Endometrial preparation for *in vitro* oocyte maturation: Early use of estrogen increases endometrial tissue and requires lower daily dosage: A cross over trial in ‘mock’ cycles

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Abstract *Purpose:* Determine if estrogen used since the beginning of the menstrual flow could improve endometrial tissue compared to standard endometrial preparation for *in vitro* maturation cycles.

Methods: Twenty polycystic ovary syndrome women were submitted to two estrogen therapy schedules: standard schedule; estrogen began on the day of planned egg retrieval (dosage was based on endometrial thickness); and long schedule; estrogen began on the first day of menstruation. No oocyte retrieval or embryo transfer was performed. Three-dimensional ultrasound was performed on the day of planned egg retrieval and one week later for endometrial evaluation.

Results: A higher endometrial thickness and volume was found in long schedule on both evaluations. The number of patients that have used 10 mg/day of estradiol was significant higher in the standard schedule (65% × 0%). No other significant difference was found.

Conclusions: The early use of estrogen improves endometrial tissue and requires lower daily dosage.

Keywords Endometrium · *In vitro* maturation of oocytes · Polycystic ovary syndrome · Ultrasonography

Introduction

In vitro fertilization (IVF) and embryo transfer are an established and successful form of treatment for infertility, with continuously improving cumulative pregnancy rates reaching 57.8% before age of 40, after 3 cycles of IVF (1). However, high success rates with IVF are achieved at the risk of 2 major complications, namely, multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). All assisted conception techniques are associated with an increase in the rate of multiple pregnancies because several embryos must be transferred to achieve high pregnancy rates. Moreover, to have several embryos available for transfer, ovarian stimulation is required, which places the patient at risk for the development of OHSS. Although there are many strategies to prevent and predict OHSS, none is universally successful. The only way to prevent OHSS reliably would be to avoid stimulating the ovaries. Because stimulated ovaries are not necessary for treatment by *in vitro* maturation (IVM) of immature oocytes, this technique has received increased attention in recent years (2, 3).

During IVM treatment, immature oocytes are retrieved transvaginally from unstimulated ovaries, matured *in vitro* for 24–48 h and then fertilized. Normally one to four embryos is transferred to the uterus 2–3 days later (4). The absence of ovarian stimulation in IVM treatment has a number of advantages, including reduction in cost and medical risks, particularly the risk of OHSS. The majority of IVM pregnancies have been reported in women with polycystic ovarian syndrome (PCOS), and pregnancy rates for women with normal ovaries appear to be lower (5). This may be related to the number of antral follicles from which immature oocytes are retrieved, which are fewer in normal than in polycystic ovaries.

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However, gonadotropin ovarian stimulation provides higher plasma levels of estrogen and consequently better endometrial preparation for embryo implantation. This fact led us to propose that the quality of the endometrial preparation may be the cause of the difference in implantation rate described for normal IVF – 35% (6) and IVM – 10%–15% (7, 8) with no evident difference between oocytes retrieved, fertilization rate, and number or quality of the embryos transferred. The only difference noted was the endometrial thickness – 5.5 mm in IVM cycles (8) against 10.6 mm in normal IVF (9).

Thus we have questioned the efficacy of the estrogen therapy used in IVM cycles for endometrial preparation that usually begins on the day of oocyte retrieval (7, 8). Our hypothesis was that estradiol used since the beginning of the menstrual flow would be able to improve endometrial thickness and volume without loss on ovarian follicle count, and then planned to test this alternative estrogen therapy versus the standard one in ‘mock’ cycles.

Methods

Sample size

Endometrial thickness of 5.6 ± 1.3 mm (mean $\pm$ standard deviation) in IVM cycles (8) was used to determine sample size. Using $\alpha = 0.05$ and a power of 80%, a difference of 20% (1.2 mm) would be detected with 20 patients per group.

Patients

Twenty patients were selected with a confirmed diagnosis of PCOS (10). PCOS should meet at least two of the following three criteria: 1. oligo- or anovulation; 2. clinical and/or biochemical signs of hyperandrogenism; 3. polycystic ovaries on ultrasound (12 or more follicles in one ovary or ovarian volume over 10 mL). Other etiologies for hyperandrogenism (such as congenital adrenal hyperplasia, hypothyroidism, Cushing’s syndrome or androgen-secreting tumors) were excluded.

