Increased Progesterone/Estradiol Ratio on the Day of hCG Administration Adversely Affects Success of *In Vitro* Fertilization–Embryo Transfer in Patients Stimulated with Gonadotropin-releasing Hormone Agonist and Recombinant Follicle-stimulating Hormone

Yu-Che Ou[1], Kuo-Chung Lan[1,2], Shiuh-Young Chang[1,2], Fu-Tsai Kung[1,2], Fu-Jen Huang[1,2]*

[1]*Chang Gung Memorial Hospital–Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, and*
[2]*Department of Obstetrics and Gynecology, Chang Gung University School of Medicine, Taoyuan, Taiwan.*

SUMMARY

Objective: We investigated the influence of premature luteinization in *in vitro* fertilization using a long protocol of gonadotropin-releasing hormone agonist (GnRHa) and recombinant follicle-stimulating hormone (rFSH), taking ovarian response into account in the definition of premature luteinization.

Materials and Methods: A total of 339 cycles of controlled ovarian hyperstimulation with rFSH and GnRHa were performed in 311 infertile couples. Premature luteinization was defined as a progesterone (P) to estradiol (E_2) ratio of > 1 on the day of human chorionic gonadotropin (hCG) administration. The P/E_2 ratio is calculated as: P (ng/mL) × 1,000/E_2 (pg/mL). Clinical outcomes were compared for the prematurely luteinized and non-prematurely luteinized groups.

Results: The mean number of retrieved oocytes, recovered mature oocytes, embryos and top quality embryos were significantly higher in the non-prematurely luteinized group than in the prematurely luteinized group. Although fertilization rates and implantation rates were similar between the two groups, the clinical pregnancy rate was higher in the non-prematurely luteinized group than in the prematurely luteinized group.

Conclusion: Premature luteinization, defined as late follicular P/E_2 ratio of > 1 in long GnRHa cycles with rFSH stimulation, adversely affected ovarian responses and clinical outcomes. It seems unrelated to preovulatory luteinizing hormone (LH) elevation and LH/hCG content of gonadotropins and could be associated with poor ovarian response and the presence of dysmature follicles. [*Taiwan J Obstet Gynecol* 2008;47(2):168–174]

Key Words: GnRH agonist, premature luteinization, progesterone to estradiol ratio, recombinant FSH

Introduction

The pathogenesis of premature luteinization in controlled ovarian hyperstimulation (COH) is still poorly understood. The role of elevated serum progesterone (P) in the late follicular phase of COH is still controversial. Elevated serum P has been reported with a wide clinical spectrum of ovarian response or *in vitro* fertilization (IVF) and clinical outcomes [1–5]. One of the major reasons for the controversy has been the diverse definitions of premature luteinization in previous literature. Most studies considered the occurrence of premature luteinization when serum P exceeded a certain level, in a range from 0.8 to 2 ng/mL [4,6–11]. Furthermore, it has been asserted that ovarian response

*Correspondence to: Dr Fu-Jen Huang, Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 123 Ta-Pei Road, Niao Sung Hsiang, Kaohsiung, Taiwan.
E-mail: huangfj@seed.net.tw
Accepted: September 20, 2007

or reserve may be of critical importance when considering premature luteinization [2]. More follicles produce more serum P. It would, therefore, be better to take into account the ovarian response, rather than the serum P level only, when considering the reasons for premature luteinization. Therefore, using the definition of P to estradiol (E_2) ratio of > 1 for premature luteinization, rather than the P concentration only, may make it possible to differentiate increased late follicular P levels due to multiple follicular secretion in women with good ovarian response from high P levels resulting from P secretion due to premature luteinization. Younis et al [2] defined premature luteinization as a P/E_2 ratio of >1 on the day of human chorionic gonadotropin (hCG) administration. Their work suggests that premature luteinization in long gonadotropin-releasing hormone agonist (GnRHa) cycles with human menopausal gonadotropin (hMG) stimulation seems to adversely affect clinical outcomes.

