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Oestrogen supplementation in gamete intrafallopian transfer (GIFT) — a prospective randomised study

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Objective. To investigate the impact of oestrogen supplementation from the early luteal to the late proliferative phase on biochemical and ongoing pregnancy rates in gamete intrafallopian transfer (GIFT).

Methods. Ninety-five patients were assigned to clomiphene citrate-human menopausal gonadotrophin (hMG)-induced GIFT cycles, with or without the use of oestrogen support (oral administration of estradiol valerate). The main outcome measures were biochemical pregnancy rate and clinical pregnancy rate.

Results. The biochemical pregnancy rate was 38.09% in the oestrogen group v. 22.9% in the control group (p = 0.096, 95% CI: 5.7 - 37.3%). The clinical pregnancy rate in the oestrogen supplementation group was 23.8% v. 14.58% in the control group (p = 0.1988, 95% CI: 10.3 - 31.7%).

Conclusion. Although the results of this study show no significant statistical difference, there is a clinical trend in favour of giving oestrogen support.

The greatest challenge currently in assisted reproductive techniques (ART) is the implantation process of the embryo. The efficiency of fertilisation is 85%, whereas fecundity for women under 30 years of age is 20 - 25%,[1] thus implantation appears to be the limiting step. Only 15% of the embryos complete the initial steps of implantation.[2] Aside from the quality of the oocyte and embryo, the quality of the endometrium plays a major role. Therefore, there is an increased interest in how to achieve adequate preparation of the endometrium in ART to guarantee optimal implantation and consequently higher pregnancy rates. In natural cycles, proliferation of the endometrium under the influence of oestrogen is characterised by thickening of the endometrium and the development of endometrial glands. In the luteal phase, oedematous formation of the stroma and coiling with maximal secretion of the endometrial glands develop under the influence of progesterone.

In ART, controlled formation of the endometrium is necessary. An adequate hormonal environment is needed for embryo-blastocyst implantation. It is possible to achieve this by supplementing a fixed dose of steroids, which result in both physiological and supraphysiological levels of oestrogen and progesterone and ultimately in good endometrial development.[3,4]

The common drugs used in ovulation induction cycles are clomiphene citrate (CC), human menopausal gonadotrophin (hMG) and gonadotrophin-releasing hormone (GnRH) agonists. When using CC for the induction of ovulation one has to take in account the anti-oestrogenic effect of this product. It has a hypo-oestrogenic effect on the endometrium.[5] CC changes follicular maturation and it results in inhibition of follicular oestrogen formation.[6] Although the ratio of growth stays the same in comparison to natural cycles, some studies have found a bigger dominant follicle and a decrease in endometrial thickness.[7,8] Dlugi *et al*.[6] described a lowered level of oestrogen in the early and mid-luteal phase, and a delayed surge in the late

luteal phase. There can also be a suboptimal receptivity in the endometrium when using CC for ovarian stimulation.[9] Although addition of oestrogen will not change the endometrial thickness,[10] it might change its functional capacity.[3] A recent study by Weigert *et al*.[11] proved the benefits of using CC in combination with recombinant follicle-stimulating hormone (FSH) and recombinant luteinising hormone (LH) as first-line treatment for induction of superovulation.

Not much is known about oestrogen supplementation for endometrial support. Prospective randomised studies on this subject mostly investigated oestrogen supplementation during the luteal phase only.[9,12-14] One can assume that this is too late for adequate preparation or could even have caused a luteolytic effect.[13]

Our aim was to investigate the supplementation of oestrogen during the follicular and luteal phase of the stimulated cycle, when using a CC protocol in combination with hMG in GIFT cycles and to monitor the eventual effect on the pregnancy rate.

Materials and methods

The study took place in the Reproductive Biology Unit, Department of Obstetrics and Gynaecology, Stellenbosch University and Tygerberg Hospital, over a period of 22 months (January 2001 - November 2002). The main indications for GIFT were unexplained infertility, ovulatory dysfunction and endometriosis. A total of 90 patients participated in the study and were randomly divided into two groups. Group A received estradiol valerate (Progynova, Shering, oral tablets 2 mg) during ovarian stimulation; group B received no oestrogen support. The mean age of patients enrolled in the study was similar in both groups (group A 33.24 and group B 32.94 years). Informed consent was obtained from all the patients.

Group A received oestrogen supplementation by oral administration of estradiol valerate. The dose given was 2 mg daily. When the endometrial thickness measured on day 9 of the cycle was below 7 mm, a supplement of 2 - 4 mg daily was added. The initial supplementation was given from day 3 of the stimulated cycle until pregnancy or onset of menstruation. Both groups received luteal support with vaginally administered progesterone (Cyclogest, 200 mg twice daily) from the day of the GIFT procedure. A biochemical pregnancy was diagnosed by the presence of ß-hCG in the woman's serum on day 12 with a significant rise over the 4 days following the GIFT procedure (doubling every 48 hours). The clinical pregnancy rate was diagnosed by ultrasound (live fetus of minimum 12 weeks' gestation). Stimulation of ovulation was achieved by administration of 100 mg of CC from day 3 to day 7 of the cycle. The patient received 2 ampoules of hMG every second day, starting from day 4 of the cycle (Perganol, Serono 75 U FSH/75 U LH). When the dominant follicle reached 18 mm and at least 2 other follicles 16 mm or more, hCG (5 000 - 10 000 IU) was administered to trigger ovulation.

The GIFT procedure took place 36 hours after hCG administration. Laparoscopy and follicle aspiration were done under general anaesthesia. The maturity of the oocytes retrieved was determined according to the criteria of Veeck[15] as being either metaphase I or II. Three metaphase II oocytes were transferred with spermatozoa by means of a catheter into the fallopian tubes (2 cm from the fimbrial end). Between 500 000 and 750 000 sperm per oocyte were transferred into each patient.