The selected patients had no previous disease and did not use any drugs during the three months preceding the study, which was conducted from August to December of 2005. These patients were under treatment for infertility and entered the study as volunteers. The study was approved by the Research Ethics Committee of the *Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo* and written informed consent was given by all subjects. These women were randomized in two groups of ten patients each (Group A and B).

Estrogen therapy

Before starting estrogen therapy, the twenty patients used medroxy-progesterone acetate, 10 mg/day, for 5 days to induce menstrual flow. In the first month the Group A was submitted to the standard schedule of estrogen therapy–estradiol valerate beginning on the day of planned egg retrieval (DPER) that was defined as 14 days after the beginning of the menstrual flow. The dosage of estradiol valerate was based on endometrial thickness: 6 mg/day if ≥ 5 mm and 10 mg/day if < 5 mm. Dehydrogesterone, 30 mg/day was used beginning 2 days after DPER (day of planned intracytoplasmic sperm injections). The Group B was submitted to the long schedule of estrogen therapy, a sequentially increasing dosage of estradiol valerate beginning on the first day of menstrual flow (2 mg/day for 4 days, 4 mg/day for 4 days and 6 mg/day until DPER and then as the standard schedule) and dehydrogesterone 30 mg/day beginning 2 days after DPER (Fig. 1). All the patients stopped the use of estradiol valerate and of dehydrogesterone one week after DPER.

After 60 days, the twenty patients used medroxy-progesterone acetate, 10 mg/day, for 5 days to promote a new menstrual flow. Then, the Group A was submitted to the long schedule and the Group B was submitted to the standard schedule.

Three-dimensional vaginal ultrasound scans (3DUS) were performed on DPER and one week later. No oocyte retrieval, fertilization or embryo transfer was done during this period (‘mock’ cycles).

Ultrasonography

All 3D scans were obtained with the Sonoacer™ 9900 apparatus (Medison Co. Ltd.), using an 8–5 MHz endovaginal

Fig. 1 Standard and long schedules for endometrial preparation. The use of estrogen of the standard schedule is based on French description for IVM endometrial preparation (7)

probe, by a single operator (WPM). First, the uterus was visualized in B mode and measurements of length, height and width were performed. The system was then switched to volume mode. After targeting the endometrium on a vertical plane by using the volume box, the slow volume acquisition setting was activated. The 3D dataset was then acquired using the slow sweep mode. Volume data were captured by holding the transducer stationary while its crystal electronically swept up to 80° for 2–3 s. The same procedure was performed to obtain a 3D dataset for each ovary. The color Doppler mode was then activated and the right uterine artery was identified. The spectral Doppler mode was enabled and a box was placed in the middle of this artery to obtain the velocity wave of blood flow. Automatic evaluation was used to determine the pulsatility index (PI) and resistance index (RI) of this vessel. The same procedure was applied to the left uterine artery and to the subendometrial arteries (arteries located in the myometrium at a distance of less than 5 mm from the endometrial transition) (11).

$PI = (\text{Peak systolic velocity} - \text{end diastolic velocity}) / \text{time-averaged maximum velocity}$ (12)

$RI = (\text{Peak systolic velocity} - \text{end diastolic velocity}) / \text{peak systolic velocity}$ (13)

All data were stored on a hard disk and CDs, and all measurements were made soon after the end of the exam by the same observer (WPM). Endometrial thickness was measured from the central part of the endometrium using the multiplanar display by placing the calipers on the myometrial–endometrial transition. Each ovary 3D dataset was analyzed in terms of follicular count and follicles were divided according to size (diameter of 2–9 mm and diameter >9 mm). VOCAL™ (Virtual Organ Computer-aided Analysis), which is an extension of the 3D-VIEW™ software (Kretz Technik, Zipf, Austria), was used to measure the endometrial and ovarian volume in the manual mode using 15° of rotation, described as a step rotation with good reproducibility (14). For these rotational measurements, two calipers were placed on the upper and lower aspects of the endometrial cavity or on the ovarian edges. When the calipers were judged to be correctly positioned, the degree of rotation was determined by selecting the rotation angle and the manual mode of measurement was entered. The borders were then traced in a clockwise fashion using a track ball until completion of 180° of rotation and the generation of a calculated volume (12 steps).