Some investigators believed that premature luteinization is associated with elevation of follicular luteinizing hormone (LH) levels [12]. Since the introduction of long GnRHa protocol, pituitary desensitization has usually been profound and endogenous LH levels have usually been low. Other investigators have suggested that the serum accumulation of hCG from hMG is responsible for premature luteinization [13]. Therefore, the use of recombinant follicle-stimulating hormone (rFSH) instead of hMG has been regarded as an excellent model for elucidating this phenomenon. Ubaldi et al [4] analyzed the IVF and clinical outcomes of women who underwent COH by using GnRHa in combination with rFSH or urinary follicle-stimulating hormone (FSH). They found that greater FSH exposure correlated with P exposure and suggested that the cause of the subtle rise in P may be related to an increased LH sensitivity of the granulosa cells of FSH-treated cycles. However, they defined premature luteinization by serum P level only. Furthermore, Adonakis et al [14] suggested that an increase in P in the late follicular phase is unrelated to any luteinizing process attributable to effects of follicular cells to LH.

If increased P/E_2 ratio in the late follicular phase could adversely alter the clinical outcomes of COH induced by a long protocol of GnRHa and hMG, this may also be true in long GnRHa cycles with rFSH administration. In this study, we sought to quantify the incidence of premature luteinization during GnRHa-assisted reproduction cycles with rFSH stimulation and to determine whether this premature increase in P/E_2 ratio is associated with adverse outcomes in terms of pregnancy and implantation.

Materials and Methods

An institutional review board approved the chart review of 311 infertile couples who underwent 339 cycles of COH with rFSH and GnRHa in our assisted reproductive technology program. The women were aged 20–40 years, and the main indications for IVF included male factors, tubal factors, unexplained infertility, and endometriosis. Patients with short protocols, dual suppression protocols, oocyte donation, and cycles incorporating hMG were excluded.

Ovarian stimulation was performed as previously described [15]. Briefly, all patients underwent a long stimulation protocol of GnRHa therapy followed by rFSH administration and transvaginal oocyte retrieval. Once adequate follicular maturation had been obtained, hCG (10,000 IU; Pregnyl; N.V. Organon, The Netherlands) was administered and oocyte retrieval was performed 35 to 37 hours later by transvaginal aspiration with ultrasound guidance. Follicular fluids and follicular washes were examined microscopically with a dissecting stereomicroscope situated inside an incubator with a controlled, humidified atmosphere of 37°C and 5% CO_2. The sperm insemination procedure in IVF cycles was performed according to our routine protocol. In brief, the oocyte–corona–cumulus complexes were cultured in IVF medium (Medicult, Denmark) 4–6 hours after retrieval in four-well multidishes (Nunc, Roskilde, Denmark) before insemination. Following the incubation, oocytes were inseminated with spermatozoa to a concentration of ~10^5 motile spermatozoa/mL in 1 mL of IVF medium. Then, oocytes were withdrawn from the insemination medium after a 2-hour exposure to spermatozoa. Each oocyte was cultured in fresh IVF medium in the four-well culture dish again. In the intracytoplasmic sperm injection (ICSI) program, the oocytes were exposed to 80 IU/mL of hyaluronidase (type VIII; Sigma Chemical Co., St Louis, MO, USA) for 5 to 6 seconds. The surrounding cumulus cells were stripped from oocytes by aspirating through a series of pipettes with decreasing inner diameters (220, 200, 180, and 160 μm) in 100-μL droplets of human tubal fluid medium. The sperm injection procedure in the ICSI cycle was based on a modified protocol as previously described [16].

After completion of the ICSI or IVF procedures, the oocytes were cultured according to our standardized *in vitro* culture procedure [17]. They were assessed for the presence of pronuclei after 16–18 hours of incubation. Fertilization was considered to have occurred when two clear pronuclei were present. If only one pronucleus was observed, a second evaluation was performed 4 hours later to determine whether the pronuclear status had changed. The developmental competence of zygotes with

Table 1. Ovarian response of controlled ovarian hyperstimulation cycles (n = 339) in the study and control groups*

	Control group (n = 202)†	Study group (n = 137)†	p
Age (yr)	32.3 ± 4.1	32.8 ± 3.6	0.392
Hormone levels			
LH on day 6 (mIU/mL)	1.4 ± 1.2	1.4 ± 0.9	0.641
LH on hCG day (mIU/mL)	1.5 ± 1.1	1.0 ± 0.6	< 0.05
Progesterone (ng/mL)	1.1 ± 0.5	1.3 ± 1.0	< 0.05
Estradiol (pg/mL)	1,827.9 ± 906.1	804.1 ± 495.9	< 0.05
Oocytes			
Oocyte (n)	7.9 ± 3.9	4.7 ± 2.9	< 0.05
Mature oocyte (n)	4.6 ± 2.4	2.8 ± 1.8	< 0.05
FSH dose (ampoule)	28.7 ± 8.3	32.2 ± 12.2	< 0.05
Duration of stimulation (d)	10.0 ± 1.9	10.4 ± 2.0	0.09

