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High doses of gonadotrophin-releasing hormone antagonist in in-vitro fertilization cycles do not adversely affect the outcome of subsequent freeze–thaw cycles

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The clinical application of gonadotrophin-releasing hormone (GnRH) antagonists instead of GnRH agonists, to prevent spontaneous premature luteinizing hormone surge during ovarian stimulation for assisted reproduction treatment has been advocated. A recent, double-blind, dose-finding study, including six dosages of the GnRH antagonist ganirelix, in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (FSH), has indicated that high doses of GnRH antagonist (1 or 2 mg once daily) are associated with a low implantation rate. This follow-up study reports on the pregnancy rate after replacement of cryopreserved embryos obtained in stimulation cycles of the above-mentioned trial. Ovarian stimulation was initiated on day 2 of the cycle, with daily injections of 150 IU recombinant FSH. Ganirelix (0.0625, 0.125, 0.25, 0.5, 1.0 or 2.0 mg) was administered once daily from stimulation day 6 onwards, up to and including the day of human chorionic gonadotrophin. Retrieved oocytes were fertilized by in-vitro fertilization (IVF) or intracytoplasmic sperm injection and a maximum of three fresh embryos was transferred. Excess embryos were frozen, and subsequently used in either natural or programmed cycles. Until June 1998, 11 ongoing pregnancies (12–16 weeks after embryo transfer) were achieved from 46 cycles in which embryos had been first frozen (23.9% per transfer). Six of these 11 patients had been treated with a high dose of ganirelix (1.0 or 2.0 mg) during the IVF cycles in which the embryos were obtained. In conclusion, our data suggest that high dosages of ganirelix do not adversely affect the potential of embryos to establish clinical pregnancy in freeze–thaw cycles.

Key words: ganirelix/GnRH antagonist/ovarian stimulation/pregnancy/recombinant human FSH

Introduction

The major threat of using only gonadotrophins for ovarian stimulation in assisted reproduction treatment is the threat of a spontaneous premature luteinizing hormone (LH) surge. Currently, most in-vitro fertilization (IVF) centres use pituitary down-regulation with gonadotrophin-releasing hormone (GnRH) agonists to prevent premature luteinization; however, pituitary desensitization requires at least 7–14 days of GnRH agonist pretreatment and results in low endogenous follicle stimulating hormone (FSH) and LH, excessive amounts of exogenous gonadotrophins, and extended duration of stimulation (Smitz *et al.*, 1992). A more rational approach would be to use GnRH antagonists, which cause an immediate blockage of the GnRH receptors on the pituitary gonadotrophs and thus treatment with the antagonist can be limited to those days when oestradiol concentrations approach the 'danger zone' for inducing an LH surge (Itskovitz-Eldor *et al.*, 1998).

Clinical experience with GnRH antagonists in IVF treatment thus far has been encouraging and demonstrates a high efficacy in preventing the LH surge. In most cases, treatment is limited to only a few days, sometimes only once in 48–72 h (Olivennes *et al.*, 1994, 1995; Nelson *et al.*, 1995; Albano *et al.*, 1996). A recent, multi-centre dose-finding study using six dosages (range 0.0625–2.0 mg) of the GnRH antagonist ganirelix in 333 patients indicated that 0.25 mg of ganirelix is the minimal effective daily dose. This dose was not only effective in preventing a premature LH rise ($\geqslant$10 IU/l), but also yielded a high implantation rate of 22% and ongoing pregnancy rate of 33% per attempt. Importantly, the six dose groups were similar in terms of the number of oocytes retrieved, as well as in the number of embryos obtained and their quality. However, very low implantation rates of respectively 8.8 and 1.5% were obtained in the two highest dose groups (1 and 2 mg), which resulted in an ongoing pregnancy rate of only 14.1% in the 1 mg group and no ongoing pregnancies in the 2 mg group (Ganirelix Dose-Finding Study Group, 1998).

The current follow-up study reports on the outcome of freeze–thaw cycles using embryos cryopreserved in stimulation cycles using recombinant FSH and ganirelix during the above-mentioned dose-finding study. These data may shed new light on the cause(s) for the less favourable results achieved in the fresh cycles with high GnRH antagonist doses.

Materials and methods

The data presented in this report were collected from subjects who participated in a multi-centre, randomized, double-blind, dose-finding study of the GnRH antagonist ganirelix (Orgalutran®, NV Organon, Oss, The Netherlands). Six dose groups were included (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 mg). A full account of inclusion and exclusion criteria, as well as the study design and outcome was recently published (Ganirelix Dose-Finding Study Group, 1998). Briefly, the

Table I. Quality of thawed embryos and their outcome after replacement

	Original ganirelix dose group (mg)						
	0.0625	0.125	0.25	0.5	1.0	2.0	Overall
Total number of frozen embryo cycles	7	8	8	7	17	11	58
Number of first frozen embryo cycles	5	8	7	7	11	8	46
Natural cycles (%)	80	50	71	86	73	75	72
Programmed cycles (%)	20	50	29	14	27	25	28
Outcome first cycles							
Good quality embryos after thawing (mean ± SD)	1.8 ±1.3	1.9 ±1.4	1.9 ±1.6	2.4 ±1.3	1.6 ±0.8	1.8 ±1.2	1.9 ±1.2
Number of embryos transferred (mean ± SD)	2.6 ±1.3	2.8 ±1.3	1.9 ±1.3	2.9 ±1.3	2.2 ±1.3	2.8 ±1.3	2.6 ±1.3
Number of miscarriages	0	0	1	1	0	0	2
Number of ongoing pregnancies	0	3	1	1	4	2	11
Ongoing pregnancy rate per cycle (%)	0	37.5	14.3	14.3	36.4	25.0	23.9

study population consisted of healthy subjects, up to 39 years of age, with regular menstrual cycles, without endocrinopathies.

