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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 esteemed colleagues,

Now that this new edition is between your hands, I sincerely hope that all the changes we have made to produce a more refined publication have been successful. Your feedback is more than welcome, and all your remarks will be taken into consideration for future updating and promotion of this respected journal.

These are very crucial, important and exciting times. We are approaching the presidential elections maybe for the first time in our lives, so, before nominating your president to- be I urge you all to review the programs of each candidate carefully in regards to the health issues, to make sure that our pressing needs for deprived patients and doctors alike are being addressed as they should be.

The activities of our society are moving at a very fast pace despite the current turmoil in the political and security departments. A meeting was held in Mahallah and another one was held in Damietta.

The annual meeting will be held within a few months and I sincerely hope to see you all there. Please make sure that all the papers and researches that you would like to submit arrive in good timing before the event.
Last but not least our prayers for our beloved country to reach the prosperity and peace it deserves.
Thank you.

Mohamed Yehia
Professor of Obstetrics and Gynecology
Ain Shams University

The role of adjuvant therapy to optimize the outcome in poor ovarian responders undergoing IVF/ICSI

Alaa wageah, MSc
Mohamed ElGharib, MD
Ezz Din Osman, MD
Iqbal Abul Hashem.Dh
Ahmed Badawy, MD FRCOG PhD
Department of Obstetrics & Gynecology,
Mansoura University

Does aspirin have a role?

The effect of adjuvant low-dose aspirin on utero-ovarian blood flow and ovarian responsiveness in poor responders undergoing IVF was evaluated and it was concluded that supplementation with low-dose aspirin failed to improve either ovarian or uterine blood flow or ovarian responsiveness in poor responders (1).

Is pretreatment with COC or progesterons worthwhile?

It was postulated that COC administration aims to suppress endogenous gonadotrophins preventing salvage of the corpus luteum from the previous cycle or a rise in progesterone with initiation of the follicular phase microdose GnRH-a and, at the same time (through its estrogen component), generate and sensitize more estrogen receptors (2).
A few RCTs have shown that COC pretreatment may be worthy for ovarian response and clinical pregnancy rates, but these data were obtained from a patient cohort which excluded poor responders (3,4). Lindheim et al. (1996) showed that COC administration prior to the GnRHa protocol was associated with higher pregnancy rates and lower cancellation rates (5). However, Duvan et al. (2008), concluded that COC pretreatment plus microdose GnRHa flare-up protocol does not offer advantages over non COC microdose GnRHa flare-up protocol among poor responder ICSI patients (6). Bendikson et al. (2006) in a retrospective study found that pregnancy outcome in GnRH antagonist protocol with and without COC were comparable (7).

Adjunctive use of Growth Hormone (GH)

The hypothesis that GH stimulates ovarian steroidogenesis, follicular development and enhances the ovarian response to FSH was proposed in 1986 (8). This action of GH is believed to be mediated via the IGF-1 that acts in synergy with FSH, amplifying its effects on granulosa cells (9). These were the theoretical basis for the introduction of GH or GH-releasing factor (GHRF) in the IVF treatment of poor responders.

Initial results in small groups of poor responders were optimistic reporting higher number of oocytes collected and improved pregnancy rates (10-14). In a RCT, similar number of oocytes, embryos and pregnancies has been reported but improvement of delivery and live birth rates after ovarian co-stimulation with GH has been noticed in 50 women older than 40 years old who have undergone ICSI (15). Kucuk et al. (2008) studied the efficacy of GnRHa long protocol with and without GH co-stimulation in poor responders and found higher fertilization rate in the group co-stimulated with GH. However, the clinical pregnancy rate was not significantly increased (16).

Kotarba et al. (2002), in a Cochrane Review, conducted a meta-analysis of the trials assessing the effectiveness of GH adjuvant therapy in poor responders, and showed no significant difference in either the number of follicles and oocytes, or gonadotrophin usage (17). In another Cochrane review, a significant improvement in live birth rate in poor responders was found with GH adjuvant therapy despite no effect in normal responders has been noticed (18). Kyrou et al. (2009) in their systematic review evaluating GH addition in poor responders stimulated for IVF based on five RCTs suggested that live birth rates are improved when GH is coadministered during ovarian stimulation for IVF in poor responders (19). Recently, Venetis et al. (2010), in their metaanalysis found that addition of GH significantly increased probability of live birth and clinical pregnancy in poor responders (20).

Addition of GH-releasing factor (GHRF)

No statistically significant difference in live birth rates was observed between patients who did or did not receive GHRF (21).

Addition of pyridostigmine

Pyridostigmine is an acetyl cholinesterase inhibitor which by enhancing the action of acetylcholine can increase GH secretion. Chung-Hoon et al. (1999) used pyridostigmine (120 mg/day orally from the day of down-regulation until the day of HCG), The results showed significant higher number of oocytes collected and improved pregnancy rates despite being statistically insignificant (22).

Adjunctive use of nitric oxide (NO)-donor (L-arginine)

Increased vascularization appears to play a critical role in the selection, growth and maturation of follicles in both natural and IVF cycles. L-Arginine, acting as a NO-donor, is a potential vasodilator. In fact, NO is derived in vivo from L-arginine by a NO-synthetase enzyme (23,24). It is also thought that NO is involved in follicular maturation and selection, possibly due to its participation in periovulatory vasodilatation (25,26). Battaglia et al., 1999, in a prospective randomized study, in which two groups of poor responders were compared, each of which was treated with the GnRHa flare-up regimen and only one group orally administered L-arginine (27). Higher numbers of collected oocytes and higher pregnancy rate were found in the L-arginine group, although the increase in pregnancy rate was not statistically significant.

Adjunctive use of glucocorticosteroids (dexamethasone)

It has been suggested that dexamethasone may affect follicular development and oocyte maturation either directly via its isoform (11bHSD) in the granulosa cells or indirectly, by increasing serum GH and consequently intrafollicular IGF-1. In addition, it may provoke immunosuppression within the endometrial microenvironment (28-30).

To our knowledge, no studies have been reported involving poor responders. In one double-blind, placebo-controlled prospective randomized study in 290 cycles of normal responders (aged <41 years), dexamethasone was administered at 1 mg/day in the long luteal protocol until the day prior to oocyte retrieval and a significantly lower cancellation rate was found (31). These findings are encouraging, as they reveal a very low incidence of poor response with the use of corticosteroids; however, the data are limited and can only be considered as preliminary.

What is the role of androgen?

It has been suggested that androgens play a role on follicular growth. Androgen receptors have been identified in the human ovary (32). The addition of androgen during the early follicular phase may have a beneficial effect on the number of small antral follicles as well as on the ovarian sensitivity to FSH. Dehydroepiandrosterone (DHEA) has been used 2 months prior to ovarian stimulation in patients who previously had a poor response with promising results (33,34).

In a study by Balasch et al. (2006) who investigated the usefulness of testosterone pretreatment in poor responders via transdermal application, it was found that this may be a useful approach for patients known to be poor responders with normal basal FSH concentrations (35). Wiser et al. (2010) evaluated the effect of DHEA supplementation on IVF data and outcomes among 33 poor-responder patients and they concluded that DHEA supplementation can have a beneficial effect on ovarian reserves for poor-responder patients on IVF treatment (36).

A RCT comparing transdermal application of testosterone preceding standard gonadotrophin ovarian stimulation to high-dose gonadotrophin in association with a minidose GnRHa protocol in poor responders concluded that pretreatment with transdermal testosterone may improve the ovarian sensitivity to FSH and follicular response to gonadotrophin treatment in previous low-responder IVF patients. This approach leads to an increased follicular response compared with a high-dose gonadotrophin and minidose GnRHa protocol (37). In contrast, another study reported that live birth/delivery rates are not improved with the addition of transdermal testosterone (38). The two above mentioned studies done by Massin et al. (2006); and Fábregues et al. (2009) were reanalyzed by Venetis et al. (2010), using a stratified analysis, clinical pregnancy rates did not differ significantly between the testosterone pretreatment group and the placebo group (20, 37).

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Is the clomifene citrate/human menopausal gonadotropin protocol cost effective in IVF/ICSI treatment?

Ahmed Gibreel MD MRCOG,
Naser Ellkanny MD,
Hamed Yousef MD,
Maged Raghib MD,
Ahmed Ragab MD,
Ahmed Badawy MD FRCOG PhD
Department of Obstetrics & Gynecology,
Mansoura University Hospitals,
Mansoura, Egypt

Abstract

Objective: The objective of this trial was to evaluate the cost/effectiveness of Clomifene Citrate/Human Menopausal Gonadotropin in comparison to GnRH agonist long protocol/HMG-HCG in treatment of IVF cycles in infertile couples.
Materials & Methods: This study prospective, controlled trial comprised a total of 88 couples undergoing IVF/ICSI treatment. Patients were divided into 2 groups: 37 couples who could not afford the cost of medications for the long protocol were recruited in group A and stimulated by the CC/HMG protocol. 51 women were recruited in group to receive the GnRH agonist long protocol. The primary outcome measure was clinical pregnancy rate per woman. The secondary outcomes were the total amount of gonadotropins, number of oocytes retrieved, and cycle cancellation rate.

Results: Seventy three women (82%) had reached embryo transfer. Only seventeen women out of the eighty eight women (19%) ended with clinical pregnancy. A total of fifteen cycles were cancelled. The cost of the cycle in the GnRHa/HMG group was significantly higher than cost of the cycle in the clomifene/HMG by 1460 EP (95% CI 1300-1600, p=0.01). The cost of pregnancy in the GnRHa/HMG group was significantly higher than the cost per pregnancy in the clomifene/HMG by 17496 EP (95% CI 16600-18400, p=0.01).

Conclusions: Clomifene/HMG protocol was associated with significantly lower pregnancy and higher cycle cancellation rate compared to the conventional long agonist protocol.
Key words: clomifene citrate, human menopausal gonadotropin, cost effective, IVF/ICSI

Introduction

Clomifene citrate was the first drug to be used for ovarian stimulation in preparation for IVF (1). It was used initially alone (1), then with gonadotropins (2,3). The high rates of cycle cancellation, due to premature LH surges, as well as the deleterious antioestrogenic effect of clomifene on the endometrium were the two main disadvantages of clomifene citrate stimulated IVF protocols (4,5). Later on, the use of clomifene citrate in IVF has been widely disfavoured after the introduction of gonadotropin releasing hormones (GnRH) agonist in IVF practices (6). However, the use of clomifene citrate in IVF was revived again following calls for milder stimulation protocols in IVF (7).

It has been estimated that the cost of medications in IVF represents approximately 50% of the total cost of IVF cycle (8). In countries where IVF treatment is only self-funded, the high cost of the long agonist protocol may hamper some patients from utilizing IVF service (9). The absence of pituitary suppressing drugs as well as the significant reduction in the number of gonadotropins ampoules in the clomiphene citrate (CC)/human menopausal gonadotropin (HMG) protocol, as reported in almost all relevant randomized trials (10-12), may point to some potential economic benefits from that protocol. Hence, we decided to investigate the genuine cost effectiveness of the CC/HMG protocol versus a long-acting GnRH agonist/HMG protocol.

Materials & methods

From December 2006 to December 2010, infertile couples who attended Mansoura Fertility Care Unit (MFCU), seeking ICSI treatment, were approached and asked to participate into the study. Our inclusion criteria included women < 39 years old undergoing their first ICSI cycle. Women with history of previous IVF/ICSI attempt(s) or with azospermic partner were excluded from the study. A total of 88 couples were included. All patients signed an informed written consent. The study was approved by the University Ethics Committee.

Women were assigned to either one of two groups. Thirty seven couples who could not afford the cost of medications for the long protocol were recruited in group A. Those women were stimulated by the CC/HMG protocol; they received CC (Clomid; Merrell Dow SA, Neuilly sur-Seine, France) 100mg daily for 5 days from day 2 of the cycle. From days 7, 150 IU/day of HMG (Merional; IBSA, Geneva, Switzerland) was administered intramuscularly (I.M). From day 7 onwards, daily vaginal ultrasound (using a 7-MHz

Correspondence
Prof. Ahmed Badawy, MD FRCOG PhD
Department of OB/GYN
Mansoura University, Mansoura, Egypt
Tel.: 0020502337070
Fax: 0020502303939
E mail: ambadawy@yahoo.com
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transducer; Medison 5220; Seol, South Korea) and twice daily urinary LH monitoring (Clearplan; Unipath Limited, Bedford, United Kingdom) were performed. Ten thousand units of human chorionic gonadotropin (HCG) (Pregnyl; NY Organon, Oss, The Netherlands) were given I.M. when two or more follicles reached 18mm in mean diameter.