**Data are presented as mean ± standard deviation; †premature luteinization was defined according to the criterion, progesterone/E_2 ratio > 1. LH = luteinizing hormone; hCG = human chorionic gonadotropin; FSH = follicle-stimulating hormone.*

two pronuclei was evaluated after a further 96 hours of *in vitro* culture. Embryos were cultured in a similar fashion to the standard protocol with the primary exception of the type of media and the duration of culture. Briefly, the embryos were placed in G1.2 medium (Scandinavian IVF Science, Gothenburg, Sweden) on day 1 post-fertilization, followed by G2.2 medium (Scandinavian IVF Science, Gothenburg, Sweden) on days 3–6. The cleaved embryos were classified according to the morphologic grading system described by Veeck [18]. The blastocysts were assigned a score according to the system of Gardner et al [19]. Grade I/II day-3 and day-8 cell embryos were defined as top-quality embryos (TQEs) [20]. Top-scoring blastocysts (TQEs) were defined as blastocysts with scores of 3AA, 4AA or 5AA [21].

Statistical analysis

Premature luteinization was defined as a P/E_2 ratio of > 1, and the ratio calculation was performed as follows: P (ng/mL) × 1,000/E_2 (pg/mL). The prematurely luteinized and non-prematurely luteinized groups were compared with respect to age, levels of LH, P, and E_2, dose of rFSH, duration of stimulation, number of oocytes retrieved, number of embryos and TQEs, implantation rate, and pregnancy rate.

Data were analyzed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA). The unpaired Student's t test, Mann-Whitney rank sum test or Fisher's exact test were used for statistical analyses as appropriate. The significance level was set at $p < 0.05$.

Results

According to the criterion of P/E_2 ratio of > 1, 137 of 339 cycles were demonstrated to have premature luteinization in the study group (Tables 1 and 2). The incidence of premature luteinization was 40.4%. Ovarian responses were demonstrated as poor with serum estradiol levels of < 500 pg/mL on hCG administration day. It was noted that there were more cases with poor ovarian response in the study group (Table 3).

The mean age was similar between the group with and the group without premature luteinization (Table 1). The level of P on the day of hCG administration was higher (1.3 ± 1.0 *vs.* 1.1 ± 0.5 ng/mL; $p < 0.05$) and the peak estradiol was lower (804.1 ± 495.9 *vs.* 1,827.9 ± 906.1 pg/mL; $p < 0.05$) in the study group. The mean level of LH on day 6 of stimulation was similar in both the control and study groups (1.4 ± 1.2 *vs.* 1.4 ± 0.9 mIU/mL; $p = 0.641$), but higher in the control group than in the study group on the day of hCG administration (1.5 ± 1.1 *vs.* 1.0 ± 0.6 mIU/mL; $p < 0.05$).

The number of oocytes retrieved (7.9 ± 3.9 *vs.* 4.7 ± 2.9; $p < 0.05$) and mature oocytes recovered (4.6 ± 2.4 *vs.* 2.8 ± 1.8; $p < 0.05$) were significantly higher in the control group than in the study group (Table 1). Fertilization rates were similar between the two groups. Regarding embryo development, embryo number (6.5 ± 3.6 *vs.* 3.9 ± 2.6; $p < 0.05$), TQE number (4.4 ± 2.7 *vs.* 2.8 ± 1.9; $p < 0.05$), transferred embryo number (2.9 ± 0.8 *vs.* 2.5 ± 1.1; $p < 0.05$) and transferred TQE number (2.5 ± 1.0 *vs.* 2.2 ± 1.2; $p < 0.05$) were significantly higher for the control group than the study group (Table 2). However, the percentage of TQE was similar for the control and study groups (67.5% *vs.* 71.7%; $p = 0.127$).

The number of days of stimulation did not differ significantly, but the total number of ampoules of rFSH used was lower in the control group than in the study group (28.7 ± 8.3 *vs.* 32.2 ± 12.2; $p < 0.05$). All patients in both groups received embryo transfers.

