Article

Luteinizing hormone affects uterine receptivity independently of ovarian function

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Abstract

Previous studies have suggested that LH, in addition to its well-known effects on the ovary, may exert direct effects on the uterus. This study evaluated the effects of mid-cycle administration of human chorionic gonadotrophin (HCG), which signals through the LH receptor, on endometrial thickness and uterine receptivity in two groups of women lacking ovarian activity and receiving embryos from an oocyte donation programme. Patients in one group still had ovulatory cycles, but their ovarian function was suppressed by pituitary down-regulation with a gonadotrophin-releasing hormone (GnRH) agonist in the embryo transfer cycle, resulting in low endogenous LH concentrations. Patients in the other group were menopausal women whose pituitary function was not down-regulated in the embryo transfer cycle and whose endogenous LH concentrations were thus high. Patients in each of the two groups were randomized into two subgroups. Patients in one subgroup were given 5000 IU of HCG 2 days before oocyte recovery in the corresponding donor. Patients in the other subgroup received placebo at the same time. Oocytes from each donor were randomly distributed between one patient from the HCG subgroup and one patient from the placebo subgroup in each patient group. Endometrial growth and secretory transformation were stimulated by sequential treatment with oestradiol valerate and progesterone. In women with low endogenous LH receiving placebo, endometrial thickness stopped increasing at the beginning of secretory transformation. Mid-cycle HCG administration resulted in a continuous increase in endometrial thickness through this period, improved the implantation rate after embryo transfer in these women (30.6 versus 20.7%) and augmented the number of multiple pregnancies. No similar stagnation of endometrial thickness and no effects of mid-cycle HCG administration on endometrial thickness, the implantation rate and the number of multiple pregnancies were found in women with high endogenous LH. It is concluded that endometrial maturation is disturbed in women with low endogenous LH but can be rescued by mid-cycle stimulation of LH receptor with exogenous HCG in the absence of ovarian activity.

Keywords: *endometrium, human, implantation, luteinizing hormone, oocyte donation, uterine receptivity*

Introduction

In spontaneously ovulating women, endometrial growth in the follicular phase of the ovarian cycle is stimulated by oestradiol secreted by ovarian follicles in response to the endogenous pituitary gonadotropins FSH and LH, whereas secretory transformation of the endometrium is controlled by progesterone secreted by the corpus luteum after the preovulatory LH peak. The effects of FSH and LH on the endometrium are thus supposed to be basically indirect, mediated by specific changes in ovarian steroid secretion. This concept is corroborated by current experience with the technique of oocyte donation (Klein and Sauer, 2002), whereby high pregnancy and implantation rates are obtained after down-regulation of pituitary FSH and LH secretion and direct stimulation of endometrial growth and secretory transformation by administration of exogenous oestrogen and progesterone (Lutjen *et al*., 1984; reviewed in Devroey and Pados, 1998).

In contrast to this conventional view, recent data have suggested that, in addition to its indirect effects mediated by

ovarian steroid hormones, LH may also act directly on the uterus both in the follicular and in the luteal phase of the cycle (Rao, 2001; Shemesh, 2001). A question then arises whether success rates in oocyte donation treatment programmes could be further improved by adding preparations with LH activity to the treatment protocol for oocyte recipients.

Regardless of potential applications in oocyte donation programmes, the mechanisms controlling uterine receptivity in women in general are largely unknown (Dominguez *et al*., 2003), and their better understanding may help improve success rates with novel approaches to ovarian stimulation for IVF as well (van der Gaast *et al*., 2002). To address this problem, studies designed in a way to evaluate uterine receptivity in assisted reproduction independently of oocyte and embryo quality are of particular interest. Assisted reproduction attempts with donated oocytes, in which uterine receptivity is stimulated, and can be evaluated, independently of ovarian stimulation and even in the absence of ovarian activity, represent a useful model for this goal.

