Human choriogonadotropin prior to controlled ovarian stimulation and *in vitro* fertilization improves implantation, and pregnancy rates

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Abstract

Purpose Our purpose was to retrospectively compare controlled ovarian stimulation(COH) in IVF cycles with administration of hCG on the day of menses (D1-hCG) with women not receiving hCG at day 1 of menses (Control).

Methods Data on maternal age, endocrine profile, amount of rFSH required, embryo characteristics, implantation and pregnancy rates were recorded for comparison between D1-hCG ($n=36$) and Control ($n=64$).

Results Dose of rFSH required to accomplish COH was significantly lower in D1-hCG. Following ICSI, more top-quality embryos were available for transfer per patient in the

D1-hCG and biochemical pregnancy rates per transfer were significantly higher in the D1-hCG. Significantly higher implantation and on-going pregnancy rates per embryo transfer were observed in D1-hCG (64%) compared to Control (41%). **Conclusions** Administration of D1-hCG prior to COH reduces rFSH use and enhances oocyte developmental competence to obtain top quality embryos, and improves implantation and on-going pregnancy rates. At present it is not clear if the benefit is related to producing an embryo that more likely to implant or a more receptive uterus, or merely fortuitous and related to the relatively small power of the study.

Capsule Administration of hCG on the start of controlled ovarian hyperstimulation (D1-hCG) significantly increases implantation and on-going pregnancy rates

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Introduction

Under normal physiological conditions human ovaries produce a single dominant follicle, from a cohort of follicles, which participates in a single ovulation each menstrual cycle. This cohort of follicles was recruited to grow during the late luteal phase of the previous menstrual cycle from a pool of follicles selected months earlier as primordial follicles. In the beginning, primordial follicles grow in a gonadotropin-independent manner through the primary and secondary follicle stage [1]. As follicles develop an *antrum* (fluid filled cavity) their development becomes reliant on follicle stimulating hormone (FSH) and is termed gonadotropin-dependent [2, 3]. Final processes during the late luteal phase of the previous menstrual cycle and menses represent important developmental phases, because they support oocyte maturation and subsequent embryonic developmental competence.

This gonadotropin-dependent process is complex and controlled by many factors including surrounding granulosa and theca cells, the oocyte [4–6], autocrine/paracrine growth factors [7, 8], and gonadotropins that govern follicle growth, oocyte development, and subsequent embryo developmental competence [9]. Although FSH is rightly considered the primary regulator of ovarian follicle growth and maturation, luteinizing hormone (LH) also exerts important modulatory actions on folliculogenesis. During the late luteal phase of the previous cycle and at the beginning of a new cycle, preantral and small antral follicles are exposed to elevated LH, which acts on theca cells to induce formation of androgens, substrates needed for granulosa cells conversion to estrogens. However granulosa cells of larger antral follicles (>10 mm) also express LH/hCG receptors and can be stimulated either by FSH or LH/hCG [10–14].

In the era of assisted reproductive technologies (ART) the application of controlled ovarian hyperstimulation (COH) with exogenous gonadotropins to override the selection of a single dominant follicle [15] has been instrumental in obtaining multiple mature oocytes capable of fertilization, embryo development, and healthy live-births. Multiple permutations of FSH and LH administration during the mid- and late follicular phases have been investigated with varying degrees of success in obtaining oocytes with embryonic developmental competence and efficiency of establishing pregnancies [16–20]. Several studies have demonstrated that administration of LH activity alone or combined with FSH can exert significant actions on folliculogenesis [10, 21]. It is hypothesized that LH activity at this phase, enhances development of

large pre-ovulatory follicles, ultimately giving rise to oocytes with increased embryonic developmental competence. Additionally, LH may stimulate atresia of small follicles through the production of androgens. Considering above mentioned concepts, it is reasonable to propose that administration of LH or hCG during menses, at the recruitment period of the final follicular wave, would enhance abilities to select fewer, yet healthier follicles containing oocytes with more developmental competence [22–24].

The objectives of this study was to retrospectively compare ovulation induction in IVF cycles with administration of hCG on the day of menses (D1-hCG) with women not receiving hCG at day 1 of menses (Control). End-point measures were follicular response to COH, exogenous hormone usage, oocyte collection, fertilization, embryo development, implantation, and pregnancy rates.

Materials and methods

This retrospective study was performed with the database from Huntington Medicina Reprodutiva of Brazil in Rio de Janeiro and Sao Paulo from 06/ 2004 to 12/2005; and it was approved by Hospital e Maternidade Santa Joana's Institutional Review Board. The authors have no conflict of interests or were sponsored by pharmaceutical industries.