Table 2. Development potential of oocytes and clinical outcome in the study and control groups

	Control group† (*n* = 202 cycles)	Study group† (*n* = 137 cycles)	*p*
Fertilization rate (%)	84.5	85.3	0.597
Embryos			
Embryos (*n*)*	6.5 ± 3.6	3.9 ± 2.6	< 0.05
TQEs (*n*)*	4.4 ± 2.7	2.8 ± 1.9	< 0.05
TQE rate (%)	67.5	71.7	0.127
Transferred embryos, (*n*)*	2.9 ± 0.8	2.5 ± 1.1	< 0.05
Transferred TQEs (*n*)*	2.5 ± 1.0	2.2 ± 1.2	< 0.05
Endometrial thickness (cm)*	1.3 ± 0.3	1.2 ± 0.3	< 0.05
Implantation rate (%)	22.5	22.4	1.0
Pregnancy rate (%)	48.5	36.5	< 0.05

**Data are presented as mean ± standard deviation; †premature luteinization was defined according to the criterion, progesterone/E_2 ratio > 1. TQE = top-quality embryo.*

Table 3. Patient distribution according to peak estradiol (E_2) level*

Peak E_2 level (pg/mL)	Control group† (*n* = 202 cycles)	Study group† (*n* = 137 cycles)	*p*
< 500	3 (1.5)	34 (24.8)	< 0.001
500–1,000	32 (15.8)	72 (52.6)	< 0.001
1,000–2,000	101 (50)	27 (19.7)	< 0.001
> 2,000	66 (32.7)	4 (2.9)	< 0.001

**Data are presented as n (%); †premature luteinization was defined according to the criterion, progesterone/E_2 ratio > 1.*

One hundred and thirty gestational sacs were demonstrated by ultrasonography after the transfer of 577 embryos in the control group, and 76 gestational sacs were noted after the transfer of 339 embryos in the study group. Procedures for the non-prematurely luteinized group yielded a similar implantation rate as those for the prematurely luteinized group (22.5% *vs.* 22.4%; $p = 1.0$). Furthermore, 98 (48.5%) clinical pregnancies were achieved in the 202 cycles without premature luteinization. In contrast, 50 (36.5%) pregnancies were achieved in 137 cycles with premature luteinization. The difference was statistically significant ($p < 0.05$).

Discussion

It has been debated for many years whether P increase in the late follicular phase of COH has a detrimental effect on the outcome of IVF. Reduced implantation and pregnancy rates were reported by some but not all investigators. The definition of premature luteinization differed and was believed to be responsible for the variable pregnancy rates in previously published literature. Most studies used an absolute P level on the day of hCG administration as an indicator of premature luteinization, and the cutoff level differed from 0.8 to 2 ng/mL [4,6–11]. Recently, Younis et al [12] defined premature luteinization as a P/E_2 ratio of > 1. This criterion could differentiate between the P level secretion from dysmature follicles and physiologic secretion from multiple healthy mature follicles. Based on this definition, they concluded that premature luteinization adversely affected clinical outcomes in long GnRHa cycles with hMG stimulation. Previous literature reported incidence of premature luteinization varying from 13% to 71%, using P only to define premature luteinization [4–10]. The incidence of premature luteinization in the present study was 40.41%, which is similar to 41% in the report of Younis et al [2], using the definition of P/E_2 ratio > 1 for premature luteinization. Thus, it is suggested that the main reason for the variation was the use of different definitions for premature luteinization.

Premature luteinization as defined by the P/E_2 ratio was more prevalent in poor ovarian responders in our study (Table 3). Perhaps it related to poor ovarian response with increased LH sensitivity, similar to the report by Younis et al [2]. This assumption deserves further evaluation. Premature luteinization may adversely affect the outcome of IVF by either altering oocyte quality or impairing endometrial receptivity. Although

