Journal of Assisted Reproduction and Genetics, Vol. 20, No. 12, December 2003 (© 2003)

Assisted Reproduction

Effects of Vaginal Progesterone Administration Starting on the Day of Oocyte Retrieval on Pregnancy Rates

Ricardo Baruffi,[1] Ana Lucia Mauri,[1] Claudia Guilhermino Petersen,[1] Valéria Felipe,[1] and José Gonçalves Franco Jr[1,2,3]

Submitted October 18, 2003; accepted October 21, 2003

***Purpose*:** Vaginal progesterone administration starting on the day of oocyte retrieval induced a decrease in uterine contraction frequency on the day of embryo transfer (ET) as compared with preovulatory values. Uterine relaxation before ET is likely to improve outcome by avoiding displacement of the embryo from the uterine cavity (Fanchin, Righini, de Ziegler, Oliviennes, Ledée, Frydman: Fertil Steril 2001;75:1136–1140). The objective of the present study was to determine whether the early use of vaginal progesterone on the day of oocyte retrieval may alter the embryo implantation and pregnancy rates.

***Methods*:** A total of 103 patients were submitted for ovarian stimulation with GnRH-a and recombinant FSH (Puregon, Organon) for the application of invasive assisted reproduction techniques (ICSI). The patients were divided into two groups in a prospective and randomized manner: Group A ($n = 51$) where application of vaginal progesterone started (Utrogestan, Besins International) at the dose of 400 mg from the evening of the day of oocyte retrieval, and Group B ($n = 52$) started to apply vaginal progesterone at the same dose but from the evening of embryo transfer (2nd day).

***Results*:** The age of Group A patients (34.2 ± 4.6) was similar ($p = 0.50$) to that of Group B patients (34.8 ± 4.9). The number of oocytes retrieved and at metaphase II from Group A patients (10.6 ± 6.9 and 7.8 ± 6.0; respectively) did not differ significantly ($p = 0.84$ and $p = 0.49$, respectively) from the number of oocytes retrieved and metaphase II from Group B patients (10 ± 5.6 and 6.7 ± 4.7, respectively). Also, there was no difference ($p = 0.48$) in number of embryos transferred to Group A patients (2.7 ± 0.8) versus Group B patients (2.7 ± 0.9). Embryo implantation and pregnancy rates for Group A patients (12.6 and 27.4%, respectively) were equal ($p = 0.98$ and $p = 1.0$, respectively) to those for Group B patients (13.4 and 28.8%, respectively).

***Conclusion*:** Vaginal progesterone at the dose of 400 mg started on the day of oocyte retrieval did not increase implantation or pregnancy rates when compared to the same dose started on the day of embryo transfer.

KEY WORDS: ICSI; implantation; progesterone; uterine contraction.

INTRODUCTION

A luteal-phase deficiency in IVF-ET cycles may result either from the use of GnRH agonists for pituitary down-regulation, leading to prolonged LH suppression, or from poor progesterone (P) production after granulosa cell removal during multiple follicular aspiration (1). Luteal phase support in stimulated IVF

[1] Center for Human Reproduction, Sinhá Junqueira Maternity Foundation, Ribeirão Preto, Sãu Paulo, Brazil.
[2] Department of Obstetrics and Gynecology, University of Ribeirão Preto, (UNAERP), Ribeirão Preto, Sãu Paulo, Brazil.
[3] To whom correspondence should be addressed at Rua D. Alberto Gonçalves, 1500-CEP, 14085-100 Ribeirão Preto, Sãu Paulo, Brazil; e-mail : crh@crh.com.br and franco@crh.com.br.

cycles is well accepted and widely used. Despite its widespread use, there is no consensus with regard to the ideal agent or protocol of administration. For those patients who are given luteal support with P, the type of preparation used varies between parenteral, oral, vaginal suppository, and vaginal gel formulations.

In the other hand, it was demonstrated that that early P supplementation before oocyte aspiration resulted in a lower pregnancy rate compared with the case of P support beginning the evening of oocyte retrieval (2). Thus, although P support clearly is benefical, the timing of initiation of this treatment can affect overall outcome.

In 1998, Fanchin *et al.* (3) observed that increased uterine contractility (UC) at the time of ET adversely affected embryo implantation and pregnancy rates in IVF. In this study, women who displayed more than 5 contractions/min had markedly lower implantation and pregnancy rates per embryo transfer (4% and 14%, respectively) than those who had less than or equal to 3 contractions/min (21% and 53%, respectively), probably because of the mechanical expulsion of embryos from the uterine cavity.

More recently, the same group (4) observed that the vaginal P administration starting 2 days before ET induces a significant reduction in uterine contraction frequency at the time of ET and this uterine relaxation may propitiate embryo permanence in the endometrial cavity and therefore assist implantation.

