

GnRH antagonists followed by a decline in serum estradiol results in adverse outcomes in donor oocyte cycles

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BACKGROUND: The aim of this retrospective study was to assess clinical outcomes using GnRH antagonists in oocyte donation cycles. **METHODS:** Between July 2000 and June 2001, 40 recipient cycles generated from donor oocytes were evaluated. Controlled ovarian hyperstimulation (COH) was started on cycle day 2 using recombinant gonadotrophins (225 IU daily). GnRH antagonist was started on cycle day 6 of COH. All recipients were synchronized to donors using GnRH agonist followed by estrogen and progesterone supplementation. Main outcome measures were days of stimulation (DOS), number of ampoules used, peak serum estradiol, number of oocytes, fertilization rate, embryo score, clinical on-going pregnancy rate and implantation rate. **RESULTS:** Thirty-seven donor cycles (93%) underwent oocyte retrieval, resulting in 36 embryo transfers. Fourteen cycles (35%) had decreased serum estradiol after initiation of GnRH antagonist. No differences were seen in numbers of FSH ampoules, DOS, peak serum estradiol, number of retrieved oocytes, fertilization rate and embryo quality. However, clinical pregnancy rate per initiated cycle [14% (2/14) versus 54% (14/26)], ongoing pregnancy rate per initiated cycle [7% (1/14) versus 46% (12/26)] and implantation rate (4 versus 24%) were all significantly less ($P < 0.05$) following a decrease in serum estradiol after initiation of GnRH antagonist. No clinical predictor, including donor age, basal day 2 FSH or estradiol, ovarian morphology or serum estradiol prior to GnRH antagonist, was predictive of a decline in serum estradiol following GnRH antagonist. **CONCLUSION:** These data demonstrate an adverse effect on clinical outcome in cycles, resulting in a decline in serum estradiol after GnRH antagonist administration. This effect was unpredictable and provided a simplified protocol for oocyte donation cycles; nonetheless, further study is needed to clarify the adverse effects of GnRH antagonists in oocyte donation cycles.

Key words: controlled ovarian hyperstimulation/GnRH antagonists/IVF/oocyte donation

Introduction

Suppression of the hypothalamic-pituitary axis to prevent a premature LH surge using either GnRH agonists or GnRH antagonists has maximized the outcomes during ovarian hyperstimulation for IVF cycles (Porter *et al.*, 1984; Neveu *et al.*, 1987; Ganirelix Dose Finding Study Group, 1998). The advantages of GnRH antagonists over GnRH agonists include immediate suppression of pituitary gonadotrophins, thereby obviating the prolonged period until pituitary suppression becomes effective. Moreover, gonadotrophin requirements, monitoring costs and the risk of ovarian hyperstimulation syndrome (OHSS) have been reported to be less (de Jong *et al.*, 1998; Ganirelix Dose Finding Study Group, 1998; The North American Ganirelix Study Group, 2001).

However, decreases in serum estradiol, pregnancy rate (PR) and implantation rate (IR) have been reported in GnRH

antagonist-stimulated cycles (Ganirelix Dose Finding Study Group, 1998; Fauser *et al.*, 1999; Felberbaum and Diedrich, 1999), suggesting an adverse effect of GnRH antagonists on either oocyte quality, embryo development or the endometrium. Oocyte donation provides a unique model to eliminate confounding variables that typically occur when comparing groups of patients undergoing IVF. The aim of the present study was to gain further insight and present preliminary results using GnRH antagonists in oocyte donation cycles, and also to assess their impact on clinical outcomes.

Materials and methods

A retrospective analysis was made of all GnRH antagonist cycles undergoing IVF-embryo transfer cycles using donated oocytes.

Subjects

Between July 2000 and June 2001, oocyte donors ($n = 32$) underwent 40 cycles using ovarian hyperstimulation with recombinant FSH (rec-FSH; Follistim; Organon) and GnRH antagonist (Antagon; Organon,

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Inc., West Orange, NJ, USA). The mean (\pm SEM) age of all oocyte donors was 27.7 ± 0.7 (range 21–36) years. All donors had normal cycle day 2–3 serum FSH levels (5.0 ± 0.6 mIU/ml) and serum estradiol levels (33.8 ± 2.8 pg/ml). The recipients [$n = 31$; mean age 43.1 ± 0.7 (range 36–54) years] underwent 40 fresh embryo transfer cycles. Severe male factor ($<1 \times 10^6$ sperm/ml) and hydrosalpinges were excluded from the evaluation, since the latter has been shown previously adversely to affect implantation in donor oocyte cycles (Cohen *et al.*, 1999).