several studies suggested that the lower pregnancy rate in premature luteinization was associated with reduced oocyte or embryo quality, inappropriate definitions of premature luteinization mean that this needs to be further evaluated [11,22]. In the present study, there were significantly lower numbers of both total oocytes and matured oocytes retrieved in the prematurely luteinized group than in the non-prematurely luteinized group. Younis et al [2] found that premature luteinization in patients with unexplained infertility was related to a lower number of follicles and indicated an early manifestation of low ovarian reserve. Low ovarian reserves contribute to the process of follicular diminution, often coupled with a decline in the number of oocytes retrieved. On the contrary, Hofmann et al [9] reported that higher serum P/E_2 ratios on the day of hCG administration were not associated with diminished ovarian reserves, as demonstrated by the clomiphene citrate challenge test or pregnancy outcomes. They also found a strong positive correlation between the P/E_2 ratio and the number of ampoules of hMG required for hyperstimulation, the peak estradiol level, and the number of mature oocytes retrieved. However, we found that the prematurely luteinized group required a higher number of ampoules of rFSH for hyperstimulation, but had lower peak estradiol levels and lower numbers of oocytes retrieved than the non-prematurely luteinized group. Our data were close to that of Younis et al [2]. The reasons for discrepancies between our results and those of Hofmann et al [1] are not clear, since both Younis et al [2] and Hofmann et al [1] used hMG for stimulation, which contained LH, hCG and possible growth factors. Özçakir et al [23] used rFSH for stimulation and found that the prematurely luteinized group had both a lower total number of oocytes and a lower number of mature oocytes retrieved, the results of which were similar to ours, except that they did not record peak estradiol levels.

The pathogenesis of premature luteinization in long GnRHa cycles is still controversial. Although pituitary desensitization with GnRHa is reported to eliminate both immunoactive and bioactive LH surges [24], a serum P rise during the late follicular phase can occur when GnRHa is combined with gonadotropins for COH in patients undergoing assisted reproductive techniques [5–7,10,11]. Its incidence has been reported to occur in 2–35% of cycles [4]. Although the long GnRHa protocol can prevent premature LH elevation in 95–98% of patients [25,26], we cannot invariably blame increased preovulatory LH levels as the sole pathogenic factor in premature luteinization.

Is there any explanation for premature luteinization in long GnRHa cycles with hMG stimulation? In comparing women with and without premature luteinization during GnRHa and hMG IVF cycles, Copperman et al [13] found higher hCG serum levels in women who experienced a serum P rise, suggesting that premature luteinization, despite pituitary suppression with GnRHa, may be due to the hCG content of hMG [27]. According to this hypothesis, the use of human rFSH or human urinary FSH in which LH activity is negligible [28] or practically absent [29] should not provoke premature luteinization. If this was the unique etiology of premature luteinization, the use of rFSH with negligible intrinsic LH bioactivity should then help avoid premature P rise. However, in the present study, we observed the occurrence of premature luteinization in patients who underwent COH by using GnRHa in combination with rFSH for assisted reproductive technology, as in the previous reports [4,23]. Some authors have proposed that the urinary hCG or LH content of the menotropins causes subtle elevations in P levels [5,13]. However, we as well as others [4,23] have used rFSH exclusively and have found a high incidence of P/E_2 ratio elevation, even though follicles were not exposed to these putative contaminants. Based on LH measurements and the use of rFSH, we can safely conclude that neither the LH nor the hCG content of the recombinant preparations is responsible for this elevation of P/E_2 ratio level.

It seems that there may be another mechanism to explain the premature luteinization in long GnRHa cycles with rFSH stimulation. Younis et al [2] suggested that premature luteinization is not necessarily an LH-dependent event and may be primarily related to an adversely affected cumulus–oocyte complex. Their LH levels on the day of hCG administration were similar for the study and control groups. In our study, the levels of LH on day 6 of ovarian stimulation were similar in both groups and, more interestingly, higher in the non-prematurely luteinized group than the prematurely luteinized group on the day of hCG administration (1.5 ± 1.1 *vs.* 1.0 ± 0.6 mIU/mL). As stated above, speculation has been that premature luteinization may be related to poor ovarian response with increased LH sensitivity [2]. Using rFSH instead of hMG could eliminate exogenous LH, hCG and growth factors in the preparation of gonadotropins, and permit a more elegant examination of the phenomenon of premature luteinization. Although the actions of FSH have been exhaustively studied and are thought to be well defined, there have been some novel twists to the FSH signal transduction. In particular, FSH stimulates the phosphorylation of kinases closely related to protein kinase A that are downstream targets of insulinlike growth factor-1 [30–32]. Thus, hyperstimulation with FSH may induce

the expression of genes other than only the aromatase pathway. These findings are consistent with those recently reported by Filicori et al [33], who found a strong positive correlation between the administered dose of FSH and follicular phase P levels.