Fifty one women were recruited in group B in whom they received the GnRH agonist long protocol which is the standard controlled ovarian hyperstimulation (COH) protocol in our unit. In brief, Decapeptyl 0.1mg/day was started on day 20 of the cycle until the day of HCG injection. After down regulation was confirmed (by serum E2 and transvaginal ultrasound), 150-225 IU of HMG/day was started for 7days, then the dose was adjusted according to the response.. From day 7, the dose was adjusted according to the follicular response. HCG (Pregnyl1; NY Organon, Oss, The Netherlands), 10 000 IU, was given when at least two follicles had reached 18 mm. Oocyte retrieval was performed 34–36 h after HCG injection, under ultrasound guidance transvaginally using single lumen needle (Labotect Labor-Technik-Göttingen GmbH, Germany). ICSI was performed by the standard technique. Embryo transfer was performed on two or three days following egg retrieval. All patients received luteal phase support with 200 mg of micronized progesterone (Utrogestan; Piette, Brussels, Belgium) daily per vaginum starting from the day of oocyte retrieval. Clinical pregnancy was defined as a visible fetal heart beat on ultrasonography. The primary outcome measure was clinical pregnancy rate per woman. The secondary outcomes were the total amount of gonadotropins used for stimulation, number of oocytes retrieved, multiple pregnancy rate, and cycle cancellation rate and severe ovarian hyperstimulation (OHSS) rate. Clinical pregnancy was confirmed when at least one fetal pole with a detectable cardiac beat could be identified in a gestational sac five weeks after embryo transfer. All cycles ended without embryo transfer, prior to oocyte retrieval for poor response or after retrieval for fertilization failure, were counted within the cancelled cycles. Calculation of the drug costs for HMG were based on the price of the HMG (Merional, IBSA, Switzerland), HCG (Pregnyl, Organon, The Netherlands), Clomifene (Clomid) in Egypt obtained from the Egyptian Ministry of Health (i.e. retail cost). Besides, a fixed fees of 2000 Egyptian Pounds paid by the couples to the Mansoura University Hospitals, Egypt.

Statistical analysis:

All statistical analysis was performed using Statistical Package for Social Science programme version 16 (SPSS). Univariate analysis was conducted to compare variables between the two groups; women on the clomifene/HMG and women on the GnRHa/HMG. Multivariate analysis was conducted to evaluate the association between the outcome (clinical pregnancy) and those factors that potentially influence the outcome. Parametric and non-parametric tests were used to compare groups depending on data whether normally distributed or not. The student “t” test, Mann-Whitney test and Chi-square tests were used whenever appropriate. All tests were two tailed with the statistical significance described at 5% significance level. Mean and standard deviation were used to describe continuous normally distributed data while the median with the range were used to describe the data when non-parametric tests were used. Numbers and percentages were used to describe nominal data. To evaluate the direction and magnitude of differences for continuous outcome measures, we deployed the correlation analysis using the Pearson or Spearman correlation coefficient, for normally distributed and skewed data, respectively. The logistic regression analysis, the forward stepwise conditional method, was deployed to calculate the odds ratio for clinical pregnancy for women on the clomifene/HMG compared to women on the GnRHa/HMG long protocol, before and after adjusting for other significant variables.

Results

A total of 88 women were recruited in this study. Seventy three women 73/88 (82%) reached embryo transfer step. Seventeen women out of the eighty eight women (19%) ended with clinical pregnancy. A total of fifteen cycles were cancelled; 5 prior to oocyte retrieval for poor follicular development and 8 for absence of embryos for transfer due to fertilization failure or cleavage arrest. Thirty seven women were stimulated by the CC/HMG protocol while fifty one women were stimulated by the long GnRH agonist/HMG protocol. The demographic and stimulation characteristics of patients in the two groups are shown in Table I. There were no differences in age, body weight causes of infertility or serum baseline FSH levels between the two groups. Data regarding the outcomes are shown in table 2. The total dose of gonadotropins used (in international units (IU), the number of oocytes retrieved, the number of embryos available, the number of embryos transferred, clinical pregnancy and cancellation rates were significantly different between the two protocols (table 2 and table 3).

Correlation Analyses demonstrated that the number of oocytes retrieved positively correlated with the number of gonadotropins ampoules used (r=0.322, P<0.001). There was a significant negative correlation between the number of oocytes retrieved and body mass index (BMI) (r=-0.159, P<0.04) as well as the duration of subfertility (r=-0.226, P=0.003). Logistic regression was deployed to calculate the odds of clinical pregnancy using the long agonist protocol compared to the CC/HMG protocol. The clinical pregnancy rate was significantly higher with long GnRHa/HMG protocol than with the Clomifene/HMG protocol (unadjusted Odds Ratio (OR) = 4.28, 95%CI 1.13-16.23). however, the pregnancy rate was found not to be significantly different between the two groups after adjustment for the total dose of gonadotropins used and the number of embryos transferred (OR =1.62; 95% CI= 0.31-8.36).

The mean cost of the cycle (Mean±SD) in the clomifene/HMG group was 2600±200 Egyptian Pounds (EP) while it was 4100±400 EP in the GnRHa/HMG group. The cost of the cycle in the GnRHa/HMG group was significantly higher than cost of the cycle in the clomifene/HMG by 1460 EP (95% CI 1300-1600, p=0.01). The mean cost per pregnancy in the clomifene/HMG group was 32400±2600 EP while it was 14900±1600 EP in the GnRHa/HMG group. The cost of pregnancy in the GnRHa/HMG group was significantly higher than the cost per pregnancy in the clomifene/HMG by 17496 EP (95% CI 16600-18400, p=0.01).

Discussion

This prospective non-randomized trial showed a significant reduction in clinical pregnancy rate in the clomifene/HMG stimulated IVF patients compared to GnRHa/HMG long agonist stimulated patients. Two randomised studies have shown the same results (10,11). Our results are in discordance with the results from some other randomised studies that showed no differences in pregnancy rates (13-18).

Our results have also demonstrated a significant increase in cycle cancellation rate within the clomifene/HMG group compared the long agonist group. These findings were in agreement with similar findings from four randomized studies (10,12,13,16). This is contrary to reports of comparable cancellation rates between the two protocols in few other randomized trials (14,17,18). There is no general consensus on when to cancel an IVF cycle prior to retrieval. Some clinicians relied on the number of 16 or 18 mm size follicles while others might rely on biochemical measures, as serum E2 level or LH level, either independently or twined to ultrasonographic criteria. This lack of consensus on the pre-retrieval criteria for cancellation may explain the inconsistency of these reports.

In agreement with almost all randomized and non-randomized trials in the literature (10,11,13,14,17). There were significant increase in the number of oocytes and embryos available for transfer with the GnRHa/HMG protocol compared to the clomifene/HMG protocol. It had been suggested that this increase in the availability of embryos, despite of the policy of small number of embryos transferred, may provide good chance for selection of embryos for transfer and cryopreservation of the surplus ones (10). The added benefit of cryopreserved embryos on the cumulative live birth rate in the long agonist protocol, compared to the CC/HMG protocol, has never been investigated.

In this study, there was a significant difference between the numbers of embryos transferred in each group. Logistic regression showed that the difference in pregnancy rates has no longer been significant after control for the number of embryos transferred. Similarly, Tummon et al. (1992) found pregnancy rate not to differ between the two protocols after controlling for the number of embryo transferred (16). The limited availability of embryos for transfer and cryopreservation after the clomifene/HMG protocol compared to the long agonist protocol had been reported by many other authors (11,12,19).

The implantation rate was not statistically different between the two protocols in our study which came in agreement with other relevant studies (12,18,20). Embryo implantation depends mainly on embryo quality and endometrial receptivity. Clomifene citrate may yield better quality embryos (21). However, clomifene citrate may also have a deleterious antioestrogenic effect of on the whole reproductive system, including the endometrium receptivity (22-25). It is not clear yet which one of these opposing factors could dominate the other and under which circumstances. This controversy and uncertainty may point to the importance of using live birth as the most important outcome in infertility studies (26). Based on the charges collected by our unit from infertile couples and costs paid by the couples for the medications in the Egyptian market, our study showed that the mean cost of a cycle with the clomifene/HMG protocol was significantly lesser than the mean cost per cycle with the long agonist protocol, however, the cost per pregnancy was three times higher than that with the standard long agonist protocol. We acknowledge the fact that a proper cost analysis should be based on calculating direct, indirect and downstream costs (27).

We are aware of the limitations in our study as our study was not a randomized study. Well-designed randomized trials are meant to provide the highest grade of evidence in absence of a systematic review and meta-analysis of randomized trials. Moreover, our study was underpowered. The small sample size of the population may make it prone to a type one (alpha) statistical error where a significant result would be detected while it is not genuinely existed.

To conclude, the clomifene/HMG protocol was associated with significantly lower pregnancy and higher cycle cancellation rate compared to the conventional long agonist protocol. The limited availability of embryos with this protocol might be responsible for the difference in pregnancy rates between the two protocols. Although the cost of per cycle was significantly reduced with the cc/HMG compared to the long protocol, the total cost per pregnancy with the former was significantly higher than with the later. More studies are warranted to measure more precisely the differences between the two protocols.

Disclosure

There is nothing to disclose for any of the authors

Legend to tables:

Table (1): Demographic characteristics for all recruited women

Variable		(N=88)
Mean age (years) ±SD		30.9 ± 3.3
Mean BMI ±SD		26.7 ± 2.2
Mean duration of sub-fertility (months) ±SD		72.7 ± 24.6
Mean basal FSH (IU/ml) ±SD		7.6 ± 1.3
Type of sub-fertility **Number of primary infertility (%)**		74/88 (84%)
Number of secondary infertility (%)		14/88 (16%)
Aetiology of sub-fertility	**Male**	23/88 (26%)
Number (%)	**Tubal**	25/88 (28%)
	Unexplained	22/88 (25%)
	Endometriosis	10/88 (11%)
	PCOS (%)	8/88 (10%)

Table (2): Comparison between CC/HMG and GnRH agonist/HMG protocols as regard cycle outcomes

	CC+HMG (N=37)	Long agonist N (51)	P value
Clinical Pregnancy Rate	3/37 (8%)	14/51(27%)	0.04*
Multiple pregnancy rate	0/3(0%)	4/14(28%)	0.57
Severe OHSS rate	0/37(0%)	2/51(4%)	0.52
Cancellation rate	11/37(29.7%)	4/51(7.8%)	0.01*
Implantation rate	3/44(7%)	18/139(13%)	0.07
Median number of oocytes retrieved [IQR]	4[2-5]	8[5-10]	< 001*
Median number of embryos available [IQR]	2[0-2]	3[2-6]	< 001*
Median number of embryos transferred [IQR]	1[1-2]	3[2-3]	< 001*

Table (3): Comparison between CC/HMG and GnRH agonist/HMG protocols as regard patient characteristics and stimulation characteristics

Parameter	CC+HMG (N=37)	Long agonist N (51)	P value
Mean age (years) ±SD	30 .7±3.2	31±3.4	0.62
Mean BMI ±SD	26.7 ±2	26.6 ±2.3	0.83
Mean duration of sub-fertility (years) ±SD	6.6 ±2.3	7.2 ±2.7	0.10
Mean basal FSH (IU/ml) ±SD	7.8 ±1.2	7.4±1.4	0.23
Type of sub-fertility {Number (%)}	Primary 33 (89%)	41(80%)	0.62
	Secondary4 (11%)	10(20%)	

Aetiology of sub-fertility Number (%)	Male	11(30%)	12(23%)	0.37
	Tubal	11(30%)	14(28%)	
	Unex-plained	9 (24%)	13(25%)	
	Endome-triosis	2(5%)	8(16%)	
	PCOS	4(10%)	4(8%)	
Mean duration of stimulation (days) ±SD		10.2± 0.9	10.5±1.1	0.18
Median amount of gonadotro-pins used in IU [IQR]		900 [750-1200]	2250 [1575-3750]	< 001*

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Clinical outcome of day 5 and day 6 blastocyst vitrification

Eman A. Elgindy, M.D.
Department of Obstetrics and Gynecology,
Zagazig School of Medicine,
Zagazig University, Egypt

Abstract

Objective:
to compare survival, clinical pregnancy and ongoing pregnancy rates of blastocysts vitrified on day 5 and those which had one day delay and vitrified on day 6.

Materials & Methods:
The study included 210 vitrified warmed cycles, 135 patients underwent vitrifivation at day 5 (group I) and 75 patients at day 6 (group II). Blastocyst survival and clinical pregnancy/embryo transfer were primary outcomes. Ongoing pregnancy/embryo transfer was the secondary outcome.

Result(s)
blastocyst post-warming survival rates were comparable between both groups (92.9% (263/283) of day 5 versus 94.9% (166/175) of day 6 blastocysts). There was no statistically significant difference between the 2 groups regarding the mean number of transferred blastocysts. Clinical pregnancy rates were 40.6% (52/128) & 43.7% (31/71) in women who undergone vitrification at day 5and day 6 respectively with no significant differences. Similarly, ongoing pregnancy rate was comparable between the 2 groups, 37.5% (48/128) versus 39.4% (28/71) in groups I & II.

Conclusion(s):
blastocysts vitrified on day 5 have the same survival, clinical and ongoing pregnancy rates of blastocysts which had one day delay and vitrified on day 6.

Key words:
Blastocyst transfer, clinical pregnancy, vitrification.

Introduction

Cryopreservation has become an increasingly important therapeutic strategy in reproductive medicine, with the birth of many infants after use of this procedure. It is important for cryopreservation in general to establish consistent outcomes, especially in terms of embryo cryosurvival to allow high chances of success in performing a frozen embryo transfer (FET). However, standard cryopreservation technologies appear to illustrate their ultimate limitations in their lack of consistency in cryo-survival. Actually, interest has shifted to vitrification as an attractive alternative to slow-freezing methodology (1) and vitrification is now the preferred method of cryopreservation in many centers (2, 3).

It is as an ultra-rapid cooling technique that is simple, potentially faster, starting to become clinically established and seems to have the potential to be more reliable and consistent than conventional cryopreservation when carried out properly (4, 5). Further, the need for controlled-rate freezing equipment, which requires routine calibration and maintenance, is eliminated. The cells are placed into the cryoprotectant, then the cells are placed in a very small volume of cryoprotectant on a special carrier, and then they are cooled at extreme rates by plunging them directly into LN2. With this method, no ice crystals form with avoidance of damage to the cells or the tissues. Actually, Lack of ice crystallization and convenience of the procedure itself are two major advantages which changed entire cryopreservation program of many centers from conventional freezing to vitrification only (2, 3).

