the concentration of solutes increases as water freezes in the medium. Slow freezing method is consuming more time up to 3 hours and requires a special freezing programming machine to provide the controlled freezing procedure [5].

2.Vitrification

Vitrification is a process which, by combining the use of concentrated solutions with rapid cooling, avoids the formation of ice. Samples reach low temperatures in a glassy state, which has the molecular structure of a viscous liquid and is not crystalline. This method has the potential advantages of being rapid to carry out and does not require controlled rate cooling apparatus. Good survival of human embryos has been demonstrated by vitrification in a number of laboratories [6]. It has been suggested

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that vitrification imparts less trauma to cells and is, therefore, a more effective means of cryopreservation of the human embryo than conventional slow freezing [7],

The general properties of cryopreservation media (CPAs) are they are mostly chemical solvents of a low molecular weight, non-toxic and can permeate cells [4]. Nowadays dimethylsulfoxide (DMSO) and propandiol (PROH) are the most successfully used, FDA approved CPAs. At the same time, they are the most widely studied CPAs for decades. In general, it is widely accepted that PROH is best used for pronucleate stage (day 1) - based cryopreservation programs, while DMSO is superior in dividing embryo stage (day 2 or 3) –based cryopreservation programs [8-10].

However, these studies were widely based on slow freezing protocols and as previously mentioned; the vitrification technique is now gaining wide popularity and is suggested to be superior for cleavage-stage embryos [11]. We hypothesized that the rule of superiority of DMSO based slow freezing program applies to DMSO based vitrification program. However, lack of comparative studies supporting this hypothesis for human embryos has led us to design this study to investigate this point.

Subjects and Methods

The study was conducted during the period from March 2009 till January 2011. The study was carried out in a private setting in Cairo, Egypt. Study protocol was approved by ethical committee of Suez Canal University. All eligible women who had undergone IVF/ICSI cycles with supernumerary good quality embryos during this period were included in the study after signing initial relevant consents. All women had long protocol IVF or ICSI cycles. They received GnRH a in the form of 0.1 mg triptorelin acetate as daily subcutaneous injection starting on day 21 to 23 of the menstrual cycle. Triptorelin acetate administration was continued until loss of follicular activity by transvaginal ultrasonography. At this stage exogenous gonadotropins was initiated and triptorelin acetate was decreased to half. When the largest 3 follicles reached 18 mm diameter, a single 10,000 IU intramuscular dose of human chorionic gonadotropins (hCG) were administered. Transvaginal follicular aspiration took place 35-36 hours later. The fertilization procedure was applied either by IVF or ICSI. Embryo transfer was performed two or three days later by replacement of morphologically best three embryos.

Supernumerary good quality embryos (class A or B [10]) were subjected to cryopreservation under the local ruling conditions. Cryopreservation procedure was applied on day 2 or 3 at 4-8 cell stage embryos. Patient-based randomization allocated frozen embryos into two groups; group A; embryos were cryopreserved with ready-to-use commercially available DMSO vitrification media & group B; embryos were cryopreserved with ready-to-use commercially available PROH vitrification media using the following protocol:

1. After exposure to equilibrium solution for 5 to 15 min embryos in group A and B were exposed to ready to use commercially available DMSO or PROH vitrification media for 60-90 seconds based on simple randomization table.
2. Embryos were then loaded in HSV Straws with two parts; inner one where the embryos were loaded and external cover to protect the inner part.
3. After loading the embryos in HSV straws, the external part of the straws were sealed by heating sealer and then immediately stored in LN2 tanks where they were completely covered with LN2 to be stored at -196 ˚C.
4. Later on, during the replacement cycle, thawing procedure was applied to the embryos of each group using ready-to-use commercially available thawing solutions. Thawing solutions are applied in 3 steps with different sucrose concentrations; 1M, 0.5M, 0M. Embryos were exposed to the media at each step for 2-3 minutes. At the last step, the embryos were exposed to physiological sucrose free solution as a final wash step. These steps allow the withdrawing of the intracellular CPA gradually and not in a sudden manner to minimize the cell shock and maintain the cell viability.
5. Embryos were then incubated in CO2 incubators, cultured in physiological cleavage media for 2 to 8 hours. Then examination for survival was performed.

The main outcome measure was embryo survival rate during thawing in replacement cycles. Survival was indicated by complete return of all embryo blastomeres to the cytoplasmic and morphological conditions existing prior to vitrification. SPSS 19 package (SPSS, Chicago, IL, USA) was used for statistical analysis. Data was expressed as means ± SD. Student t, chi square and factorial ANOVA tests were used when appropriate. Significant values were set at $p<0.05$ level.

Results

Overall, 66 women were eligible for the study and had undergone thawing of embryos in 66 replacement cycles. All participants were undergoing a replacement cycle after a failed first fresh cycle. A total of 212 embryos were included with 114 embryos in group A and 98 embryos in group B.

Table 1 shows a comparison between the two groups as regards to female age, number of oocytes retrieved, oocyte maturity, the fertilization rate and the division rate. The only significant difference was shown in fertilization rate (56.2% vs. 47.6% for group A and group B respectively, p=0.04).

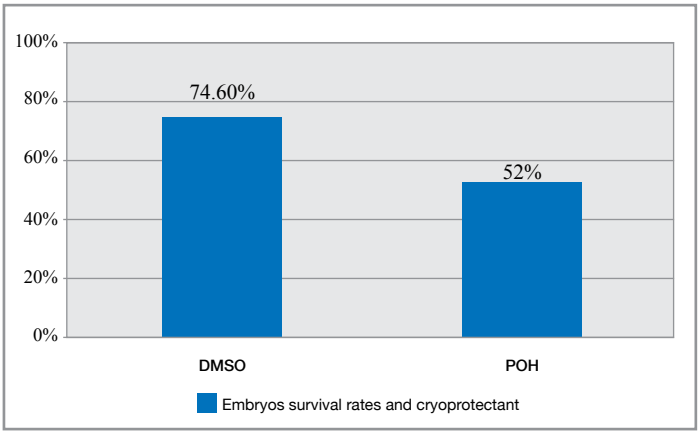
Table 1: Cycle characteristics of the two groups

	DMSO(n=36)	PROH(n=30)	P
Age	28.9 ± 4.6	29.67±5.2	0.51
Retrieved oocytes	16.77±4.3	18.55±3.6	0.40
Mature oocytes	14.43±2.3	14.08±3.1	0.83
Fertilized oocytes	10.9±1.8 (65.2%)	8.86 ±2.3 (47.6%)	0.04 (S)
Divided embryos	10.7±2.4 (64%)	8.83±1.4 (47.5%)	0.05

S: Significant at 0.05

After thawing survival rate was examined in the two groups. 85 out of 114 embryos (74.6%) survived group A, in contrast to 51 out of 98 embryos (52%) for group B, the difference was highly significant, $p < 0.005$. Figure 1

Figure 1: Post-thawing survival rate in the two groups.



To avoid bias, the following factors: age, number of retrieved oocytes, number of mature oocytes, number of fertilized oocytes and number of divided oocytes that were retrieved at fresh cycles, were tested in relation to number of survived embryos in replacement cycles via factorial ANOVA test. None of the mentioned factors significantly affected the number of survived embryos (table 2).

Table 2: ANOVA test relating different influencing factors to number of survived embryos in the two groups

	Group A		Group B	
	F	P	F	P
Age	0.549	0.892	1.128	0.413
No. retrieved oocytes	0.791	0.69	0.889	0.609
No. mature oocytes	0.439	0.952	1.46	0.243
No. fertilized oocytes	1.54	0184	2.043	0.087
No. divided oocytes	1.626	0.157	2.007	0.092

We investigated embryo survival per stage of embryo development. For 4-cell stage , 29 out of 43 embryos (67.4%) survived in group A, in contrast to 25 out of 41 survivor embryos in group B, (60.9%), p=0.13. For 6-cell stage, 34 out of 44 embryos (77.2%) survived in group A, in contrast to 19 out of 35 survivor embryos in group B (54.2%), p=0.02. For 8-cell stage, 22 out of 27 embryos (81.4%) survived in group A, in contrast to 7 out of 22 survivor embryos in group B (31.8%), p=0.01. Table 3

Table 3.Survival rate per stage of embryo development

	Group A	Group B	p
4-cell stage	67.4%	60.9%	0.13
6-cell stage	77.2%	54.2%	0.02 S
8-cell stage	81.4%	31.8%	0.01 S
Fertilized oocytes	10.9±1.8 (65.2%)	8.86 ±2.3 (47.6%)	0.04 (S)

S: Significant at 0.05

Disscussion

The role of cryopreservation in assisted reproduction has long been a milestone in IVF structure, with 60% of cycles having extra embryos suitable for freezing and up to 18% of total IVF cycles in USA are non-donor frozen cycles [12]. IVF centers usually adopt one of two strategies for embryo replacement upon having a good number of fertilized oocytes. The choice is principally based on local expertise and preference of individual centers. The 1st strategy is based on 1st day post oocyte collection (pronucleate stage) embryo freezing. It usually entails keeping five 2 PN embryos for fresh replacement and freezing the extra embryos for future replacement. The 2nd strategy is based on divided embryo (day 2, 3 or 5) freezing. It usually entails choosing the best 1-3 embryos for fresh embryo transfer and freezing the extra good quality dividing embryos for further replacement cycles. This strategy is more widely accepted by most of the centers. Traditionally, with the use of slow freezing protocols, the two most widely used cryoprotective agents were 1,2 propandiol (PROH) and Dimethylsulfoxide (DMSO), with plethora of evidence suggesting superiority of PROH for pronucleate stage freezing and DMSO for dividing stage embryo freezing programs [8-10]. Van der Elst et al [10]in 1995 have conducted a randomized controlled trial on different protocols of slow freezing, two of them were using each of the two cryoprotective additives solely and the third was a combination of the two. The study proved superiority of Dimethylsulfoxide based freezing protocol on the other two protocols as regards to embryo survival as well as pregnancy per transfer rates. Their suggested explanation was a change of membrane permeability and surface to volume ratio in multicellular as opposed to unicellular embryos, in addition to difference in freezing temperature favor Dimethylsulfoxide for freezing of divided embryos. Other explanations were suggested by Brian Wowk [13] that unnecessary formation of hydrogen bond between POH molecules and proteins less active sites causing the risk of a complete lethal dehydration and over consumption of POH but DMSO is leaving these sites for some water molecules preventing the lethal complete dehydration. Also, crowding occurs due to over-reaction between 1,2 propandiol and protein molecules weakens the hydrogen bonds that in return minimizes the activity of 1,2 propandiol as a cryoprotective agent , thus, allowing the protein molecules to regain its chemical activity leading to a lethal effect..

Vitrification, as a method of cryopreservation, has been gaining wide acceptance during the last decade. It entails rapid cooling after emersion with concentrated cryoprotective agents, avoiding the formation of ice and possible cell trauma related to over dehydration or possible rehydration. Good evidence has demonstrated survival of human or animal embryos comparable or even better to conventional slow freezing method [6, 7, 14].

As the technique entails use of high concentration of cryoprotective agent, it is expected that type of cryoprotectant agent is of utmost importance to achieve this tissue equilibrium. Several agents, including dimethylsulfoxide, acetamide, and 1,2propandiol propylene glycol have been used without evidence of superiority of one agent on the others [7, 15]. To our knowledge, this is the first study comparing two cryoprotective agents during vitrification. As a pragmatic study, our results concentrate on embryo survival, rather than pregnancy rate in order to avoid the need to control for many confounding factors that could affect pregnancy, especially as we adopt a strategy of dividing embryo freezing with all patients enrolled in this study having failed their fresh cycles. Our results show a significantly higher survival rate in embryos subjected to dimethylsulphoxide as a cryoprotective agent in comparison to those subjected to 1,2 propandiol (74.6% vs.

52% respectively). Interestingly, the difference was more apparent with advancement of stage of embryo development. It is logic to think that the superior protective effect of dimethylsulphoxide over 1,2 propandiol on dividing cells using slow freezing protocol applies also with the higher concentration used for vitrification. This appears to be more evident with higher stages of embryo development. However, this point needs further validation.