This study was undertaken to examine whether mimicking the ovulatory LH peak in women receiving embryos in artificial, anovulatory cycles (oocyte donation) affects the endometrial thickness and the implantation rate. Human chorionic gonadotrophin (HCG), which binds to the same receptor as pituitary LH with a somewhat higher affinity (Huhtaniemi and Catt, 1981), was used as the source of LH activity. The specificity of the exogenous LH action was evaluated by comparing the effects of HCG treatment in ovulating women after deep pharmacological suppression of endogenous LH and in anovulatory women with physiological or premature menopause in whom pituitary LH secretion was not suppressed and was high at the time of embryo transfer.

Materials and methods

Design

This study involved 526 IVF attempts performed in 526 infertile couples receiving embryos from an oocyte donation programme. The study was carried out between January 2000 and December 2002. Repeated attempts for the same couples were not included. The attempts were divided into two groups according to whether the oocyte recipient did or did not ovulate (**Figure 1**). Ovulating patients were arbitrarily defined as those who had undergone at least one IVF attempt with their own oocytes no more than 1 year ago and from whom at least one oocyte was recovered during this attempt. Non-ovulating patients were defined as those who simultaneously fulfilled the following criteria: basal (cycle days 1–3) serum FSH concentration was >30 IU/l, basal serum inhibin B concentration was <15 pg/ml, and no follicles were apparent in the ultrasound picture of the ovaries.

Patients in each of the two groups were randomized to receive mid-cycle HCG treatment or placebo (**Figure 1**). The randomization was based on the oocyte-sharing policy used in the oocyte donation programme. According to this policy, oocytes from one donor are shared between two recipients selected from a waiting list. For each donor, one of the oocyte-sharing recipients received mid-cycle HCG treatment and the other received placebo. The decision of which of the oocyte-sharing recipients would receive HCG and placebo respectively was based on the alphabetical order of the first letter of the patient's surname. When the surname of both oocyte-sharing recipients began with the same letter, the first letter of the first name made the difference. Accordingly, the effect of exogenous HCG administration on endometrial growth and receptivity could be evaluated independently of oocyte quality in women with very low concentrations of endogenous LH (ovulating women after pituitary down-regulation) as well as in those with very high concentrations of endogenous LH (menopausal women without pituitary down-regulation).

Informed consent was obtained from all participants for this study.

Oocyte donor preparation

Oocyte donors were healthy volunteers aged between 19 and 28 years. All medical interventions related to donor preparation were performed at MAR&Gen (Granada, Spain). Details of oocyte donor preparation in this programme have been published previously (Tesarik *et al*., 2002). Briefly, ovarian stimulation was carried out with the use of urinary and recombinant gonadotrophin preparations after pituitary down-regulation with a GnRH agonist administered in the luteal phase. Beginning with day 5 of ovarian stimulation, gonadotrophin doses were adapted individually according to serum oestradiol and LH concentrations and to the number and size of ovarian follicles determined by vaginal ultrasound every other day. Ovulation was induced with 10,000 IU of HCG (Profasi; Serono, Rome, Italy, or Gonadotrophine chorionique; Organon, Eragny sur Epte, France) when at least six follicles measuring ≥18 mm in diameter were observed. Oocytes were recovered by transvaginal route under ultrasound guidance 36 h later.

Oocyte recipient preparation

The preparation of oocyte recipients was started in their country of origin (France or Spain) according to the same protocol. All recipients subsequently presented at MAR&Gen

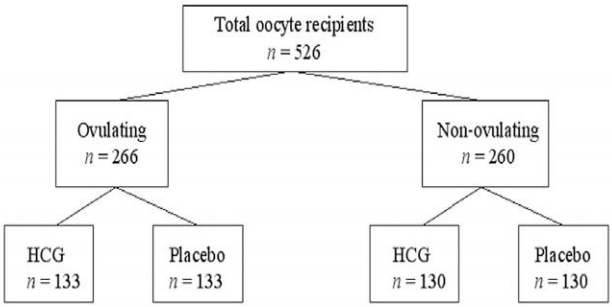

Figure 1. Schematic representation of patient allocation to different treatment and placebo groups.