Medical records from 123 women fulfilling inclusion criteria, including: (i) aged 20–38 years; (ii) infertility attributable to tubal factor, severe male factor or unexplained factor; (iii) at least one failed previous assisted reproduction treatment attempt; (iv) regular, spontaneous menstrual cycles of 25–35 days; (v) acceptable follicular phase serum concentrations of FSH (≤ 10 IU/l), LH (≤ 13.5 IU/l) and estradiol ($E2 \leq 60$ pg/mL); (vi) body mass index (BMI) ≤ 30 kg/m²; (vii) and presence of both ovaries and normal uterine cavity were selected to the study.

Patients were excluded from the study when the following conditions were reported: (i) polycystic ovarian syndrome (PCOS); (ii) previous history of ovarian hyperstimulation syndrome (OHSS); (iii) previous history of intolerance to any of the agents used in the study; (iv) clinically significant conditions/disease or active substance abuse; (v) abnormal gynecological bleeding of unknown origin; and (vi) male partner with leukoospermia ($> 2 \times 10^6$ /mL) or clinical signs of infection detected in a semen analysis within the last 2 months.

Patients were subscribed to:

- A D1-hCG group ($n=36$), if they received 250 μ g of recombinant (r)hCG (Ovidrel, Serono Labs) on day 1 and started ovulation induction on day 3 of the menstrual cycle, when a new ultrasound scan and measures of FSH, LH, hCG, and E2 were performed.

B Control group ($n=64$), if they did not receive hCG on day 1 and started ovulation induction on day 3.

Both groups were stimulated with conventional antagonist protocols [21], briefly patients were started on day 3 with r-FSH (Gonal-F, Serono Labs.) and monitored with serial transvaginal ultrasounds to monitor and control follicular growth and endometrial thickness. On day 5 of stimulation (day 7 of menstrual cycle) until the end of the stimulation protocol, adjustments of r-FSH doses were allowed. When the leading follicle reached 14 mm, 0.25 mg of GnRH-antagonist (Cetrotide; Serono Labs) was administered daily until final follicular maturation. When at least three mature follicles with mean diameter ≥ 17 mm were detected on the ultrasound 250 μ g of rhCG (Ovidrel; ovulatory-hCG) were administered. This was followed by transvaginal ultrasound-guided oocyte retrieval approximately 35–36 h later.

Following oocyte aspiration cumulus cells were removed and all mature (metaphase II, MII) oocytes were inseminated by intracytoplasmic sperm injection (ICSI) as previously described [25]. At 16–20 h post-insemination oocytes/presumptive zygotes were assessed for evidence of fertilization. Zygotes with two pronuclei and two polar bodies were considered normally fertilized and transferred into embryo growth media (P1 + 10% serum substitute supplement; Irvine Scientific), overlaid with mineral oil, and cultured under humidified conditions in 5% CO₂ and air at 37°C. Embryo development was assessed every 24 h. On day 3 after ICSI a final embryo grade was assigned based on morphological classifications comprised of blastomere number, blastomere size and shape, and percentage of fragmentation. Embryos that contained 8 blastomeres and less than 20% fragmentation on the day of transfer were considered top-quality embryos.

Selected embryos were transferred into modified human tubal fluid (HTF-Hepes + 50% serum substitute supplement; Irvine Scientific), loaded into a Sydney IVF embryo transfer catheter of 23 cm (Cook IVF USA), and transferred into the uterine lumen under trans-abdominal ultrasound guidance. Luteal phase support was provided by daily administration of 600 mg of vaginal progesterone (Utrogestan®; laboratories Besins-Isvoresco, Paris, France) associ-

ated with 4 mg of 17 β -estradiol (Estrofem®; Nordisk laboratories Bagsvaerd, Denmark). Serum β -hCG levels were measured 12 days after embryo transfer.

Main outcome measures were i) IU of r-FSH used for controlled ovarian hyperstimulation, ii) follicle diameter 36 h before oocyte pick-up, iii) number of oocytes collected per patient, iv) number of mature oocytes (MII) per patient, v) fertilization rate (2 pronuclei and 2 polar bodies/MII oocytes), number of “top quality embryos” on day of embryo transfer, biochemical pregnancy rate (positive β -hCG/transfer), clinical pregnancy rate (fetal heart and gestational sac/transfer), and implantation rate (fetal heart and gestational sacs/number of embryos transferred), birth rate (number of births/number of embryo transferes), multiple pregnancy rates (number of multiple pregnancies/number of pregnancies). Statistical analyses were carried out by Chi-square, T-Test, ANOVA followed by Tuckey’s test for means, and Z test for two proportions as required. Values were considered significantly different if $P<0.05$.

