Efficacy of low-dose human chorionic gonadotropin (hCG) in a GnRH antagonist protocol

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Purpose: To examine the efficacy of low-dose hCG using a GnRH antagonist protocol.

Methods: Prospective randomized study was performed at the Kyono Ladies Clinic. One hundred ninety-two women (<40 years old, <3 previous cycles) were randomly assigned to GnRH agonist (buserelin) long protocol (LP, $n = 66$), GnRH antagonist (cetrorelix) with no low-dose hCG protocol (NhCGP, $n = 63$), or GnRH antagonist with low-dose hCG protocol (hCGP, $n = 63$).

Results: The hCGP was associated with reduced total amounts of FSH, increased oocyte maturation rate, high-quality day 3 embryos rate, and number of frozen embryos. Ovarian hyperstimulation syndrome (OHSS) tended to be lower in the GnRH antagonist protocol. Pregnancy and implantation rates did not differ significantly between study groups.

Conclusions: Daily low-dose hCG supplementation in the late follicular phase could improve the outcome in FSH based-GnRH antagonist protocol. This protocol, however, does require further modifications, including determination of the optimal doses for hCG and gonadotropin pretreatment.

KEY WORDS: ART; GnRH agonist; GnRH antagonist; late follicular phase; low-dose hCG.

INTRODUCTION

Both GnRH agonists and antagonists have played important roles in reducing the incidence of premature LH surges and assisted reproductive technology (ART) (1–3). However, a Cochrane review of five preliminary randomized studies indicated a trend towards slightly lower implantation and pregnancy rates in the GnRH antagonist group (3). Early follicular phase FSH prevalence is essential to follicle recruitment, whereas increments in LH are critical for dominant selection and maturation at the time of declining FSH levels (4). Filicori *et al.* (5–9) demonstrated that selective addition of LH activity in

the form of low-dose hCG in the GnRH agonist-long protocol 1) reduced recombinant FSH/hMG consumption, while the ICSI outcome was comparable to traditional controlled ovarian stimulation (COS) regimens; 2) stimulated follicle growth and maturation independent of FSH administration; 3) was associated with a reduced number of small preovulatory follicles; 4) did not cause premature luteinization; and 5) resulted in a more estrogenic intrafollicular environment. The impact of exogenous LH supplementation or suppression on COS in the GnRH-agonist-long protocol is still controversial. We examined the efficacy of LH activity in the form of low-dose hCG, especially in a GnRH antagonist protocol. The present study compares LH suppression in 1) FSH based-GnRH agonist long protocol (LP), 2) FSH based-GnRH antagonist protocol (NhCGP), and 3) LH containing daily low-dose hCG supplementation in the mid-late follicular phase, GnRH antagonist protocol (hCGP).

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MATERIALS AND METHODS

A randomized, prospective study was performed at the Kyono Ladies Clinic after obtaining approval from the Clinical Ethics Committee. All subjects (192 women; <40-years old with body mass index (BMI) <27 kg/m²), who underwent COS between January and September 2004, signed an informed consent prior to participating in our study. The patients were then randomly assigned through selection of sealed envelopes to one of three treatment groups. All patients received oral contraceptive (Planoval, Takeda, Japan) for 2–3 weeks before COS and were injected with an initial dose of pure urinary human FSH (uhFSH) (Fertinorm P; Serono, Switzerland) ranging from 225 to 300 IU/day. The treatments included long protocol (LP, *n* = 66), uhFSH after midluteal pituitary desensitization with nasal GnRH agonist (900 µg/day Buserelur, Aventis, Japan); no low-dose hCG protocol (NhCGP, *n* = 63), a full dose of uhFSH administered until reaching a follicular diameter of 14 mm, at which point Fertinorm P dosage was increased to 300 IU/day and GnRH antagonist (Cetrorelix, Serono, USA) was initiated; or low-dose hCG protocol (hCGP, *n* = 63), using the same protocol as NhCGP until reaching a follicular diameter of 14 mm, at which point Fertinorm P dosage was decreased to 75 IU/day and Cetrorelix (0.25 mg/day) with 200 IU/day of hCG (hCG; Mochida, Japan) was initiated. Intramuscular injection of hCG (10,000 IU) was given when at least three follicles of 18 mm diameter were obtained. Oocytes were retrieved by transvaginal ultrasound-guided aspiration 36 h after hCG administration. All embryo transfers (ETs) were performed 3 days after oocyte retrieval using a Wallace catheter. A good-quality embryo was defined as an embryo at the six to eight cell stage with blastomeres of uniform size and shape, and with fragmentation <20%. The luteal phase was supported with 400 mg/day of micronized progesterone (Prometrium, Solvay, USA) administered intravaginally, and with an additional 2 mg of estradiol (E2 valerate, Barr, USA) administered orally on the day of ET. These treatments were continued until either serum pregnancy tests yielded negative results or embryonic heartbeat was confirmed on ultrasonography. No more than two embryos were transferred. Clinical pregnancy was defined by gestational sac (GS) at 6 weeks gestation, and fetal cardiac activity (FCA) was confirmed at 7 weeks gestation through ultrasonography.