Statistical analysis was performed using Student's *t*-test to compare the age of the patients in both groups. Fisher's exact test was used to compare the outcome of pregnancy. A *p*-value of < 0.05 was considered significant. The biochemical pregnancy rate was measured according to maternal serum ß-hCG level.

Results

The mean age was comparable in both groups: group A 33.24 ± 3.35 standard deviation (SD) versus group B 32.94 ± 3.51 SD ($p = 0.69$). Biochemical pregnancy rates were 38.09% in group A and 22.9% in group B (Table I). Clinical pregnancy rates were 23.8% in group A and 14.85% in group B (Table II). The Fisher's exact test for the one-sided hypothesis was not significant for biochemical pregnancy ($p = 0.096$, 95% CI: 5.7 - 37.3%) or for clinical pregnancy ($p = 0.1988$, 95% CI: 10.3 - 31.7%).

Table I. Biochemical pregnancy rate (BPR) in the oestrogen supplementation group (A) and control group (B)

Group	No. of patients	Age (yrs)	Biochemical pregnancy (*N*)	BPR (%)
A	42	33.24 (3.35)	16	38.09
B	48	32.94 (3.51)	11	22.9

Table II. Clinical pregnancy rate (CPR) in the oestrogen supplementation group (A) and control group (B)

Group	No. of patients	Age (yrs)	Clinical pregnancies (*N*)	CPR (%)
A	42	33.24 (3.35)	10	23.8
B	48	32.94 (3.51)	7	14.85

Discussion

In our study we wished to investigate whether the supplementation of oestrogen from the early proliferative phase to the late secretory phase would improve the endometrial environment for embryo implantation and thus the pregnancy rate in CC-hMG-stimulated GIFT cycles. This experimental prospective randomised study included 90 patients.

Our results showed a higher pregnancy rate in the group where supplementation was given (biochemical pregnancy rate group A 38.09% v. group B 22.9%, clinical pregnancy rate group A 23.8% v. group B 14.58%), but these findings

did not reach statistical significance.

Few prospective randomised studies have investigated the value of oestrogen supplementation in ART. Aside from our study, only one study by Jung and Roh[16] investigated the use of oestrogen support from the early proliferative phase to the late secretory phase of the endometrium. The setting of this study was in GnRH-hMG-stimulated *in vitro* fertilisation (IVF)-embryo transfer (ET) cycles. The ongoing pregnancy rate in the oestrogen group was 48.3% v. 25.9% in the control group and it was concluded that oestrogen supplementation during a whole cycle increased the endometrial receptivity for the transferred embryos.

On the other hand, two studies[12,13] investigated the use of oestrogen combined with progesterone as luteal support in ART. Both these studies used GnRH-hMG as stimulation in IVF cycles. Smitz *et al*.[12] found comparable pregnancy rates in both protocols (29.5 v. 29.1%). There was, however, a higher preclinical pregnancy loss in the group where no oestrogen was given. Lewin *et al*.[13] gave oestrogen supplementation earlier in the luteal phase to counter a possible luteolytic effect by oestrogen on the corpus luteum (day 1 v. day 6) and also found no advantage (pregnancy rate 26.5 v. 28%).

The importance of oestrogen support during ovulation induction is controversial. As mentioned, oestrogen plays a critical role in the formation of the endometrium in natural cycles. Aside from its major role in the proliferative phase, it also primes the endometrium for the luteal phase by the further proliferation of the basal cell layer and the induction of P-receptors,[17] thereby ensuring the capacity of the endometrium to become secretory. It also plays a mediating role for P-receptors in the luteal phase.[18] When the oestrogen levels are insufficient because of oestrogen depletion at the onset of the cycle, an insufficient number of P-receptors will be induced and thus luteal insufficiency can be present. Therefore, an adequate level of oestrogen should be present at the beginning of the cycle in ovulation induction to ensure sufficient priming of the endometrium.

In most studies, oestrogen supplementation was given only in the luteal phase in combination with progesterone luteal support. The duration of this regimen could be too short at that stage and it might be too late for the endometrium to be primed by oestrogen for the induction of progesterone receptors.[17-19] It also may be too late to overcome the oestrogen decline in the luteal phase and even cause luteolysis of the corpus luteum, possibly by initiating local prostaglandin overproduction.[19] The use of oestrogen supplementation in the luteal phase could be beneficial in a selected group of patients with low oestradiol levels before hCG administration,[20,21] or in a group where the use of hCG is contraindicated because of increased risk of ovarian hyperstimulation syndrome.[14]

When specifically using CC for ovulation induction, as in this study, oestrogen could counter the hypo-oestrogenic effect of this drug.[22] Elkind-Hirsch and co-workers[23] concluded that oestrogen supplementation at the beginning of the cycle, combined with progesterone supplementation in the luteal phase improved and normalised the alterations in endometrial morphology caused by CC. A study by Hurd *et al*.[9] investigated the use of oestrogen support versus no support in a CC-stimulated protocol for IVF-ET. They also found a significantly higher pregnancy rate per retrieval for the oestrogen and progesterone support group.

In conclusion, our study supports the hypothesis that oestrogen supplementation could play an important role in ART. Although we did not find a significant difference in pregnancy outcome one has to take note of the clinical trend that was seen in favour of oestrogen supplementation. There are not enough studies in the literature to support the concept of administrating oestrogen throughout the whole cycle in ART. More studies in this area are needed to clarify these findings. In future our study as well as that of Jung and Roh,[16] and hopefully other studies will shed more light on this important subject if a meta-analysis is attempted.

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