(Granada, Spain) at latest 1 day before oocyte recovery from the corresponding donor. Ovulating oocyte recipients were given a long-acting GnRH agonist preparation (triptorelin, Decapeptyl 3.75 mg; Ipsen Pharma, Barcelona, Spain) in the luteal phase to suppress pituitary FSH and LH secretion. When the suppression was complete (serum oestradiol <45 pg/ml), endometrial growth was stimulated with increasing doses of oral oestradiol valerate (Progynova; Schering, Madrid, Spain) as described (Tesarik *et al*., 2002). This treatment was started 2 days before the beginning of gonadotropin administration in the corresponding donor. The first day of oestradiol valerate administration is considered as day 1 of the embryo transfer cycle. One day before oocyte recovery from the corresponding donor (day 15 of the embryo transfer cycle), stimulation of secretory transformation of the recipient's endometrium was started with the use of vaginally administered micronized progesterone (Utrogestan; Laboratoires Besins-Iscovesco, Paris, France). Embryo transfer was performed 3 days after oocyte recovery from the donor and 4 days after the beginning of progesterone treatment of the oocyte recipient (day 19 of embryo transfer cycle).

In addition to these treatments, patients belonging to the HCG-supplemented groups (**Figure 1**) received an intramuscular injection of 5000 IU of HCG (Profasi or Gonadotrophine chorionique) 2 days before oocyte recovery from the corresponding oocyte donor (day 14 of embryo transfer cycle). Patients forming the placebo groups received intramuscular injection of solvent only at the same time.

Endometrial thickness was measured by vaginal ultrasound on days 6, 14 and 19 (embryo transfer day) of the embryo transfer cycle. It was defined as the minimum distance between the echogenic interfaces of the myometrium and endometrium measured in the plane through the central longitudinal axis of the uterine body (Friedler *et al*., 1996). On the same days, serum concentrations of oestradiol and LH were determined.

Assisted reproduction techniques

All assisted reproduction techniques were performed at MAR&Gen (Granada, Spain). IVF was assisted by intracytoplasmic sperm injection (ICSI) in all cases. The preparation of oocytes for ICSI, the ICSI procedure, in-vitro culture of sperm-injected oocytes, zygotes and embryos, and embryo transfer were performed as described (Tesarik *et al*., 2002). According to the couple's decision, two to four embryos were transferred in each treatment attempt. All embryos transferred scored as good-morphology ones both at the pronuclear zygote stage, using the previously described scoring system (Tesarik and Greco, 1999; Tesarik *et al*., 2000), and at the cleavage stages. Good-morphology cleaving embryos were characterized as embryos with equal-sized blastomeres (with the exception of 3-cell embryos for which the presence of one bigger and two smaller, equal-sized blastomeres was considered a feature of good morphology), <10% of intrazonal space occupied by cell fragments and the absence of multinucleated blastomeres. Embryo transfer was performed 3 days after oocyte recovery. Implantation rate was calculated by dividing the number of gestational sacs with heartbeat detected by vaginal ultrasound by the number of embryos transferred in each group of patients.

Table 1. Basic characteristics and clinical parameters of patients involved in individual groups.

Patient characteristics	*Ovulating women*		*Non-ovulating women*	
	HCG	*Placebo*	*HCG*	*Placebo*
Number of patients	133	133	130	130
Age (years)[a]	40.2 ± 1.8	40.4 ± 1.9	41.7 ± 2.4	41.8 ± 2.5
Body weight (kg)[a]	58.4 ± 3.1	58.7 ± 3.0	59.6 ± 3.2	59.4 ± 3.3
GnRHa	Yes	Yes	No	No
Time from GnRHa to embryo transfer (days)[a]	24.9 ± 2.7	25.1 ± 2.8	–	–
No. embryos transferred per patient[a]	2.6 ± 0.2	2.5 ± 0.2	2.6 ± 0.2	2.7 ± 0.2

[a]Values are mean ± SEM.
Differences between the HCG and placebo groups are not significant ($P > 0.05$).