Total FSH dose, blood E₂ level at hCG injection, small follicle count at ovum pick-up (OPU), number of retrieved oocytes, oocyte maturation rate, fertilization rate, day 3 high-quality embryo rate, and number of transferred embryos were compared between protocols. Next, clinical pregnancy rate and frequency of OHSS were investigated. OHSS was defined according to the classification proposed by Golan *et al.* (10). Follicle size was classified as small (diameter < 10 mm), medium (diameter 10–14 mm), or large (diameter > 14 mm).

Various ART-relevant biological parameters were compared for the three protocols, using an overall *F*-test for one-way analysis of variance. If the overall *F*-test yielded statistically significant results (*P* < 0.05), further pair comparisons of the groups were made using the least significant difference *t*-test procedure. Rates of maturation, day 3 high-quality embryo, embryo implantation and clinical pregnancy for the three groups were compared using the chi-square test and Yates method.

RESULTS

Causes of infertility in the 192 patients are shown in Table I. No significant differences in mean age, BMI, duration of infertility, number of previous cycles or day 2/3 hormone levels were found between

Table I. Basic Characteristics of Patients Participating in COS With Long Protocol of Buserelin (LP), and Those Participating in COS With Antagonist (NhCGP) and Antagonist With 200 IU hCG (hCGP) Protocols With Cetrorelix

Variable	LP (<i>n</i> = 66)	NhCGP (<i>n</i> = 63)	hCGP (<i>n</i> = 63)
Previous IVF cycles	0.6 ± 0.2	0.7 ± 0.3	0.6 ± 0.3
Age (years)	32.3 ± 2.8	32.6 ± 2.9	33.3 ± 3.1
Duration of infertility (months)	46.6 ± 16.8	42.4 ± 12.5	48.3 ± 14.6
Body mass index (kg/m ²)	21.3 ± 3.2	20.8 ± 4.6	21.1 ± 4.3
Basal hormone level (day 2/3)			
LH	1.9 ± 1.3	2.7 ± 1.8	2.3 ± 1.5
FSH	5.2 ± 1.7	7.0 ± 4.0	7.0 ± 2.4
E ₂	24.3 ± 17.6	25.7 ± 13.7	24.8 ± 12.1
T	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2
Etiology of infertility, (<i>n</i>)			
Tubal factor	18 (27.3)	19 (30.2)	21 (33.3)
Ovulatory factor	4 (6.1)	5 (7.9)	5 (7.9)
Male factor	32 (48.5)	30 (47.6)	29 (46.0)
Endometriosis	4 (6.1)	2 (3.2)	2 (3.2)
Unexplained factor	8 (12.2)	7 (11.1)	6 (9.5)

Note. All listed characteristics revealed no significant differences. Values in parentheses are percentages.