With the introduction of sequential culture media in ART, and driven by the large increase in the rate of multiple pregnancies arising from earlier-stage ET, extended culture to the blastocyst stage has become more common. The best available evidence suggests that the probability of pregnancy, implantation and live birth rates after fresh IVF is significantly higher after blastocyst-stage embryo transfer as compared to cleavage-stage embryo transfer (6, 7). However, possibility of some embryos not developing into blastocysts in vitro and as a result cancellation of embryo transfer should be considered. So, blastocyst transfer policy should be applied in good prognosis patients (6, 7). With this concept, many centers have shifted to blastocyst transfer. Consequently, the need to cryopreserve human blastocysts is also increasing. Although the results achieved by conventional slow freezing seem successful (8-10), clinical results with blastocyst cryopreservation have not necessarily been consistent, owing to the higher potential for damaging ice crystal formation in traditional slow-freezing protocols. So, there have been an increasing number of reports of successful human blastocyst vitrification (11-15).

Generally, if there is failure in achieving pregnancy after initial transfer of fresh blastocysts, surplus vitrified blastocysts would be transferred in a subsequent cycle.

Moreover, there have been suggestions that, fresh BT cycles might be canceled for patients who have exhibited poor endometrial receptivity or ovarian hyperstimulation syndrome. Under such circumstances, all available fresh blastocysts would be vitrified for transfer in a subsequent cycle (16). Importantly, previous investigators have found superior implantation rates with fresh transfers occurring at day 5 as compared with day 6. They reported an almost doubled clinical pregnancy and implantation for fresh day 5 blastocyst compared with fresh day 6 blastocysts (17). The one-day delay in expansion was considered in itself an indication of inferior viability.

A pertinent question is whether extra blastocysts which were vitrified on day 5 or the ones which had required 6 days to reach expanded blastocyst and vitrified on day 6 have the same or different embryonic developmental potential upon warming. So, the objective of the current study is to compare survival, clinical pregnancy and ongoing pregnancy rates of blastocysts vitrified on day 5 and those which had one day delay and vitrified on day 6.

Materials & Methods

From October 2007 to November 2010, 210 vitrified-warmed BET cycles were evaluated. 135 women had undergone blastocyst vitrification on day 5 and 75 on day 6. All patients included used standard long protocol for controlled ovarian stimulation (COS) and underwent ICSI. In our program, women who have ≥ 4 grade one embryos (i.e. regular symmetrical blastomeres with no fragmentation) on day 3 after retrieval (18) are counseled for extended culture and BET. Ovarian stimulation was performed as previously reported (19).

Embryo Scoring

Embryos reaching the blastocyst stage, whether on day 5 or day 6, were graded by using the system of Gardner and Schoolcraft (20). Blastocysts were given a number based on the degree of expansion and hatching status (from 1 to 6): 1 = early blastocyst: the blastocele accounts for less than one-half of the volume of the embryo; 2 = blastocyst: the blastocoel occupies more than one-half of the volume of the embryo; 3 = full blastocyst: the blastocoel fills the embryo completely; 4 = expanded blastocyst: the blastocoel is now larger than the early embryo, and the zona pellucida has begun to thin; 5 = hatching blastocyst: trophectoderm (TE) cells have begun to herniate through the zona pellucida; and 6 = hatched blastocyst: the blastocyst has completely escaped the zona pellucida. For blastocysts regarded to be full blastocysts and onward (grades 3–6), a second scoring step was performed under an inverted microscope to assess the inner cell mass (ICM) and the TE. For the ICM, the following descriptions are used: A = tightly packed with many cells; B = loosely grouped with several cells; and C = very few cells. For the TE, the following grading is used: A = many cells forming a cohesive epithelium; B = few cells forming a loose epithelium; and C = very few large cells. Extra blastocysts were only considered for vitrification if they were regarded to be full blastocysts and onward (grades 3–6), Inner cell mass (ICM) scored A-B and trophoectoderm (TE) scored A-B.

Protocol for Vitrification and Warming

Vitrification of blastocysts was undertaken using the Cryoloop carrier system (Vitrolife, Sweeden) after a two-step loading with cryoprotectant agents at 24°C. Briefly, blastocysts were placed in equilibration solution, which is the base medium (HEPES-buffered solution with 20% serum supplement; Irvine Scientific, USA) containing 7.5% Ethylene glycol (EG) and 7.5% DMSO. After 8–13 minutes, the blastocysts were washed quickly in vitrification solution, which is the base medium containing 15% DMSO, 15% EG, and 0.5 mol/L sucrose. These 2 solutions were to be used in sequence according to the step-wise microdrop vitrification protocol. Importantly, blastocysts were exposed to the vitrification solution ≤30 seconds. From last microdrop, 1-3 blastocysts in < 1uL media was loaded into the Cryoloop carrier, capped under the LN2 with the cryovial immerged in the LN2 till final storage.

Patients not achieving a clinical pregnancy returned for a frozen blastocyst transfer cycle. All women received letrozole (Femara, Novartis), one tablet (2.5 mg)/day, starting from day 3 of the cycle for 5 days. When dominant follicle reached ≥18mm and endometrium thickness≥8mm, 10000 IU of HCG were given (day 0). Vaginal administration of progesterone (cyclogest, Florham Park, NJ) was initiated on day HCG+3 (usually 4 days before the frozen blastocyst transfer was scheduled).

On day of vitrified blastocyst transfer, to remove the cryoprotectants, blastocysts were warmed and diluted in a two-step process. With the Cryoloop submerged in LN2, the protective cap was removed and placed directly into a pre-warmed (approximately 30°C) organ culture dish containing thawing solution (HEPES buffered solution containing gentamycin sulphate, 1.0 mol/L sucrose and 20% serum supplement).After 1 minute, blastocysts were transferred to dilution solution (HEPES buffered solution containing gentamycin sulphate, 0.5 mol/L sucrose and 20% serum supplement) for 4 minutes. Then, blastocysts were transferred to the washing solution (HEPES buffered solution containing gentamycin sulphate and 20% serum supplement) for 9 minutes and then returned to the culture medium (Sage Blastocyst Medium) until transfer. Whether vitrification was performed on day 5 or day 6, one to three blastocysts were transferred into the patient's uterus on day HCG+7.

Serum β-hCG tests were performed two weeks after ET and transvaginal ultrasound (US) were scheduled three weeks later to confirm a clinical pregnancy. Spontaneous abortion was defined as the spontaneous loss of a clinical pregnancy before 20 completed weeks of gestational age (21). Clinical pregnancy rate was defined as the number of clinical pregnancies expressed per 100 embryo transfer cycles (21). On-going pregnancy rate was defined as the number of clinical pregnancies, continuing beyond 20 weeks of gestation and expressed per 100 initiated embryo transfer cycles.

Outcome measures

Blastocyst survival and clinical pregnancy/embryo transfer were primary outcomes. Ongoing pregnancy/embryo transfer was the secondary outcome.

Data were statistically described in terms of mean ± standard deviation (SD), frequencies (number of cases) and relative frequencies (percentages) when appropriate. Analysis was carried out by means of a X2 test using computer programs Excel version 7 (Microsoft Corporation, NY, USA). Statistical significance was defined as $P<0.05$.

Results

The study included 210 vitrified warmed cycles, 135 patients underwent vitrification at day 5 (group I) and 75 patients at day 6 (group II).Table 1 shows the mean age and clinical outcome of patients who completed the vitrified blastocyst transfer program. No significant differences could be observed regarding age in the two groups. Of 135 women who had vitrification at day 5, 128 women underwent warmed BET (94.8%, 128/135). Meanwhile, 71 of the 75 women who had vitrification at day 6 had undergone warmed BET (94.7%, 71/75) with no significant differences between the 2 groups. Regarding the blastocyst post-warming survival rates, 92.9% (263/283) of day 5 blastocysts and 94.9% (166/175) of day 6 blastocysts survived after warming and this difference was not significant. 260 blastocysts were transferred in first group, while 145 blastocysts were transferred in second group with no statistically significant difference between the 2 groups regarding the mean number of transferred blastocysts. Clinical pregnancy rate was 40.6% (52/128) in women who undergone vitrification at day 5 and was 43.7% (31/71) among those who had vitrification at day 6 with no significant differences. Similarly, ongoing pregnancy rate was comparable between the 2 groups, 37.5% (48/128) versus 39.4% (28/71) respectively in groups I & II.

Discussion

Data from the present study suggest that, blastocysts which had shown one day delay and vitrified on day 6, results in similar survival, clinical and ongoing pregnancy rates when transferred in subsequent cycles compared to transfer of blastocysts vitrified on day 5.

Previous studies have demonstrated that fresh embryos reaching the blastocyst stage and transferred on day 5 had a significantly higher pregnancy rate than those blastocyst embryos transferred on day 6 (17). We recently performed a study (submitted for publication) upon174 patients who had undergone BET on day 5 and 22 participants who did not have expanded blastocysts on day 5 and were left for one day, and all developed expanded blastocysts and had undergone BET on day 6. Blastocysts transferred on day 5 implanted at nearly twice the rate of blastocysts transferred on day 6 (40% vs. 19%, $P < 0.05$). Pregnancy rates were also almost twice as high in day 5BET {106/174 (60.9%)} than those undergoing day 6BET {7/22(31.8%)}. Similarly, ongoing pregnancy/live-birth rates were also higher in first group{91/174(52.3%)} than in those undergoing day 6BETgroup{6/22(27.3%)} Actually, Shapiro et al. present provocative retrospective data suggesting that synchrony of embryo and endometrial development may be an important factor in pregnancy rates following blastocyst transfer (17).

So, the transfer of blastocysts which had shown one day delay in expansion and transferred on day 6 might result in embryo-endometrial dyssynchrony. Moreover, it might be suggested that, the more slowly developing blastocysts could be innately compromised to some extent. Importantly, Embryos that were vitrified on day 6 were required to be expanded blastocysts and, before they were transferred, must have survived the warming process. These requirements may have selected better-quality embryos than day 6 blastocysts transferred in the fresh cycle. It appears that, there is profound clinical value in knowing they can be vitrified as late as day 6, successfully warmed and result in ongoing pregnancy. Additionally, it is plausible that a more synchronous transfer of these warmed blastocysts contributed to the good outcome.

In accordance with current study findings, Richter et al, suggested that blastocysts cryopreserved on day 6 resulted in similar pregnancy rates when transferred to artificially prepared endometrium in cryopreserved cycles or in donor egg cycles, compared to transfer of blastocysts cryopreserved on day 5 (22). So, with the reported high clinical and ongoing pregnancy rates following vitrified-warmed transfer of day 6 blastocysts, it might be a good policy to encourage vitrification of supernumerary embryos reaching the blastocyst stage beyond day 5. In the meantime, this study should stimulate further investigation in this field in the ongoing quest to improve outcomes from in vitro fertilization and ICSI.

There are other issues with vitrification that need further discussion. Concerns about introduction of high concentrations of cryoprotectant, which are necessary to prevent mechanical damage from ice, exist with vitrification. The problem of cryoprotectant toxicity is an immediate and practical one, just as it is to a lesser extent in classic slow- cooling procedures. Extremely rapid cooling allows a decrease to be made in the concentration of the cryoprotectant and thereby a reduction in potential toxicity (23).

The greatest advantages of vitrification have been seen in chill-sensitive cells such as oocytes and blastocysts (24). The main characteristic of the blastocyst is its fluid-filled cavity, the blastocele. It has been reported that, with increasing volume of the blastocelic cavity, the survival rate drops with vitrification. This is thought to be due to insufficient permeation of cryoprotectant into the blastocelic cavity, such that residual water may promote ice crystallization during the vitrification process. Several articles report that survival rates in cryopreserved expanded blastocysts could be improved by artificial reduction of the blastocelic cavity (12-14, 25-26). In our protocol and others (5, 16), we proceeded without any opening in the zona pellucida before vitrification independent of the size of the blastocelic cavity. The previous concern appears theoretical rather than practical and proceeding without blastocele collapse spares extra-procedure with a comparable survival and PR (5, 16).

Another concern has been made that fungi, bacteria, and viruses are able to survive in LN2 (27-29). Given that with vitrification the cells are directly plunged into LN2, they therefore have direct contact with LN2 and so the question arises as to whether the LN2 has to be sterilized because it may be a possible source of contamination. Use of clean LN2 for the initial vitrification step, followed by sealing of the carrier, seems to address the concern of potential contamination during cryostorage. To further reduce fears of contamination, it is possible to store material from potentially infectious patients separately from seemingly noninfectious samples. Therefore, it is important to perform routine screening tests for viral infections, including hepatitis B and C, on all couples undergoing infertility treatment. In the event that a couple screens positive, we offer vitrification of blastocysts. Even though we consider the risk of cross contamination during storage to be almost infinitesimal, in such cases we nevertheless recommend placing embryos in specially designated tanks, or shipping them off-site. It is worth noting that to date no viral, fungal, or bacterial contamination event has been described from many publications related to vitrification since 1985.

So, concerns about vitrification are well defined, limited in number, and easily surmountable. In general, with much shorter protocols, vitrification [1] is able to be undertaken on a more flexible basis by laboratory staff, [2] allows for the potential reduction in personnel time needed during the entire vitrification process, [3] simplifies laboratory techniques for cryopreservation in human ART, and [4] may enable more optimal timing of embryo cryopreservation, e.g., individual blastocysts may be cryopreserved at their optimal stage of development and expansion. Interest levels will inevitably rise, given the potential benefits of vitrification. This in turn will drive its development to higher levels of clinical efficiency and utilization (1, 31-32).