Results

Forty eight and sixty five IVF cycles were commenced in D1-hCG and control groups. Maternal age, FSH, LH, and E2 concentrations at the beginning were not significantly different between D1-hCG and control group. Additionally, the average commencing daily dose of recombinant FSH administration was similar, but the total amount of recombinant FSH required to accomplish follicular maturity was significantly lower in patients treated with D1-hCG. Data is summarized in Table 1. Also, ultrasound measures of total number of follicles per patient (12.4 ± 3.5 vs. 10.2 ± 5.0 ; $p=0.2$) and follicles per patient greater than 18 mm on the day of maturational/ovulatory hCG (1.3 ± 1.5 vs. 2.2 ± 2.4 ; $p=0.2$) were not significantly different between groups.

Despite lower amounts of r-FSH used in women treated with D1-hCG, similar numbers of retrieved oocytes and mature oocytes were obtained (Table 2). Following ICSI, no statistical significance in percentage of MII oocytes that survived injection, normally fertilized eggs, and cleaved rate of zygotes was observed (Table 2), although the

Table 1 Comparison of maternal age and serum measures of FSH, LH, and E2 before ovarian stimulation and doses of r-FSH at start of stimulation and amount used in both groups. Values are means $\pm$ SD

	D1-hCG	Control	P value
Maternal age	34.7 $\pm$ 3.9	34.5 $\pm$ 4.3	0.8
FSH (IU/ml)	5.9 $\pm$ 3.5	6.8 $\pm$ 2.6	0.5
LH (IU/ml)	4.6 $\pm$ 3.1	4.9 $\pm$ 1.4	0.8
E2 (pg/ml)	42.9 $\pm$ 31.4	32.0 $\pm$ 17.6	0.3
Initial r-FSH dose (IU/day)	221.7 $\pm$ 6.7	231.8 $\pm$ 8.0	0.9
Total r-FSH used (IU/COH)	1,731 $\pm$ 722	2,096 $\pm$ 704*	<0.05

Table 2 Comparison of oocyte collection, maturity, fertilization and cleavage following controlled ovarian hyperstimulation with exogenous hormones in women treated with D1-hCG or Controls. Top quality embryo defined as eight cells or greater with $\leq 20\%$ fragmentation on Day 3

	D1-hCG	Control	P value
Total oocytes / patient (mean $\pm$ SD)	11.0 $\pm$ 5.2	11.2 $\pm$ 5.0	0.9
MII oocytes / patient (mean $\pm$ SD)	8.5 $\pm$ 4.2	8.9 $\pm$ 4.0	0.6
Normally fertilized rate (%)	84%	81%	0.4
Cleavage rate (%)	81%	81%	1.0
Blastomeres on Day 3 (#/embryo; mean $\pm$ SD)	6.6 $\pm$ 2.2	7.2 $\pm$ 1.3*	<0.05
Top Quality Embryos on Day 3 (#; mean $\pm$ SD)	1.0 $\pm$ 0.2*	0.5 $\pm$ 0.5	<0.001

average number of blastomeres per day 3 embryos from D1-hCG was statistically reduced compared to Controls (6.6 $\pm$ 2.2 vs. 7.2 $\pm$ 1.3 respectively; $p<0.05$). Grade or degree of fragmentation was similar between groups. Interestingly and with statistical significance, more top-quality embryos (8-cells, <20% fragmentation) were available to be transferred per patient in the D1-hCG group (0.95 $\pm$ 0.2 vs. 0.51 $\pm$ 0.5; $p<0.001$).

Number of embryos transferred was similar between treatment groups, but biochemical pregnancy rates were significantly higher in the D1-hCG group (Table 3). One miscarriage occurred within the D1-hCG and no miscarriages in the Control group. Nevertheless significantly higher on-going pregnancy rates per embryo transfer were seen in the D1-hCG group compared to Controls (23/36=64% versus 26/64=41% respectively, $p<0.05$). Additionally, patients with D1-hCG had significantly higher implantation rates compared to without D1-hCG, however birth, multiple pregnancy and abortion rates were similar between groups (Table 3). No patients in either arms of the study developed symptoms of ovarian hyperstimulation syndrome (OHSS).