Table II. Clinical Parameters of Ovarian Stimulation for Three Groups of Patients

Variable	LP	NhCGP	hCGP
E ₂ level at hCG injection (pg/mL) ^a	2764 ± 1221.4 ^b	1872.0 ± 700.5 ^{b,c}	2551.4 ± 1565.0 ^{b,c}
Total FSH amount (IU) ^a	2358.9 ± 424.5 ^b	2201.2 ± 511.3 ^{b,c}	1819.5 ± 435.5 ^{b,c}
Days of FSH administration (days)	9.2 ± 1.1	8.9 ± 1.7	8.8 ± 1.5
No. of oocytes ^d	14.3 ± 7.8 ^b	11.2 ± 6.2 ^b	11.2 ± 7.1 ^b
Maturation rate (%) ^e	79.3 (747/942) ^f	73.3 (508/693) ^{f,g}	81.6 (577/707) ^g
Fertilization rate (%)	73.6 (550/747)	77.0 (391/508)	73.1 (422/577)
D3 high quality embryo rate (%) ^e	24.1 (111/460) ^g	32.1 (118/368)	36.1 (137/380) ^g
ET cancellation rate (%)	12.1 (8/66)	10.0 (6/63)	6.3 (4/63)
Pregnancy rate (GS) (%)	63.8 (37/58)	36.8 (21/57)	40.7 (24/59)
Pregnancy rate (FCA) (%)	56.9 (33/58)	36.8 (21/57)	39.0 (23/59)
Implantation rate (%)	35.3 (41/116)	25.2 (27/107)	24.3 (28/115)
Miscarriage rate (%)	16.2 (6/37)	10.5 (2/21)	9.1 (2/24)
OHSS rate (severe)	9.1 (6/66)	1.6 (1/63)	1.6 (1/63)
No. of frozen embryos ^h	3.0 ± 3.3 ⁱ	1.8 ± 2.0 ^{i,j}	2.0 ± 2.6 ^{i,j}

^a*P* = 0.001 by *F*-test for one-way ANOVA.^b*P* = 0.001 by *t*-test, for comparison of LP and NhCGP, LP and hCGP.^c*P* = 0.01 by *t*-test, for comparison of NhCGP and hCGP.^d*P* = 0.017 by *F*-test for one-way ANOVA.^e*P* = 0.001 by chi-square test.^f*P* = 0.01 by Yates method, for comparison of LP and NhCGP.^g*P* = 0.001 by Yates method, for comparison of NhCGP and hCGP, LP and hCGP.^h*P* = 0.039 by *F*-test for one-way ANOVA.ⁱ*P* = 0.001 by *t*-test, for comparison of LP and NhCGP, LP and hCGP.^j*P* = 0.033 by *t*-test, for comparison of NhGP and hCGP.

groups. In addition, causes of infertility did not differ significantly after group randomization. The majority of patients successfully completed ovarian stimulation and received hCG (LP, 100%; NhCGP, 98%; hCGP, 100%). In addition, most patients underwent oocyte retrieval (LP, 100%; NhCGP, 98%; hCGP, 100%).

Various COS-associated parameters are presented in Tables II and III. No significant differences in rates of fertilization, implantation, clinical pregnancy, miscarriage, ET cancellation, or severe OHSS rate were noted between the groups. Serum E₂ levels at hCG injection are significantly lower in NhCGP than in LP and hCGP. Total gonadotropin was lower for hCGP than for LP or NhCGP (*P* = 0.001). Maturation rate was higher for hCGP than NhCGP (*P* = 0.01), while the day 3 high-quality embryo rate was higher for hCGP than LP (*P* = 0.001). The hCGP displayed a

significantly decreased small follicle count at OPU compared to NhCGP or LP (*P* = 0.001 each). The number of frozen embryos were higher for hCGP than for NhCGP (*P* = 0.033).

The number of oocytes and frozen embryos were higher for LP than for the others (*P* = 0.001).