In conclusion, blastocysts vitrified on day 5 have the same survival, clinical and ongoing pregnancy rates of blastocysts which had one day delay and vitrified on day 6.

Table I: Vitrification/warming data of blastocysts vitrified on day 5 and day 6.

	Day 5(n=135)	Day 6(n=75)	P
Age (years)	32 ± 3.1	31.8 ± 3.9	0.84
Transfer cycles	128 (94.8%)	71 (94.7%)	1
Survived blastocysts	263/283 (92.9%)	166/175 (94.9%)	0.41
Blastocysts transfer	2.03 ± 0.5	2.04 ± 0.4	0.88
CP/ET	52(40.6%)	31(43.7%)	0.67
OP/ET	48(37.5)	28(39.4%)	0.78

CP=clinical pregnancy, ET=embryo transfer, OP= ongoing pregnancy. Data presented as mean ± SD unless otherwise specified. P > 0.05 non-significant

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A Prospective, Randomized, Comparative Clinical Study of the efficacy of Letrozole and Clomiphene Citrate as adjuvants to Follicle-Stimulating hormone in Superovulation

Zakaria F. Sanad MD, Osama A. El.Kelani MD
Obstetrics and Gynecology Department , Menofiya University, Egypt

Abstract

Objective: To compare the efficacy of the aromatase inhibitor letrozole and clomiphene citrate (CC) as adjuvants to follicle-stimulating hormone (FSH) in superovulation.

Materials & Methods: A total of 120 patients with unexplained or mild male factor infertility were randomized to receive either letrozole or CC as adjuvants to FSH. From day 3 to 7 of the cycle 2.5 mg/d letrozole or 100 mg/d CC were administered followed by 75 IU/d FSH starting on day 7 until the day of human chorionic gonadotropin (hCG). Ovulation was triggered with 10,000 IU of hCG when the leading follicle (s) reached 18 mm in diameter. A single intrauterine insemination (IUI) was performed 36 h later. Ovarian (estradiol (E2) levels and number of follicles) and endometrial (endometrial thickness) response and pregnancy outcome were the main measures.

Results: The number of mature preovulatory follicles (1.9 + 0.7 vs. 2.2 + 0.5, P<0.0001) and the peak E2 level (791 + 163 vs. 1137 + 192 pg/ml, P<0.0001) were significantly higher in the CC group than in the letrozole group. A significantly higher endometrial thickness was observed on the day of hCG in the letrozole group (9.1 + 1.2 vs. 7.7 + 1.4 mm, P<0.0001). There was no significant difference between groups in clinical pregnancy rates (38.4% in the letrozole and 31.3% in the CC groups).

Conclusions: The aromatase inhibitor letrozole appears to be a good alternative to CC in superovulation therapy.

Key Words: Aromatase inhibitor, letrozole, clomiphene citrate, FSH, superovulation.

Introduction

Typically, patients with unexplained or mild male factor infertility are offered superovulation combined with intrauterine insemination (IUI) as first-line therapy to enhance fecundity(1-3).

The effectiveness of clomiphene citrate (CC), a non-steroidal agent that has both estrogenic and antiestrogenic effects, in inducing ovulation is well established(4). In anovulatory women, the use of CC is widely accepted as the first-line therapy because of its low cost and easy administration(5). Its use is associated with a high ovulation rate of 60% - 80%, but with a lower pregnancy rate of about 50%(4,5). However, several disadvantages have been described, including potential negative effects on the cervical mucus(5,6), uterine blood flow(7), the endometrium(8), embryo development(9), and overall pregnancy outcome(4,5,10).

Gonadotropin preparations, either urinary or recombinant, have been used to stimulate ovulation in women who are resistant to CC as well as to stimulate the production of several mature follicles in conjunction with assisted reproduction technologies(11). Unfortunately, multiple gestation, ovarian hyperstimulation syndrome (OHSS), inconvenience, and higher treatment cost are drawbacks of these compounds(12).

Recently, it has been suggested that a new group of highly selective aromatase inhibitors, including letrozole; that suppress estrogen biosynthesis may successfully substitute for CC in superovulation regimens(13-15). Aromatase inhibitors have a reduced half-life (about 2 days) compared with CC (>2 weeks) which exerts a central estrogen-depletion effect of long duration(16). Letrozole increases endogenous gonadotropin secretion, but unlike CC, it does not lead to estrogen receptor (ER) depletion(13,14). It could therefore decrease the requirement for gonadotropins without adverse effects on peripheral tissues such as the endometrium(17,18).

The objective of the present study was to compare the efficacies of the aromatase inhibitor letrozole and CC as adjuvants to gonadotropin stimulation in superovulation combined with IUI therapy in couples with unexplained or mild male factor infertility.

Correspondence
Dr\ Osama Elkelani, Faculty of medicine, Menofiya university, Shebin Elkom, Menofiya Governorate, Egypt
T: +2 010 00 41 723
Fax: +2 048 22 33 521
E.mail: oelkilani@yahoo.com

Material and methods

This prospective randomized clinical study included couples with unexplained or mild male factor infertility treated between June 2002 and May 2005 in the Department of Obstetrics and Gynecology, Menofiya University and its outpatient clinic. All patients underwent standard infertility investigations including early follicular phase transvaginal ultrasonography (TV/US) and measurement of serum FSH, TSH, and prolactin, confirmation of tubal patency by hysterosalpingography and pelvis normality by laparoscopy and semen analysis(19).

Inclusion criteria were female age <35y, >1 year infertility, patent fallopian tubes, a normal uterine cavity, a basal day 3 serum FSH level <12 mIU/mL, and the presence of at least 10 million rapidly motile sperm/mL (mild male factor). The local Ethics Committee approved the study. Patients were counseled individually about the study and protocols by a resident coordinator. Patients who elected to participate gave their written informed consent before participation.

All couples underwent a maximum of 3 cycles of treatment. Patients were randomized using a computer-generated random table into 2 groups:

1. The letrozole group (59 patients, 147 cycles) who received 2.5 mg/day letrozole (Femara, Novartis) from days 3 to 7 of the cycle.
2. The CC group (61 patients, 159 cycles) who received 100mg/day CC (Clomid, Serono) from days 3 to 7 of the cycle. All physicians were blinded to allocation.

Protocol

After either letrozole or CC, all patients received 75 IU/day FSH (Fostimon, IBSA) starting on day 7 (sequential manner) until the day of hCG administration. All patients underwent baseline TV/US in the early follicular phase to confirm absence of ovarian cysts which were defined as any sonolucent structure with a mean diameter > 15 mm(20).

Subsequent US scans were performed on day 9 of the cycle and then daily after the mean diameter of the largest follicle reached 16 mm. At each US scan, the internal diameter of each visible follicle was measured in two planes and the average diameter was calculated. In addition, the endometrial thickness, defined as the maximum distance between the echogenic interfaces of the endometrial-myometrial junctions, was measured in the plane through the central longitudinal axis of the uterus(21). Ovulation was triggered with 10,000 IU of hCG (Choriomon, IBSA) when the leading follicle(s) reached 18 mm in diameter. Serum estradiol (E2) levels were measured on day 3 and the day of hCG administration.

A single IUI was performed 36 h after hCG administration by using a Labotect ET catheter (Labotect GmbH, Göttingen). Semen has been processed by wash/centrifugation in Earle's salt solution (Biochrom AG, Berlin) and suspensions of motile spermatozoa were prepared in a final volume of 0.5 mL. The luteal phase was supplemented with vaginal micronized progesterone (400mg/day, Prontogest pessaries, IBSA). Serum β-hCG was measured 14 days later. A pregnancy was established by visualization of a gestational sac by 6 weeks gestation using TV/US.

Statistical Analysis

For statistical analysis, a commercially available statistical package SPSS version 13 (SPSS, Chicago, IL) was used. We evaluated the total number and size of the follicles, endometrial thickness and type, the number of gonadotropin ampoules and the dose, mean E2 level, pregnancy rate (chemical and clinical), and miscarriage rate. When the assumption of normality was met, mean differences between the two groups were analysed using a Student's t-test. To evaluate differences between proportions (e.g., pregnancy rates) a X2 test was used. Results are expressed as mean + SD unless otherwise indicated. P values below 0.05 were considered as statistically significant

Results

A total of 120 infertile couples (59 in letrozole and 61 in CC groups) completed the treatment cycles (147 in letrozole and 159 in CC groups). Demographic characteristics showed no significant differences between groups (Table 1). The number of follicles >10 mm on cycle day 9 and the number of follicles >16 mm on day of hCG were significantly higher in the CC group than in the letrozole group (Table 2). The total dose of FSH and the peak E2 level (on the day of hCG) were significantly higher in the CC group than in the letrozole group. The day of hCG was significantly earlier and, the endometrial thickness was significantly higher in the letrozole group than in the CC group (Table 2). There was no significant difference between groups in pregnancy rates (38.4% in the letrozole and 31.3% in the CC groups). In contrast, the abortion rate was significantly higher in the CC group than in the letrozole group (Table 2).

Table (1): Demographic characteristics of patients treated with letrozole + FSH and CC + FSH

Variable	Letrozole + FSH (n=59)	CC + FSH (n=61)	P-Value
Age (ys)	29.7 + 3.5	28.5 + 3.7	0.14+
Weight (Kg)	67.5 + 5.2	68.2 + 6.1	0.58+
Height (m)	1.64 + 0.3	1.61 + 0.4	0.71+
BMI (Kg/m2)	26.4 + 3.1	27.3 + 4.1	0.27+
Duration of infertility (ys)	3.6 + 0.6	3.4 + 0.7	0.18+
Day 3 FSH (mIU/mL)	6.8 + 1.7	7.2 + 1.5	0.27+
Day 3 E2 (pg/mL)	51.3 + 3.9	49.7 + 4.2	0.08+
Unexplained infertility (n)	48	52	0.63+
Mild male factor infertility (n)	11	9	

+ = Not significant BMI= Body mass index

Table (2): Ovarian-endometrial response and pregnancy outcome

Variable	Letrozole + FSH (n=147)	CC + FSH (n=159)	P-Value
No. of follicles >10 mm on cycle day 9	2.7 + 1.2	3.1 + 1.4	0.008**
No. of follicles >16 mm on day of hCG	1.9 + 0.7	2.2 + 0.5	<0.0001**
Total dose of FSH (IU)	357 + 51	371 + 46	0.01*
Peak E2 on day of hCG (pg/mL)	791 + 163	1137 + 192	<0.0001**
Day of hCG	11.8 + 0.9	12.2 + 0.7	<0.0001**
Endometrial thickness on day of hCG (mm)	9.1 + 1.2	7.7 + 1.4	<0.0001**
Chemical pregnancy rate per cycle	29/147 (19.7%)	27/159 (17%)	0.56+
Chemical pregnancy rate per couple	29/59 (49.2%)	27/61 (44.3%)	0.7+
Clinical pregnancy rate per cycle	23/147 (15.6%)	19/159 (11.9%)	0.4+
Cumulative clinical pregnancy rate per couple	23/59 (38.4%)	19/61 (31.3%)	0.44+
Abortion rate	3/23 (13%)	8/19 (42.1%)	0.04*
Multiple pregnancy	1/23 (4%)	3/19 (15.8%)	0.3+

+ = Not significant *** = Significant** ****= Highly significant**

Discussion

Superovulation combined with IUI has been used to enhance fecundity for couples with unexplained or mild male factor infertility(1-3). Because of its antiestrogenic action, the use of CC has been associated with adverse effects mainly on the quality of the cervical mucus and on the endometrial development(22). Higher doses and prolonged use of CC may also aggravate these effects(23,24). Nevertheless, gonadotropin therapy is associated with a significant cost, inconvenience, and discomfort to the patient due to the need for injections, higher risk for multiple pregnancy and increased risk of OHSS(25). Recently, aromatase inhibitors have been used in ovarian stimulation protocols(13,14,26). By blocking the conversion of androgens to estrogens (E), they eliminate rapidly the circulating E2, thus releasing the hypothalamic/pituitary axis from negative feedback. As a result, there is an increase in the production and release of FSH, which is readily available to stimulate follicular growth. Furthermore, acute E withdrawal leads to increased peripheral activin production which further stimulates pituitary FSH production and secretion(27). Androgen accumulation within the follicular microenvironment up-regulates FSH receptor expression(28) as well as ovarian insulin-like growth factor 1 (IGF-1) levels, both of which act synergistically to augment FSH action and promote follicular development(29,30).

In our study, the mean number of mature follicles (>16mm) on the day of hCG was significantly higher in the CC group than in the letrozole group. This is in agreement with Mitwally and associates (2005)(18) who found CC treatment to be consistently associated with development of more ovarian follicles than with letrozole and Fatemi et al.(31) in their pilot study which demonstrated lower E levels and fewer follicles in the letrozole group. In contrast, Barroso et al. (2006)(32) found similar number of mature follicles on day of hCG in CC and letrozole groups.

The total dose as well as the cost of FSH required was significantly higher and the day of hCG was significantly later in the CC group than in the letrozole group. In a prospective nonrandomized study, Mitwally and Casper(17) compared letrozole +FSH, CC+FSH, and FSH alone in women with unexplained infertility undergoing superovulation and IUI. The authors concluded that similar to CC, letrozole reduced the required FSH dose for superovulation without the undesirable antiestrogenic effects sometimes observed with CC. The same authors compared letrozole +FSH and FSH alone and demonstrated improved ovarian response in the combination group as evidenced by a reduced dose of gonadotropins as well as a higher number of mature follicles(14).