As it was unpractical to randomize embryos, we opted to patient-based randomization at the outset of the study. This has led to inability to control for all possible influencing factors from the start. As a result, we ended with slightly unequal arms of the study (114 vs. 98 embryos for dimethylsulphoxide and 1, 2 propandiol groups) and slightly different fertilization rate for the two groups. However, it is our policy to freeze only good quality embryos. When all confounding factors, including patient age and fertilization rate were tested for effect on embryo survival, none had shown any significant effect.

In conclusion: Dimethylsulphoxide based medium used for vitrification/thaw process of day2 or day 3 divided embryos demonstrated a significant higher embryo survival rate than that of 1,2propandiol based vitrification medium.

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Follicular Fluid Activin A and Leptin Are Not Correlated With IVF Outcome Measures

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ABSTRACT

Objective: The study is designed to evaluate the relationship between follicular fluid (FF) Leptin, Activin A and IVF outcome.
Subjects & Methods:
Prospective observational study measuring FF Leptin and Activin A in 90 patients undergoing ICSI excluding women having PCOS. FF samples collected at oocyte retrieval from follicles > 18 mm in diameter were analyzed for Leptin and Activin A using ELISA and results were correlated to ICSI outcome.

Results: For the whole study population, mean FF Leptin level was 45.2 ng/ml and mean BMI was 25.3 kg/m². FF Leptin had a positive correlation with BMI. The mean FF Activin A was 880.8 pg/ml. 29/90 patients (32.2%) achieved pregnancy, 25 of them (86.2%) had more than 50% grade A embryos on Day 2 in contrast to 33 patients (54.1%) in the non-pregnant group (P <.02). FF Leptin and Activin A did not correlate to day 2 embryo quality or pregnancy outcome FF total Activin A level did not relate to FF Leptin level or number of oocytes collected. Low or high FF Activin A or Leptin levels did not affect fertilization, pregnancy or embryo quality.

Conclusion: Neither FF Activin A nor FF Leptin levels can be used to predict IVF outcome measures.

Key Words: Activin A, Leptin, follicular fluid, intracytoplasmic sperm injection, pregnancy.

Introduction

Assisted reproduction is a complicated process involving multiple stages of ovarian stimulation, ovum pick up, fertilization, embryo cleavage and implantation. The ultimate goal of all these procedures is achievement of a viable intra uterine pregnancy as a step of achievement of a healthy baby. Good quality of all reproductive components and primarily embryos has a positive impact on success rates. Definitely, better selection of embryos is one of the greatest challenges in IVF [1]. A morphological approach of choosing good quality embryos at 2-8 cell stage based on number, equality of size and percent fragmentation has been the method of choice of day 2 or 3 embryo transfer [2].

Activins are disulphide-linked dimeric glycoproteins belonging to the TGF-b superfamily. Other members include inhibin and follistatin. Dimerization of b subunits alone gives rise to three forms of activin referred to as activin A (bA-bA), activin AB (bA-bB) and activin B (bB-bB) [3]. While inhibin and activin exert their actions mainly through a negative feedback effect on pituitary FSH, activins, mainly activin A exert their effects through local autocrine and paracrine effects on granulosa cells through action on specific receptors [4]. Evidence from in vitro and animal studies suggests that activin effects are mainly stimulant to granulosa cell proliferation and maturation, steroidogenesis and oocyte maturation [5]. Activin can promote FSH receptor expression on undifferentiated rat granulosa cells, evidence that can explain the transformation of follicle from late pre-antral to early antral stage [6]. Once granulosa cells have acquired functional FSH receptors, their proliferation and differentiation would be driven mainly by FSH, but modulated by other extrinsic and locally produced factors including insulin, growth hormone and leptin [7]. Activin A was shown to be higher in follicular fluid of follicles containing high quality oocytes of IVF cycles [8].

Leptin, a protein secreted from adipose and many other tissues of mammals was found to a greater extent in people with a high body mass index [9]. Leptin was shown to be involved in various reproductive aspects including initiation of puberty, fertility and preg-

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nancy. Oestrogen was shown to induce leptin secretion. Obesity per se was found to have detrimental effects on conception whether in natural or assisted reproduction cycles [10]. Serum leptin at various stages of IVF cycles, in addition to follicular fluid leptin were suggested as predictors for IVF outcome with some conflicting results. Serum and follicular fluid leptin were consistently rising during IVF cycles with consistent correlation between serum leptin level on day of oocyte retrieval and follicular fluid leptin. Anifandis et al. reported that elevated follicular fluid leptin concentration was associated with reduced ovarian response, follicle maturation, embryo quality and response [11]; however, others failed to prove those associations [12, 13]. The aim of this work is to study a correlation between follicular fluid activin A and leptin and the various IVF outcome measures.

Subjects & Methods

The study was carried out in a private IVF center setting in Cairo, Egypt. Patients less than 42 years with day2-3 FSH <10IU/L were included in the study excluding patients with polycystic ovarian syndrome to nullify its effect on follicular development and IVF outcome. Ninety women were included in the study after signing initial relevant consents and after obtaining the ethical approval from Suez Canal University ethical committee. All women had long protocol ICSI cycles as per the center protocol. They received GnRHa in the form of 0.1 mg triptorelin acetate as daily subcutaneous injection starting on day 21 to 23 of the menstrual cycle. Triptorelin acetate administration was continued until loss of follicular activity by transvaginal ultrasonography. At this stage exogenous gonadotropins were initiated and triptorelin acetate was decreased to half. When ≥3 follicles reached 18 mm diameter or more, a single 10,000 IU intramuscular dose of human chorionic gonadotropins (hCG) was administered. Transvaginal follicular aspiration took place 35-36 hours later under sedation/general anesthesia.

All large mature follicles (>18 mm) were aspirated into empty sterile tubes and oocytes noted carefully to follow them up after fertilization and embryo development. Samples with blood contamination or with flushing fluid were excluded.The fluid was used only if it contains good quality (grade I) oocyte and follicular fluid was centrifuged at 1500 r.p.m. for 15 minutesand the supernatants were frozen at -70 C for future analysis [11]. ELISA tests were used for measuring leptin and activin A as described elsewhere [12, 14]. Body mass index was evaluated on day of oocyte recovery. Standard ICSI procedure for all cases as per the IVF center protocolwas carried out only to metaphase II (M II) oocyteswith daily evaluation of fertilization and embryo grading. Embryos were graded on day 2 and 3 according to the number of blastomeres (<5 or >5), equality of size and degree of fragmentation, giving a score of 1 or 2 for each item. Embryos were scored 'A' for those achieving a score of 5-6 and 'B' for those scoring less.

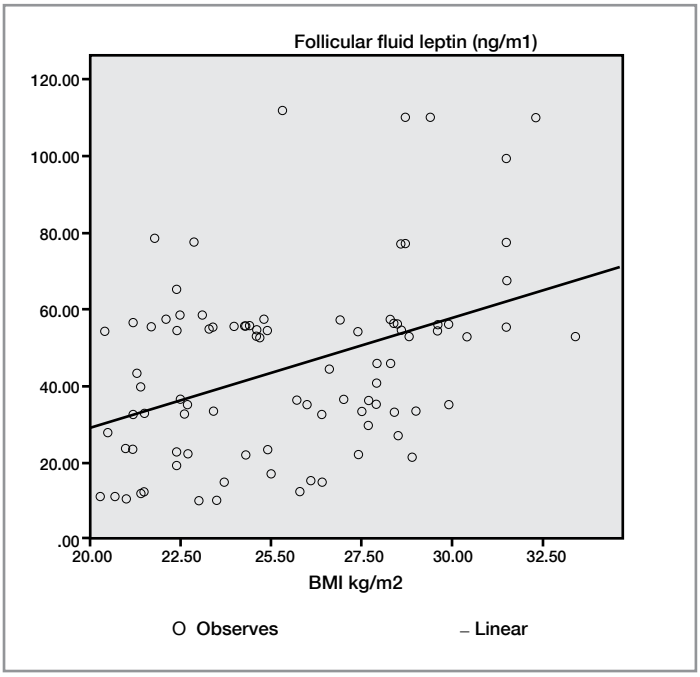
For statistical purposes, Day 2 embryo quality was considered good when the majority of embryos were grade A and poor when the majority was grade B. Two embryos (or 3 embryos in women over 40 years) were transferred in early (day 2or 3) and late (day 5) ET respectively. ET was performed using labotect catheter (Germany) under ultrasound guidance. Luteal phase support was achieved by 400 mg vaginal progesterone. Pregnancy was confirmed by quantitative β-hCG after 2 weeks of oocyte retrieval followed by sonographic confirmation of cardiac activity 2 weeks later.

SPSS 19 package (SPSS, Chicago, IL, USA) was used for statistical analysis. Data was expressed as means ± SD. Student t, chi square and Pearson correlation test were used when appropriate. Significant values were set at $p<0.05$ level.

Results

A total of 90 women were counseled, consented, and enrolled into the study from October 2010 till April 2011. There were no patient drop-outs or cycle cancellation. The indications for treatment were: Male factor: 34 patients (37.7%), tubal factor: 14 patients (15.5%), endometriosis: 12 patients (13.3%), unexplained infertility: 18 patients (20%) and combined factors: 12 patients (13.3%).The whole study group age ranged between 23 to 41 years with a mean of 29.6 ± 5.1 years. All women were nulliparous. No difference in the total dose of gonadotropins was found between the pregnant and the non-pregnant groups.

For the whole group, mean follicular fluid (FF) leptin level was 45.2 ± 26.7 ng/ml and BMI was 25.3 ±3.3 kg/m2. FF leptin had a positive correlation with body mass index ($r=0.4$, $p<0.005$) (Fig 1). Mean follicular fluid activin was 880.8 ± 954pg/ml. No correlation between follicular fluid activin and oocyte number (Pearson correlation: 0.089 $p=0.4$) or follicular fluid leptin (Pearson correlation: 0.15 $p=0.15$).



Pregnancy rate for the whole group was 32.2% (29/90). Table 1 shows patient characteristics in pregnant and non-pregnant groups. No significant differences were shown regarding age, duration of infertility. Body mass index, follicular fluid leptin or activin as well as fertilization rate were not significantly different in the two groups. The only significant difference was in the pregnant group having significantly better quality embryos on day 2 ($p=0.02$).

Table (1): The demographic parameters of the patients before Table 1: patient characteristics according to pregnancy state

	Pregnant (N=29)	Not pregnant (N=61)	All patients (N=90)	*P value
Age (years)**	28.9 ±4.6	30.3 ±5.3	29.6± 5.1	0.61
Months of Infertility **	54.6 ± 23.6	57.8 ± 29.5	56.7 ± 27.6	0.64
BMI (kg/m²)**	24.9 ± 3.6	25.7 ± 3.1	25.5 ± 3.3	0.25
FF leptin (ng/ml)**	43.5 ± 24.8	45.7 ± 23.1	45.2 ± 26.7	0.62
Activin A (pg/ml)**	782.2 ± 368.2	925.2 ± 338.3	880.8 ± 354.6	0.53
Day 2 good emb. quality	25 (86.2%)	33 (54.1%)	58 (64.4%)	0.02 (S)
Fertilization rate **	65.7 ± 12.7	65.8 ± 22.2	65.7 ± 19.7	0.90

** mean ± SD S: Significant at 0.05 level BMI = Body Mass Index; FF = Follicular Fluid

Looking at the embryo quality, follicular fluid leptin and activin were not significantly different in the two groups of 'good' or 'bad' embryo quality on day 2; (34.4 ± 21.5 vs. 37.2 ± 20.7ng/ml for leptin and 825.2 ± 725.4 and 895.4 ± 912.3 for activin A, P = 0.5 and 0.62 respectively).

The data were classified according to follicular fluid leptin of ≤ and >60 ng/ml into low and high FF leptin respectively (table 2). There were no significant differences between the two subgroups as regards to patients' age or duration of infertility, number of oocytes retrieved, fertilization rate, good quality embryo rate and pregnancy rate. Also, mean FF activin was not significantly different between the two subgroups.

Similarly, the data were classified according to follicular fluid activin of ≤ and >1000 pg/ml into low and high FF activin respectively (table 2). There were no significant differences between the two subgroups as regards to patients' age or duration of infertility, number of oocytes retrieved, fertilization rate, good quality embryo rate and pregnancy rate.Also, mean FF Leptin was not significantly different between the two subgroups.