DISCUSSION

The present results indicate that E₂ levels at time of hCG injection, number of oocytes retrieved, and number of frozen embryos were significantly higher for LP than for the other protocols tested. Likewise, implantation, clinical pregnancy, ongoing pregnancy, and severe OHSS rates tended to be higher for LP compared to the other protocols, although no significant differences were identified. With regards to the GnRH antagonist protocols, however, hCGP displayed an improved maturation rate, reduced number of small follicles, increased number of frozen embryos, and improvement in total FSH cost. Moreover, the hCGP group had improved day 3 high-quality embryo rate compared to LP. Significantly lower E₂ levels at hCG injection for FSH-based NhCGP indicated that the LH suppression was too strong, whereas hCGP- and FSH-based LP reflected the appropriate LH suppression.

Table III. Follicle Counts at OPU

Variable	LP	NhCGP	hCGP
Large follicle	8.3 ± 3.8	6.9 ± 3.8	6.9 ± 4.3
Medium follicle	11.1 ± 7.2	8.2 ± 6.5	10.1 ± 6.8
Small follicle ^a	5.0 ± 4.1	5.2 ± 4.6	3.2 ± 2.9

Note. *P* = 0.001 by *t*-test, for comparison of LP and hCGP; *P* = 0.001 by *t*-test, for comparison of hCGP and NhCGP.

^a*P* = 0.0079 by *F*-test for one-way ANOVA.

Concomitant FSH, with an immediate drop in serum LH, might lead to follicle atresia at the time of antagonist administration.

Hillier reported that follicle recruitment is FSH dependent, and follicles do not form LH receptors until the mid-follicular phase when they measure 10 mm in diameter on ultrasound (11). Chappel and Howles have also reported that endogenous LH levels of 1.5–10 mIU/mL are necessary for theca cell activity and follicle growth. However, LH suppression to below 1.5 mIU/mL may occur with prolonged GnRH agonist use, and could affect follicle growth and estradiol production (12). Some authors have shown that diminished LH activity may lead to decreased follicular estradiol production, and that LH may be necessary in cases of GnRH downregulated cycles (13–15). Recently, multiple novel roles of LH have been proposed. LH may affect IVF results by determining oocyte quality, influence uterine receptivity via ovarian estradiol secretion, or have direct effects on endometrial and uterine vessels (16–18).

Tesaric *et al.* (19) showed that hCG administration increased endometrial thickness on the day of ET, and improved implantation rate in recipients whose pituitary was down regulated with a GnRH agonist or who received embryos through an oocyte donation program. A dose-determining study previously showed a direct correlation between increasing GnRH antagonist dose, ganirelix, and decreasing serum LH levels in patients undergoing recombinant FSH (rFSH) stimulation alone. Consistent with a decline in LH activity was a significant and profound decline in implantation and ongoing pregnancy rates compared with patients receiving 0.25 mg or less (20). In our study, despite better embryo quality, we reported lower implantation and clinical pregnancy rates after GnRH antagonist stimulation since the GnRH antagonist poorly influenced on uterine receptivity. We may need more powerful exogenous LH in the late follicular phase as well as stronger luteal support. Closer examination of an appropriate start time and dosage in the GnRH antagonist protocol, which results in both good-quality embryos and uterine receptivity, is necessary.

Kolbianakis *et al.* have suggested that high concentrations of LH or exposure to high levels of LH and E2 in the early follicular phase may exert detrimental effects on the success of ovulation induction and conception, thus functioning as a causal factor in early pregnancy loss (21–24).

This is feasible either by introducing antagonist administration earlier in the cycle or pretreating

patients with the oral contraceptive pill, thus suppressing gonadotropins at the start of stimulation. In PCOS patients treated with rFSH, if, at any point during stimulation, they developed ≥ 4 follicles between 8 and 13 mm in mean diameter with no other larger follicle, the patients were randomized to continue treatment with 1) placebo, 2) 225 IU of recombinant LH (rLH)/daily, or 3) 450IU rLH/daily until one of the follicles reached 18 mm in diameter. At this point, 10,000 IU of hCG was administered. Although results did not reach significant level, there was a clear trend showing that follicle sizes were distributed between 11 and 21 mm in the placebo group, in contrast with the two rLH groups in which one follicle was large and secondary follicles were clustered in sizes generally below 15 mm. High LH dose administration led to atresia, supporting the ceiling theory (25). In the patients of hypogonadotropic hypogonadism, the European Recombinant Human LH Study Group (26) reported that a daily dose of 75 IU rLH was effective in promoting optimal follicular development and maximal endometrial growth in the majority of women.