The estrogen levels in women on aromatase inhibitors were found to be 2-3 times lower than those reported in CC cycles, however, endometrial thickness was greater in the aromatase inhibitor cycles(33). In our study, despite significantly lower E2 levels in the letrozole group, endometrial thickness was significantly higher in the letrozole group than in the CC group. This is in accord with the findings of Fisher et al.(34) who compared the effects of CC and letrozole on normal ovulatory women and the findings of Mitwally and Casper(17) who compared letrozole +FSH, CC + FSH, and FSH alone in women with unexplained infertility. Other investigators reported similar endometrial thickness in women treated with letrozole or CC(35).

In numerous studies, the endometrial thickness after ovarian stimulation has been correlated to the chance of conception(36,37). Most investigators agree that an endometrial thickness of at least 6 mm is necessary for successful implantation(36). In contrast to CC, aromatase inhibitors do not bind to ERs, therefore their use is not associated with ER depletion. Furthermore, it has been shown that elimination of E2 from the circulation leads to up-regulation of ERs in the endometrium(38). It has been speculated that E deprivation during the use of aromatase inhibitors may lead to a subsequent increase in endometrial sensitivity to E, which accelerates the endometrial proliferation and development and improves blood flow(39). In support to this notion, Cortinez et al.(40) concluded that letrozole induced high midluteal progesterone, leading to both a normal endometrial histology and development of pinopodes, considered to be relevant markers of endometrial receptivity.

We couldn't detect any significant difference in pregnancy rate per cycle as well as in the cumulative pregnancy rate per couple between letrozole and CC groups. This is consistent with the findings of Barroso et al.(32) and Al-Fozan et al.(35). However, the miscarriage rate in our study was significantly higher in the CC group than in the letrozole group. This is in accord with the findings of several investigators(35,41) and may have been due to the different mechanisms of action of letrozole and CC(35). We postulate that this might be due to the relatively short half-life of letrozole, which allowed complete endometrial recovery before implantation.

In conclusion, the present study provided further evidence for beneficial effects of the use of the aromatase inhibitor letrozole in combination with FSH in couples with unexplained and mild male factor infertility undergoing superovulation/IUI therapy. Benefits observed in this study were the achievement of a lower number of mature follicles, a lower dose of FSH required, a higher endometrial thickness as well as a similar pregnancy rate and a lower miscarriage rate compared with CC plus FSH. However, further prospective and randomized studies are needed to establish a potential beneficial effect on pregnancy outcome.

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Is immediate post-partum curettage of the endometrium accelerate recovery from Preeclampsia–Eclampsia? Five Years Experience in.... will be started with Does.

A Ragab MD1, M Raghib MD1, A Badawy MD, FRCOG1, R Barakat MD1, A El-Samanoudy2
Department of Obstetrics/ Gynecology, Mansoura University Hospitals, Mansoura, Egypt1
Department of Biochimestry, Mansoura Faculty of Medicine, Mansoura, Egypt2

Abstract

Objectives: to evaluate the effect of immediate postpartum curettage in pre-eclampsia and eclmapsia women, on rapid resolution of clinical and laboratory indices, duration of stay in obstetric intensive care unit, and also morbidities associated with eclampsia.

Materials & Methods: in this prospective randomized case control study, 420 pre-eclamptic or eclamptic women with singleton pregnancy, were diagnosed from 24 weeks onward, involved. These cases were subdivided into two groups, first, (220 cases) underwent immediate postpartum curettage, while the second, (200 cases) comprised the control group, who were not submitted to immediate postpartum curettage.

Results: significant improvement was noted in the mean arterial blood pressure (MAB), urine output, renal and liver functions (creatinine, uric acid, and SGPT, SGOT levels respectively) and also in the platelet count in the study group compared to those in the control group. Average duration of 52.6 + 5.12 hours hours hospital stay in the study group was significantly lower than 78.2 + 3.12 in the control group (P = 0.002). Five percent of women in the study group developed serious complications, mainly eclampsia, renal or liver impairment in the postpartum period in comparison to 24.6% in the control group.

Conclusion: immediate postpartum curettage is a safe and effective procedure and can accelerate recovery from pre-eclampsia or eclampsia, consequently affecting the incidence and severity of post-partum complications.

Key words: eclampsia, curettage, postpartum.

Introduction

Preeclampsia (PE), especially those with early-onset and severe PE, is a leading cause of maternal and perinatal morbidity and mortality [1, 2]. PE affects 5–7% of first pregnancies and recurs in 13–18% of subsequent pregnancies [3–5]. Severe PE, developing remote from term (less than 34 weeks), represents around 25% of all cases of PE and is associated more likely to recur in a subsequent pregnancy [6, 7], and is associated with a higher rate of maternal morbidity than PE developing near term [8]. Approximately 50,000 women die worldwide each year from eclampsia; a severe form of complicating PE [9]. Most of these deaths are from developing countries. The presence of a toxin, that acts as a pressor substance (hysterotonin) in the decidua and amniotic fluid of women has been suggested to be responsible for the multiplicity of clinical expression [10].
To affect a rapid cure, the chorionic villi must be expelled or surgically removed [11, 12]. Resolution of eclampsia occurs only with delivery and subsequent removal of functioning trophoblastic tissue [13]. Accelerated recovery from the disease process following delivery could avert associated serious and life threatening maternal complications and shorten the time required for intensive care stay and hospitalization. The present study evaluates the effect of immediate postpartum curettage on the resolution of clinical and laboratory indices associated with eclampsia.

Patients and methods

The protocol of this study was approved by the local ethical committee in Mansoura Faculty of Medicine, Egypt. All the included group gave informed consent before starting the study. The study included 420 singleton pre-eclamptic – eclamptic women in the period from (April 2007 to April 2011) in Mansoura teaching hospital attending obstetric care and intensive care units with complications of severe PE. All patients were subjected to complete history taking, general, abdominal, local examination (if needed). Laboratory investigations (hematological, renal, hepatic and urine analysis) and fundus examinations. After termination of pregnancy, 220 women were randomly selected and then subjected to immediate post-partum gentle curettage by the largest possible curette (170 Preeclampsia and 50 eclamptic patients). The other group, 200 patients (160 preeclampsia and 40 eclamptic) were not subjected to immediate postpartum gentle curettage (control). Exclusion criteria were any patients with previous cardiovascular, renal,

hepatic diseases, or those with hypertension prior to pregnancy, and patients with a previous history of convulsions whatever the etiology. Close observation of all patients during the post partum period included the clinical parameters (blood pressure measurement, the degree of consciousness), the ICU stay time, the occurrence or the recurrence of fits, hematological, renal and hepatic parameter as well as fundus examinations. The data obtained antenatal and post-natal then analyzed.

Statistical analysis

Obtained data were statistically analysed using Statistics Package for Social Sciences computerized package (SPSS 11 Inc., Zonguldak Karaelmas University, Zonguldak, Turkey) using chi-squared test to compare differences in rates and P-value < 0.05 was considered significant.

Table (1) : gestational age in both groups:

Gestational age (weeks)	Study group (n=220)	Control group (n=200)
24-30	40	50
30-34	70	55
35-37	95	85
38-39	15	10

The mean gestational age 31. 5±3.2 weeks in both the study and control groups

Table (2): Clinical and laboratory parameters on admission and also mode of delivery

Patient characteristics (Clinic, lab. investigations and mode of delivery)	Study group (n=220)	Control group (n=200)
MAB (mmHg)	159 ±3.5	157 ±2.9
Mean systolic blood pressure (mmHg)	169 ±3	165 ±4
Mean diastolic blood pressure (mmHg)	108 ±4	107 ±5
SGOT	68 ±1	66 ±3
SGPT	42 ±3	41 ±2
Serum creatinine	1.3 ±0.2	1.2 ±0.3
Platelet count	120.000 ±25	125.000 ±20
Serum uric acid	5.2 ±2.1	5.1 ±2.5
Patient from rural areas	150	160
Patient from urban areas	70	80
CS	125	130
Vaginal delivery	95	70

There no great difference in the data obtained from both the study “n=220” or the control group “n=200’ even in the mode of delivery

Table (3): post operative evaluation of MAB after 12, then 24 hours

Hours after delivery	Study group	Control	P value
12	110.3	115.7	0.03
24	101.2	110.6	-

Despite MAB is still high in both groups 12 hours after delivery “110.3 and 115.7 in the study and control groups respectively” but it is much decreased in the study group after 24 hour “10.1 compared to 110.6 in the control group”.

Table (4): Post operative fits

Time elapse after operation	Study	Control
24 hours	2	8
After 24 hours	-	3

No fit was encountered after24 hours of delivery in the study group, while encountered in 3 patients of the control group.

Table (5): Liver and renal function after 24 and 48 hours

	Study (24 hours)	Control	Study (48 hours)	Control
SGOT	68 ±3	66 ±1	39 ±2	45 ±4
SGPT	41 ±1	40 ±2	25 ±5	35 ±3
Creatinine	1.2 ±0.2	1.1 ±0.2	0.7	0.9
Uric acid	5.1 ±0.2	5 ±0.2	4.9 ±0.1	5 ±0.1

Liver and renal functions were noticed to be decreased from first 24 hours and markedly after 48 hours in the study than the control group. The hospital stay time before discharge 52.6 + 5.12 hours in the study compared to 78.2 + 3.12 in the control group.

Discussion

A majority of cases came from rural areas (150 in the study and 160 in the control groups nearly 73.8%), this might be explained by ignorance about the importance of ante-natal care as the majority of cases (85.5%) were un-booked. Similar findings have been reported by Chandra et al [14]. Antenatal care plays a significant role in early detection and management of pregnancy induced hypertension and prevention of eclampsia. Mean gestational age in our patients was nearly the same in both groups (31.5±3.2 weeks) and this is nearly similar to that reported by Magann et al [15]. In our study, clinical and lab data “SGOT, SGPT, serum creatinine, uric acid, platelet count were nearly similar in both groups in almost equal magnitude at the time of admission. On the other hand, 24 hours postpartum, the MAP in the study group was significantly (P 0.03) reduced compared to that in the controls. The average time in hours taken for MAP to reach 105 mm Hg or less was 40 ± 3.15 hours in the study group whereas 86 ± 5.34 hours in the controls. Reestablishment of renal functions was noted to be rapid in those of the study group compared to controls (as evident by creatinine level, 1.2 ±0.2 after 24 hours and 0.7 after 48 hours in our studied patients while was 1.1 ±0.2 after 24 hours and 0.9 after 48 hours). Again; an adequate and higher urinary output in postpartum period leads to rapid disappearance of excessive extra-vascular extracellular fluid and edema, and thus to accelerated recovery from the disease process, the notice which was documented in our study group. Also values of liver functions (SGOT and SGPT) in curetted subjects recorded a more rapid reversal to normal compared to those in controls. The difference in liver renal function was obvious from the second day but highly significant at 72 hours, as that found by Chandra et al [14] and also Fejgin and Charles [15]. Meanwhile; Alkan et al [16] observed that uterine curettage performed in the postpartum period had favorable effects on blood pressure, platelet count, and urinary output and also helped in faster recovery from severe preeclampsia but with no difference between the curetted and non curetted groups with regard to liver function values. On the contrary Magann et al [17] found no significant difference at all in liver function and renal function tests at 24 hours postpartum. In our study we have found no significant diferenec in uric acid levels after 24 and 48 hours in both groups, contrary to Witlin et al [18] that reported uric acid levels being more accurately reflect the severity of as well as recovery from pre-eclampsia/eclampsia. Eventually we think that postpartum uterine curettage is useful for patients with severe preeclampsia that require faster recovery. In the present study, follow-up of the patients after the procedure and hospital stay time is much decreased than usual.

Conclusion

Immediate postpartum curettage is a safe and effective procedure which accelerates recovery from preeclampsia and eclampsia, hence averting complications and decreasing the mortality and morbidity associated with this pregnancy related serious complications.

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Single versus double intrauterine insemination (IUI) in women with idiopathic subfertility

Osama M Warda, Maged R Elshamy, Adel S Helal, Hosam Goda
Obstetrics and Gynecology Department, Faculty of Medicine
Mansura University

Abstract

Objective: To compare pregnancy rates per treatment cycle of controlled ovarian hyperstimulation (COH) among patients receiving COH with single IUI with patients receiving two IUIs and those who practice regular sexual intercourse

Materials & Methods: All of the 300 studied women were diagnosed as idiopathic infertility and their husbands were having normal seminogram. They were divided into 3 subgroups each 100 women:G1; received induction of ovulation and advised to have a normal and frequent regular intercourse during the period around ovulation. G2; received induction of ovulation and subjected to IUI after 30 hours of HCG injection which is given in a dose of 10,000 iu intramuscular based on ultrasonic monitoring of folliculogenesis. G3; received induction of ovulation and subjected to intrauterine insemination after 30 hours and 42 hours of HCG injection.

Results: A total of 300 patients were randomized among groups1, 2, 3 (100 women per group) cycles). Data analysis demonstrated no significant differences among 3 groups with respect to age, Body mass index, Mean ovarian volume ,LH ,FSH, PRL ,E2 or Testosterone levels (table -1). However, Our result showed a pregnancy rate 2% in the first group, 10% in the second group and 11% in the 3rd group with statistically significant difference between single and also double IUI and normal frequent intercourse without insemination but no statistical difference between single and double IUI as regards pregnancy rate (table -2)

Conclusion: We concluded that double insemination not beneficial more than single insemination in spite of increasing the number of motile sperm.

