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An artificially induced follicle stimulating hormone surge at the time of human chorionic gonadotrophin administration in controlled ovarian stimulation cycles has no effect on cumulus expansion, fertilization rate, embryo quality and implantation rate

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In the spontaneous menstrual cycle, the mid-cycle gonadotrophin surge causes maturation of the cumulus-oocyte complex, mucification of cumulus cells and expansion of the cumulus oophorus, resumption of meiosis and maturation of the cytoplasm of the oocyte. Whether this is an effect purely of luteinizing hormone (LH) or whether follicle stimulating hormone (FSH) also plays a role is unknown. The effect of an artificially induced FSH surge at the time of human chorionic gonadotrophin (HCG) injection on maturation of the cumulus-oocyte complex was investigated in a prospective randomized double-blind trial. Twelve patients underwent controlled ovarian hyperstimulation [long gonadotrophin-releasing hormone agonist (GnRHa)/human menopausal gonadotrophin (HMG) protocol] for in-vitro fertilization (IVF) treatment. At the time of HCG administration, six patients received a bolus injection of FSH (450 IU i.m.); the other six patients received a placebo. The peak plasma concentrations of FSH of the experimental group were compared with the peak values of FSH obtained at the mid-cycle gonadotrophin surge of the natural cycle of a group of 12 volunteers to validate the bolus injection of FSH. Maturation of the cumulus-oocyte complex was quantified by measuring the expansion of the cumulus, by the fertilization rate and the implantation rate. The quality of the embryos was scored according to the average morphology score. The bolus injection of FSH mimicked the mid-cycle gonadotrophin surge. The mean peak value of FSH (12.9 IU/l) in the experimental group was fully comparable with the mean peak value of FSH (10.0 IU/l) of the mid-cycle gonadotrophin surge in the natural cycle. No effect of a bolus injection of FSH on

the maturation of the cumulus-oocyte complex or any other outcome variable was found. It is not advantageous to combine the final HCG injection with a bolus injection of FSH in GnRHa/HMG stimulated cycles.

Key words: FSH surge/human/IVF/maturation cumulus-oocyte complex

Introduction

In the spontaneous menstrual cycle the mid-cyclic gonadotrophin surge causes the final event of the ovulatory process, maturation of the cumulus-oocyte complex, follicle rupture and luteinization. The cumulus oophorus mucifies and expands. The oocyte resumes meiosis till the second metaphase and becomes capable of being fertilized (Vermeiden and Zeilmaker, 1974; Thibault and Levasseur, 1988). During the mid-cyclic gonadotrophin surge, both luteinizing hormone (LH) and follicle stimulating hormone (FSH) are secreted. In stimulated cycles using gonadotrophin-releasing hormone agonist (GnRHa), the human chorionic gonadotrophin (HCG) injection fully mimics LH, but the FSH surge is absent.

Many animal experiments have suggested a role for FSH in the maturation of the cumulus-oocyte complex (Eppig, 1980; Behrman *et al.*, 1988; Buccione *et al.*, 1990; Chen *et al.*, 1994; Schramm and Bavister, 1994; Byskov *et al.*, 1997). It has been shown in mice that FSH, and not LH, stimulates cumulus expansion and mucification *in vitro* by stimulating granulosa cells to synthesize and secrete hyaluronic acid (Chen *et al.*, 1994). FSH stimulates plasminogen activator, causing lysis of connective tissue and leading to ovulation in mice (Reich *et al.*, 1985). There is also some evidence from in-vitro studies that FSH affects maturation of the cumulus-oocyte complex in humans (Gomez *et al.*, 1993). The concentrations of FSH in follicular fluid correlate with the degree of maturation of the cumulus-oocyte complexes (Laufer *et al.*, 1984; Suchanek *et al.*, 1994), suggesting an in-vivo relationship. This raises the question whether induced elevated plasma FSH concentration during the final pre-ovulatory maturation events, comparable with the elevated concentration of plasma FSH during the mid-cycle gonadotrophin surge, has a positive effect on the final maturation processes of the cumulus-oocyte complex in women treated with ovarian hyperstimulation.

The aim of this study was to assess the effect of a bolus injection of FSH concomitant with the HCG injection on cumulus expansion, fertilization rate, embryo quality and implantation rate.