Marrs *et al.* and Humaidin *et al.* reported that rLH supplementation seems to benefit treatment outcome for women above 35 years of age (27, 28). While use of LH appears beneficial in patients with low-basal LH levels or in the late follicular phase of COS, appropriate dosages remain under discussion.

The longer plasma half-life of hCG and the greater its potency (roughly six to eight times that of LH), enables a highly effective and more stable occupancy of LH/hCG receptors (29, 30). rLH (300–750 IU) and low-dose hCG (200 IU) seem to be capable of maintaining E₂ secretion, as well as stimulating follicle development and maturation even when FSH administration is discontinued (6,31). Serum hormone concentrations measured immediately before high-dose hCG (10,000 IU) were found to diverge, with serum hCG, E₂, and T significantly higher in the hCG (200 IU) alone group and serum FSH higher in the no hCG group, while serum LH and P levels did not (9). Optimal basal LH levels appear to be 1.5–10 mIU/mL (12) while optimal follicular development depends on minimum LH exposure or “LH threshold.” When endogenous LH secretion is absent, a daily injection of 75 IU rLH can deliver a sufficient LH supply; Whereas, the concept of an LH ceiling defines an upper stimulation limit. Loumaye *et al.* (32) has demonstrated that rLH alone can trigger follicular growth arrest in 4/6 of hypogonadotropic hypogonadism patients and 5/12

of PCOS patients, suggesting the existence of an “LH ceiling” during late follicular maturation. When only the impact on oocyte number and developmental competence are taken into account, exogenous LH during ovarian stimulation may be beneficial in some women but detrimental to others. The level of serum LH before stimulation or on day 5 of stimulation may become useful criterion when distinguishing between these conditions, but the optimal cutoff values still remain to be determined (18).

We should classify and consider 1) early (first to fifth of COS) or mid-late (sixth to hCG injection or 14 mm in follicular diameter) follicular phase, 2) GnRH agonist-long protocol (mild-middle, profound LH suppressed), GnRH agonist-short protocol, or GnRH antagonist protocol (≤ 0.25 mg or >0.25 mg), 3) response type (normal, poor, high, hypo-hypo) (age of <35 or ≥ 35), 4) influence on oocyte quality and/or uterine receptivity (fresh cycles or HRT cycles), and 5) pretreatment (OCP or Luteal E_2 valerate or GnRH antagonist 3 mg). The optimal time and dosage of exogenous LH should also be determined according to serum LH levels fundamentally, making reference with previous COS data and quality of oocytes and embryos if possible.

For example, the addition of 50–200 IU of hCG or 75–150 IU rLH may be advisable in patients with hypogonadotropic hypogonadism and in patients who suffer profound pituitary and ovarian desensitization with very low or detectable serum LH concentrations after GnRH antagonist administration. In addition, 50–200 IU low-dose hCG or 75–225 IU rLH should prove useful for maintaining appropriate blood LH levels from the mid-follicular phase (day 6 of stimulation) of the GnRH antagonist protocol.

Although immunoreactive LH and bioactive LH concentrations are related, differences are often observed. In such cases, we should consider the number, sensitivity, and variation of LH receptor (33).

Pretreatment for basal antral follicle synchronization is important, as are FSH and LH. In this study, we pretreated with oral contraceptive pills. Fanchin *et al.* (34, 35) reported that pretreatment with luteal E_2 (E_2 valerate 4 mg) or GnRH antagonist (cetrorelix 3 mg) reduce antral follicle growth rate and improves size homogeneity. Coordination of follicular development optimizes ovarian response to r-hFSH/GnRH antagonist protocols and may represent an attractive approach to improve fertility outcomes.