Discussion

Controlled ovarian stimulation with exogenous gonadotropins is routinely employed in ARTs to manage and treat infertility during IVF/ICSI cycles. The goal of COH is to

obtain from a cohort of follicles multiple oocytes with embryonic developmental competence and ability to establish viable pregnancies, thus overcoming IVF technical/biological inefficiencies. Considering the complex temporal changes in FSH and LH levels during the normal menstrual cycle, their roles in regulating multiple events during folliculogenesis are not completely understood. Recently it has been suggested that addition of LH during final stages of COH could modulate folliculogenesis, promoting a better interaction between the theca and granulosa compartments [21, 22, 26]. It is also well recognized that LH activity could be provided in various ways; with human derived LH contained in human menopausal gonadotropin (hMG), recombinant LH (rLH) or human derived or rhCG.

In the present study, we demonstrated in a group of patients at the same age and ovarian profile, documented by similar gonadotropin and estradiol concentrations, a novel approach to test ovarian stimulation using administration of rhCG at the 1st day of menses (D1) before commencing rFSH for COH. Human choriogonadotropin represents an efficient way of administering LH activity because both hormones bind to the same receptor (LH/hCG receptor)[27] and exert comparable actions. Thus a primary goal of the present study was to attempt to mimic the “LH wave” present at the beginning of normal cycles. A normal US in day 1 and the 12 withdrawn due to ovarian cysts were probably accomplished by the LH wave after the hCG administration.. In addition, the longer plasma half-life of hCG and its greater potency (roughly six to eight times that of LH) [28] which permits highly effective and more stable occupancy of LH/hCG receptors [10], may be beneficially enhancing FSH half-life [22]. Within this study, administration of D1-hCG prior to COH with rFSH resulted in comparable follicular development and oocyte outcomes compared to Controls with significantly less rFSH usage, suggesting an enhanced initial FSH activity, possibly due to an extended half-life. Thus, D1-hCG appears to improve selection of developmentally capable follicles, supports their growth, and decreases the amount of FSH needed to achieve a complete follicular maturation. Importantly, it has been suggested that low-dose hCG may only stimulate growth and support maturation of larger follicles (greater than 12–14 mm) [14]. However, our findings suggest that

Table 3 Comparison of embryos transferred, biochemical and on-going pregnancy rates, and implantation rates following controlled ovarian hyperstimulation with exogenous hormones in women treated with D1-hCG or Controls

	D1-hCG	Control	P value
Embryos transferred (mean $\pm$ SD)	3.5 $\pm$ 1.1	3.6 $\pm$ 1.1	0.7
Biochemical pregnancy/ET (%)	67*	41	0.02
On going pregnancy/ET (%)	64*	41	0.04
Implantation (%)	33*	21	0.03
Birth/ET(%)	47	34	0.2
Abortion/ET (%)	16	6	0.1
Multiples/pregnancies (%)	23	27	0.7

interactions of hCG with granulosa cell LH/hCG receptors, in the initial days of stimulation, is capable to early follicular support, may overcome atresia, and enhances selection of viable follicles [29]. Moreover, despite the use of rhCG, these regimens of low-dose hCG with the discontinuation of FSH, or the presently described D1-hCG protocol, can substantially reduce the cost of ovulation induction for ART [22, 26]. Such results are of significant practical importance in an era of desired efficiency of infertility treatment at the lowest economic cost.

Available information suggests that LH activity as well as the administration of variable quantities of low-dose hCG can cause significant reductions in the occurrence of small pre-ovulatory follicles [21, 22], and a recent meta-analysis indicates that the LH action during the ovarian stimulation produces a 4% increase in the live birth rate [30]. Recruitment of a cohort of small antral follicles, from which the ovulatory follicle will emerge, occurs at the time of luteal regression, sometimes referred to as secondary recruitment [3, 31, 32]. It is likely that these follicles are not a homogeneous cohort, but represent follicles of similar size but in different stages of development and potentially containing oocytes with differing embryonic developmental potential. One could speculate that administration of D1-hCG may synchronize early development of this cohort of follicles giving rise to oocytes with increased developmental competence. Such an idea is supported by presented data where mature oocytes from D1-hCG and Control treatments demonstrated comparable and normal fertilization rates leading to the transfer of similar numbers of embryos; yet greater production of top-quality embryos (8 cells and less than 20% of fragmentation) in the D1-hCG patient group. As mentioned earlier, a better quality embryo is an indicator of enhanced oocyte developmental competence. In addition, we found further evidence to support this idea by looking at efficiency of mature oocyte to gestational sac ratio; that is the number of MII required for each gestational sac. This ratio was significantly higher in the D1-hCG treatment group compared to Controls. Yet, several studies consider top quality embryos those with higher implantation ability [33]. Check et al. [34] analyzed a single embryo transfer data basis and reported that six, seven or eight-cell embryos produces similar pregnancy rates. Consequently, the translation of D1-hCG's better quality embryos into a significantly improved biochemical pregnancy rate, implantation rate, and on-going pregnancy rate per transfer might still under debate and scientific testing.