Materials and methods

Patients

In order to assess the effect of a bolus injection of FSH at the time of HCG administration, a prospective randomized double-blind trial was performed. Patients were fully informed of the objective and practical outlay of the experiment and had given written informed consent. Twelve patients participated in the experiment. All received conventional ovarian hyperstimulation using a long protocol GnRHa/HMG (human menopausal gonadotrophin) until the minimum criteria for HCG administration were met. Using sealed envelopes, randomization of the patients into the experimental or placebo group was effected on the day of HCG administration. The assignment of the groups was withheld from patients, medical and nursing staff and laboratory personnel. The code was broken after all patients had fully completed the in-vitro fertilization (IVF) treatment cycle and the results of the pregnancy test were known. The experimental group (FSH+ group) received HCG 10 000 IU i.m. plus a bolus injection of 450 IU FSH i.m., a dose designed to mimic the natural mid-cycle FSH surge, based on pharmacokinetic data (Le Contonnec *et al.*, 1993). The control group received HCG 10 000 IU i.m. plus an injection of saline i.m.

The patients in this study were between 18 and 38 years of age, and had a diagnosis of isolated tubal pathology. Patients with polycystic ovary syndrome, endometriosis externa grade II or more or oligomenorrhoea were excluded from this study. The partners exhibited normal semen parameters (the yield of fertile spermatozoa after Percoll 40/90 centrifugation was $>5.0 \times 10^6$ per ejaculate, and a recovery of at least 10%). Couples with infertility caused by immunological factors (immunobead test $>20\%$), were excluded from the study.

On day 2 or 3 of the menstrual cycle (CD 2 or 3) a transvaginal sonographic examination was performed to exclude the presence of ovarian cysts. In order to exclude pregnancy, a blood sample was obtained. When plasma HCG levels were <5 IU/l patients commenced an oral contraceptive containing 30 µg of ethinyl oestradiol and 150 µg of levonorgestrel (Microgynon 30; Schering, Berlin, Germany) on CD 3, daily for 21 days. After 2 weeks, triptoreline acetate medication 100 µg s.c. per day (Decapeptyl; Ferring, Malmö, Sweden) was initiated (CD 17). The first day of the withdrawal bleeding (2–4 days after completion of the oral contraceptive regime) was CD 1 of the next cycle. Again, the presence of ovarian cysts was excluded by transvaginal sonography (CD 2).

Ovarian hyperstimulation with 225 IU HMG (Pergonal; Ares-Serono Laboratories Ltd, Welwyn Garden City, UK) was initiated on CD 3. The ovarian response was monitored using transvaginal ultrasonography and oestradiol determinations. When the minimum criteria for ovarian hyperstimulation were met (the largest follicle 18 mm and at least three follicles >16 mm in diameter; serum oestradiol >1500 pmol/l) 10 000 IU of HCG (Profasi; Ares-Serono Laboratories Ltd, Welwyn Garden City, UK) were administered i.m. Concomitantly with the HCG administration, the experimental group received 450 IU (six ampoules) of FSH i.m. (Metrodin; Ares-Serono Laboratories Ltd, Welwyn Garden City, UK), whereas the control group received a placebo (saline). Plasma FSH values were determined (Amerlite FSH assay monoclonal 2nd IRP 78/549; Amersham, UK) 5 min before (FSH 1), and 30 min (FSH 2), 10 h (FSH 3) and 34 h (FSH 4) after HCG injection.

The estimation that 450 IU FSH mimicked the mid-cycle FSH surge was validated by comparing the plasma FSH concentration of the experimental groups with a study also performed in our centre. Twelve volunteers were monitored daily for one complete menstrual cycle. Blood samples were obtained and LH and FSH were measured.

Table I. Definition of cumulus oophorus expansion

Score	Stage of cumulus expansion
1	Very compact cumulus and corona
2	Compact cumulus and corona
3	Expanded fluffy cumulus and expanded corona radiata
4	Fully expanded cumulus, radial corona
5	Expanded cumulus, corona incomplete
6	Incomplete corona, clumping of cumulus cells
7	Totally denuded oocyte

The mid-cycle gonadotrophin surge was detected and the highest measured FSH concentration was compared with the plasma concentration of FSH of the experimental groups.