In conclusion, the present study shows that premature luteinization, defined as late follicular P/E_2 ratio of > 1 in long GnRHa cycles with rFSH stimulation, adversely affected ovarian responses and clinical outcomes. It apparently does not seem to be linked to the preovulatory LH elevation and LH/hCG content of gonadotropins. It could be associated with poor ovarian response and the presence of dysmature follicles. However, longer series are needed to better address this issue.

References

1. Hofmann GE, Khoury J, Michener C. Elevated serum progesterone-to-estradiol ratio during gonadotropin stimulation for intrauterine insemination or *in vitro* fertilization is not associated with diminished ovarian reserve. *Fertil Steril* 2002;78:47–50.
2. Younis JS, Matilsky M, Radin O, Ben-Ami M. Increased progesterone/estradiol ratio in the late follicular phase could be related to low ovarian reserve in *in vitro* fertilization-embryo transfer cycles with a long gonadotropin-releasing hormone agonist. *Fertil Steril* 2001;76:294–9.
3. Fanchin R, Righini C, Olivennes F, Ferreira AL, de Ziegler D, Frydman R. Consequences of premature progesterone elevation on the outcome of *in vitro* fertilization: insights into a controversy. *Fertil Steril* 1997;68:799–805.
4. Ubaldi F, Camus M, Smitz J, Bennink HC, Van Steirteghem A, Devroey P. Premature luteinization in *in vitro* fertilization cycles using gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle-stimulating hormone (FSH) and GnRH-a and urinary FSH. *Fertil Steril* 1996;66: 275–80.
5. Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R. Premature elevation of plasma progesterone alters pregnancy rates of *in vitro* fertilization and embryo transfer. *Fertil Steril* 1993;59:1090–4.
6. Edelstein MC, Seltman HJ, Cox BJ, Robinson SM, Shaw RA, Muasher SJ. Progesterone levels on the day of human chorionic gonadotropin administration in cycles with gonadotropin-releasing hormone agonist suppression are not predictive of pregnancy outcome. *Fertil Steril* 1990;54: 853–7.
7. Silverberg KM, Burns WN, Olive DL, Riehl RM, Schenken RS. Serum progesterone levels predict success of *in vitro* fertilization-embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. *J Clin Endocrinol Metab* 1991;73:797–803.
8. Check JH, Chase JS, Nowroozi K, Dietterich CJ. Premature luteinization: treatment and incidence in natural cycles. *Hum Reprod* 1991;6:190–3.
9. Hofmann GE, Bentzien F, Bergh PA, Garrisi GJ, Williams MC, Guzman I, Navot D. Premature luteinization in controlled ovarian hyperstimulation has no adverse effect on oocyte and embryo quality. *Fertil Steril* 1993;60:675–9.
10. Givens CR, Schriock ED, Dandekar PV, Martin MC. Elevated serum progesterone levels on the day of human chorionic gonadotropin administration do not predict outcome in assisted reproduction cycles. *Fertil Steril* 1994;62: 1011–7.
11. Harada T, Yoshida S, Katagiri C, Takao N, Ikenari T, Toda T, et al. Reduced implantation rate associated with a subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotrophin-releasing hormone agonist and gonadotrophin. *Hum Reprod* 1995;10:1060–4.
12. Younis JS, Simon A, Laufer N. Endometrial preparation: lessons from oocyte donation. *Fertil Steril* 1996;66:873–84.
13. Copperman AB, Horowitz GM, Kaplan P, Scott RT, Navot D, Hofmann GE. Relationship between circulating human chorionic gonadotropin levels and premature luteinization in cycles of controlled ovarian hyperstimulation. *Fertil Steril* 1995;63:1267–71.
14. Adonakis G, Deshpande N, Yates RWS, Fleming R. Luteinizing hormone increases estradiol secretion but has no effect on progesterone concentrations in the late follicular phase of *in vitro* fertilization cycles in women treated with gonadotropin-releasing hormone agonist and follicle-stimulating hormone. *Fertil Steril* 1998;69:450–3.
15. Huang FJ, Chang SY, Tsai MY, Kung FT, Wu JF, Chang HW. Determination of the efficiency of controlled ovarian hyperstimulation in the gonadotropin-releasing hormone agonist-suppression cycle using the initial follicle count during gonadotropin stimulation. *J Assist Reprod Genet* 2001;18:91–6.
16. Tsai MY, Huang FJ, Kung FT, Lin YC, Chang SY, Wu JF, Chang HW. Influence of polyvinylpyrrolidone on the outcome of intracytoplasmic sperm injection. *J Reprod Med* 2000;45:112–20.
17. Huang FJ, Huang HW, Lan KC, Kung FT, Lin YC, Chang HW, Chang SY. The maturity of human cumulus-free oocytes is positively related to blastocyst development and viability. *J Assist Reprod Genet* 2002;19:555–60.
18. Veeck LL. Preembryo grading. In: Veeck LL, ed. *Atlas of the Human Oocyte and Early Conceptus*, Volume 2. Baltimore, MD: Williams and Wilkins, 1991.
19. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* 2000;73:1155–8.
20. Lan KC, Huang FJ, Lin YC, et al. The predictive value of using a combined Z-score and day 3 embryo morphology score in the assessment of embryo survival on day 5. *Hum Reprod* 2003;18:1299–306.
21. Huang FJ, Lan KC, Kung FT, et al. Human cumulus-free oocyte maturational profile and *in vitro* developmental potential after stimulation with recombinant versus urinary FSH. *Hum Reprod* 2004;19:306–15.
22. Schoolcraft W, Sinton E, Schlenker T, Huynh D, Hamilton F, Meldrum DR. Lower pregnancy rate with premature luteinization during pituitary suppression with leuprolide acetate. *Fertil Steril* 1991;55:563–6.