While this supposed embryo quality would suggest that D1-hCG is influencing folliculogenesis, oogenesis, and subsequent embryonic developmental competence, we can not exclude the potential of other non-ovarian targets for D1-hCG action. Considering the action of D1-hCG at the ovarian level, at least two hypotheses can be proposed for

enhanced oocyte embryonic developmental competence, improved implantation and pregnancy rates. First, recall that follicular development in women occurs in a wave-like fashion. Baerwald and coworkers [31] observed nonrandom wave-like changes in follicle number and diameter and confirmed that women exhibit two or three waves of folliculogenesis during interovulatory intervals. Only the final wave of follicle development was ovulatory, whereas all preceding waves were anovulatory. Thus, a potential influence of D1-hCG may be to promote synchrony of folliculogenesis, resulting in synchronized intra-follicular hormonal /growth factor milieu that enhances oocyte developmental competence. This may occur through hCG-induced theca cell intra-ovarian androgen production that may synergize with FSH deprivation to hasten atresia and the demise of smaller ovarian follicles [35, 36]. Future experiments investigating this hypothesis might focus on viability and degree of apoptosis in oocyte-associated cumulus cells harvested from follicles derived from COH with and without D1-hCG.

A second hypothesis of how D1-hCG might act at the ovarian level is that follicular paracrine factors stimulated by LH-like activity could impart enhanced oocyte developmental competence. The primary shortfall in this hypothesis is the lack of knowledge regarding specific ovarian-derived factors that are essential and/or stimulatory to oocyte developmental competence. Luteinizing hormone, and thus presumably hCG, can stimulate granulosa cells of antral follicles to produce EGF-like factors [7], and theca cells to produce insulin-like growth factors [37]. These factors are known to influence oocyte cytoplasmic maturation [38, 39] which may translate into effects on subsequent embryonic developmental competence. Based on DNA microarray analyses of ovarian genes during the preovulatory period, Kawamura and colleagues [40] have found major increases in BDNF (brain-derived neurotrophic factor) expression after LH or hCG stimulation. Brain-derived neurotrophic factor is a member of the neurotrophin family widely expressed in the central nervous system and is important for neuronal survival and differentiation. In the ovary, BDNF was found to be essential for early folliculogenesis [40–42]. This is an example of a LH-activity responsive paracrine factor important for folliculogenesis. It would be interesting to utilize a similar microarray strategy to identify differentially expressed genes within granulosa / cumulus cells in response to D1-hCG.

As mentioned previously D1-hCG administration may also have action beyond influencing ovarian function and preventing ovarian hyperstimulation syndrome. Luteinizing hormone/hCG receptors are expressed in many reproductive organs and within specific regions within these organs. Endometrial tissues from numerous species have been reported to contain LH/hCG receptors [43–45]. In the

human and nonhuman primate LH/hCG receptor expression appears to be cycle-dependent with secretory endometrium expressing higher levels compared to proliferative endometrium [46, 47]. Interesting, addition of hCG to human uterine fibroblasts and decidualized cells resulted in increased expression of LH/hCG receptors [48]. Human choriogonadotropin has been suggested to be a mediator of endometrial receptivity with potential mechanism of action through insulin-like growth factor binding protein-1 and/or regulation of endometrial angiogenesis through vascular endothelial growth factor. Filicori and coworkers [23] have proposed that low-dose hCG may be capable of enhancing uterine receptivity by enhancing endometrial quality and stromal fibroblast function. Whether D1-hCG administration prior to COH is acting through an endometrial pathway to enhance receptivity, implantation, and pregnancy rates remains to be investigated.

In summary, our comparison of regular COH to COH with the administration of D1-hCG prior to COH in management of infertility by IVF/ICSI resulted in comparable follicle development and number of mature oocytes harvested; yet required less rFSH for COH and thus reduced the cost of an IVF cycle. In addition, D1-hCG treatment was superior to no D1-hCG for providing increased number of top-quality day 3 embryos that had significantly greater implantation, resulting in significantly enhanced on-going pregnancy rates. Although, the multiple pregnancies were similar between groups replacing only 2 embryos in D1-hCG treatments might reduce multiples. While results for this trial are encouraging, future prospective randomized studies should confirm our results and focus on detailing mechanisms of action for this D1-hCG enhancement of oocyte developmental competence and increased pregnancy success.

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