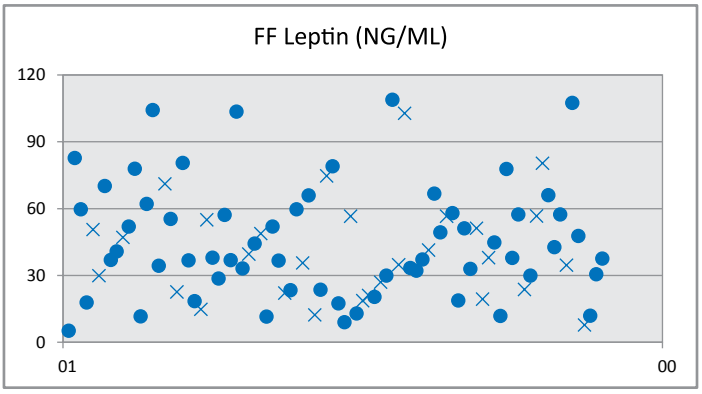
Table 2: Low and high leptin and activin A in the study group.

	Leptin (ng/ml)			Total Activin A (pg/ml)		
	Low (no = 74)	**High (no = 16)**	***P value**	**Low (no = 65)**	**High (no = 25)**	***P value**
Age (years)**	30.3 ±5.7	29.6 ±5.1	0.7	31.6 ±5.7	28.9 ±5.1	0.5
Months of Infertility **	50.2 ± 22.3	55.4 ± 24.3	0.4	52.2 ± 23.6	54.8 ± 21.3	0.4
No. of oocytes collected **	10.09 ± 5.5	11.88 ±6.5	0.9	12.95 ± 5.3	11.8 ±7.2	0.67
Fertilization rate **	65.6 ± 20.7	62.9 ± 23.5	0.6	64.6 ± 21.7	65.7 ± 16	0.83
Day 2 good emb. quality	50 (67.5%)	8 (50%)	0.1	41 (63%)	17 (68%)	0.63
Pregnancy	25(33.8%)	4 (25%)	0.1	23 (35.3%)	6 (24%)	0.09
FF level **	FF Activin 904.3 ± 442	FF Activin 796 ± 491	0.2	FF Leptin 36.9 ± 28.5	FF Leptin 27.3 ± 14.1	0.1

** Significant at 0.05 level* *** mean ± SD*

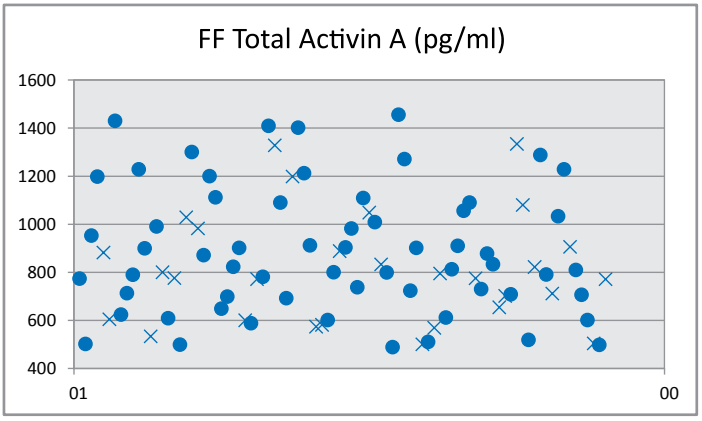
Figures 2 and 3 show the distribution of pregnancy among patients with different levels of Leptin and Activin A respectively. The occurrence of pregnancy did not correlate to the level of the hormones.

Figure 2: Low and high follicular fluid Leptin and pregnancy



Leptin level of ≤ 60 ng/ml is considered low
• = Non pregnant •= pregnant

Figure 3:
Low and high follicular fluid Activin A and pregnancy



Activin A level of ≤ 1000 pg/ml is considered low
• = Non pregnant X= pregnant

Discussion

Leptin and activin are two hormones that do have some role in folliculogenesis and the local regulations of follicular development and therefore were studied to try to prove or not any association between them and the ART parameters and outcome.Ninety patients were enrolled, of them 29 (32.2%) achieved pregnancy. Patients' characteristics were similar in the pregnant and non-pregnant groups as regards to age, duration of infertility and body mass index.The only significant difference was in the pregnant group having significantly better quality embryos on day 2 which was in accordance with other studies [15, 16]. We defined good embryo quality at day 2 as those patients having 50% or more of their embryos of grade A.

Consistent with other studies [17, 18], follicular fluid leptin was positively correlated with body mass index. Obesity was defined by WHO as having a BMI of more than 30 km/m2 and obese women are almost three times more likely than non-obese women to have some degree of infertility [19]. This could be either related to obesity itself or due to associated polycystic ovarian syndrome that is associated with obesity in more than 50% of its population [20]. For that reason women with PCOS were excluded from the study.

Follicular fluid leptin was not found to be correlated with pregnancy or indeed any other ART outcome measures. This result was observed in other studies [21, 21] although other investigators reported an association between FF leptin and IVF/ICSI outcome [10, 11]. Leptin can affect follicular development through a central (hypothalamic-pituitary) and end organ (ovary and endometrium) effects [22].

When the group was further divided to those with high and low FF leptin (≤and >60 ng/ml) – this cut of point was based on the observation by Anifandis et al, 2005 [10] that below this level poor embryo quality and IVF failure are expected –there was no significant differences between the two subgroups as regards to the outcome measures namely fertilization, pregnancy or day 2 good embryo quality rates. This was consistent with other reports [12, 13].

Lau et al (1999) found that level of Activin A was higher in follicles containing good quality oocytes, however this increase did not reach significant difference and failed to show an association with the fertilizing ability of the oocytes [8]. We tested this hypothesis by measuring the level of Activin A in large follicles containing good quality oocytes. There was no significant difference in Activin A levels in pregnant and non-pregnant groups.

The group was further classified into low and high Activin A level subgroups for those having FFactivin A ≤ and > 1000 pg/ml respectively – the choice of the cut of point 1000 pg/ml was arbitrary -, again, there were no significant differences in any of the outcome measures namely fertilization, pregnancy or day 2 good embryo quality rate. This could be explained by the fact that available Activin A is tightly bound to follistatin, a cysteine rich glycoprotein locally secreted by theca cells that was found to have a high binding affinity to Activin A [7] and was suggested to neutralize its biological activity in distant target tissues [23]. Our results suggest that this binding affinity is also functioning in local follicular fluid medium.

A hypothesis that follicular fluid Activin A affects follicular fluid Leptin synthesis was tested. We failed to find a correlation between FF Activin A and Leptin levels. Whether there was actual no relation or an effect is neutralized by follistatin binding requires further validation by testing the effect of purified FF Activin A on FF Leptin.

In conclusion, the local hormonal milieu in the ovary can only be examined by measurement of follicular fluid hormones. This can give us an idea about the hormonal influence on the developing follicles subjected to the controlled ovarian stimulation. In our study, there was no correlation between FF Activin A, Leptin and the IVF outcome measures as well as no correlation between FF Activin A and Leptin.

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Cesarean Section Scar Endometriomas: Immunohistochemical Staining of Estrogen Receptor-alpha, and CD34

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Abstract

Objective: Cesarean section scar endometriomas (CSSEs) are believed to be the result of direct inoculation of the subcutaneous tissues or abdominal fascia with endometrial cells during surgery as well as higher immune tolerance during pregnancy. The ectopic endometrium exhibits multiple subtle, but biologically important, molecular abnormalities favoring increased production of estrogen, cytokines, prostaglandins, and metalloproteinases.

Patients and Methods: The present study was undertaken to immunohistochemically study the expression of the nuclear estrogen receptors (ERs) alpha and cytoplasmic endothelial cell markers CD34 in 34 cases of CSSEs, 27 cases of ovarian endometriomas (OEs) compared with 18 cases with late proliferative endometrium (PE) as control group

Results: The incidence of CSSEs is 0.39% in Mansoura university hospitals, the mean total score (TS) of ERs-alpha and CD34 assessed by mean vascular density (CD-34 MVD) were significantly increased ($p<0.001$) in cases of CSSEs and OEs versus the control group. There was a statistically significant increase in the TS of ERs-alpha ($p<0.001$) in cases of OEs versus CSSEs. On the other hand, there was no statistically significant difference in CD-34 MVD between cases of CSSEs and OVs. In cases with CSSEs, there was no significant correlation (r 0.212, p 0.229) between TS of ERs-alpha and CD-34 MVD. No significant correlations were noted between either ERs or CD-34 MVD and age of the patients, parity, number of prior CSs, duration since the last CS(s) and size of CSSEs.

Conclusion: CSSEs are a multifactorial disease, both ERs alpha and CD34 may play a role in the pathogenesis and maintenance of endometriosis. Obstetricians should keep in mind measures to prevent transmission of endometrial cells during CS.

Key words: Cesarean section scar endometriomas, ovarian endometriomas, immunohistochemical staining, CD34, ERs-alpha.

Introduction

Robert Mayer in 1903 was the first, who described the presence of endometriosis in the postoperative scar (1). Cesarean section scar endometriomas (CSSEs) was believed to be the result of direct inoculation of the abdominal fascia or subcutaneous tissues with endometrial cells during surgery (2). Other proposed theories are: higher immune tolerance during pregnancy and autoantibody formation (3,4). Circulating blood cells originating from bone marrow can differentiate into endometriotic tissues at various sites (5).

Although endometriosis is a nonmalignant disorder, the ectopic endometrium has the capacity to adhere, attach, and implant. It exhibits multiple subtle, but biologically important, molecular abnormalities, including the activation of oncogenic pathways or biosynthetic cascades favoring increased production of estrogen, cytokines, prostaglandins, and metalloproteinases(6).

Estradiol enhances the survival or persistence of endometriotic tissues. Moreover, it aggravates the pathological processes (e.g., inflammation and growth) and the symptoms (e.g., pain) associated with endometriosis. The predominant expression of ERs-alpha may be essential for the development and growth of peritoneal and ovarian endometriosis (7). There are scarce informations about ERs alpha in cases of CSSEs. Direct and indirect evidences have suggested that angiogenesis is a prerequisite for the development of endometriosis, and activation of angiogenesis for adequate blood supply is essential for the survival of the normal as well as ectopic endometrium (8-10)

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CD34, a sialomucin-type glycophosphoprotein, has potentially important roles in blood vessel formation in both embryos and adults. Under all circumstances, CD34 have been shown to facilitate cell migration, it seems to act as a signaling molecule. In endometriosis, it appears that, up to 37% of the micro vascular endothelium of the ectopic endometrial tissue originates from endothelial progenitor cells (11). CD34 were elevated in cases of endometrial carcinoma, and endometrial hyperplasia, than benign endometrium (12), yet it has not been studied before in cases of OEs and CSSEs.

The present study was undertaken to study the expression of ERs-alpha and CD34 in tissue biopsies from CSSEs and ovarian endometriomas (OEs) compared with late proliferative endometrium as control.

Subject & Methods

In the present study, 61 formalin-fixed, paraffin-embedded tissue biopsies obtained from Egyptian women during the period from June 2007 till May 2012, were immunohistochemically studied. Of these, 34 cases were CSSEs their data were retrieved from the archival materials of department of pathology and department of gynecology, Mansoura university hospitals. The clinico-pathological parameters (e.g. age, parity, symptoms as cyclic or non cyclic pain, size of endometriomas, number of prior CSs and the time interval between CS and the surgical excision) were retrieved from the hospital patient files, as well as the histopathological reports. It was not possible to check CS status whether it was selective or elective at delivery. Also, the presence of associated pelvic endometriosis in cases of CSSEs was not possible to assess.

The other 27 ones were patients with OEs underwent ovarian cystectomy or oophorectomy via either laparotomy or laparoscopy at Mansoura university hospitals. The control group included 18 age-matched cases with late proliferative endometrium, they have regular menstrual cycles and had not received any hormone therapy in the prior 6 months.

Exclusion criteria included: Patients with previous uterine surgery (e.g. myomectomy, hysterotomy, ectopic pregnancy, and previous tubal surgery), other abdominal operations as appendectomy, other extra-pelvic sites for endometriomas (e.g. episiotomy scar), and prior hormonal therapy 3-6 months before surgery and recurrent endometrioic lesions. Mansoura ethical committee approved the study.