Transvaginal ultrasound guided puncture for oocyte retrieval took place 35 h after HCG injection. Luteal support was given with three daily doses of 200 mg micronized progesterone administered intravaginally (Progestan; Organon, Oss, The Netherlands).

Methods

The follicular aspirates were transferred to Petri dishes, and the oocyte–cumulus complexes were identified. A random sample of maximally 12 oocyte–cumulus complexes per patient was used for this study. After ovum retrieval the oocyte–cumulus complexes were spread with a glass pipette, and cumulus expansion was assessed. The expansion of the cumulus oophorus was expressed with a number ranging from one to seven (see Table I). Each oocyte was recorded on videotape for ~10 s, and video recordings were also made of the embryos at day 1 and day 2 after ovum pick-up (VHS super tape). The oocytes and embryos in this study were incubated separately in Earle's medium plus 1% human serum albumin (HSA), at 37°C under 5% CO₂ in air.

The semen was processed using Percoll 40/90 discontinuous gradient centrifugation. Insemination with 50–100 000 progressively motile spermatozoa took place 2–6 h after ovum retrieval. At day 1 after ovum retrieval, inspection for fertilization took place. At days 2 and 3 embryo quality was scored using the average morphology score as described earlier (Roseboom *et al.*, 1995). Embryo transfer took place either at day 2 or day 3 after ovum retrieval. In all patients three embryos were transferred.

Fertilization rate was expressed as the number of fertilized oocytes divided by the total number of oocytes per patient. Implantation rate was defined as the number of implantations (observed as embryonic cardiac activity at ultrasonography) divided by the number of transferred embryos. A vital pregnancy was defined as an ongoing pregnancy resulting in live birth.

Statistics

Mean values per patient plus standard deviations were given for age, FSH 1, FSH 2, FSH 3, FSH 4, number of oocytes, cumulus expansion, fertilization rate, average morphology score, number of embryos and implantation rate. A correlation matrix was constructed, and a Mann–Whitney Rank Sum test was performed.

Results

The mean age of the patients was 35.5 years in both groups. The mean plasma FSH value just prior to HCG administration in the FSH+ group and placebo group was 8.0 ± 2.2 IU/l and 7.8 ± 0.7 IU/l respectively. The values obtained in the two groups 30 min, 10 and 34 h after HCG administration are

Table II. Mean values \pm SD ($n = 6$) per patient per treatment group. FSH+, i.m injection 450 IU FSH just before the final human chorionic gonadotrophin (HCG) injection. Placebo, saline injection just before the final HCG injection

	FSH+	Placebo
Number of patients	6	6
Age (years)	35.5 (± 1.1)	35.5 (± 1.9)
FSH 1 (prior to HCG)	8.0 IU/l (± 2.2)	7.8 IU/l (± 0.7)
FSH 2 (30 min after HCG)	8.0 IU/l (± 2.1)	7.5 IU/l (± 0.6)
FSH 3 (10 h after HCG)	12.9 IU/l (± 2.8)*	6.6 IU/l (± 0.7)*
FSH 4 (34 h after HCG)	10.0 IU/l (± 1.9)*	4.5 IU/l (± 0.5)*
No. oocytes per patient	14.3 (± 5.8)	13.8 (± 4.3)
Cumulus expansion score	3.4 (± 0.8)	3.1 (± 0.6)
Fertilization rate (%)	74.8 (± 13.8)	66.7 (± 17.9)
Morphology score	0.47 (± 0.6)	0.34 (± 0.3)
No. embryos per patient	11.2 (± 5.8)	9.0 (± 2.8)
Vital pregnancies (% per patient)	3 (50)	4 (67)
Implantation rate (%)	16.6 (± 18.1)	44.2 (± 40.2)

*Statistically significant difference between the two groups, $P < 0.004$.

shown in Table II. By 10 h after HCG, mean plasma FSH was 12.9 ± 2.8 IU/l and 6.6 ± 0.7 IU/l in the FSH+ and placebo groups respectively. The FSH values 10 and 34 h after HCG were significantly different between the two groups ($P < 0.004$). Of the 12 volunteers in whom the FSH concentrations were monitored during one menstrual cycle, one had no mid-cycle gonadotrophin surge, and so her values were not included. The mean of the highest mid-cycle FSH plasma concentrations of the other 11 volunteers was 10.0 ± 4.2 IU/l. The mean of the lowest plasma FSH concentration just before the mid-cycle gonadotrophin surge was 3.5 ± 0.8 IU/l ($P < 0.0015$).