Keywords: idiopathic infertility, intrauterine insemination, IUI

Introduction

Intrauterine insemination (IUI) is one of the most commonly performed treatments for infertile or hypofertile couples. Although the technique was first reported by Dickinson in 1921 (1), it was not until the 1980s when IUI started to become popular. Over the past twenty-five years, there has been a substantial amount of research evaluating this method. As in much of infertility, methodological problems preclude clear conclusions. In particular, well-planned randomized controlled trials are rare. However, the data available allow to scientifically treat patients even if the science is not perfect (2).

The success rates of IUI depends on the use or non use of ovarian stimulation , the number of insemination per treatment cycle , different methods of timing ovulation and different sites of insemination (3). Semen preparation offers sperms with progressive motility while seminal fluid and dead sperms are removed. Although there are alternative methods of insemination like intravaginal, intracervical using cap, intratubal or direct intraperitoneal insemination, IUI appears to be the preferred method in most studies (4)

Male factor of infertility affects between 25 -50% of infertile couples. Many couples with male infertility are not absolutely infertile but are subfertile (5). It would be cost effective to start with less invasive and less expensive method before proceeding to more complicated treatment. IUI was shown to be effective in a wide range of sperm abnormalities causing male factor subfertitliy (6).

Intra uterine insemination with or without controlled ovarian hyper stimulation (COH) is one of the treatment modalities offered to couples who have tried to conceive for at least one year . Insemination is less stressful, invasive and expensive than IVF and similar procedures. It is therefore often used when a male partner is subfertile, or when the reason a couple is not becoming pregnant is unknown (7)

Superovulation with intrauterine insemination is a treatment modality used in unexplained infertility and mild male infertility. Increasing the efficiency of the technique has always been an interest of research. Double intrauterine insemination has been suggested to increase efficiency (8)

Osama M. Warda, MD
Assistant professor of OB/GYN
Mansura faculty of medicine
Address: Obstetrics & gynecology dept. Mansoura University hospital- Mansura
E-mail : om2warda@yahoo.com, osamawarda@gmail.com
Mobile: 0106 615 32 12

2012

Materials & Methods

Three hundred consented women attending the outpatient department of fertility care unit of Mansura University Hospital during the period from January 2008 to December 2009
were enrolled in the study. The main complaint is subfertility.

Thorough history taking, clinical examination, abdominal and pelvic ultrasound, HSG and \or laparoscopy for tubal patency as well as routine laboratory investigations (reproductive hormones and thyroid functions) are performed to all the studied women to exclude systematic or metabolic diseases. All patients with endocrinal disease or under hormonal therapy were excluded from the study.

All of the studied women were diagnosed as idiopathic infertility and their husbands were having normal seminogram. They were divided into 3 subgroups each 100 women: First group; received induction of ovulation and advised to have a normal and frequent regular intercourse during the period around ovulation. Second group; received induction of ovulation and subjected to IUI after 30 hours of HCG injection which is given in a dose of 10,000 iu intramuscular based on ultrasonic monitoring of folliculogenesis. Third group; received induction of ovulation and subjected to intrauterine insemination after 30 hours and 42 hours of HCG injection.

Semen Preparation: Semen for insemination, either intrauterine or in vitro, must be prepared to remove seminal plasma products and/or select the healthier population of sperm prior to use. Traditionally, a double wash technique is performed, with or without subsequent swim-up to isolate the motile fraction if necessary.
Semen samples were prepared in human tubal fluid media supplemented with 5% human serum albumin (HSA; location 1) with the double sperm wash (SW) procedure.

The IUI Procedure: The patient placed in lithotomy position. The cervix is exposed and gently wiped with cotton ball soaked in warm sterile saline, and then the insemination catheter is attached to a 1 ml syringe. The sperm sample is loaded into the catheter at a volume of 0.3 – 1 ml then the catheter is gently passed through the cervical canal into the uterine cavity & semen sample is slowly expelled. We avoid touching the fundus of the uterus as it might cause uterine contraction.

Statistical analysis was performed using SPSS statistical computer program. Comparison between two groups was via the use of Pearson uncorrected test. $P < 0.05$ was considered significant .chi – square used for non parametric values.

Discussion

Ovarian stimulation is a key element of different types of subfertility treatment (9). It has been shown that the number of fertilized oocytes achieved directly correlates with the chance of achieving a pregnancy (10). Intrauterine insemination with or without superovulation is the initial step in assisted reproductive technologies (8).

Timing of IUI is an important factor that may affect its success. This is due to the fact that the sperms probably survive for a shorter period after IUI, since they are not deposited in the cervical crypts as in the case of normal coitus. Timing of ovulation by the LH rise is more accurate than the LH peak itself. Insemination is preferred the day after the initiation of LH surge or 36 hours after administration of HCG (10).

Although many studies showed no difference between single versus double insemination (8-10), other studies showed that double insemination may result in a significant increase in pregnancy rates specially in couples with low sperm count or male factor infertility (11).

Our result showed a pregnancy rate 2% in the first group, 10% in the second group and 11% in the 3rd group with statistically significant difference between single and also double IUI and normal frequent intercourse without insemination but no statistical difference between single and double IUI as regards pregnancy rate. Our result in agreement with Contineau et al (9) who stated that double IUI showed no significant benefit over single IUI in the treatment of subfertile couples with husband semen. Also Ransom et al (12) found that no significant difference in pregnancy rates (11 and 14 %) between single and double IUI of partners. But contrarily Deary AJ et al (13) stated that the switch from single to double inseminations has resulted in improved pregnancy outcomes.

We concluded that double insemination not beneficial more than single insemination in spite of increasing the number of motile sperm.

Table 1: Patients' characterisctics of the study groups

	Group1	Group2	Group3	p value
Age in years		28 ± 3	27±4	NS
Height in cm	165 ±4	168 ± 3	166 ± 3	NS
Weight in kg	66 ± 2	65 ± 3	66 ± 3	NS
Body mass index (kg\m2)	24.3± 0.5	23.1 ± 0.8	24.4 ± 0.1	NS
Mean ovarian volume in ml	6.1± 0.2	6.3 ± 0.3	6 ± 0.1	NS
LH (IU\L)	6.5± 0.3	6.7±0.5	6.6 ± 0.1	NS
FSH (IU\L)	5.5 ± 0.2	6 ±0.1	5.8 ± 0.1	NS
PRL (ng\ml)	12 ± 0.8	13 ± 0.1	12.5 ±0. 2	NS
E2 (pgm\ml)	71 ± 6	70 ± 5	70 ± 4	NS
Testosterone	0.5± 0.02	0.5± 0.03	0.4 ± 0.06	NS

Table 2: pregnancy rate per cycle (PRC) in women in the 3 groups

Group 1	Group 2	Group3	P value
2%	10%	-	0.037
2%	-	11%	0.021
-	10%	11%	1

PRC in G1= 2%, in G2= 10%, in G3= 11%

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Can protein Z deficiency predict pregnancy outcome?

H Gouda MD, Raghib M MD
Department of Obstetrics and Gynecology,
Mansoura University Hospitals,
Mansoura, Egypt.

Abstract

Objecive: The objective of this was to determine if preeclampsia (PE), intrauterine growth restriction "IUGR"; or intra-uterine fetal death "IUFD" are associated with changes in maternal plasma concentrations of protein Z.

Methods: This is a prospective case control study which comprised of 130 pregnant women. They were divided into 4 groupd : Severe preeclampsia group (n=40), unexplained IUGR group (n=25), Unexplained IUFD group (n=25) and 40 women with normal pregnancy as a control group. Cross sectional maternal plasma protein Z concentrations were measured by a sensitive and specific immunoassay.

Results: There was a statistical significant difference regarding protein Z between control group and complicated pregnancies (p=0.047). Protein Z levels were significantly lower in severe preeclamptic patients was than that of the other study groups p<0.0001. However, there was no significant difference in the maternal plasma protein Z concentration between patients with unexplained IUGR or intrauterine fetal demise.

Conclusion: Preeclampsia is associated with significantly lower maternal median plasma concentration of protein Z than normal pregnancy and also a high rate of protein Z deficiency was observed in patients with IUGR and fetal demise.

Keywords: preeclampsia, intrauterine growth restriction, fetal demise, protein Z dependent protease inhibitor (ZPI).

Introduction

During pregnancy the alterations in the coagulation system are considered to be adaptive mechanisms for prevention of bleeding at the time of delivery [1-2]. Normal pregnancy is associated with excessive thrombin generation [3] and a tendency for platelets to aggregate [4]. Thrombosis, however, has been proposed as a mechanism of disease in preeclampsia (PE) [5, 6], intrauterine growth restriction (IUGR) [7-8], stillbirth, recurrent pregnancy losses [9], and preterm delivery [10-11]. There is good evidence to support this view including an excessive rate of thrombotic lesions in the placental villi and decidual vessels in patients with these pregnancy complications [12-13]; and higher maternal plasma concentrations of thrombin-anti-thrombin complexes in patients with PE, and small-for-gestational age neonates [14-15].

Protein Z is a single chain vitamin-K-dependent plasma glycoprotein formed in the liver and contributes to the inhibition of activated factor X (FXa), limiting thrombin generation and thus inhibiting coagulation [16]. Protein Z is a co-factor of the protein Z-dependent protease inhibitor (ZPI) [17]. In the absence of protein Z, the activity of ZPI is reduced by more than 1,000-fold, thus, protein Z deficiency has been associated with a procoagulant state [17, 18]. Therefore, protein Z deficiency has been reported with poor pregnancy outcome as it is incriminated in the pathophysiology of early fetal losses between the 8th and 15th weeks' gestation, as well as late pregnancy complications including PE, IUGR, IUFD, preterm delivery (19). Anti-protein Z antibodies have also been proposed as a possible underlying mechanism leading to low plasma PZ concentrations that brought the patient to the previous mentioned pregnancy related complications (20).
In this prospective cross sectional controlled study, we aimed to compare the level of maternal plasma protein Z in normal and abnormal pregnancy and correlate that with the pregnancy outcome.

Patients and methods

This prospective case control cross sectional study comprised of 130 patients among those attending he obstetric unit in Mansoura University Hospitals, a tertiary referral center, in the period from May 2009 –September 2011. The study group included patients with severe PE (N=40); patients who had fetuses with unexplained IUFGR and delivered neonates small for gestational age (N=25); patients with unexplained fetal demise (N=25); and gestational age matched women with a normal pregnancy and normal outcome (N=40) which served as a control group. An informed consent was taken from all patients

Correspondence
Dr. Maged Raghib, MD
Department of OB/GYN
Mansoura University, Mansoura,
Egypt
Tel 0020502268830
Fax 0020502268840

before being included in the study and the protocol of the study was approved by Mansoura University ethics committee. We excluded from the study al patients with multiple pregnancies, those who have lupus anti-coagulant, vitamin K deficiency, vitamin K antagonist treatment, chronic essential hypertension or chronic renal disorders, fetuses with congenital defects and/ or chromosomal anomalies.

In this study, severe PE was defined as diastolic blood pressure ≥110 mmHg or systolic blood pressure ≥160 mmHg and/or proteinuria ≥3+ by dipstick. Unexplained IUGR was defined as ultrasonographic fetal biometry less than the fifth percentile gestational age in the absence of apparent etiology. This is proved postpartum by SGA when a birth weight is below the 10th percentile [21]. Birth weight percentiles for gestational age were classified in four groups as follows: 1) ≤5th percentile; 2) 5th-10th percentile; 3) 10th – 90th percentile; and 4) >90th percentile [21]. Unexplained fetal demise was defined as a fetal death occurring after 19 weeks of gestation and proved by ultrasound examination when the fetus is visible without cardiac activity.

All assays were done on the first diagnosis of the condition (PE, IUGR or IUFD) after 20 weeks of gestation [22]. Assays were done at a corresponding mean gestational age in the control group. All venous blood samples were collected into plastic tubes containing anhydrous salt of trisodium citrate anticoagulant solution (3.2 at a ratio of 9 volumes blood to one volume citrate). The samples were centrifuged at 1300g for ten minutes at 4°C and the plasma was used to perform the global coagulation tests and a part stored at -70°C until assay. Prothrmbin time was estimated according to, using Thrombol S Kit from Dade Behring (Germany) (23). Activated partial thromboplastin time (APTT) was estimated according to the method reported by Procter and Rapaport (24), using pathromtin SL Kit from Dade Behring (Germany). Fibrinogen assay was done according to the method of Clauss (25) using multifibrin-U Kit from Dade Behring (Germany). Concentrations of protein Z in maternal plasma were determined by sensitive and specific immunoassays obtained from Diagnostica Stago (Asnieres-sur-Seine, France). The protein Z immunoassay utilizes the quantitative sandwich enzyme immunoassay technique. Protein Z deficiency was defined as maternal plasma concentrations ≤5th percentile [22] of the normal pregnancy group (≤ 1.59µg/mL).

Statistical analysis:

The statistical package used was SPSS (statistical package for social science) version 12 (SPSS Inc., Chicago, IL USA). As protein Z plasma concentrations were not normally distributed; thus, Kruskal–Wallis and Mann–Whitney U tests were used for comparisons among groups. The Chi-square was used to compare categorical variables and p value < 0.05 was considered statistically significant.