Immuno-histochemical staining

From each paraffin block, 3 sections each of 4 μm-thicknesses were taken. One section was stained with Hematoxylin and Eosin for revision of the histopathological diagnosis; the criterion for diagnosis of endometrioma was the presence of endometrial glands and/or stromal cells in the tissues. The other 2 sections were dewaxed using xylol for 15 min, rehydrated in an alcohol-raw, and subjected to antigen retrieval on a high setting for 10 min in a pressure cooker in sodium citrate buffer (pH 6.0) containing citric acid 0.1 M and sodium citrate 0.1 M in distillate aqua. After cooling, the slides were washed twice in phosphate buffer solution (PBS). Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxide (Merck) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with "diluted normal serum" for 20 min at room temperature. Sections were evaluated by two expert pathologists, who were blinded about the clinical data of patients. Results from both were collected and the mean of the results was used.

ERs-alpha was diluted in diluting medium (Dako, Glostrup, Denmark) for a further 30 min and repeated washing with PBS, visualization was performed with DAB for 8-10 min. The slides were further counterstained with Mayer's acidic Hematoxylin and washed in an alcohol-raw (50-98%). Negative controls were performed by replacing the primary antibody with normal mouse serum. Positive cells showed a brownish nuclear reaction (fig 1A, 2A), ERs-alpha was evaluated by the total score (TS) (13). The TS is the sum of intensity score (IS) and proportion score (PS). IS was graded as 0 point = no staining reaction, 1 point = weak staining, 2 points = moderate and 3 = strong and PS was graded as 0 point = cell nuclei completely negative, 1 point = 1% positive nuclei, 2 points = >1-10% positive nuclei, 3 points = >10 -33% positive nuclei, 4 points = >33-66% positive nuclei, 5 points = > 66-100% positive nuclei.

CD34 Primary CD34 antibodies were prediluted. The slides were then incubated for 1 hour at 37°C and kept at 4°C in a humid chamber, after washing the sections with PBS, biotinylated anti-mouse IgG was applied to slides, followed by incubation and rinsing with a stream of PBS. Conjugated antibodies were visualized with Diaminobenzidine (DAB) chromogen stain. Sections were counterstained with Mayer's Hematoxylin for 1–2 minutes, dehydrated and mounted. For each case, a negative control was applied by replacing the antibody by PBS or nonimmune serum. Brown cytoplasmic staining of CD34+cells was considered positive reaction (fig 1B, 2B). Each brown stained cell or cell cluster that was clearly separated from adjacent microvessels, endometrial cells and other connective tissue elements were considered as a single countable microvessel. Initially, the most vascularised tumor areas containing the greatest number of capillaries and small venules (so called neovascular hot spots) were selected under low power (x40 and x100) using a light microscope. Five hot spots were taken, high-power (x400) fields were then chosen randomly, and the number of microvessels in each high power field was counted in each sample (14). Vessels characterized by thick muscular walls or with lumen greater than 20μm in diameter were excluded from the count. Mean vascular count (MVC) was calculated as the mean of the 5 values obtained. CD-34 MVD was calculated by dividing the MVC of the examined fields on the high-power field area which is 0.74 mm2.

Statistical analysis was carried out via Statistical package for social Science (SPSS) version 17 program on windows XP. Qualitative data were represented in the form of number and frequency, while quantitative data were represented in the form of mean ± standard deviation (mean±SD). Kolmogrov-smirnov test was used to test normality of quantitative data. Student's t test was used to compare groups. Whereas, Pearson's correlation test was used to determine correlation between variables. Results were considered significant if p value less than or equal 0.05.

Results

During the study period, 37 cases with histologically confirmed abdominal wall endometriomas, 3 cases were excluded: one case following ectopic pregnancy, and one case following abdominal hysterectomy, and one was recurrent lesion and 34 cases were diagnosed with CSSEs, among 10,136 women underwent CS, giving an incidence of CSSEs about 0.34% (table 1). The mean age of the patients at the time of surgical excision in CSSEs was (28.8 + 6.09) years range (22-42), in patients with OEs, it was (29.11 + 5.19) years range (20 – 41), while in women with PE, it was (29.72+7.31) years range (18-44) years. There was no statistically significant difference between all groups.

In CSSEs, the mean number of parity was (2.56 ± 1.11), with a range from 1 to 6, the mean number of prior CSs was (2.12 ± 0.91), with a range from one to four, 25 patients (61%), had one prior CS. All patients complained of scar nodule(s), cyclic pain in the affected area, relating to the menstrual period was present in 23 patients (67.7%). Non cyclic pain in the scar area, without any relationship with the men¬strual period, was reported by 11 patients (32.3%). The mean duration of between CS(s) and surgical excision was (30.88± 12.07) with a range from (9-58) months.

The mean size of CSSEs, defined the largest single diameter, (5.36 ± 1.39) ranged from (2.5-6.7) cm. Histopathologically in 30 cases (91%), the endometrial glands and stroma were within in a background of fibro adipose tissue (subcutaneous), while in 3 cases skeletal muscle fibers were present (subaponeurotic) (table 1).

ERs-alpha were expressed in all tissue biopsies both in the endometrial glandular and stromal cells in all groups. The mean TS of ER-alpha was increased significantly ($p<0.001$) from the OEs (5.52 + 1.40), than CSSEs (4.53 + 1.26), versus the control group (2.85+ 1.38). Interestingly, there was a statistically significant increase ($p <0.005$) among cases of OEs versus CSSEs (table2). The mean CD-34 MVD was increased significantly ($p <0.001$) in cases with CSSEs (45.50+ 19.67) than control group (28.50+13.81). Also, it was increased significantly ($p <0.001$) in cases with OEs (41.30+17.57) than control group. However, there was no statistically significant difference ($p <0.44$) between cases of CSSEs and OEs (table 2).

In cases with CSSEs, there was no significant correlation ($r = 0.212$, $p =0.229$) between TS of ERs-alpha and CD-34 MVD. Also, no significant correlations were noted between either TS of ERs-alpha or CD-34 MVD, age of the patients, size of CSSEs, number of prior CS(s) (table 3). There was no statistically significant change between either TS of ERs-alpha or CD-34 MVD in relation the type of pain or size of CSSEs (data not shown). In CSSEs, scar lesion(s) were excised > 20 months in 28 patients, and in 6 patients < 20 months after prior CS(s), there was a significant increase ($P < 0.05$) in both CD-34 MVD and TS of ERs-alpha and duration > 20 months (table 4).

Discussion

The incidence of CSSEs in the present study was 0.34%, Nominato et al (15) reported the incidence of scar endometrioma in 0.2% women submitted former to CS, others reported an incidence of 0.29%, and a relative risk of 27.3 for the occurrence of surgical scar endometriosis following CS(4). Our results were higher probably due to different selection criteria as we selected only cases with CSSEs. The average age of the patients in the present study was 28 years, it was younger than that reported by (16), this may be due to tendency toward earlier marriages and consequently earlier deliveries in our patients. We did not find any correlation between the number of parity and the occurrence of CSSEs, these results were in agreement with (16). On contrary low parity may increase the risk of CSSEs (17). The number of prior CSs did not increase the incidence of CSSEs, these were in agreement with (17, 18). In 23 patients (67.7%) cyclic pain was present, this was concordant with others (16, 19).

ERs alpha in CSSEs and OVs ERs-alpha were immunohistochemically expressed both in the glandular and stromal cells in all studied groups. The mean TS of ER-alpha was increased significantly ($p<0.001$) in the cases of OEs, than control group. These results were in agreement with others (7,17). Also, it was increased significantly ($p<0.001$) in the cases of CSSEs, than control group. Our study was among the first ones to address ERs-alpha in CSSEs. Interestingly, there was a statistically significant increase ($p <0.001$) among cases of OEs versus CSSEs. The exact causes of these findings were unknown it may indicate that estradiol may be more important in the pathogenesis OVs rather than CSSEs, other factors may contribute to the development of CSSEs. Also, there was a lack of literature data confirming relation of hyperestrogenemia to scar endometriomas. These findings highlight the importance of estradiol both in the pelvic and CSSEs. It is well known estradiol enhances the survival or persistence of endometriotic tissues, it aggravates the pathological processes (e.g., inflammation and growth) and the symptoms (e.g., pain) associated with endometriosis. Moreover, ERs are ligand-dependent transcriptional factors, which can bind to different DNA sites to initiate the expression of specific genes. In addition, indirect mechanisms through contacts with DNA-bound transcription factors have reported (7).

CD-34 MVD in CSSEs and OVs Vascular density was quite heterogeneous in any given tissue sections of the various histological types, some areas being vascular and other areas remaining relatively avascular. The mean CD-34 MVD was increased significantly ($p<0.001$) in cases with OEs than control group. In vitro study confirmed these results in mice (20). The mean CD-34 MVD was increased significantly ($p<0.001$) in cases with CSSEs than control group. There was no statistically significant difference ($p >0.05$) between cases of CSSEs and OEs. The present study is among the first ones that study the expression of CD 34 in CSSEs. The potential causes of increase in CD34 in both OEs and CSSEs may be due to chronic overstimulation of endothelial cells leading to cell activation and proliferation leading to angiogenesis. Regardless of its possible causes, angiogenesis is of paramount importance in the growth and survival of endometriotic lesions as ectopic lesions require nutritional supply to maintain proliferation and to invade into ectopic sites within the host. Thus, CD34 may appear to be part of the complex interplay of sialomucins contributing to the maintenance of endometriosis.

The potential significance of angiogenesis, it may allow for the identification of patients at high risk of recurrence after surgical excision who may benefit from aggressive surgical procedures as well as postoperative therapy. Also, the potential antiangiogenetic therapy as a method of treatment of endometriosis. In cases with CSSEs, there was no significant correlation between TS of ERs-alpha and CD-34 MVD. This highlight the concept that both pelvic and CSSEs are a multifactorial disease. Also, there was no significant correlations between either of CD-34 MVD nor ERs-alpha and the size of CSSEs. Increased expression of both and CD-34 MVD and ERs-alpha in tissue biopsies of CSSEs more than 20 months after prior CS, may indicate that both factors are important both in the pathogenesis and progression of OEs and CSSEs and may explain the aggressiveness nature of endometriosis.

Since the results of the present study supports the theory of iatrogenic cell transportation, as well as in the face of increasing rates of cesarean deliveries, obstetricians should adopt measures to prevent CSSEs, first of all unnecessary CS(s) should be avoided and, if CS is performed careful surgical techniques as (1) Perform selective CSs after the onset of spontaneous labor, whenever possible, instead of elective CSs as the onset of labor marks the termination of pregnancy- induced immune tolerance to the implanted endometrial cells. Wicherek et al.,(4) stated that performing CS(s) without the presence of labor conditions more than doubles the risk in relation to situations in which cervical ripening and uterine contractions are present. (2) Shielding the wound by a quadrangular bandage during placental extraction and during curettage of the uterine cavity and immediately discard swabs or sponges used for cleaning the uterine cavity (21). (3) Avoid penetration of the endometrium during suturing the myometrium as reported by (22). (4) Failure to close the parietal and visceral peritoneum in the CS may be related to greater rates of CSSEs (23), although an

evidence based obstetrics recommend during cesarean section to leave the visceral peritoneum unsutured. We advise multicenter randomized control to substantiate or refute these steps. (4) Thorough washing the abdominal wall via irrigation with a salt solution before definitive closure (24). (5) Also, it is recommended not to use the same surgical material and the same in¬struments as used in hysterorraphy, when suturing other abdominal wall layers as stated by(25). (6) Ongoing use of high doses of progesterone during the first six months after CSs in order to decrease the occurrence of endometriosis at the surgical site (26). (7) Prolonged breast feeding is well known protecting factor because of causing hypoestrogenic status that does not support endometriosis development (26). Although, there are no randomized controlled trials that can support these maneuvers, we advise adopting these steps during the surgical procedures.

We can conclude that CSSEs is a multifactorial disease, both ERs alpha and CD34 may play a role in its pathogenesis and maintenance. Obstetrician should keep in mind measures to prevent transmission of endometrial cells during CS.

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Prevalence of Polycystic Ovary Syndrome among Fertile and Infertile Women in Minia Governorate, Egypt

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Abstract

Objective: To demonstrate the prevalence of polycystic ovary syndrome PCOS in fertile and infertile women in Minia Governorate, Egypt.