Table 2. Effects of mid-cycle HCG administration in ovulating women after pituitary down-regulation on serum oestradiol and LH concentrations and endometrial thickness on different days of the embryo transfer cycle[a].

HCG treatment	*Serum oestradiol (pg/ml)*			*Serum LH (IU/l)*			*Endometrial thickness (mm)*		
	Day 6	Day 14	Day 19	Day 6	Day 14	Day 19	Day 6	Day 14	Day19
Yes (n = 133)	65 ± 6	217 ± 20	218 ± 20	0.16 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	5.8 ± 0.4	8.2 ± 0.7	9.7 ± 0.9
No (n = 133)	67 ± 5	221 ± 20	218 ± 19	0.13 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	5.8 ± 0.4	8.1 ± 0.6	8.2 ± 0.8
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.01

[a]Data are mean ± SEM.

Statistics

The statistical significance of differences in hormone concentrations and endometrial thickness between patients receiving mid-cycle HCG and placebo was determined with the use of analysis of variance and Student's *t*-test. Differences in the pregnancy and implantation rate between these groups of patients were evaluated by Fisher's exact test.

Results

Evaluation of group homogeneity

There were no differences in the patients' age, body weight and the number of embryos transferred between the mid-cycle HCG and placebo groups (**Table 1**). In ovulating patients, in whom GnRH agonist was used for pituitary desensitization, the duration of desensitization before embryo transfer was also similar for both groups (**Table 1**). Within each category of patients, characterized by the presence or absence of ovarian activity, the mid-cycle HCG and placebo groups can thus be considered as homogeneous with regard to the basic patients' characteristics and clinical parameters of the treatment attempts analysed.

Embryo transfer cycles with pituitary down-regulation

No differences in serum oestradiol and LH concentration, determined on days 6, 14 and 19 of the embryo transfer cycle, were found between the women receiving mid-cycle HCG and placebo, respectively (**Table 2**). Endometrial thickness was also comparable in both groups on days 6 and 14, but it was significantly higher in the HCG group on day 19 of the cycle (**Table 2**). The difference between the HCG and the placebo group on day 19 was related to endometrial thickness failing to increase further between days 14 and 19 in the placebo group, which contrasted with the continuous increase through day 19 observed in the HCG group (**Table 2**).

The implantation rate after transfer of similar numbers of embryos (2.6 and 2.5, respectively) was higher in the group of patients receiving mid-cycle HCG as compared to the placebo group (**Table 3**). The number of multiple pregnancies in the mid-cycle HCG group (29: 27 twin and two triplet) was also higher ($P < 0.001$) as compared with the placebo group (three, all twins). However, the pregnancy rate was similar in both groups (**Table 3**).

Table 3. Effect of mid-cycle HCG administration in ovulating women after pituitary down-regulation on uterine receptivity after transfer of embryos obtained by fertilizing oocytes from young donors.

HCG treatment	*Embryos transferred*	*Gestational sacs with heartbeat*	*Implantation rate (%)*	*Pregnancy rate*
Yes (n = 133)	346	106	30.6[a]	75/133 (56.4)[b]
No (n = 133)	333	69	20.7[a]	66/133 (49.6)[b]

Values in parentheses are percentages.
[a,b]$P < 0.01$, $P > 0.05$ respectively.

Table 4. Effects of mid-cycle HCG administration in non-ovulating, menopausal women without pituitary down-regulation on serum oestradiol and LH concentrations and endometrial thickness on different days of the embryo transfer cycle[a].

HCG treatment	*Serum oestradiol (pg/ml)*			*Serum LH (IU/l)*			*Endometrial thickness (mm)*		
	Day 6	Day 14	Day 19	Day 6	Day 14	Day 19	Day 6	Day 14	Day19
Yes (n = 130)	68 ± 6	218 ± 16	219 ± 18	26 ± 2	27 ± 3	28 ± 3	5.7 ± 0.4	8.1 ± 0.6	9.3 ± 0.7
No (n = 130)	66 ± 5	218 ± 17	220 ± 20	25 ± 3	28 ± 3	29 ± 3	5.6 ± 0.5	8.3 ± 0.5	9.5 ± 0.8
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

[a]Data are mean ± SEM.