Side effects

Weight gain was evaluated by weighing the patients on DPER and one week after on both schedules. Breast pain, headache and nausea were evaluated by applying a questionnaire on

DPER and one week later on the two schedules, with the patients describing these symptoms as absent, mild (patient feel this symptom but it did not annoy her), moderate (the side effect annoyed her but did not interfere with her activities) and intense (the side effect have interfered with her activities).

Statistical analysis

Data were analyzed statistically using the GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, California, USA). Data regarding endometrial volume and thickness, ovarian follicle count, uterine volume, ovarian volume, PI and RI of uterine and subendometrial arteries, and weight were analyzed by the Kolmogorov-Smirnov test to determine the normality of distribution. The paired *t* test was applied to compare the two schedules both on DPER and one week later. The proportion of patients who reached a minimum satisfactory endometrial thickness (8.0 mm) and volume (2.5 mL) one week after DPER and the proportion of patients who used 6 mg or 10 mg of estradiol valerate after DPER of both schedules were analyzed by Fisher's exact test. It was given numeric values to the side effects: 0 if absent; 1 if mild, 2 if moderate and 3 if intense. Then we have compared the side effects of the two estrogen therapies by Wicoxon matched pairs test. A *p* value <0.05 was considered to be statistically significant.

Results

We found that the use of estrogen since the beginning of the menstrual flow (long schedule) promoted an increase in endometrial thickness and volume both on DPER and one week later, as shown in Fig. 2.

No dominant follicle (>9 mm) was found. The ovarian follicular count did not differ significantly on both days (power of 90% to detect a difference of 4 follicles) as did not all the other variables analyzed by the paired *t* test, as shown in Table 1.

No significant difference was noted in the potential side effects of estrogen therapy (breast pain, headache and nausea) both on DPER and one week later analyzed by Wicoxon matched pairs test, although on DPER of the standard schedule the patients were not using estrogen.

After DPER, thirteen subjects were using a dosage of 10 mg/day of estradiol on the standard schedule, as opposed to none on the long schedule (65% standard schedule *versus* 0% long schedule; *p* < 0.0001 by Fisher's exact test). The others were using 6 mg/day due to an endometrial thickness ≥5 mm on DPER.

There was a significant difference in the number of subjects that reached the minimum endometrial thickness and

Fig. 2 Endometrial thickness and volume on day 14 and 21 of standard and alternative estrogen therapies used for endometrial preparation. Transversal bar represents the mean. Value of p was obtained by paired t test

volume described as satisfactory for embryo implantation - endometrial thickness of 8 mm (9) and endometrial volume of 2.5 mL (15): 3 patients (15%) on standard schedule *versus* 20 patients (100%) on long schedule; $p < 0.0001$ by Fisher's exact test.

Discussion

The process of embryo implantation in the uterus, although not entirely defined, seems to be determined by embryo quality, endometrial receptivity and synchronization between embryonic and endometrial development. In natural cycles, following fertilization in the fallopian tube 24–48 h after ovulation, the zygote migrates through the fallopian tube until it reaches the uterine cavity at the morula stage four days after ovulation (16). On the next day, the blastocyst form, sheds its zona pellucida, superficially apposes, and adheres to the endometrium. Although the initial apposition is

unstable, adhesion involves increased physical interactions between embryo and uterine epithelium (17). Implantation occurs only during the “window of implantation” (18) that is temporally confined to days 20–24 of a normal ovulatory cycle. Implicit in successful implantation is the concept of endometrial receptivity, which has been defined as “the temporally and spatially unique set of circumstances that allow for successful implantation of the embryo” (19). Thus, a potential mean of improving the implantation rate in IVF involves the evaluation and potential manipulation of endometrial receptivity which is under hormonal regulation.