Patients & Methods: 1450 patients visiting outpatients' clinics of Minia Maternity University Hospital were classified into two groups; group (I), included 620 fertile women and group (II) included 830 infertile women. All patients were searched for ovulatory disorders and manifestations of hyperandrogenism. Trans-vaginal ultrasound was done for all patients. Free testosterone (free T) was done only for patients with hyperandrogenism (336).

Results: The prevalence of PCOS was 27.4%. The prevalence was 14% and 37.5% in fertile and infertile women respectively. The percentages of ovulatory disorder, hirsutism and PCO in infertile patients with POCS were 73.3%, 60.4% and 79.4% respectively. There was significant correlation between prevalence of PCOS and increased BMI ($r=0.221$ and $P = 0.001$).

Conclusions: PCOS represents a major health and reproductive problems in women of the reproductive age.

Key words: prevalence, PCOS, hirsutism, free testosterone and anovulation.

Introduction

PCOS is one of the most common endocrine disorders affecting women of reproductive age. Epidemiological studies have reported that the prevalence ranged from 6.5% to 8% using biochemical and/or clinical criteria, (1, 2) and this prevalence increased to 20% or more in ultrasound-based studies. (3, 4)

There is evidence that the prevalence of PCOS differs in populations with increased risks of insulin resistance and metabolic disease (5, 6). Other studies in Australia have concluded that this prevalence increased in women with obesity, hyperinsulinism, diabetes, dyslipidemia and a history of low birth weight. (7, 8) The most frequent presentations of women with PCOS are infertility, menstrual irregularity, hirsutism, and/or other outward signs of androgen excess such as acne or alopecia. A guide to the diagnosis also include metabolic disturbances such as obesity insulin resistance, dyslipidemia, and hypertension. Due to these adverse clinical and metabolic complications, considerable effort remains regarding what collection of symptoms constitutes a diagnosis of PCOS. (9)

This condition should promote early diagnosis and management because there is strong evidence that women with PCOS may suffer from infertility, dysfunctional uterine bleeding, metabolic syndrome, type II diabetes, and cardiovascular disease. There are also some studies concluded that women with PCOS are at increased risk of obstructive sleep apnea, depression, nonalcoholic fatty liver disease, and certain cancers. (10, 11) The aim of this study was to demonstrate the prevalence of polycystic ovary syndrome in fertile and infertile women in Minia Governorate, Egypt.

Patients and methods

This cross sectional observational analytic study was conducted on 1450 women visited the out patient clinics of Minia Maternity University Hospital in the period between January 2010 and April 2011. Patients were classified into 2 groups; group I included fertile patients while group II included infertile patients.

Inclusion criteria for fertile group were as follow: middle aged female, on IUCD for contraception and her last delivery was for at least 2 years. An inclusion criterion for

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group II was primary or secondary infertility for variable periods. Patients were excluded from the study if they were pregnant or breast-feeders, if they did not accept to complete the study steps, if they reported being menopausal, using hormone replacement therapy or hormonal contraception, if they had a hysterectomy or oophorectomy, presence of any other etiologies of androgen excess, known hypothyroid patients and patients missing information about cycle regularity. Written informed consents were taken from every participant in this study after the approval of the ethical committee of the department of Obstetrics and Gynecology and Faculty of Medicine, Minia University. Information was collected about age, parity, menstrual cycle frequency and regularity.

For all women, body weight, and height were measured. Body mass index was calculated as weight in kilograms divided by the height in meters squared (kg/m2). Features of hyperandrogenism as hirsutism, acne or androgenic alopecia were searched for. All women (n = 1450) were subjected to trans-vaginal ultrasound scans of the ovaries using 5 MHz intra-cavitary vaginal probe, (Sonoace 9900, Medison, Seoul, Korea) on the second or third day of her spontaneous or progesterone induced menstrual cycles. Venous blood samples were obtained from patients with clinical features of hyperandrogenism (n = 336) at the same day as the ultrasound was performed All sera were stored at -80°C until the time of measurements.

We adopted the Rotterdam diagnostic criteria (9); PCOS was defined by the presence of two or more of the following; clinical and/or biochemical hyperandrogenism, menstrual disorders and polycystic ovaries. The clinical assessment of hirsutism is subjective, and it is important to consider the patient's perception of unwanted hair growth in addition to the perceived rate and timing of hair growth onset. Hirsutism was referred to the growth of coarse, dark hair in areas without hair at all or where fine hair typically grows or, and takes male pattern distribution; above the lip and on the chin, chest, abdomen, and back. Free testosterone (free T) was used to assess hyperandrogenism (9). Polycystic ovaries were identified by vaginal ultrasound, conducted in the follicular phase or when hormonal assessment showed no follicular activity. A positive finding of polycystic ovaries required either 12 or more follicles measuring 2–9 mm in diameter, or increased ovarian volume (10 cm) in at least one of the ovaries. (12) Idiopathic hirsutism IH was defined more strictly as diagnosable in women who have 1) hirsutism, 2) normal ovulatory function and 3) normal androgen profile.

Statistical methods:

Statistical analyses were performed using SPSS version 16 (SPSS, Chicago, IL, USA). P value less than 0.05 were considered statistically significant.

Results

The current study included 1450 patients, they were classified into 2 groups, group I included 620 fertile women, while group II included 830 women complaining of infertility. Demographic and anthropometric criteria of study populations are summarized in table (1)

Table (I): Demographic and anthropometric data of the study populations:

	Group I (n=620)	Group II (n=830)	P- Value
Age (years)	20-37 (28.2±4.8)	18-35 (25.3±5.1)	< 0.001*
Parity	1-7 (2.5 ± 2)	0-2 (0.8 ± 0.5)	< 0.001*
Wight (kg)	49 -112 (56.31 ± 11.28)	45 -122 (67.43 ± 10.43)	< 0.001*
Height (cm)	140-177 (161.78 ± 6.04)	143-175 (166.23 ± 5.32)	< 0.001*
BMI (kg/m2)**	22.5–36.6 (26.2 ± 3.4)	19.5–37.6 (25± 3.95)	< 0.001*

* P values are highly significant ** BMI: body mass index

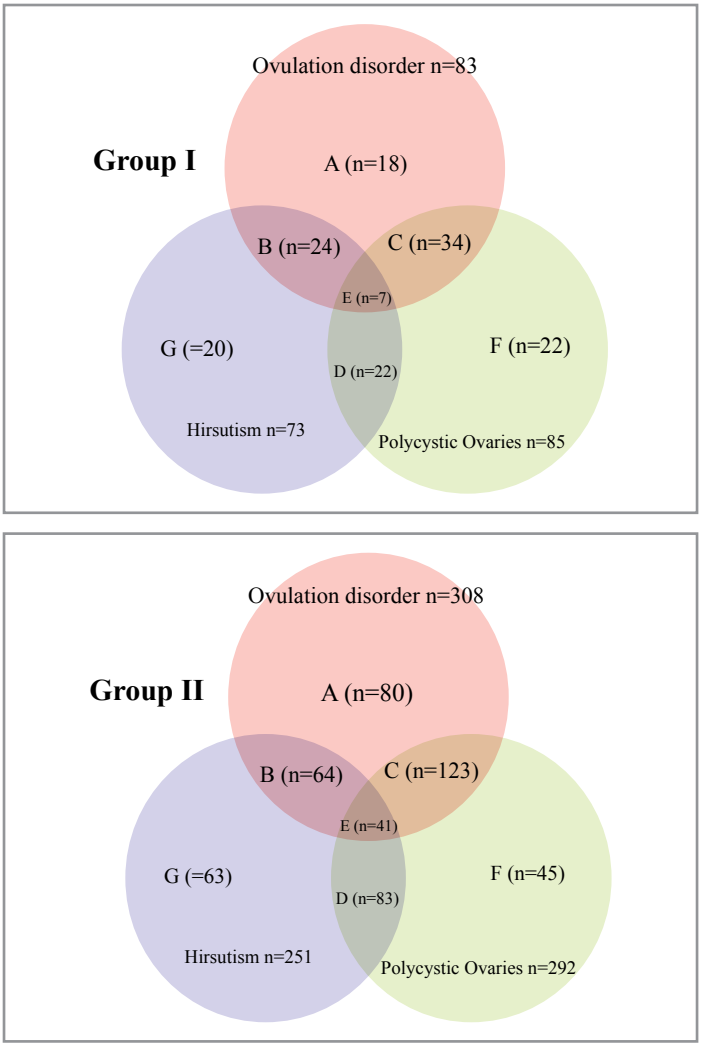

Figure (I): Distribution of the diagnostic criteria in the study population

A= ovulatory disorder alone
B= ovulatory disorder and hirsutism
C= ovulatory disorder and PCO
D= hirsutism and PCO
E= ovulatory disorder, hirsutism and PCO
F= PCO alone **G**= hirsutism alone

Table II: Distribution of the diagnostic criteria in the study population:

Results	Group I (n=620)	Group II (n=830)	P- Value*
Oligo/ anovulation	83 (13.4%)	308 (37.1%)	< 0.001
Hirsutism	73 (11.7%)	263 (31.7%)	< 0.001
Free T (pmol/L)	49 ± 7.7(18-60)	51 ± 9.6 (20-63)	0.0657**
PCO	85 (13.7%)	292 (35.25%)	< 0.001
PCOS	87 (14%)	311 (37.5%)	< 0.001

* p value < 0.05 is significant and
p value<0.001 is highly significant
** t-test with unequal variance

From Figure (1), the prevalence of PCOS in fertile group (B+C+D+E) was 87/620 (14%), while in infertile group; the prevalence was 311/830 (37%). Analysis of the data of the infertile group showed that the percentage of ovulation disorders (B+C+E) in patients with PCOS was 73.3% (228/311), while the percentage of hirsutism (B+D+E) was 60.4% (188) and percentage of PCO (C+D+E) was 79.4% (247). 12 Patients (16%) presented with hirsutism only of the fertile group had normal free T they were diagnosed as idiopathic hirsutism IH, while 30 out of 263 patients (11.4%) presented with hirsutism had normal free T in the infertile group and diagnosed as IH

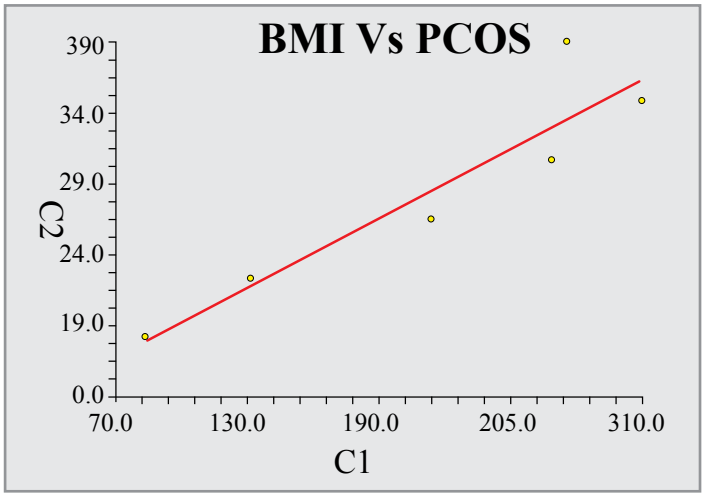

Figure (II): Correlation between BMI and number of patients with PCOS

C1 represent PCOS cases in group II, C2 represent BMI (body mass index) r=0.221 and P = 0.001.The number of women with PCOS increased with higher BMI (correlation coefficient was 0.221 and P = 0.001) as shown in figure 2

Discussion

In the current study the prevalence of PCOS was 14% among fertile women, while in patients complaining of infertility the prevalence was 37% using the Rotterdam diagnostic criteria in a random sample of an Egyptian population. The reported prevalence of PCOS in various geographic regions varies between 2.2% and 26%. (2, 6, 14-17). The prevalence was 2.4% among 915 Southern Chinese women recruited through the offer of a free medical examination using Rotterdam diagnostic criteria (15); the prevalence was 6.5% in 154 white Spanish women during blood donation using national institute of health (NIH) criteria. (18) The cumulative prevalence of PCOS was 6.6% using the NIH definition among women undergoing pre-employment physical examinations in the United States, (2); the prevalence of PCOS was reported to be 17.8% among 978 South Australian women, who were recruited in a retrospective birth cohort study using Rotterdam diagnostic criteria (14). Among 157 women with type II diabetes in Esfahan-Iran, the prevalence of PCOS was 8.2%. (19) It was clear that, the PCOS prevalence depends on the recruitment process of the study population and criteria used for its definition; the Rotterdam diagnostic criteria increased its prevalence by 2 times versus NIH criteria, as reported before. (20-22)

The main bewildering questions emerged in the 14 % of fertile group who had full picture of PCOS, were about health complications that may be concealed in those patients, their past reproductive history; pregnancy might occur during episodes of occasional ovulation and their future reproductive performance; they may be the substrate of future secondary infertility.