CONCLUSION

This GnRH antagonist protocol with low-dose hCG supplementation reduces total FSH dose requirements, improves the rates of oocyte maturation and day 3 high-quality embryos, and increases number of frozen embryos.

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REFERENCES

1. Alvano C, Felberbaum RE, Smits J, Riethmuller-Winzen H, Engel J, Diedrich K, Devroey P: Ovarian stimulation with hMG: Results of a prospective randomized phase III European study comparing the luteinizing hormone-releasing hormone (LHRH)-antagonist cetrorelix and the LHRH-agonist buserelin. European Cetrorelix Study Group. *Hum Reprod* 2000;15:526–531
2. Ludwig M, Katalinic A, Diedrich K: Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. Meta analysis. *Arch Gynecol Obstet* 2001;265:175–182
3. Al-Inany H, Aboulghar H: GnRH antagonist in assisted reproduction: A Cochran review. *Hum Reprod* 2002;17:874–885
4. Zeleznik AJ, Hillier SG: The role of gonadotropins in the selection of the preovulatory follicle. *Clin Obstet Gynecol* 1984;27:927–940
5. Filicori M, Cognigni GE, Taraborrelli D, Spettoli D, Ciampaglia W, de Fatis CT, Pocognoli P: Luteinizing Hormone Activity Supplementation Enhances Follicle-Stimulating Hormone Efficacy and Improves Ovulation Induction Outcome. *J Clin Endocrinol Metab* 1999;84:2659–2663
6. Filicori M, Cognigni GE, Tabarelli C, Pocognoli P, Taraborrelli S, Spettoli D, Ciampaglia W: Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. *J Clin Endocrinol Metab* 2002;87:1156–1161
7. Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Ferlini F, Perri T, Parmegiani L: Comparison of controlled ovarian stimulation with human menopausal gonadotropin or recombinant follicle stimulating hormone. *Fertil Steril* 2003;80:390–397
8. Filicori M, Cognigni GE, Gamberini E, Parmegiani L, Troilo E, Roset B: Efficacy of low-dose human chorionic gonadotropin alone to complete controlled ovarian stimulation. *Fertil Steril* 2005;84:394–401
9. Filicori M, Fazleabas AT, Huhtaniemi I, Licht P, Rao ChV, Tesarik J, Zygmunt M: Novel concepts of human chorionic gonadotropin: Reproductive system interactions and potential in the management of infertility. *Fertil Steril* 2005;84:275–284
10. Golan A, Ron-EI R, Herman A, Weinraub Z, Soffer Y, Capsi E: Ovarian hyperstimulation syndrome: An update review. *Obstet Gynecol Surv* 1989;44:430–440