COH protocol

Prior to cycle stimulation, all oocyte donors received 1–2 months treatment with oral contraceptives (Mircette; Organon, Inc.) for cycle synchronization. Following a withdrawal bleed, ovarian stimulation was started on day 2 with three ampoules per day of rec-FSH for the first 4 days of treatment, and adjusted according to ovarian response. From cycle day 6 of stimulation, daily injection of GnRH antagonist (0.25 mg, s.c.) was added. All cycles were monitored using transvaginal ultrasound and serum estradiol levels, starting on cycle day 5 of stimulation. When three or more follicles reached 18–20 mm, hCG was given to trigger ovulation. Transvaginal oocyte aspiration was performed 36 h later, under ultrasound guidance. Recipients were synchronized to an oocyte donor using a regimen of oral micronized estradiol and i.m. progesterone (Sauer *et al.*, 1995).

Following fertilization, embryos were assessed and assigned using a standardized scoring system [cell number \times symmetry (symmetric = 3; slightly asymmetric = 2; asymmetric = 1) \times fragmentation ($<10\%$ = 4; 10–20% = 3; 20–30% = 2; $>30\%$ = 1)]. After 72 h, the embryos were transferred transcervically to the recipient's uterus under ultrasound guidance. Pregnancy was confirmed by serial beta-hCG measurement at 9 and 12 days after embryo transfer.

Table I. IVF-embryo transfer results for oocyte donors receiving GnRH antagonist

Parameter	Value ^a
No. of ampoules	41.1 ± 1.7
Days of stimulation	10.4 ± 0.2
Estradiol on day of hCG (pg/ml)	1719 ± 156
No. of oocytes retrieved	17.3 ± 1.5
FR (%)	71 ± 3.2
No. of embryos transferred	3.9 ± 0.2
Clinical PR per embryo transfer (%)	40 (16/40)
Ongoing PR per embryo transfer (%)	33 (13/40)
IR (%)	17.3 ± 4.0

^aValues are mean \pm SEM.

FR = fertilization rate; IR = implantation rate; PR = pregnancy rate.

Hormone assays

Serum samples were assayed on cycle day 2 for FSH (DPC, Los Angeles, CA, USA) and estradiol (DPC) using CIA Immulite kits. The intra- and inter-assay coefficients of variation (CV) were 5.4 and 8.1% for FSH, and 6.3 and 6.4% for estradiol.

Statistical analysis

Statistical analyses were performed using the SPSS statistical package. A chi-square and a *t*-test were used to assess differences between groups, and stepwise logistic regression was used to assess correlations. A *P*-value < 0.05 was considered statistically significant.

The measured outcomes included dose of gonadotrophins, days of stimulation, serum estradiol levels, number of oocytes, fertilization rate (FR), embryo score, IR and pregnancy rate (PR). Clinical pregnancies were defined as the presence of a fetal heart beat on ultrasonographic examination. Ongoing pregnancies were defined as pregnancies after 12 weeks from the embryo transfer.

Results

Overall, 37 donor cycles underwent oocyte retrieval (93%), and this resulted in 36 (90%) embryo transfers. One cycle was cancelled due to a poor response, and two others surged on the day of retrieval (fluid in cul-de-sac and collapse of dominant follicles). One embryo transfer was not performed due to fluid identified in the uterine cavity on the day of transfer. Numbers of oocytes, FR, numbers of embryos transferred, clinical PR, on-going PR and IR are detailed in Table I. No cases of severe OHSS were encountered.

Following GnRH antagonist administration, 14 donor cycles (35%) had a decrease in serum estradiol prior to hCG administration. Thirteen of these (93%) showed a decrease in serum estradiol at >3 days after GnRH antagonist administration. When compared to cycles without any decline in serum estradiol ($n = 26$), no differences in age, day-2 FSH and estradiol and cycle stimulation characteristics (including the number of ampoules used), days of stimulation, cycle day-5, -7, -8 and peak serum estradiol and number of retrieved oocytes were seen (Table II).

The numbers of cases requiring ICSI, as well as FR, embryo transfer score and the number of embryos transferred and cryopreserved, were similar in both groups after GnRH antagonist treatment (Table III). However, the clinical PR per initiated cycle [14% (2/14) versus 54% (14/26), $P = 0.05$], the

Table II. Comparison of cycle stimulation for oocyte donors receiving GnRH antagonist, and the effect on serum estradiol (E_2)

	Decrease in serum E_2 after GnRH antagonist ($n = 14$)	Normal rise in serum E_2 after GnRH antagonist ($n = 26$)	<i>P</i>
Days of stimulation	10.8 ± 0.4	10.2 ± 0.3	NS
No. of ampoules	44 ± 3.8	39.3 ± 1.9	NS
No. of MII oocytes	16.3 ± 2.8	17.8 ± 1.7	NS
Cycle day-5 serum E_2	350 ± 81	335 ± 39	NS
Cycle day-7 serum E_2	1058 ± 247^a	557 ± 116^b	NS
Cycle day-8 serum E_2	816 ± 186^c	926 ± 98^d	NS
Peak E_2	1515 ± 253	1846 ± 197	NS

Values are mean \pm SEM.