In the present study, there was significant positive correlation between women with PCOS and their BMI (r=0.221 & P = 0.001). This is in agreement with previous studies (14, 16, 23) that reported women with PCOS had the highest median BMI.

Hirsutism is the commonest clinical manifestation of androgen excess in PCOS. In the present study, 73 patients (11.7%) of the fertile women had hirsutism, only 32 % of those women (24/73) had cycle irregularities while the rest of them presented with regular cycles. 12 (16%) patients had IH. While in the infertile group, hirsutism was diagnosed in 31.7% and IH was diagnosed in 30 cases out of 263 women. It is important to note that hirsutism was found in 60.9 % (53/87) and 60.4% (188/311) of patients with PCOS in fertile and infertile groups respectively.

The reported prevalence of idiopathic hirsutism IH varies from 5-29%. (24). Overall 60–75% of patients with PCOS will have hirsutism (25) but there is wide variation based on ethnicity and degree of obesity. Its assessment should therefore be ethnic specific. Most studies have examined Caucasian and African-American women.(26) East Asian women have a lower prevalence of hirsutism (27) while the prevalence and severity of hirsutism in women with PCOS of Southern Asian origin is greater when compared to Caucasians.(28) The prevalence of PCOS in women with hirsutism is 75–80%, whereas 20–40% with acne alone have PCOS. About 10% of women with alopecia only will have PCOS.(29, 30)

In the current study, menstrual irregularities were found in 13.4 % in the fertile group compared to 37.1% in the infertile group (P < 0.001) but ovulatory disorder constituted 74.7% and 73.3% of patients with PCOS in fertile and infertile groups respectively. Approximately 75% of women with PCOS have menstrual irregularities suggestive of anovulation. This includes oligomenorrhea and amenorrhea. However 20–30% of oligoanovulatory women with PCOS can present with apparent eumenorrhoea (i.e. subclinical oligoanovulation). Therefore eumenorrhoeic women with other features of PCOS should have multiple determinations of serum progesterone (drawn between Day 20–24 of their menstrual cycle) to accurately categorize their ovulatory status. (31) In the present study PCO morphology (PCOM) was diagnosed in 13.7% and 35.2% of both study groups.

The term polycystic ovary to describe this morphology is a misnomer because there are no dominant cysts or follicles larger than 10 mm because of anovulation. A study from Cape Town demonstrated no correlation between the severity of ovarian morphology and the endocrine or metabolic manifestations of PCOS. (32) PCOS is a functional disorder that does not depend on the pres-

ence of polycystic ovaries, and the absence of PCOM does not exclude the diagnosis. Approximately 20–30% of asymptomatic women < 35 years of age will have PCOM; many studies proved that about 20% of these women will actually have PCOS by NIH definition. Conversely, 10–25% of patients with PCOS by NIH definition will not have PCOM on ultrasonography.(9, 25, 29, 31)

Up to 30% of females with normal androgens and normal menses can have PCOM. (12, 33) It has also been suggested that some women with PCOM and normal ovulatory cycles may have higher LH, androgen and insulin levels as well as lower SHBG levels when compared with control women without PCOM. (34) This phenotype may therefore represent the mildest form of PCOS. PCOS represents a major health and reproductive problems in women of the reproductive age in Minia Governorate. We recommend follow up of fertile patients with PCOS to answer the above listed questions.

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Comparison between Hysterosalpingography and Saline Infusion Sonography in Patients with Recurrent Failed Implantation in Intra-Cytoplasmic Sperm Injection

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Abstract

Objective: Abnormal uterine findings are reported in up to 50% of women with recurrent implantation failure. Hysterosalpingography is commonly used in evaluation of these patients. The introduction of saline infusion sonohysterography has improved diagnosis of endometrial pathologies. Aim of work was to compare accuracy of Hysterosalpingography and Saline infusion sonohysterography in diagnosing uterine pathologies among infertile women with failed intra-cytoplasmic sperm injection.

Subject & Methods: 118 women with recurrent implantation failure underwent hysterosalpingography and saline infusion sonohysterography. The reports were reviewed and findings including submucous fibroids, intrauterine septum, synechia and polyps were compared to those obtained by hysteroscopy. Sensitivity, specificity, and accuracy of procedures were measured.

Results: Regarding submucous fibroids, Hysterosalpingography had sensitivity, specificity and accuracy 80%, 96% and 92.9% respectively, whereas, Saline infusion sonohysterography had sensitivity, specificity and accuracy 95.2%, 98% and 97.5% respectively. Regarding intrauterine septum, Hysterosalpingography had sensitivity, specificity and accuracy 85%, 95.3% and 93.7% respectively whereas Saline infusion sonohysterography had sensitivity, specificity and accuracy 89.5%, 97.1%, and 95.9% respectively. Regarding intrauterine synechia, Hysterosalpingography had sensitivity, specificity and accuracy 75.5%, 94.4% and 88.1% respectively. Saline infusion sonohysterography had sensitivity, specificity and accuracy 70.8%, 97.7%, and 88.1% respectively. Regarding endometrial polyps, Hysterosalpingography had sensitivity, specificity and accuracy 64.7%, 97.7% and 85.5% respectively. Saline infusion sonohysterography had sensitivity, specificity and accuracy 66% and 97.7% and 86.1% respectively.

Conclusion: Saline infusion sonohysterography is comparable, in sensitivity, specificity and accuracy, to Hysterosalpingography in evaluation of uterine abnormalities.

Key word: Recurrent ICSI, hysterosalpingography, saline infusion sonohysterography

Introduction

Approximately 15% of couples are affected with subfertility, of which up to 20% remain unexplained. Uterine cavity abnormalities can be a contributing cause of subfertility and recurrent implantation failure. Uterine cavity assessment has been suggested as a routine investigation in the evaluation of subfertile women. (1)

The success of in-vitro fertilization (IVF) treatment is low. Failure of IVF treatment is generally due to embryonic, uterine or transfer factors, but remains unexplained in most cases. A number of interventions have been proposed to improve IVF outcome, many of which may not be evidence-based and their efficacy is uncertain. One of the common investigations proposed following IVF failure is to re-evaluate the uterine cavity. (2)

Traditionally, hysterosalpingography (HSG) has been the most commonly used technique in the evaluation of uterine cavity. The introduction of saline infusion sonohysterography (SIS) has significantly improved sonographic diagnosis of various endometrial pathologies. This procedure entails instillation of warm saline into the uterine cavity transcervically to provide enhanced visualization of the endometrium during transvaginal ultrasound examination. (3)

We aimed to compare the accuracy of HSG and SIS for diagnosing uterine pathologies among infertile women with recurrent failed intra-cytoplasmic sperm injection (ICSI).

The sensitivity, specificity, positive, negative predictive values and accuracy for HSG and SIS were determined for the diagnosis of endometrial pathology.

Subjects & Methods

A prospective interventional study was introduced to the emer- This study included one hundred and fifty infertile women with unexplained recurrent implantation failure (defined as at least two failed previous ICSI cycles, during which good quality embryos were transferred (4)). A written consent was obtained from all candidates and the study was approved by the medical ethics committee of Ain Shams University. All patients were subjected to HSG, SIS and diagnostic hysteroscopy (DH) which is the gold standard investigation.

The HSG was performed under fluoroscopy in an outpatient office setting at least 48 hours after menses had ceased. The patients were routinely premedicated with Hyoscine Butylbromide 10 mg (Buscopan®, Boehringer Ingelheim) prior to the procedure. The patient was placed in a lithotomy position, and a sterile Graves speculum was inserted to expose the ectocervix. Using a tenaculum, to fix and apply traction on the cervix, the cervical os was cannulated with a Leech Wilkinson Uterine Canula of suitable size. In order not to obscure the lower uterine segment, the Graves speculum was withdrawn slowly and carefully, not to dislodge the uterine canula. Ten cc of urographin contrast were injected intrauterine with fluoroscopic control (OEC 9800, General Electric Company, Fairfield, CT). A combination of pulse fluoroscopy (8 frames per second) and continuous fluoroscopy were used with automated exposure control. Static image capture was achieved by use of the fluoroscopic last image hold feature. Images of early and maximal opacification of the uterine cavity, fallopian tubes, and peritoneal contrast spillage were obtained.

SIS was performed by the same operator, during the follicular phase of the cycle. In lithotomy position, the ectocervix was exposed using a sterile warm Collin speculum (to facilitate its removal during the procedure). After cleaning with povidone-iodine, a sterile 5-F catheter, with an occlusive balloon, was flushed with sterile saline solution before being inserted through the cervical os. A ring forceps was used for advancement of the catheter approximately 5–10 cm to position the tip beyond the endocervical canal and not touching the uterine fundus. The speculum was removed while the catheter was left in place. Next, transvaginal sonographic sagittal and coronal or transverse scanning of the pelvis, adnexae, and uterus was performed during instillation of sterile saline solution. Various amounts (5–20 ml) of saline solution were used; only 2–5 ml are needed to distend the uterine cavity adequately. A study was considered normal when serial sagittal and coronal views of the distended endometrial cavity failed to reveal any distortion, cavitary defect, or undistended regions. Intracavitary defects were described and a likely diagnosis was suggested.

DH was performed using rigid hyteroscope 5.2 mm (Karl Storz, Germany). The scope was advanced under direct visualization. The uterine cavity was distended with normal saline, installed from a 500 ml bag wrapped in a pressure bag connected to a manometer and pumped to 120–200 mmHg.(5) Either positive or negative findings were recorded, and applicable therapeutic procedures were completed. The presence of fibroids, an intrauterine septum, intrauterine synechia, or endometrial polyps were reported. In the absence of these findings, the cavity was described as normal.

Statistical analysis:

All data were transferred to IBM cards using IBM personal computer, analyzed with statistical program for social science "SPSS V11.0, SPSS Inc., Chicago, IL, USA". The data obtained were expressed as descriptive statistics (mean ± standard deviation, range and percentage). Chi-squared test was used for the analysis. A P-value of <0.05 was considered statistically significant. Considering hysteroscopic findings as the gold standard confirmatory test, sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated.

Results

Of the 150 patients, only 118 patients continued the study. Their age ranged between 19 and 42 years with a mean±SD 32.81±4.32. Five patients had previous pregnancies while 113 were nulligravida. Seventy nine patients had two failed ICSI attempts while the other 39 patients had more than two failed ICSI procedure.