Results

The study comprised of 130 patients in total. They were divided into control group of 40 normal pregnancies and 3 case groups of 90 patients according to pregnancy complications. Age of patients in both groups ranged from 18-38 years with no statistical significant difference between both groups (27.6 + 6.27 vs 29.43+ 5.07). There were no differences between both groups as regards gravidity, parity or previous pregnancy complications and the mean gestational age at the time of examination (Table 1).
Table (2) for protein z assay shows a statistical significant difference between control group and complicated pregnancies (p=0.047). Protein Z levels were significantly lower in severe preeclamptic patients was than that of the other study groups p<0.0001. However, there was no significant difference in the maternal plasma protein Z concentration between patients with unexplained IUGR or intrauterine fetal demise.

Table (1): Clinical characteristics of the study and control groups

	Normal pregnancy group (n=40)	Complicated pregnancy group (n=90)	p
Age (years)	27.6 + 6.27	29.43+ 5.07	0.60
Gravidity	1.93 + 1.31	2.07 + 1.33	0.09
Parity	0.93 + 1.01	0.5 + 0.89	0.07
Gestational age (weeks)	30.2 + 4.31	28.9 + 5.11	0.11

Table (2): Protein Z concentration in normal and complicated pregnancies

	Control group (n=40)	Complicated pregnancies (n=90)			p
Protein Z (µgm/ml)	2.3± 0.31 (1.4 -3.2)	1.85± 0.41 (03 -5.4)			0.047
		PE	**IUGR**	**IUFD**	
		1.6 ± 0.43 (0.3-3.3)	2.3 ± 0.62 (0.3-3.8)	2.85 ± 0.21 (0.3-5.4)	< 0.0001

Discussion

There are inconsistent reports regarding the changes in plasma concentrations of protein Z in women with PE. While some authors have reported that there is no significant difference in the median plasma concentrations of protein Z between patients with PE and women with normal pregnancy, [20] others have reported that the median plasma protein Z concentrations are significantly lower in women with PE, SGA, and preterm delivery than those with normal pregnancies [26, 27]. However, the authors did not analyze each complication independently; therefore, the association between PE and changes in protein Z plasma concentration is not clear [27].

Normal pregnancy is characterized by an increased plasma concentration of protein Z, which has been proposed to be part of a compensatory mechanism for the increased concentration of factor X and perhaps for the increased thrombin generation [26]. Preeclampsia is associated with an exaggerated hypercoagulable state and excessive thrombin generation, [27] as determined by higher maternal plasma concentrations of TAT complexes [28-30] and lower antithrombin III concentrations [31, 32] than patients with a normal pregnancies. Moreover, patients with PE who delivered preterm have a higher rate of thrombotic lesions in the decidua and in the placental villi [12] than normotensive patients with indicated or spontaneous preterm delivery [10, 12]. Therefore, it is possible that an exaggerated procoagulant state may account for the lower plasma concentration of protein Z among women with preclampsia.

In this study, there was a significantly lower level of protein Z in women with PE than in women with normal pregnancy outcome (OR 22.65, 95% CI 6.79-116.82). In a previous report, [18] the rate of protein Z deficiency defined as the 10th percentile of the normal population in women with PE was not significantly different from the rate observed in women with normal pregnancy. In contrast, we found that 25% = 10/40 of the patients with PE had protein Z deficiency; this difference may be attributed to the relatively larger sample size of patients with PE included in our study, different definition of protein Z deficiency (<5th percentile of the normal pregnant population) and differences in the study population. Protein Z deficiency has been reported in non-pregnant women [22] as well, suggesting that in some of the patients protein Z deficiency may precede the clinical presentation of PE, and a low maternal plasma concentration of protein Z can be a risk factor for the subsequent development of PE in a subset of patients. There were no significant differences between the median maternal protein Z plasma concentrations of patients who delivered an SGA neonate or had fetal demise in comparison to women with normal pregnancy; these results are consistent with a previous report by Bretelle et al. [18]. In contrast, a recent study reported that patients with adverse pregnancy outcome, including PE, SGA, recurrent unexplained vaginal bleeding, and preterm parturition, had lower mean plasma concentrations of protein Z than patients with normal pregnancy outcome in all three trimesters [27]. In our study we observed low protein Z level in 40% = 10/25 cases of IUGR.

The finding that women with fetal demise have a higher rate of protein Z deficiency than women with normal pregnancy is in harmony with Bretelle et al. report [18]. Of interest, the rates of protein Z deficiency that were observed in women with normal pregnancy (2.5%) and those with fetal demise (40%) in the current study, are similar to those reported in non-pregnant women with normal obstetric history, and with a history of previous fetal loss between 10-15 weeks gestation [33]. The similarity in the rate of protein Z deficiency between pregnant and non-pregnant women in both groups (those who had a normal pregnancy and those with fetal demise) suggests that a subset of women in the latter group might have a predisposing protein Z deficiency.

Moreover, we have proposed that pregnancy could be considered as a stress test to the hemostatic system [34]. Thus, the physiologic hypercoagulable state that accompanies pregnancy may facilitate the occurrence of thrombotic events of the placenta and adverse pregnancy outcome (i.e. fetal demise) in potentially thrombophilic patients that were clinically "silent" in the non-pregnant state [34]. In addition, Gris et al [22] reported that six out of eight patients with protein Z deficiency had one parent who is also protein Z deficient; [22] thus, the possibility that in some cases protein Z deficiency may be inherited. The process which is not searched in our study and needs further genetic investigations.

The results of this study indicate that PE, IUGR and fetal demise are associated with maternal protein Z deficiency; however, only patients with PE have a lower median maternal plasma concentration of protein Z that may be secondary to a higher activation of the coagulation system in patients with this pregnancy complication.

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Prevalence of antithyroid antibodies in patients with unexplained infertility

Maged R Elshamy MD1, Hosam Z Elhafez MD2
1 Department of Obstetrics & Gynecology, 2Department of Clinical Pathology
Faculty of medicine, Mansoura University, EGYPT

Abstract

Objective: To evaluate the relative prevalence of antithyroid antibodies in unexplained infertility compared to other types of infertility.
Patients and methods: The study comprised of 96 subfertile couples and 80 parous women attending outpatient clinic in Mansoura University Hospital. There were 30 patients had tubal causes, 16 patients had PCOS, 10 patients had endometriosis and 40 had unexplained infertility. Antithyroid auto-antibodies (antithyrglobulin and antimicrosomal) were estimated in the sera of all patients.
Results: There were no significant differences in the frequency of antimicrosomal antibodies and antithyroglobulin in subfertile cases compared to the controls (25% and 22% versus 12% and 10%, P value 0.22 and 0.12, respectively). Subgroup analysis had revealed significantly higher frequency of women with positive antimicrosomal and antithyroglobulin antibodies in couples with unexplained infertility compared to fertile controls.
Conclusion: A significantly higher proportion of thyroid auto-antibodies existed in women with unexplained subfertility compared to the fertile women.
Key words: Subfertility, thyroid antibodies, unexplained infertility

Introduction

Unexplained infertility is defined as failure to conceive after at least one year of continuous unprotected sexual intercourse in which the standard infertility testing revealed no cause for this failure. The prevalence of unexplained subfertility was estimated to be approximately 10 -20 % of all cases of infertility (1). The reason for unexplained infertility is enigmatic and several hypothetical causes, however, had been described. Abnormalities in gametes, implantation failure, hostile cervical mucus and autoimmune disorders were all suggested to play a role (2-4).

Autoimmunity has been linked to several reproductive conditions including premature ovarian failure, unexplained infertility and recurrent pregnancy loss (2, 4-6). Antithyroid antibodies had not been infrequently isolated from women with subfertility (7, 8). A number of antithyroid antibodies have been isolated from women with thyroid dysfunction (9). Thyroid peroxidase (Tpo) is a glycoid protein present on the thyroid cell surface. It is responsible for the iodination of tyrosine residues on thyroglobulin (TG) as well as the intramolecular coupling reaction of iodinated tyrosine leading to the formation of thyroxine and triiodiothyronine (10, 11). Antithyroglobulin antibodies (ATA) and antithyroid peroxides antibodies (microsomal antibodies or AMA) had been isolated from women with unexplained infertility as well as women with premature ovarian failure (12).

NICE guidelines for subfertility management do not recommend screening for thyroid dysfunction in a rather asymptomatic subfertile woman. Neither do they recommend screening for autoimmunity in unexplained subfertility (http.www.NICE.com). Nevertheless, these recommendations based on limited number of studies investigating the associations between antithyroid antibodies and unexplained infertility. Hence, we decided to perform this case-control study to assess the prevalence of thyroid antibodies in subfertile women and evaluate the relative prevalence of thyroid antibodies in unexplained infertility compared to other types of infertility.

Corresponding author
Maged ragheb Elshamy
Assistant professor of Obstetric and Gynecology,
Obstetric and Gynecology Department,
Mansoura University Hospital,
Mansoura, EGYPT
Tel 050 2232553
Fax 0502267016
E mail dr_maged66@yahoo.com

2012

Patients and Methods

The study comprised of 96 subfertile couples among those attending Mansoura University Hospital outpatient clinic and a private practice setting during the period from September 2006 till September 2010. Eighty parous women, matched for age, were recruited as a control group. The protocol of this study was approved by the local ethical committee of the institution and all participants gave an informed consent before inclusion in the study. Women aged 40 years or more, women with past or current history of endocrine or autoimmune disease and women with history of recurrent miscarriage were excluded from the study. All patients included in the study were subjected to hysterosalpingography for tubal patency, midluteal serum progesterone for confirming ovulation, laparoscopy and semen analysis for their partners. There were 30 patients had tubal causes, 16 patients had PCOS, 10 patients had endometriosis and 40 patients were diagnosed to have unexplained infertility. Unexplained infertility was defined as inability to conceive for 1 year or more whenever normal semen analysis, positive ovulation (mid-luteal phase progesterone), and

patency of both fallopian tubes was documented. Aged-matched control group comprised of 80 women below 40 years old and had at least one living baby. Women in the control group were excluded if they had experienced any previous difficulty in conceiving for one year or more, past or current history of endocrine or autoimmune disease or history of recurrent miscarriage.

Hormonal assay for FSH, LH, TSH, T3, T4 was done using electrochemiluminescent immunoassay technique according to the method of Beastall et al (1987) , while prolactin was determined according to the method of Fahie –Wilson and Soule (1997) (13, 14) . Antithyroid autoantibodies (antithyrglobulin and antimicrosomal) were estimated in all patients by the indirect fluorescent (IFA) technique. The reaction occurs in two steps. The first is the interaction of thyroid antibodies in patient's sera with thyroid substrate. The second is the interaction of FITC labeled anti human immunoglobulin with thyroid antibodies attached to the thyroid tissue producing apple – green staining in a positive assay (15).

Statistical analysis:

Statistical analysis was performed using the Statistical Package for Social Science version 16.0 (SPSS Inc., Chicago, IL, USA). Data were checked for normality in distribution and using Student t test (t), chi-square test ($\chi 2$), Mann-Whitney (U) test where appropriate. When a quantitative analysis of the data was performed, groups were compared by analysis of variance with Schef˝fe's post hoc analysis. Significance was defined as $P \leq 0.05$.

Results

Data regarding differences in the demographic and basal line features between both infertile group (cases) and fertile one (control) are presented in Table 1. The mean age, height, weight, BMI, serum basal FSH and LH values did not differ significantly between the two groups. The median serum TSH level was significantly higher in the subfertile group compared to the controls.

The percentages of individuals positive for antimicrosomal antibodies and antithyroglobulin antibodies are shown in Table 2. There were no significant differences in the frequencies of antimicrosomal antibodies and antithyroglobulin in subfertile cases compared to the controls (25% and 22% versus 12% and 10%, p value 0.22 and 0.12, respectively). Subgroup analysis had revealed significantly higher frequencies of women with positive antimicrosomal and antithyroglobulin antibodies in couples with unexplained infertility compared to fertile controls (Table 3).

Discussion

In this study, there were higher proportions of positive thyroglobulin and micriosomal autoantibodies in infertile group although the difference was not statistically significant when compared with the control group.

The results of this study point out to an association between unexplained subfertility and the presence of anti-thyroid antibodies. It remains to be determined whether these antibodies might have played a role in the low reproductive performance of these couples or not. Two plausible hypotheses had been suggested for the presence of these antibodies; a phenomenon accompanying immune system activation by specific autoantigens or by immune activation initiated by viral and bacterial inflammation.

Our results were in agreement with Poppe et al (2003) who reported a higher proportion of positive antibodies in sera of women of couples diagnosed with subfertility compared with fertile controls, however, the difference has yet to reach the statistical level of significance(9). Moreover, Abalovich et al (2007), reported no significant difference of thyroid autoantibodies between a group of infertile patients and a control group (16). This is in contrast to Grassi et al (2001) who found a high prevalence of thyroid autoantibodies in infertile patients (17). Nevertheless, the significantly higher prevalence of thyroid autoantibodies in women with an overall poor reproductive performance was highlightened in more studies though the exact mechanism of action remains unknown (18, 19).

In our series, there were a higher proportion of thyroid antibodies in women with unexplained subfertility. This finding supports similar findings by other researchers (8, 20), however, it remains difficult to recommend any change in current management, whether screening or treatment, of idiopathic infertility based on our findings. The small sample size in our study as well as the nature of our study as a case control study may render it prone to some methodological outflaws. Moreover, there is almost no evidence that the use of any specific treatment, as immune modulator for example, could improve the prognosis. Nevertheless, based on the relatively big difference (effect size) in the proportion of these antibodies in women with unexplained infertility compared to fertile women, we do recommend more research in this area.