Table 5. Effect of mid-cycle HCG administration in non-ovulating, menopausal women without pituitary down-regulation on uterine receptivity after transfer of embryos obtained by fertilizing oocytes from young donors.

HCG treatment	*Embryos transferred*	*Gestational sacs with heartbeat*	*Implantation rate (%)*	*Pregnancy rate*
Yes (n = 130)	338	105	31.1[a]	74/130 (56.9)[b]
No (n = 130)	348	109	31.3[a]	78/130 (60.0)[b]

Values in parentheses are percentages.
[a,b]$P > 0.05$.

Embryo transfer cycles without pituitary down-regulation

Serum oestradiol and LH concentrations were comparable in the women receiving mid-cycle HCG and placebo, respectively (**Table 4**). However, as compared with pituitary down-regulated women (**Table 2**), serum LH concentrations, measured on days 6, 14 and 19 of the embryo transfer cycle, were high (**Table 4**).

No difference in endometrial thickness was found between the mid-cycle HCG and placebo groups on any cycle day on which this examination was done (**Table 4**), including day 19 on which a significant difference in favour of the HCG-receiving group was detected in embryo transfer cycles with pituitary down-regulated women (**Table 2**).

Unlike the pituitary down-regulated women, both the pregnancy rate and the implantation rate after transfer of comparable numbers of fresh embryos (2.6 and 2.7, respectively) were similar in the mid-cycle HCG and placebo groups (**Table 5**). The number of multiple pregnancies was also similar in the mid-cycle HCG (28: 25 twin and three triplet) and placebo (29: 27 twin and two triplet) groups.

Discussion

In assisted reproduction attempts endometrial thickness is usually evaluated conjointly with ovarian follicular development until the ovulation-inducing injection of HCG is administered. By analogy, endometrial thickness of oocyte recipients in oocyte donation treatment attempts is usually not monitored beyond the day on which endometrial secretory transformation with exogenous progesterone is started. Patients with satisfactory endometrial thickness at the outset of progesterone treatment are considered to have a good prognosis for embryo implantation.

The results of this study show that the measure of endometrial thickness performed at the beginning of secretory transformation does not necessarily predict the situation on the day of embryo transfer. This is particularly true for treatment attempts in which endogenous LH secretion has been profoundly suppressed by pharmacological pituitary down-regulation. In fact, a stagnation of endometrial thickness at the beginning of the pseudo-luteal phase (in the absence of endogenous LH peak and the corpus luteum) is a typical finding in pituitary down-regulated women. This study is the first to show that this decrease can be prevented by the mid-cycle administration of HCG, which facilitates continuous growth of endometrium through this period.

In contrast to pituitary down-regulated women, stagnation of endometrial thickness at the beginning of the pseudo-luteal phase was not observed in women with premature or physiological menopause who showed high concentrations of endogenous LH and in whom pituitary down-regulation was not performed. Moreover, the application of exogenous HCG had no effect on the endometrial thickness in these women.

These data have several important physiological and clinical implications. First, the prevention by exogenous HCG of the stagnation of the endometrial thickness in the early pseudo-luteal phase of women in whom ovarian activity is blocked by pituitary down-regulation means that the effect of HCG must be independent of ovarian function and thus probably direct on the uterus. LH receptor, which is the physiological target of HCG (Huhtaniemi and Catt, 1981), is expressed in both epithelial and stromal cells of the human endometrium, and the expression level is maximal during the luteal phase (Reshef *et al*., 1990). Moreover, an experimental study with porcine endometrial explants has shown that biological activity of LH receptor (determined by measuring LH-induced prostaglandin production) reaches its maximum between days 14 and 16 of the oestrous cycle (Stepien *et al*., 1999). It has also been shown in the cow that pulses of LH continue after ovulation, in the early luteal phase (Rahe *et al*., 1980), and the response to these pulses in uterine cells may thus be facilitated by the simultaneous increase in LH receptor expression level. Because HCG has a lower clearance and a higher affinity for LH receptor, as compared with LH, a single mid-cycle HCG administration may represent a sufficiently strong stimulus to mimic this early luteal LH activity in pituitary down-regulated women. The absence of effects of mid-cycle HCG administration in women with high tonic serum LH concentrations corroborates the conclusion that the HCG effects observed in this study are indeed mediated by LH receptor.