Figure (1): Comparison between DH, HSG and SIS as regards detection of specific intrauterine lesions:

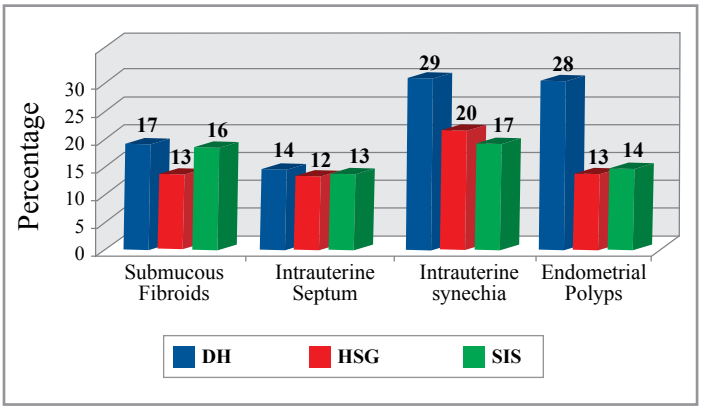

Table (1): Comparison between HSG and SIS as regards sensitivity and specificity for intrauterine lesions:

	Sensitivity			Specificity		
	HSG (%)	SIS (%)	P	HSG (%)	SIS (%)	P
Submucous Fibroids	80	95.2	>0.05	96	98	>0.05
Intrauterine Septum	85	89.5	>0.05	95.3	97.1	>0.05
Intrauterine synechia	75.5	70.8	>0.05	94.4	97.7	>0.05
Endometrial Polyps	64.7	66	>0.05	97.7	97.7	>0.05

Table (2): Comparison between HSG and SIS as regards positive predictive value and negative predictive value for intrauterine lesions:

	Positive predictive value			Negative predictive value		
	HSG (%)	SIS (%)	P	HSG (%)	SIS (%)	P
Submucous Fibroids	83.1	91	>0.05	95.1	99	>0.05
Intrauterine Septum	77.3	85	>0.05	97.1	98	>0.05
Intrauterine Synechia	87.2	94.4	>0.05	88.4	85.7	>0.05
Endometrial Polyps	94.3	94.3	>0.05	82.5	83.3	>0.05

Table (2): Comparison between HSG and SIS as regards positive predictive value and negative predictive value for intrauterine lesions:

	HSG (%)	SIS (%)	P
Submucous Fibroids	92.9	97.5	>0.05
Intrauterine Septum	93.7	95.9	>0.05
Intrauterine Synechia	88.1	88.1	>0.05
Endometrial Polyps	85.5	86.1	>0.05

Discussion

Structural abnormalities of the uterus may affect the reproductive outcome by interfering with implantation and causing spontaneous miscarriage. Abnormal uterine findings are reported in as many as 50% of women with recurrent implantation failure (6)

Hysteroscopy is generally considered to be the gold standard in the diagnosis of intrauterine pathologies, including endometrial polyps, submucous myomas, intrauterine adhesions and uterine septa.(7) Recently, the use of contrast media such as saline with transvaginal sonography is increasingly used to improve the delineation of uterine cavity abnormalities.(3)
In this study we compared HSG and SIS as regards rate of detection, sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the test in diagnosing uterine abnormalities. Hysteroscopic findings were the gold standard and reference.

This study showed that hysteroscopy had a better diagnostic capability compared to HSG and SIS, diagnosing submucous fibroid, intrauterine septum, intrauterine synechia and endometrial polyps with a percentage of 17%, 14%, 29% and 28% respectively, while HSG showed percentages of 13%, 12%, 20% and 13% whereas, SIS showed percentages of 16%, 13%, 17% and 14% for the same lesions respectively (Figure 1).

Several studies have compared DH, HSG and SIS in detecting uterine abnormalities. One study comparing HSG with hysteroscopy reported sensitivity of 81% and a specificity of 80% with a false negative rate of 9% and a false-positive rate of 22%.(8) Another study conducted to assess the diagnostic reliability of hysteroscopy and HSG, demonstrated HSG to have a sensitivity of 79% and a specificity of 82%, with an 18% false positive rate and a 19% false-negative rate. They concluded that even though HSG is mainly used for the assessment of tubal patency, it has a secondary role in the assessment of the uterine cavity.(9)

Furthermore, SIS has been found to be sensitive, specific and accurate in identifying intrauterine abnormalities such as polyps, submucosal fibroids, adhesions, septa and uterine anomalies. One study even showed that SIS had the same diagnostic accuracy as hysteroscopy for endometrial polyps.(10) Another study comparing SIS with hysteroscopy reported 87.5% sensitivity, 100% specificity, 100% positive predictive value and 91.6% negative predictive value for the detection of any cavity abnormality with SIS as compared with hysteroscopy.(11)

On the other hand, the accuracy of HSG in assessment of the uterine cavity integrity in infertile patients has been reported to be rather disappointing. The sensitivity and specificity were described to be 79–98% and 15–82%, respectively (12) and similar studies have also shown that hysteroscopy had a better diagnostic accuracy than SIS.(13)

In this study, as regards submucous fibroids, HSG had a sensitivity and specificity 80% and 96% respectively. Positive predictive value, negative predictive value and accuracy were 83.3%, 95.1% and 92.9% respectively, whereas, SIS had a sensitivity and specificity 95.2% and 98% respectively, positive predictive value, negative predictive value and accuracy of 91%, 99% and 97.5% respectively. These results are in agreement with Erdem et al. were SIS had a Positive predictive value, negative predictive value and accuracy of 91%, 99% and 97.5% respectively.(14)

In this study as regard intrauterine septum, HSG had a sensitivity and specificity of 85% and 95.3% respectively. Positive predictive value, negative predictive value and accuracy were 77.3%, 97.1% and 93.7% respectively whereas SIS had a sensitivity and specificity 89.5% and 97.1% respectively. Positive predictive value, negative predictive value and accuracy were 85%, 98% and 95.9% respectively. As regard intrauterine synechia, HSG had a sensitivity and specificity of 75.5% and 94.4% respectively. Positive predictive value, negative predictive value and accuracy were 87.2%, 88.4% and 88.1% respectively. SIS had a sensitivity and specificity 70.8% and 97.7% respectively. Positive predictive value, negative predictive value and accuracy were 94.4%, 85.7% and 88.1% respectively. Similar studies have shown HSG and SIS to have a sensitivity of 75% in the detection of intrauterine adhesions.(10)

In this study as regard endometrial polyps, HSG had a sensitivity and specificity 64.7% and 97.7% respectively. Positive predictive value, negative predictive value and accuracy were 94.3%, 82.5% and 85.5% respectively. SIS had a sensitivity and specificity 66% and 97.7% respectively, Positive predictive value, negative predictive value and accuracy of 94.3%, 83.3% and 86.1% respectively, which was in accordance with several studies, one showing sensitivity and specificity of 100% and 91.8% respectively,(14) and another study which showed that SIS had a sensitivity and negative predictive value 100% for endometrial polyps.(15)

Finally, in conclusion SIS is comparable to HSG in diagnosis of uterine abnormalities, but with the advantage of lack of ionizing radiation and of lower cost, more feasibility, and outpatient procedure with better tolerability; it can replace HSG as a primary diagnostic test for uterine anomalies prior to DH.

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Value of Oral Contraceptive Pill Pretreatment before GnRH Antagonist Ovarian Stimulation Protocol on The Outcome of IVF/ICSI

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Abstract

Objective: To evaluate the effect of oral contraceptive pill (OCP) pretreatment in gonadotropin-releasing hormone (GnRH) antagonist ovarian stimulation protocol on the outcome of IVF/ICSI regarding number of retrieved oocyte, oocyte maturation rate, fertilization rate, good quality embryo rate, cycle cancellation rate, pregnancy rate and clinical abortion rate.

Subjects & Methods:
A total of 84 patients, in a prospective controlled clinical trial, had ICSI using GnRH antagonist protocol during the period from February 1st, 2009 to September 30th, 2011 were included in this prospective randomized trial. We compared the IVF outcomes between OCP pretreated (n=43) and no pretreatment group (n=41) in gonadotropin-releasing hormone (GnRH) antagonist ovarian stimulation protocol.

Results: The mean duration of ovarian stimulation and mean amount of gonadotropins in OCP pretreated group was significantly higher than that of no pretreatment group (11.7 ± 2.3 vs. 9.8 ± 1.8 days and 2720.4 ± 1165.0 IU vs. 2295.8 ± 1121.1 IU). The mean number of retrieved oocytes and oocyte maturation rate was significantly higher in OCP pretreated group (10.9 ± 5.3 vs. 7.5 ± 5.2 and 90.8% vs. 73.3%). The number of total gained embryos and the good quality embryo rate was also significantly higher in OCP pretreated group (7.9 ± 0.9 vs. 4.2 ± 1.0 and 69.9 % vs. 48.7%). Fertilization rate was also higher in OCP pretreated group (84.9 ± 0.2% vs. 70.5 ± 0.3%). The implantation and pregnancy rate were higher, although not reaching statistically significant level, in OCP pretreated group (11.6% vs. 10.7% and 45.4% vs. 36.2%).

Conclusion: OCP pretreatment before GnRH antagonist protocol for IVF appears to have reliable benefits in terms of IVF outcomes regarding number of retrieved oocytes, oocyte maturation rate, fertilization rate, and good quality embryo rate. But, it also has a weak point in respect to longer stimulation duration and increased gonadotropin consumption. The OCP pretreated cycle in GnRH antagonist protocol is more advantageous, despite not reaching statistical significant level, in respect to pregnancy rate, and cycle cancellation rate. Well controlled, large scaled studies are needed to support effectiveness of OCP pretreatment before starting GnRH antagonist ovarian stimulation protocol for IVF/ICSI.

Keywords: Gonadotropin-releasing hormone antagonist; Oral contraceptive pill pretreatment; Ovarian stimulation; In vitro Fertilization

Introduction

Gonadotropin-releasing hormone (GnRH) antagonists have been widely used after its first introduction in assisted reproductive technologies to prevent a premature luteinization [1]. GnRH antagonist protocols are preferred for poor responders because of shorter duration and use of lower amount of gonadotropins for ovarian stimulation as compared with traditional GnRH long agonist protocols [2]. However, it induces insufficient synchronization of follicular cohort development and lack of flexibility in the starting day of ovarian stimulation, which is less likely in GnRH agonist long protocols [3]. For getting over these limitations, several pretreatments have been applied [4-7]. Among them, oral contraceptive (OCP) pretreatment has been reported to induce higher numbers of oocytes retrieved compared to no pretreatment group in GnRH antagonist cycles [8].

The effect of this intervention on the probability of pregnancy has so far been examined only in a small randomized controlled trial (RCT) [4]. However, prior to adopting a modification in an already established protocol of treatment such as the daily GnRH antagonist protocol [5], its effect on the probability of pregnancy needs to be evaluated.

In this study, we aimed to evaluate the effect of OCP pretreatment in in vitro fertilization (IVF) cycles using GnRH antagonists by comparison between OCP-pretreated and non-treated groups.

Subjects & Methods

A total of 84 patients indicated for ICSI using GnRH antagonist protocol during the period from February 1st, 2009 to September 30th, 2011 were included in this prospective randomized trial. Patients were prospectively selected from our IVF center

Inclusion criteria were: age <39 years; ≤3 previous assisted reproduction (ART) attempts; body mass index (BMI) of 18–29 kg/m2; regular menstrual cycles; no polycystic ovaries according to Rotterdam definition; no endometriosis > stage II; basal hormonal levels of FSH (<10 IU/l) and LH (<10 IU/l) at initiation of stimulation for the non-OCP group and at initiation of OCP in the OCP group; and no previous poor response to ovarian stimulation. Poor ovarian response was characterized either by cancellation of the cycle due to poor follicular development after at least 10 days of gonadotropin stimulation, or by retrieval of less than five cumulus–oocyte– complexes (COCs) at oocyte retrieval.

The study population were divided into 43 patients used OCP pretreatment in previous menstrual cycle before starting GnRH antagonist protocol for IVF (OCP pretreated group), and 41 patients used no medication before GnRH antagonist protocol for IVF (no pretreatment group). Randomization was done using computer-generated program and all IVF cycles carried out in our center

In OCP pretreated group, daily OCP (Yasmin®, Bayer Schering Pharma AG, Berlin, Germany) was applied from the first day of previous menstrual cycle. after OCP discontinuation, the ovarian stimulation was done with gonadotropin from the second day of menstrual cycle as usual as extensively described. Briefly, the dose of gonadotropins was determined on an individual basis according to the age, Day 3 FSH value and echographic characteristics of the ovaries. Patients underwent serial transvaginal ultrasound starting on Day 6 of ovarian hyperstimulation. The patients underwent pituitary downregulation with daily GnRH antagonist (Cetrotide®, Merck-Serono, Geneva, Switzerland) from mid or late follicular period of this cycle applied when dominant follicle reached to 12 or 13 mm. When two or more follicles reached 16 - 18 mm in diameter 10,000 IU of hCG (Choriomon, IBSA, Lugano3, Suisse) was administered. Trans-vaginal ultrasound guided oocyte pick-up (OPU) was performed 34-36 hours later and then, maturity and quality of retrieved oocytes was evaluated. Embryo transfer was performed 48–72 h after the oocyte collection.

The IVF outcomes such as retrieved oocyte number, oocyte maturation rate, fertilization rate, good quality embryo rate, cycle cancellation rate, pregnancy rate and clinical abortion rate were compared between OCP pretreated and no pretreatment group. Clinical pregnancy was defined as the ultrasonographic demonstration of an intrauterine gestational sac 4 weeks after embryo transfer.