The objective of this study was to determine whether the early use of vaginal progesterone on the day of oocyte retrieval may alter the embryo implantation and pregnancy rates.

MATERIAL AND METHODS

A total of 103 patients were submitted to ovarian stimulation with GnRH-a and recombinant FSH (Puregon, Organon) for the application of invasive assisted reproduction techniques (FIV/ICSI). Ovarian stimulation before the ICSI procedure consisted of blockade of the 2nd phase with nafarelin acetate at the dose of 400 μg/day (Synarel, Searle). After 14 days of treatment with the analogue and the establishment of blockade, administration of recombinant FSH (Puregon, Organon) was started at a fixed dose of 150–300 IU for a period of 7 days. On the eighth day of ovarian stimulation, we started monitoring follicular development only by vaginal ultrasound and the doses of FSH were adapted to the ovarian response. When at least three follicles measuring $\geq$17 mm in diameter were observed, hCG was administered at the dose of 5000–10,000 IU (5).

Oocytes were collected from the follicles by ultrasound-guided transvaginal puncture 34–36 h after hCG. After identification in follicular fluid, the oocytes were classified according to maturity. The cumulus–corona complex was removed by exposure to a hyaluronidase type IV solution (H-4272, Sigma Chemical Co., USA) at the concentration of 40 IU/ml. The denuded oocytes were incubated in IVF-50 medium (Scandinavian IVF Science AB, Sweden) until the time for ICSI. A discontinuous gradient of Sperm-Prep-100TM (Scandinavian IVF Science AB, Sweden) was used for separation of spermatozoa from seminal fluid in the 40 and 90% fractions.

ICSI was performed under an inverted Eclipse TE300 microscope equipped with a Hoffman lens system and coupled to automatic micromanipulators and injectors. A 10% polyvinylpyrrolidine solution (PVP-ICSI-100, Scandinavian IVF Science AB, Sweden) diluted in IVF-50 was used to immobilize the spermatozoa. The ICSI procedure was performed according to the technique described by Svalander *et al.* (6).

The patients were then divided into two groups in a prospective and randomized manner on the day of oocyte retrieval, by drawing lots, using a randomization table previously elaborated for the study, Group A ($n = 51$) started application of vaginal progesterone (Utrogestan, Besins International) at the dose of 400 mg from the evening of the day of oocyte retrieval, and Group B ($n = 52$) started to apply vaginal progesterone at the same dose but from the evening of embryo transfer (2nd day). The pregnancy test was performed on the 14th day after embryo transfer and clinical pregnancy was confirmed during the 6th week by the presence of a gestational sac and embryo with a heart beat. Data were analyzed statistically by the Student's t test, Mann–Whitney, and Fisher exact tests.

RESULTS

The age of Group A patients (34.2 ± 4.6) was similar ($p = 0.50$) to that of Group B patients (34.8 ± 4.9). The number of oocytes retrieved and at metaphase II from Group A patients (10.6 ± 6.9 and 7.8 ± 6.0; respectively) did not differ significantly ($p = 0.84$ and $p = 0.49$, respectively) from the number of oocytes retrieved and metaphase II from Group B patients (10 ± 5.6 and 6.7 ± 4.7, respectively). Also, there was

Table I. Clinical and Laboratory Results

	Group A	Group B	*p*
Cycles	51	52	
Age	34.2 ± 4.6	34.8 ± 4.9	0.50
Retrieved oocytes	10.6 ± 6.9	10.0 ± 5.6	0.84
Oocytes in metaphase II	7.8 ± 6.0	6.7 ± 4.7	0.49
Embryos transferred	2.7 ± 0.8	2.7 ± 0.9	0.48
Implantation rate	12.6%	13.4%	0.98
Pregnancy rate/ transfer	27.4%	28.8%	1.00

no difference ($p = 0.48$) in number of embryos transferred to Group A patients (2.7 ± 0.8) versus Group B patients (2.7 ± 0.9). Embryo implantation and pregnancy rates for Group A patients (12.6% and 27.4%, respectively) were equal ($p = 0.98$ and $p = 1.0$, respectively) to those for Group B patients (13.4% and 28.8%, respectively) (Table I).

DISCUSSION

It has been demonstrated that myometrial contractile activity influences embryo implantation, possibly through mechanical displacement of embryos, in both animals and humans (3). In the present study, the authors have shown that a considerable fraction of IVF–embryo transfer patients have persistently high uterine contraction frequency at the time of noncavitating embryo transfer, 4 days after HCG administration. Moreover, the observation of high-frequency uterine contractions (>5.0 contractions/min) at the time of embryo transfer is associated with markedly lower implantation and pregnancy rates per embryo transfer (4 and 14% respectively) as compared to cases with low-frequency contractions (3.0 contractions/min; 21 and 53% respectively). This may result in the mechanical expulsion of embryos from the uterine cavity.