The relationship between endometrial thickness and uterine receptivity in assisted reproduction treatment attempts remains a controversial issue, and there also is uncertainty as to the days of the embryo transfer cycle on which endometrial thickness should be measured to predict the chance of pregnancy (reviewed in Friedler *et al*., 1996). Only a few studies reported changes in endometrial thickness between ovulation-inducing HCG injection and embryo transfer in assisted reproduction attempts (Rabinowitz *et al*., 1986; Imoedemhe *et al*., 1987; Gonen *et al*., 1989), showing a continuous endometrial growth in the postovulatory period. However, these studies were performed with ovarian stimulation regimens that did not use pituitary down-regulation. As compared with these early studies, several alterations in endometrial morphology and morphometry were found in the periovulatory period of ovarian stimulation cycles using pituitary down-regulation with GnRH agonists (Bourgain *et al*., 1994; Meyer *et al*., 1999). In all these studies, however, preovulatory LH surge was mimicked by the ovulation-inducing HCG administration. So far as is known, the present study is the first to analyse the early secretory phase of human endometrium in the absence of any significant stimulation of the uterine LH receptor. Previous studies comparing pregnancy rates in ovulating and postmenopausal recipients (Sauer *et al*., 1995; Lydic *et al*., 1996; Paulson *et al*., 2002) have reported similar pregnancy rates, but they have not addressed the possible role of LH in uterine receptivity. In the present study the pregnancy rates were also comparable in ovulating and postmenopausal recipients, irrespective of exogenous HCG administration. However, the higher implantation rates observed in all postmenopausal recipients and in HCG-supplemented ovulating recipients as compared with ovulating recipients receiving placebo led to a higher number of multiple pregnancies in these groups. Our data suggest that a direct action of LH or HCG on uterine LH receptors is needed to support both endometrial growth and uterine receptivity in the implantation window.

None of the biochemical markers for uterine receptivity proposed so far has been proven clinically useful (Domínguez *et al*., 2003), and the mechanism by which LH or HCG can directly affect this function is not known. The expression of oestrogen and progesterone receptors in the human endometrium has been reported to be altered in ovarian stimulation cycles using GnRH agonists (Hadi *et al*., 1994; Bourgain *et al*., 2002) in which serum concentrations of LH during the follicular phase are relatively low. Moreover, both morphologic maturation and biochemical modifications typical of receptive endometrium can be induced in the monkey model by in vivo treatment with HCG, and these effects are inhibited by a progesterone receptor antagonist (Srisuparp *et al*., 2001). Signalling through LH receptor in uterine cells may thus primarily affect the expression level of progestone and perhaps oestrogen receptors, which might be necessary for the optimal transformation of the endometrium in the early secretory phase. However, a number of other signalling molecules, including the cytokines leukemia inhibiting factor, colony-stimulating factor-1 and interleukin-1, integrins, glycodelin and mucin 1 (MUC1) (Lindhard *et al*., 2002; Herrler *et al*., 2003), are involved in the endometrial function at the time of implantation, and their action may be modulated by LH. In addition to the effects on the endometrium, observed in this study, HCG has also been shown to increase uterine artery blood flow in both intact and ovariectomized gilts (Ziecik *et al*., 1996). Further study is needed to explain the mechanism of LH effects on the human uterus and to determine the optimal substitution treatment protocols to support uterine receptivity in both ovarian stimulation and oocyte donation treatment cycles.

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