Statistical analysis was performed using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA). Each variable was presented as mean ± standard deviation. Student's t -test and Chi-square test were used wherever appropriate. P -value of < 0.05 was considered statistically significant.

Results

The mean age and body mass index in OCP pretreated and non-pretreated groups were similar (34.6 ± 3.1 vs. 35.1 ± 3.5 years and 22.3 ± 2.6 kg/m2 vs. 21.7 ± 1.9 kg/m2). The basal follicle stimulating hormone (FSH) level (10.2 ± 3.7 IU/mL vs. 9.1 ± 2.9 IU/mL) was also similar between two groups. The primary infertility rate tended to be higher in non-pretreated group (66.1% vs. 55.4%) but not statistically significant. The mean duration of infertility was longer in control group (5.1 ± 2.7 years vs. 4.7 ± 2.3 years) but, also, not statistically significant (Table 1).

	OCP pretreated group (n = 43)	No pretreatment group (n = 41)	P - value
Age of female (yr)	34.6 ± 3.1	35.1 ± 3.5	NS
BMI (kg/m2)	22.3 ± 2.6	21.7 ± 1.9	NS
Duration of infertility (yr)	4.7 ± 2.3	5.1 ± 2.7	NS
Primary infertility (%)	55.4	66.1	NS
Secondary infertility (%)	44.6	33.9	NS
Basal serum FSH (mIU/mL)	10.2 ± 3.7	9.1 ± 2.9	NS

Table 1: Comparison of baseline characteristics in both study groups

	OCP pretreated group (n = 43)	No pretreatment group (n = 41)	P - value
Duration of COH (day)	11.7 ± 2.3	9.8 ± 1.8	0.0001
Dosage of gonadotropin (IU)	2720.4 ± 1165.0	2295.8 ± 1121.1	0.054
E2 on hCG day (pg/mL)	1170.8 ± 1267.3	1086.0 ± 877.5	NS
EM thickness on hCG day (mm)	10.4 ± 2.6	10.0 ± 2.4	NS
Number of retrieved oocyte	10.9 ± 5.3	7.5 ± 5.2	0.053
Number of matured oocyte	9.9 ± 4.3	5.5 ± 4.1	0.052
Oocyte maturation rate (%)	90.8	73.3	0.052
Number of total gained embryo	7.9 ± 0.9	4.2 ± 1.0	0.053
Good quality embryo rate (%)	69.9	48.7	0.054
Number of transferred embryo	2.6 ± 0.9	2.3 ± 1.0	NS
Fertilization rate (%)	84.9 ± 0.2	70.5 ± 0.3	0.017
Implantation rate (%)	11.6	10.7	NS
Pregnancy rate/embryo transfer (%)	45.4	36.2	0.304
Clinical abortion rate (%)	41.7	42.1	NS
Cycle cancellation rate (%)	13.5	17.5	NS

Table 2: Comparison of IVF/ICSI outcomes in both study groups The mean duration of ovarian stimulation in OCP pretreated group was significantly longer than that of no pretreatment group (11.7 ± 2.3 vs. 9.8 ± 1.8 days). Mean amount of gonadotropins for controlled ovarian stimulation in OCP pretreated group was higher than that of control group (2720.4 ± 1165.0 IU vs. 2295.8 ± 1121.1 IU). The mean number of retrieved oocytes and oocyte maturation rate was significantly higher in OCP pretreated group than that of no pretreatment group (10.9 ± 5.3 vs. 7.5 ± 5.2 and 90.8% vs. 73.3%). The number of total gained embryos and the good quality embryo rate was also significantly higher in OCP pretreated group than that of control group (7.9 ± 0.9 vs. 4.2 ± 1.0 and 69.9 % vs. 48.7%). Fertilization rate was also higher in OCP pretreated group (84.9 ± 0.2% vs. 70.5 ± 0.3%). The implantation and pregnancy rate were higher, although not reaching statistically significant level, in OCP pretreated group (11.6% vs. 10.7% and 45.4% vs. 36.2%). The clinical abortion rate also showed no significant difference between two groups. The cycle cancellation rate tended to be lower in OCP pretreated group than OCP non-treated group but not statistically different.

Discussion

The present study was scheduled to evaluate the effect of oral contraceptive pill (OCP) pretreatment in gonadotropin-releasing hormone (GnRH) antagonist ovarian stimulation protocols on the outcome of IVF/ICSI regarding number of retrieved oocytes, oocyte maturation rate, fertilization rate, good quality embryo rate, cycle cancellation rate, pregnancy rate and clinical abortion rate.

The retrieval of good quality oocyte is a very important factor to achieve pregnancy in infertile women undergoing IVF/ICSI. To gain good quality embryo, growth of finely matured oocyte is firstly needed. To get more matured oocytes, the synchronized growing of follicles is one of important factor. During COH, most of the early antral follicles are required to grow coordinately in response to exogenous gonadotropins thus accomplishing simultaneous functional and morphological maturation [10].

Marked discrepancies of follicular size at the end of COH may be counterproductive since they imply that a substantial fraction of FSH-sensitive follicles fail to undergo satisfactory maturation. This phenomenon potentially reduces the number of viable oocytes and embryos and the probability of conception. Selection of good embryos for transfer depends on embryo cohort size: implications for the `mild ovarian stimulation' debate [10]. The number of embryos available for transfer predicts successful pregnancy outcome, especially in older women with normal ovarian hormonal reserve testing [11]. Low maturation rate of oocytes in GnRH antagonist cycles was thought to be due to older ages of patients receiving the antagonist protocol, or asynchronous follicular development and a limited number of dominant follicles due to ovarian stimulation without pituitary suppression in GnRH antagonist protocols.

Asynchronous multi-follicular growth during COH may be a direct consequence of size heterogeneities of early antral follicles during the early follicular phase [12]. Luteal estradiol administration strengthens the relationship between day 3 FSH and inhibin B levels and ovarian follicular status [13]. Some follicles are able to respond to lower FSH levels than others by their intrinsic sensitivity to FSH, and start their development during the late luteal phase [14]. Since larger follicles are more FSH responsive than are smaller follicles, exogenous gonadotropin administration is likely to intensify further size discrepancies of growing follicles during COH [15]. Follicular development begins during the luteal phase of the human menstrual cycle. Hence, COH protocols such as midluteal long protocol, suppression of luteal FSH secretion could prevent untimely and uncoordinated development of FSH sensitive follicles during the luteal-follicular transition and faster follicular growth synchronization during COH can be obtained [16].

However, this luteal suppression of FSH cannot be achieved in GnRH antagonist COH protocols. Therefore, marked follicular size discrepancies would be occurred in GnRH antagonist COH cycles.

OCP pretreatment might exert a suppressive effect on the cohort of existing follicles. Fanchin et al. (2003) showed that luteal E2 administration synchronizes the follicular cohort and is associated with more follicles and oocytes retrieved [13]. As demonstrated by Van Heusden et al. (1999), OCP is able to suppress the luteofollicular transition and the endogenous FSH rise occurs 3 days after OCP withdrawal [17]. The same effect is described by De Ziegler et al. (1998) after E2 withdrawal [18]. In another study, OCP pretreatment in GnRH antagonist cycles in low responders, also, resulted in improving ovarian response by intrinsic gonadotropins before COH [19].

In the present study, the baseline characteristics of IVF cycles between both groups were comparable. In OCP pretreated group it presented improvement of fertilization rate and gained more number of fertilized embryo than that of OCP non-treated group even if longer duration and larger used dose of gonadotropin for ovarian stimulation.

According to previous meta-analysis regarding OCP pretreatment in GnRH antagonist cycles [20], OCP pretreatment was associated with an increased gonadotropin consumption and increased duration of stimulation without improvement of ongoing pregnancy rate. There were many other studies which concerned to OCP pretreatment and IVF outcomes in GnRH antagonist cycles. Among them, Kolibianakis et al. [21], reported that OCP pretreated GnRH antagonist COH cycles have no significant benefit in ongoing pregnancy rates and moreover results in a significantly higher early pregnancy loss of compared to non-OCP cycles. In another systemic review and meta-analysis analyzed by Griesinger et al. [22], OCP pretreatment in GnRH antagonist for COH have no significant benefit in increasing ongoing pregnancy rates. A recent study focused on compromised group like as low responders [23]. The study showed higher number of retrieved and matured oocytes, and fertilized oocytes in OCP pretreatment group in low responders which was defined as elevated basal FSH level (>8.5 mIU/mL), and/or antral follicle count <5. In the present study, the number of gained embryo and oocyte fertilization rate were higher in cycles of OCP pretreatment.

In conclusion, OCP pretreatment before GnRH antagonist protocol for IVF appears to have reliable benefits in terms of IVF outcomes regarding number of retrieved oocytes, oocyte maturation rate, fertilization rate, and good quality embryo rate. But, it also has a weak point in respect to longer stimulation duration and increased gonadotropin consumption. The OCP pretreated cycle in GnRH antagonist protocol is more advantageous, despite not reaching statistical significant level, in respect to pregnancy rate, and cycle cancellation rate. Well controlled, large scaled studies are needed to support effectiveness of OCP pretreatment before starting GnRH antagonist ovarian stimulation protocol for IVF/ICSI.

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News & Views

Live Operative Workshops In Gynecologic Endoscopy: Issues And Problems

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During the last quarter of the 20th century, and especially during the last decade, there has been a paradigm shift in the methods for performance surgery. For many procedures, the "invasiveness" involved has been dramatically reduced resulting in superior outcomes manifested as improved survival, fewer complications, and quicker return to functional health and productive life. However, the highly technical nature of gynecologic endosurgery and the rapid rate with which new instruments are introduced, require a high intensity of surgical training. One way to accomplish this issue is the implementation of "live operative workshops".

The popular program in any surgical meeting especially in the field of gynecologic endoscopy is the live operative workshop. Live workshops allow the audience to observe surgical anatomy and technique. They provide an opportunity for interaction and an excellent platform to learn newer surgical skills. Advancements in video technology have made it possible to transmit good quality images and this is even truer for minimally invasive endoscopic gynecologic procedures. It is therefore not surprising that these workshops play an important role in surgical education. It has become easier to demonstrate new procedures, display newer instruments and popularize recent treatments. Many hospitals and surgeons are increasingly using this medium as an advertisement to build a reputation.

Transcontinental video demonstrations are possible and one need not go across seven seas to observe a new surgical technique. But in this enthusiasm, are we truely compromise patient safety, privacy and patient human rights? Is a true consent available? Is there an element of subtle coercion? Do the patients get honest and unbiased information? And finally, who will be responsible for complications which may arise out of these procedures, especially when they are performed by "master surgeons" who come from foreign countries?. It is equally distressing to see patients from the underdevloped world, being subjected to "experimental procedures". It is not uncommon to see surgeons from the developed world to try out a new surgical technique before performing it in their own institution and on their countrywomen. Is it not unethical to allow our population to be used as guinea pigs?.

I am not questioning the usefulness of live workshops but I believe we should all be concerned about these issues. It is important to remember that the American College of Surgeons and the American College of Obstetricians and Gynecologists have banned live procedures during their meetings.

It is true that unless we take adequate steps to council our patients adequately, ensure their safety and protect their privacy, we risk unethical censure. The patients should have an absolute indication for the procedure and no compromization should be permissible just to accommodate the "impatient surgeon and an industry driven procedure". The faculty should impress upon the enthusiastic audience that the workshop serve a limited purpose, there being no substitute for in-service training. Self-propagation, financial gain and advertisement should not be the motive behind these workshops. As a policy, we must provide guidelines and device a mechanism which can audit all workshops. It is imperative that the "concept of see one, do one and teach one" be discarded.

We do believe that the degree of training and experience strongly correlate with complication rates and the success of gynoendosurgery is very much dependent on the surgeon's skills. However, future training programs must find a way to include participation in a substantial number of surgical procedures which is essential for acquiring proper technical skills and for gaining adequate knowledge of patient selection, preparation for surgery, and postoperative care. Perhaps the use of recent computer programs, graphics, animations, and recent multimedia virtual systems will facilitate the development of virtual simulator trainers to enhance the ability to learn and master new complex endoscopic operations.

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