As UC are postulated to be hormone-regulated, in 2000 Fanchin *et al.* (7) investigated the role of plasma oestradiol and progesterone concentrations on UC during ovarian stimulation for IVF. A total of 59 women were studied on the day of administration of HCG and embryo transfer. Plasma oestradiol and progesterone concentrations were measured, and 5 min ultrasound scans of the uterus were digitized with an image analysis system to assess UC frequency and direction. Cycles were sorted according to whether progesterone concentrations on the day of embryo transfer were ≤100 ($n = 34$) or >100 ($n = 25$) ng/mL. On the day of HCG, UC frequency was similar in both groups at 4.5 ± 0.2 and 4.6 ± 0.3 UC/min (mean ± SE) respectively. On the day of embryo transfer, UC frequency remained steady in the low progesterone group, whereas it decreased (3.5 ± 0.2 UC/min) in the high progesterone group ($p < 0.001$), and was negatively correlated with progesterone concentrations ($r = -0.56$; $p < 0.001$). No influence of oestradiol on UC was noticed. These observations confirm the utero-relaxing effects of progesterone in the nonpregnant uterus and could support the administration of progesterone before embryo transfer to increase tissue concentrations and improve the outcome of IVF.

More recently, Fanchin *et al.* (4) investigated whether uterine contractility at the time of ET can be reduced by early onset of luteal support with progesterone administered vaginally. Eighty-four women undergoing 84 GnRH-a and FSH/hCG cycles for IVF-ET were studied. Vaginal progesterone was randomly started on the day of oocyte retrieval (Group A, $n = 43$) or on the evening of ET (Group B, $n = 41$). On the day of hCG administration and just before ET, 2-min sagittal uterine scans were obtained by ultrasound and digitized with an image analysis system for assessing uterine contraction frequency. Whereas uterine contraction frequency was similar in both groups on the day of hCG (4.6 ± 0.3 and 4.5 ± 0.3 contractions/min, respectively), only women in Group A showed decreased uterine contraction frequency on the day of ET (2.8 ± 0.2 vs. 4.2 ± 0.3 contractions/min). The authors concluded that the vaginal progesterone administration starting on the day of oocyte retrieval induced a decrease in uterine contraction frequency on the day of ET as compared with preovulatory values. Uterine relaxation before ET is likely to improve IVF-ET outcome by avoiding the displacement of embryos from the uterine cavity.

In our study we want to determine whether the early use of vaginal progesterone on the day of oocyte retrieval may alter the outcome of ICSI program by reduction of UC. However, embryo implantation and pregnancy rates for those patients beginning the P from the evening of oocyte retrieval (12.6 and 27.4%, respectively) were equal ($p = 0.98$ and $p = 1.0$, respectively) to those beginning the P from the evening of embryo transfer (13.4 and 28.8%, respectively). However, the acceptance of a H_0 hypothesis (no difference between groups) when the hypothesis in reality is false would induce a so called type II error (β-error) and to avoid this type of error it is advisable to increase the number of patients studied. To detect a difference in the pregnancy rates between the two treatment groups (keeping the current rates in both

groups), with a power of 80% and significance level of 5%, a total of 16.330 of each group were required.

In conclusion, vaginal progesterone at the dose of 400 mg started on the day of oocyte retrieval did not increase implantation or pregnancy rates when compared to the same dose started on the day of embryo transfer.

REFERENCES

1. Chnatilis SJ, Zeitoun KM, Patel SI, Johns DA, Madziar VA, McIntire DD: Use of crinone vaginal progesterone gel for luteal support in in vitro fertilization cycles. Fertil Steril 1999;72:823–829
2. Sohn SH, Penzias AS, Emmi AM, Dukey AK, Layman LC, Reindollar RH: Administration of progesterone before oocyte retrieval negatively affects the implantation rate. Fertil Steril 1999;71:11–14
3. Fanchin R, Righini C, Oliviennes F, Taylor S, de Ziegler D, Frydman R: Uterine contractions at the time of embryo transfer alter pregnancy rates after in vitro fertilization. Hum Reprod 1998;13:1968–1974
4. Fanchin R, Righini C, de Ziegler D, Oliviennes F, Ledée N, Frydman R: Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. Fertil Steril 2001;75:1136–1140
5. Franco JG Jr, Baruffi RLR, Coelho J *et al*: Prospective randomized comparison of ovarian blockade with nafarelin versus leuprolide during ovarian stimulation with recombinant FSH in an ICSI program. J Assist Reprod Genet 2001;18:593–597
6. Svalander P, Forsberg AS, Jakobsson AH, Wikland M: Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995;63:828–837
7. Fanchin R, Ayoubi JM, Olivennes F, Righini C, de Ziegler D, Frydman R: Hormonal influence on the uterine contractility during ovarian stimulation. Hum Reprod 2000;15(Suppl 1):90–100