Stimulation cycles

Ovarian stimulation was initiated on day 2 of the menstrual cycle, with 150 IU recombinant FSH (rFSH, Puregon®, NV Organon) for 5 days. Ganirelix was administered from day 6 of rFSH onwards, until and including the day of human chorionic gonadotrophin (HCG) when at least three follicles ⩾17 mm diameter were observed by sonography. During ganirelix treatment, the rFSH dose was adjusted depending on the individual ovarian response. To induce ovulation, 10 000 IU of HCG (Pregnyl®, NV Organon) was given. Oocytes were retrieved transvaginally 36 h later, fertilized [either by conventional IVF or by means of intracytoplasmic sperm injection (ICSI)] and up to three embryos were transferred on day 2–5 post-retrieval. Excess embryos were cryopreserved.

Thaw cycles

Embryos were frozen in eight out of the 13 IVF centres that took part in the dose-finding study. In three IVF centres no embryos were stored and in two German centres only two pronuclear (2PN) oocytes were frozen which were not included in this analysis. Data were collected from 65 subjects who did not become pregnant after replacement of fresh embryos, and for whom spare embryos were cryopreserved. By June 1998, 46 patients had had at least one embryo transfer using thawed embryos. For the first replacement of thawed embryos, 72% of the patients underwent a natural cycle and 28% underwent a 'stimulated' cycle during which thawed embryos were replaced. These programmed cycles were performed according to the individual site's clinical routine. Ten out of these 46 patients had two frozen embryo cycles and one patient had four frozen embryo cycles. For three patients the quality of thawed embryos was insufficient for embryo transfer.

Results

By June 1998, 46 patients had had at least one embryo transfer of frozen–thawed embryos. The mean age of these patients was 30 years (range 22–36) and 52% suffered from primary infertility.

The number of subjects who had embryo transfer in each dose group ranged between 5 (0.0625 mg) and 11 (1.0 mg) (see Table I). The mean (±SD) number of transferred embryos was 2.6 ± 1.0 (range 2.2–2.9). The first frozen embryo cycle resulted in 11 ongoing pregnancies (23.9%) 12–16 weeks after embryo transfer and included nine singleton and two twin pregnancies. In total, 11 and eight transfers were performed in subjects who had been treated with 1 mg and 2 mg ganirelix respectively. These transfers resulted in four ongoing pregnancies (36.4%) in the 1 mg group and in two ongoing pregnancies (25.0%) in the 2 mg group. In addition, one subject (0.25 mg) had a miscarriage without proof of a vital fetus and one subject (0.5 mg) had a miscarriage after proof of a vital fetus. In total, 10 subjects who first had a frozen embryo cycle continued with a second frozen embryo cycle, but none of them became pregnant. One patient had four frozen embryo cycles and a singleton ongoing pregnancy was established after her last transfer.

Discussion

The clinical outcome of thawed embryo transfers may be taken primarily to indicate the quality of cryopreserved embryos. Thus, the overall ongoing pregnancy rate of 23.9% achieved in first thawing cycles with cryopreserved embryos obtained after ovarian stimulation with GnRH antagonist treatment reflects a good clinical outcome. In comparison, the ongoing pregnancy rate for freeze–thaw cycles in a large prospective randomized multicentre study (Out *et al.*, 1995) with rFSH (Puregon®) and urinary FSH (Metrodin®) was only 14.4 and 7.5% in the two groups respectively (Jones *et al.*, 1997), although this study included several thawing cycles per patient.

Theoretically, the lower success rates of fresh embryo transfers from stimulation cycles with higher doses of ganirelix may be due to a direct adverse effect of the GnRH antagonist on the oocyte/embryo quality. The morphological quality of oocytes and embryos obtained after exposure to relatively high or low doses of ganirelix was comparable. However, assessment of embryo quality based on morphological features cannot be used to reflect the true potential of an embryo to develop or to implant. Therefore, additional data should support this finding, particularly because implantation rates varied considerably between the dose groups. Our current data strongly support the possibility that there is no negative effect of a high ganirelix dose on embryo developmental potential, since the overall ongoing pregnancy rate of freeze–thaw cycles was relatively high (23.9%) and six of the 11 established ongoing

pregnancies were obtained from embryos exposed to 1.0 or 2.0 mg of ganirelix in the cycles during which they were created.

A closer look at the dose finding study indicates that both LH and oestradiol serum concentrations on the day of HCG administration were inversely correlated with the ganirelix dose used (Ganirelix Dose Finding Group, 1998). Therefore, one might consider that the low pregnancy rate in the high dose groups may have been caused by an aberrant hormonal milieu during the late follicular phase. However, data analysis did not reveal a direct relationship between serum LH or oestradiol concentrations and clinical outcome. A direct effect on the endometrium by relatively high doses of GnRH antagonist cannot be excluded, since human endometrial GnRH receptors have been identified very recently (Dong *et al.*, 1998; Raga *et al.*, 1998), but so far, their physiological relevance as well as their possible interaction with GnRH antagonists is unknown.

In summary, we report for the first time the pregnancy outcome of thaw cycles of embryos obtained after ovarian stimulation with rFSH using a GnRH antagonist to prevent premature LH surges. The ongoing pregnancy rate appeared to be good and not related to the dose of GnRH antagonist, suggesting that there is no direct negative effect of the GnRH antagonist on the quality of oocytes and embryos.

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