To conclude, our study has shown no difference in the proportion of antithyroglobulin antibodies and antimicrosomal antibodies in the sera of subfertile women compared to fertile controls. However, subgroup analysis has revealed a significantly higher proportion of these antibodies in women with unexplained subfertility compared to the controls. The implications of our finding on clinical practice remain unclear and more research is warranted.

Table 1: comparison between subfertile women and parous women as regard age, BMI, TSH level, LH level, FSH level

groups	Subfertile women (cases) n=96	Parous women (controls) n=80	P value
Age (years)	24 ± 3	26 ± 4	0.8
Body mass index(BMI) Mean±SD	23.5 ± 0.5	24. 3 ±0.6	0.4
TSH (MIU \ ML) Median [interquartile range]	7.23±1.1	1.87±0.4	0.02
LH (IU\L) Mean±SD	21 ± 0.5	21 ± 0.7	0.8
FSH (IU\L) Mean±SD	5.3 ± 0.2	5.1 ± 0.7	0.7

Table 2: Thyroid antibodies in infertile patients and control group

Groups	Group A n = 96	Group B n = 80	P value
Number and percentage of cases with positive antimicro-somal antibodies	30 (25 %)	12 (15%)	0. 22
number and percentage of cases with positive antithyro-gobulin antibodies	26 (22%)	8 (10 %)	0. 12

Table 3: Thyroid antibodies in unexplained infertility patients and in the control group

Groups	Unexplained infertility patients n =40	Control group n = 80	P value
Antimicrosomal antibodies number and percentage of positive cases	18 (50 %)	12 (15 %)	0.005*
antithyrogobulin number and percentage of positive cases	14(38.88 %)	8 (10 %)	0.009*

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News & Views

These news were compiled by Dr. Mahmoud Shawer, FRCOG

1. Carbetocin at caesarean section

Carbetocin is a synthetic analogue of oxytocin with a longer biological half life. As such it may have advantages over the standard 5 IU intravenous dose of oxytocin given at caesarean section once the baby is delivered. The routine 5 IU of oxytocin is frequently augmented by a further infusion of the drug prophylactically against post-partum haemorrhage in patients the surgeon considers to be at high-risk.

A report by Attilakos et al (BJOG 2010; 117:929-36) showed that 100 mg of carbetocin was more effective than an ampoule of 5 IU oxytocin in reducing the need for additional oxytocin infusions. There were no differences in the incidence of haemorrhage or blood transfusion requirements. Despite its higher cost carbetocin may find a place in the armamentarium of uterotonic agents used in first world obstetrics but continuous oxytocin infusion and rectal mesoprostol will delay its entry to our country.

2. Genetic susceptibility to breast cancer

It is seductive to believe that genetic testing could hold the key to breast cancer risk prediction. There are ten environmental factors that influence risk: age at menarche, parity, age at first birth, breastfeeding, menopausal status, age at menopause, use of hormone replacement, body mass index, height and alcohol consumption.

There are also a dozen single nucleotide polymorphisms (SNPs) that are known to be associated with breast cancer risk so it might be possible to marry the environmental factors with the SNPs to come up with a gene-environment rubric that would really identify those at risk. To test this hypothesis Travis et al (Lancet 2010;375:2143-51) used the data from the UK Million Women Study to investigate over 7000 women who developed breast cancer and see if combining environmental plus SNP information lead to a definable interaction that would be a useful predictor of risk.

Unfortunately it did not reveal any significant combinations of the possible 120 interactions despite formidable statistical pyrotechnics so genome-wide association studies are unlikely to yield progress (Narod Lancet 2010;375:2123-4). It is exactly a decade since the human genome was mapped and although a scientific success in decoding our 3 billion base pairs, the clinical benefits remain scarce. Maybe the next 10 years will be more fruitful.

3. Surgery for stress urinary incontinence

As women age their chances of urinary incontinence increase until about one in three will have her lifestyle affected. The social cost can be expressed by patients saying "Incontinence doesn't kill you, but it takes your life away". An article in JAMA (Goode et al 2010; 303: 2172-81) and a commentary by Wagner and Subak (2184-5) give data about the magnitude of the problem and the options available that may be helpful to those trying to address their difficulties with their doctor's help.

The number of operations for stress urinary incontinence has increased markedly over the last few years. Not only are women living longer and anticipating a higher quality of life but the procedures available offer more choice, are simpler and are less likely to cause complications.

The changes came 15 years ago when the synthetic midurethral sling was introduced using tension-free vaginal tape (TVT). Unlike the Burch urethropexy or the suburethral fascial sling the new operation was less invasive, did not require a traditional abdominal incision and could be performed as an out-patient procedure. This retropubic TVT proved immensely popular because of its simplicity and excellent results but randomised trials of its efficacy were slow in following. Its "obvious" advantages remained unchallenged until, 5 years later a different type of midurethral sling appeared that was not placed in the retropubic space but through the obturator foramen. It was suggested that it had advantages over the TVT in that it had fewer complications and bladder perforation was less common.

There is a clinical impression that the two procedures are roughly equivalent in efficacy in terms of their outcomes and complication rates (Rogers NEJM 2010;362; 2184-5). Clinical equivalence is a challenge to prove but Richter et al (NEJM 2010; 362: 2066-76) did carry out such a trial comparing midurethral retropubic and transobturator slings. They recorded objective and subjective measurements of cure rates after one year as well as the complication rates and the need for additional treatment. The research showed remarkably similar findings for the 300 women in each group giving objective positive results around 80% at 12 months follow-up. The subjective results were around 60% with the retropubic sling being slightly more satisfactory than the transobturator sling.

The retropubic sling had more complications, often related to mesh exposures or bladder perforations whereas the transobturator sling gave rise to more neurological side effects such as numbness and weakness. Given that up to one third of women having a procedure for incontinence require another manoeuvre at some time in their lives, there is no guarantee that the long term outcomes will be equivalent. It seems both operations are comparable in efficacy and complication rates after one year so other considerations can be taken into account for individual cases.

In Egypt and other countries with limited resources, cheaper tapes cut from surgical mesh are used frequently with no reported complications. Transobturator tapes do provide very good results and are most often successful when there has been no previous incontinence surgery and where the woman did not suffer from prior urge incontinence

4. Neonatal care

Perinatal mortality rates have improved with more advanced neonatal care and neonatal intensive care units (NICU) in our country – in Cairo and other cities - are accepting earlier and earlier gestationally aged infants for treatment. In sophisticated NICUs there is about a 75% survival rate of babies born between 24 and 27 weeks and 6 days gestation but there is a high prevalence of neurodevelopmental problems in survivors – approximately 50%. Naturally all statistics improve as the neonate's age approaches 28 weeks

Neonatologists are constantly reviewing their strategies, especially those of pulmonary function support. Two articles by the SUPPORT Group (NEJM 2010;362:1959-69 & 1970-9) address the issues of intubation plus surfactant and the percentage oxygen saturations that are best for these tiny infants. It seems that nasal continuous positive airway pressure (CPAP) is a viable alternative to immediate intubation plus surfactant administration so it is worth considering even if later intubation does become necessary.

Answering the second question – that of target oxygen saturations – has proved problematic. Keeping levels between 85 & 90% resulted in fewer cases of severe retinopathy of prematurity but more babies died compared with the group whose oxygen saturations were kept between 90 & 95%. It is also essential to provide long-term follow up on neurodevelopmental outcomes as many subtle characteristics only become apparent in later years.

There is clear evidence that resuscitation of 22 -23 weekers is very rarely successful and should only be undertaken after counselling which must consider that non-survivors will have to endure long periods of intensive care and this is part of a "hidden morbidity" especially in our private practice and that survivors will, more likely than not, suffer permanent cerebral damage (Swamy et al Arch Dis Child 2010;95:F293-4).

5. Maternal vitamin A

Adequate maternal levels of vitamin A are essential in early pregnancy for normal lung development in the fetus. Vitamin A regulates growth through cell proliferation and differentiation and children born to mothers in areas of deficiency may suffer from suboptimal alveolar development.

In the 1990s mothers in Nepal were given vitamin A, beta-carotene or placebo as part of a trial on the effects of supplementation on pregnancy outcomes and then 10 years later their children had their pulmonary function studied (Checkley et al NEJM 2010;362:1784-94). There were improved lung function results in those whose mothers (and as newborns) had received vitamin A compared with those given beta-carotene or placebo as measured by their forced expiratory volume. Populations experiencing chronic vitamin A deprivation should be provided with supplementation antenatally as well as subsequently through the child's school years for optimal lung development.

On the other side of the coin, vitamin A supplementation has been shown not to reduce maternal mortality in Ghana (Kirkwood et al Lancet 2010;375:640-9). Although it has one of the highest maternal mortality rates in the world, the people of Ghana seldom suffer from night blindness which is a manifestation of vitamin A deficiency so it may be that supplementation did not benefit the women in a clinically discernable manner.

Supplementation is not going to be the magic bullet which will allow deprived nations to achieve their Millennium Development Goat of a 75% reduction in maternal mortality ratios by 2015. Progress toward the goals is reported by Hogan et al (Lancet 2010;375:1609-23) and deserves to be read by all those concerned about women's health globally

6. OCs & mortality

One of the longest running surveys is the UK General Practitioners' study of the effects of oral contraceptives (OCs). Forty years ago GPs started tracking the health of OC users and a control group of non-users to see of OCs were linked to increased or decreased mortality rates (Hannaford et al BMJ 2010;340:c927). Initial reports suggested an increased risk of cardiovascular problems in older women and smokers but the latest data show users to be at a lowered risk compared with never-users. There were fewer in deaths from cancer and circulatory disease leading to an overall reduction in all-cause mortality of 52 per 100 000 woman years.

7. Cardiovascular disease prevention

As women age their cardiovascular disease risk becomes more similar to that of men. Indicators of risk such as dyslipidaemia or elevated C-Reactive protein levels have been successfully used in men as triggers for the initiation of preventative medication – like statins. Evidence that primary prevention in women is now starting to appear (Mora et al Circulation 2010; 121: 1069-77) and rosuvastatin looks a promising agent.

In the JUPITER trial nearly 7 000 women over the age of 60 with hematological risk factors were allocated to rosuvastatin or placebo. The statin did significantly reduce coronary events in women in much the same ration as it did for men – results which are supported by meta-analyses of primary prevention statin trials. It seems that sex differences for CVD do become less far apart with age and medical interventions have a similar protective effect.

- **Hysterectomy and urinary symptoms**
  The effects of hysterectomy on urinary tract symptoms are difficult to evaluate. After recovering from the operation women generally feel better and report an improved quality of life but long-term follow up has suggested a predisposition to urinary symptoms. Whether this increased incidence of symptoms is due to natural aging processes or the operation is a matter of debate.

A study from Finland adds some clarity (Heliovaara-Peippo et al BJOG 2010;117:602-9). The researchers randomised women with a mean age of 43 years to either hysterectomy or the lev-

onorgestrel releasing intrauterine system (LN-IUS) for the management of the menorrhagia. Diligent follow-up over the next decade of their lives allowed deductions to be made as to the effect of hysterectomy on urinary tract symptoms. Detailed questionnaires were filled in at baseline, 6 and 12 months, 5 years and 10 years with over 90% of participants completing the programme.

They found that those allocated to hysterectomy had more urinary tract infections and used more medication for urinary incontinence than those treated with LN-IUS. The sensation of incomplete bladder emptying was also more common in the surgically treated women allowing the trial to conclude that a hysterectomy increases the risks of women experiencing incomplete emptying, lower urinary tract infections and stress incontinence.

8. Post-operative DVT

Men and women are at increased risk of venous thromboembolism postoperatively. This is well known and preventative measures should be used in all women undergoing major gynaecological surgery. Evidence is accumulating that the risk lasts longer than two weeks after surgery – and may extend to day-care surgery.

Sweetland et al (BMJ 2009;339:b4583) report on data derived from the UK Million Women Study and show that the risk of venous thromboembolism (VTE) reaches its peak 3 weeks after surgery and remains elevated for 12 weeks.

A middle-aged woman having an operation is 70 times more likely to be admitted to hospital with a VTE than someone not operated on. This is in the first 6 weeks after inpatient surgery and the risk is still present for another 6 weeks thereafter. These facts are “a wake-up call to all surgeons” say Cohen (BMJ 2009;339:b4477) because most prophylaxis is confined to hospital stays or the week thereafter, missing the most at risk period. Even the latest figures are probably an under-estimate as most VTEs are undiagnosed, untreated and managed outside of hospitals.

The UK National Institute for Health and Clinical Excellence (NICE) has published recommendations for all patients in hospital and estimate only half of those who should received prophylaxis actually get it. The summary by Hill et al (BMJ 2010;340:C95) enumerates the following risk factors: cancer patients, age over 60 years, admission to critical care, dehydration, thrombophilia, obesity, common comorbid medical conditions such as heart disease, metabolic, endocrine or respiratory pathology, infections or inflammatory diseases, a personal or close family history of VTE, hormone use or smoking.

Preventative strategies include practical measures of mobility and hydration, mechanical devices to aid circulation plus drugs like low molecular weight heparin (or unfractionated heparin). These drugs should be continued till “the patient is no longer at increased risk of VTE”. Given the most recent data this is clearly longer than has previously been thought. It is essential that the active discharge management should be given to every woman leaving hospital after surgery.

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For further information about Mirena® please contact:

Bayer HealthCare
Scientific Office

6, El Shawarby St., Cairo, Egypt
Tel.: +202 23909351, 23904907
Fax: +202 23936770
E-mail: ma.pv.me@bayer.com