No differences were found between the two experimental groups in number of oocytes retrieved per patient, average degree of cumulus expansion, mean fertilization rate, mean number of embryos, average embryo morphology score, implantation rate or pregnancy rate (see Table II).

In the FSH group, three vital pregnancies occurred and three singletons were born. In the placebo group, four vital pregnancies and one biochemical pregnancy occurred, and there were one triplet, two twins and one singleton live births. The average implantation rate per embryo per patient was 16.6 ± 18.1 in the FSH group and 44.2 ± 40.2 in the placebo group.

In the correlation matrix, no correlations were found between any of the parameters. By example, the correlation coefficient of plasma FSH concentrations (FSH3) and the degree of cumulus expansion was 0.30 (not significant).

Discussion

A bolus injection of 450 IU FSH mimics the natural mid-cycle FSH surge. The peak plasma FSH values (FSH3) measured in the FSH+ group were fully comparable with the peak values measured in the natural cycle (mean values 12.9 ± 2.8 IU/l versus 10.0 ± 1.9 IU/l). The plasma FSH values 10 and 34 h after HCG administration (FSH3 and FSH4) were

significantly higher in the group that received a bolus injection of FSH compared with the placebo group. Despite the significant differences in FSH values between the two groups, no significant differences could be found in cumulus expansion, fertilization rate, embryo morphology and implantation rate. No correlation was found between plasma FSH values and average cumulus expansion per patient.

The bolus injection of FSH had no measurable effect. This can be explained by the observations that the FSH concentration at the time of the final maturation of the cumulus-oocyte complex is not related to plasma FSH concentration in follicular fluid. Pre-ovulatory follicles can accumulate FSH (Laufer *et al.*, 1984; Bernardus, 1993; Suchanek *et al.*, 1994), and have their own regulatory mechanism by which their cumulus-oocyte complexes are exposed to different concentrations of FSH.

In the FSH+ and placebo groups, the mean plasma FSH concentration before the final HCG injection was ~ 8 IU/l (Table II). This is approximately two times higher than the mean plasma FSH concentration in the spontaneous cycle before the mid-cycle gonadotrophin surge (3.5 IU/l). In cycles with ovarian stimulation, FSH is available in relative excess at the time of the final HCG injection. This FSH concentration does not induce the final maturation of the cumulus-oocyte complex. In patients in whom ovum retrieval was attempted without bioavailability of HCG, no mature cumulus-oocyte complexes were obtained (Ndukwe *et al.*, 1996). It must be concluded that HCG is essential for the triggering of the final maturation process of the cumulus-oocyte complex. However, the degree of maturation of the cumulus oophorus is correlated with the follicular fluid concentration of FSH (Laufer *et al.*, 1984; Suchanek *et al.*, 1994) and not to the concentration of any other hormone (oestradiol, progesterone, testosterone or HCG) present in the follicular fluid (Enien *et al.*, 1995). This is a strong indication for a permissive or synergistic role of FSH in the maturation process of the cumulus-oocyte complex.

There is, however, an alternative explanation. The accumulation of FSH in follicular fluid is an indicator of follicular 'health', of the potency of the follicle to respond to the mid-cycle LH surge or to final HCG injection. Accumulation of FSH and maturation of the cumulus-oocyte complex thus represent two independent phenomena, each reflecting the competency of the follicle.

The pregnancy rate and the implantation rate in this study were high (Table II). This can partly be attributed to chance (the group is small). The group of patients included in this study were infertile due to isolated tubal pathology, which has been shown to have a high probability of pregnancy after IVF treatment (Roseboom *et al.*, 1995). The implantation rate found in the placebo group was high, though not statistically different from the implantation rate in the FSH group (16.6 versus 44.2%, $P = 0.21$). From a scientific point of view, a larger study would be justified in order to assess whether an excess of FSH at the time of HCG administration has a negative effect on implantation. However, such a study cannot be justified clinically.

Since no beneficial effect of a bolus injection of FSH at the time of HCG administration could be found on any outcome

variable, it is not advantageous to mimic the natural mid-cycle FSH surge in IVF cycles by giving a bolus injection of FSH.

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