^a $n = 7$; ^b $n = 8$; ^c $n = 7$; ^d $n = 18$.

MI = metaphase II.

Table III. Comparison of recipient outcomes and effect on serum estradiol (E₂) after GnRH antagonist for oocyte donors

	Decrease in serum E ₂ after GnRH antagonist (n = 14)	Normal rise in serum E ₂ after GnRH antagonist (n = 26)	P
ICSI cases (%)	43 (6)	54 (14)	NS
FR (%)	72 ± 5	71 ± 4	NS
Embryo score	246 ± 32	247 ± 24	NS
No. of embryo transfers	3.5 ± 0.4	4.1 ± 0.2	NS
Cases with cryopreservation (%)	36 (5)	46 (12)	NS
No of embryo(s) cryopreserved	9.2 ± 2.0	7.3 ± 1.0	NS
Clinical PR per initiated cycle (%)	14 (2)	54 (14)	0.05
Ongoing PR per initiated cycle (%)	7 (1)	46 (12)	<0.05
IR (%)	4.2 ± 3	24 ± 5	<0.05

Values are mean ± SEM unless otherwise stated.

Values in parentheses are numbers of patients.

FR = fertilization rate; IR = implantation rate; PR = pregnancy rate.

ongoing PR per initiated cycle [7% (1/14) versus 46% (12/26)], and IR (4 ± 3% versus 24 ± 5%) (both $P < 0.05$) were significantly less if the serum estradiol was decreased following the initiation of GnRH antagonist treatment.

In a stepwise regression analysis, age, ovarian morphology, baseline FSH, estradiol, days of stimulation, number of ampoules used and cycle day-5 serum estradiol were shown not to be significant predictors of any estradiol response to GnRH antagonist treatment.

Discussion

The presence of GnRH receptors outside the pituitary has been identified at the level of the ovarian follicle and endometrium (Dekel *et al.*, 1988; Emons *et al.*, 1993). It has been suggested that GnRH antagonists have a role in interacting with these extra-pituitary receptors and may adversely impact on folliculogenesis and embryo development, as reflected by lower serum estradiol concentrations, ongoing PR and IR rates compared with conventional GnRH agonist use (Ganirelix Dose Finding Study Group, 1998; Fauser *et al.*, 1999; Felberbaum and Diedrich, 1999).

To discern the impact on folliculogenesis and endometrial level is difficult in conventional IVF cycles. However, the ovum donation model allows for the study of isolated parameters that may affect outcome, by standardizing for embryo quality and endometrial receptivity. Overall, in the present study, a decrease in serum estradiol, which was seen in almost one-third of the cycles, resulted in a significant reduction in pregnancy outcome following GnRH antagonist treatment. While these adverse effects were not seen with respect to cycle stimulation or embryo quality, it still suggests an adverse effect of GnRH antagonists on folliculogenesis and embryo development that cannot be seen morphologically. As such, the plateau or decrease in serum estradiol in the late follicular phase of some cycles may be the result of over-suppression of LH by GnRH antagonists that, seemingly, is important in oocyte maturation of some—but curiously not all—cycles. The specific role of LH in folliculogenesis and oocyte maturation is unclear. However, it is believed that LH is necessary to stimulate androgen substrate by the theca cells (Adashi, 1996; Gougeon, 1996) and in the late follicular phase

acts in synergy with FSH to support follicular growth (Erickson *et al.*, 1979). It is possible that the use of gonadotrophins with both LH and FSH preparations would eliminate this effect.

The unpredictable effect of GnRH antagonists is puzzling. Decreases in serum estradiol could not be predicted based on parameters such as age, basal FSH/estradiol, ovarian morphology or cycle day-5 estradiol. Interestingly, eight donors underwent a repeat cycle using the same protocol. Two of these women who had a decrease in serum estradiol following GnRH antagonist treatment in their first cycle resulting in no pregnancies did not show this effect in their second cycle, the results being one spontaneous abortion and one ongoing pregnancy. Three donors who had a normal response in their first cycle that resulted in two ongoing pregnancies, showed a decline in their second cycle that resulted in one spontaneous abortion.

The use of GnRH antagonists has facilitated short and simple treatment, and is particularly attractive for oocyte donors where prolonged pituitary suppression and a risk of OHSS are significant issues. However, such use of GnRH antagonists has an unpredictable effect on estradiol production during follicular recruitment that appears adversely to affect pregnancy outcome if a decline in serum estradiol occurs. Further study is needed to clarify the effect of GnRH antagonists on serum estradiol, and how this may impact on pregnancy outcomes.

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