In normal IVF cycles there is endogenous production of estradiol by the ovaries due to exogenous gonadotropin stimulation and only progesterone supplementation is needed. But in endometrium preparation for egg donation and IVM there is a need for estrogen supplementation to improve endometrial receptivity. An adequate period of estrogen administration is essential to achieve a normal secretory

Table 1 Results obtained on day of planned egg retrieval (DPER) and one week later (OWL) of the two estrogen schedules

	Standard DPER	Long DPER	Standard OWL	Long OWL
End thickness (mm)	5.11 ± 1.50*	9.96 ± 2.22*	7.41 ± 2.24*	10.52 ± 2.43*
End volume (mL)	1.31 ± 0.58*	3.32 ± 0.83*	2.08 ± 0.53*	3.55 ± 0.89*
R Ovarian FC	13.95 ± 4.22	13.90 ± 4.88	13.85 ± 4.31	14.0 ± 5.15
L Ovarian FC	13.85 ± 4.44	14.2 ± 3.89	13.55 ± 3.32	13.5 ± 3.89
Uterine Volume (mL)	51.3 ± 17.7	51.9 ± 16.8	52.1 ± 17.4	53.6 ± 17.12
R ovarian volume (mL)	14.2 ± 2.76	14.1 ± 2.20	14.2 ± 2.70	14.0 ± 2.73
L ovarian volume (mL)	13.5 ± 2.72	14.0 ± 3.93	13.5 ± 2.73	13.3 ± 3.41
R uterine artery RI	0.81 ± 0.08	0.83 ± 0.07	0.81 ± 0.08	0.83 ± 0.07
R uterine artery PI	2.26 ± 0.63	2.38 ± 0.55	2.22 ± 0.83	2.31 ± 0.70
L uterine artery RI	0.82 ± 0.07	0.82 ± 0.08	0.80 ± 0.08	0.81 ± 0.09
L uterine artery PI	2.29 ± 0.61	2.35 ± 0.66	2.20 ± 0.81	2.34 ± 0.79
Sub-End artery RI	0.64 ± 0.09	0.65 ± 0.07	0.66 ± 0.06	0.63 ± 0.06
Sub-End artery PI	0.99 ± 0.26	1.03 ± 0.19	1.06 ± 0.27	1.0 ± 0.14
Weight (kg)	66.2 ± 8.85	66.4 ± 8.85	66.85 ± 8.59	67.05 ± 8.62

Note. Data are given as mean ± SD; FC = follicular count; End = endometrial; R = right; L = left.

* $p < 0.001$ (paired t test)

endometrium that facilitates implantation. It has been demonstrated that short (6 days) (20) or prolonged (5 weeks) (21, 22) exposure of the endometrium to estrogen allows normal endometrial maturation and permits great flexibility for endometrial synchronization. On the other hand, lower serum estradiol concentrations and higher early abortion rates were observed in patients after short estrogen exposure (5–10 days) (23).

The results of our study demonstrate that a very short (2 days) estrogen therapy habitually used for endometrial preparation in IVM before the use of progesterone (3, 4, 7) was not able to increase the endometrial thickness and volume as did a longer period. In our study, one week after the day of planned egg retrieval (probably 1 or 2 days after embryo transfer) only 15% (3 patients) of the women submitted to the standard schedule achieved 8 mm of endometrial thickness and 2.5 mL of endometrial volume, as opposed to 100% (20 patients) in the long schedule. Kovacs *et al.* (9) analyzed 402 pregnancies in 1228 IVF/ICSI and found that only 5 occurred with an endometrial thickness <8 mm. Yaman *et al.* (15) demonstrated that “Pregnancy rate in patients with an endometrial volume >2.5 mL was significantly higher than that in patients with an endometrial volume <2.5 mL”. Although many centers performing IVM give hCG prior to immature oocyte retrieval (4, 7) with a probable effect on the receptivity of the endometrium since hCG induces morphological and functional decidualization of the endometrium (23, 24), this was not evaluated in our study since we were not testing endometrial receptivity but only the effect of a longer estrogen therapy on endometrial thickness and volume.

Our study also demonstrated that estrogens used since the beginning of menstrual flow did not reduce the ovarian follicle count or ovarian volume, events that would be deleterious for oocyte retrieval. Another advantage of early estradiol administration is a reduction in the estradiol valerate dosage. In the long schedule the endometrium was already developed on DPER and so no patient used 10 mg/day estradiol valerate, as opposed to 65% (13 patients) of the women on the standard schedule. This is of great importance since these patients usually maintain this dosage until the 12th week of pregnancy (7). Nevertheless, more studies are needed to prove that the early use of estradiol improves endometrial receptivity and implantation rate and does not interfere with oocyte retrieval.

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