11. Hillier SG: Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. *Hum Reprod* 1994;9:188–191
12. Chappel S, Howles C: Re-evaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. *Hum Reprod* 1991;6:1206–1212
13. Fleming R, Chung CC, Yates RWS, Coutts JRT: Purified urinary follicle stimulating hormone induces different hormone profiles compared with menotrophins, dependent upon the route of administration and endogenous luteinizing hormone activity. *Hum Reprod* 1996;11:1854–1858
14. Gordon UD, Harrison RF, Fawzy M, Hennelly B, Gordon AC: A randomized prospective assessor-blind evaluation of luteinizing hormone dosage and in vitro fertilization outcome. *Fertil Steril* 2001;75:324–331
15. Ferraretti AP, Gianaroli L, Magli MC, D'angelo A, Farfali V, Montanaro N: Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. *Fertil Steril* 2004;82:1521–1526
16. Rao CV: Multiple novel roles of luteinizing hormone. *Fertil Steril* 2001;76:1097–1100
17. Semesh M: Actions of gonadotrophins on the uterus. *Reproduction* 2001;121:835–842
18. Tesarik J, Mendoza JK: Effects of exogenous LH administration during ovarian stimulation of pituitary down-regulated young oocyte donors on oocyte yield and developmental competence. *Hum Reprod* 2002;17:3129–3137
19. Tesarik J, Hazout A, Mendoza C: Luteinizing hormone affects uterine receptivity independently of ovarian function. *Reprod Biomed Online* 2003;7:59–64
20. Garirelix ose Finding Study Group: A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon®). *Hum Reprod* 1998;13:3023–3031
21. Kolibianakis EM, Albano C, Kahn J, Camus M, Touraye H, Van Steirteghem AC, Devroey P: Exposure to high levels of luteinizing hormone and estradiol in the early follicular phase of gonadotropin-releasing hormone antagonist cycles is associated with a reduced chance of pregnancy. *Fertil Steril* 2003;79:873–880
22. Stanger JD, Yovich JL: Reduced in-vitro fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. *Br J Obstet Gynecol* 1985;92:385–393
23. Howles CM, Macnamee MC, Edwards RG, Goswamy R, Step-toe PC: Effect of high tonic levels of luteinizing hormone on outcome of in-vitro fertilization. *Lancet* 1986;2:521–522
24. Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS: Influence of serum luteinizing hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. *Br Med J* 1988;297:1024–1026
25. Serono Study No. 7313: Data on file, 1998, Geneva, Switzerland.
26. The European Recombinant Human LH Study Group: Recombinant human luteinizing hormone (LH) to support recombinant follicle stimulating hormone (FSH) -induced follicular development in LH-and FSH-deficient anovulatory women: A dose-finding study. *J Clin Endocrinol Metab* 1998;83:1507–1514
27. Marrs R, Meldrum D, Muasher S, Schoolcraft W, Werlin L, Kelly E: Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. *Reprod BioMed Online* 2003;8:175–182
28. Humaidan P, Bungum M, Bungum L, Anderson CY: Effect of recombinant LH supplementation in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with recombinant FSH: An opening study. *Reprod BioMed Online* 2004;8:635–643
29. Stokman PG, de Leeuw R, van den Wijngaard HA, Kloostermanboer HJ, Vemer HM, Sanders AL: Human chorionic gonadotropin in commercial human menopausal gonadotropin preparations. *Fertil Steril* 1993;60:176–178
30. The European Recombinant LH study Group: Human recombinant luteinizing hormone is as effective as, but safer than, urinary human chorionic gonadotropin in inducing final follicular maturation and ovulation in in vitro fertilization procedures: Results of a multicenter double-blind study. *J Clin Endocrinol Metab* 2001;86:2607–2618
31. Sullivan M, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ: Ovarian responses in women to recombinant follicle stimulating hormone and luteinizing hormone (LH): A role for LH in the final stages of follicular maturation. *J Clin Endocrinol Metab* 1999;84:228–232
32. Loumaye E, Engrand P, Shoham Z, Hillier SG, Baird DT on behalf of the recombinant LH study group. Clinical evidence of an LH ceiling effect induced by administration of recombinant human LH during the late follicular phase of stimulated cycles in World Health Organization type I and type II anovulation. *Hum Reprod* 2003;18:314–322.
33. Takahashi K, Kurioka H, Ozaki T, Kanasaki H, Kohsaka M, Miyasaki K, Karino K: Increased prevalence of luteinizing hormone β -subunit variant in Japanese infertility patients. *Hum Reprod* 1998;13:3338–3344
34. Fanchin R, Salomon L, Castero-Branco A, Olivennes F, Frydman N, Frydman R: Luteal estradiol pre-treatment coordinates follicular growth during controlled Ovarian hyperstimulation with GnRH antagonists. *Hum Reprod* 2003;18:2698–2703
35. Fanchin R, Castelo A, Kadoch IJ, Hosny G, Bagirova M, Frydman R: Premenstrual administration of gonadotropin-releasing hormone antagonist coordinates early antral follicle sizes and sets up the basis for an innovative concept of controlled ovarian hyperstimulation. *Fertil Steril* 2004;81:1554–1559