23. Özçakir HT, Levi R, Tavmergen E, Göker EN. Premature luteinization defined as progesterone estradiol ratio > 1 on hCG administration day seems to adversely affect clinical outcome in long gonadotropin-releasing hormone agonist cycles. *J Obstet Gynaecol Res* 2004;30:100–4.
24. Cedars MI, Surey E, Hamilton F, Lapolt P, Meldrum DR. Leuprolide acetate lowers circulating bioactive luteinizing hormone and testosterone concentrations during ovarian stimulation for oocyte retrieval. *Fertil Steril* 1990;53:627–31.
25. Ron-El R. Improved pregnancy rate in IVF/ET by combined long-acting GnRH analogue and gonadotropins. In: Vickery BH, Lunenfeld B, eds. *GnRH Analogues in Reproduction and Gynecology*, Volume II. Dordrecht/Boston/London: Kluwer Academic Publishers, 1990:167–71.
26. Penzias AS, Shamma FN, Gutmann JN, Jones EE, DeCherney AH, Lavy G. Nafarelin versus leuprolide in ovulation induction for *in vitro* fertilization: a randomized clinical trial. *Obstet Gynecol* 1992;79:739–42.
27. Stokman PGW, de Leeuw R, van den Wijngaard HAGW, Kloosterboer HJ, Vemer HM, Sanders ALM. Human chorionic gonadotropin in commercial human menopausal gonadotropin preparations. *Fertil Steril* 1993;60:175–8.
28. Mannaerts B, de Leeuw R, Geelen J, Van Ravestein A, Van Wezenbeek P, Schuurs A, Kloosterboer H. Comparative *in vitro* and *in vivo* studies on the biological characteristics of recombinant human follicle-stimulating hormone. *Endocrinology* 1991;129:2623–30.
29. Shaw RW, Ndukwe G, Imoedemhe DA, Bernard AG, Burford G. Twin pregnancy after pituitary desensitisation with LHRH agonist and pure FSH. *Lancet* 1985;326: 506–7.
30. Gonzalez-Robayana U, Falender AE, Ochsner S, Firestone GL, Richards JS. Follicle-stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-induced kinase (Sgk): evidence for A kinase-independent signaling by FSH in granulosa cells. *Mol Endocrinol* 2000;14:1283–300.
31. Richards JS. New signaling pathways for hormones and cyclic adenosine 3′,5′-monophosphate action in endocrine cells. *Mol Endocrinol* 2001;15:209–18.
32. Richards JS, Russell DL, Ochsner S, et al. Novel signaling pathways that control ovarian follicular development, ovulation and luteinization. *Recent Prog Horm Res* 2002;57: 195–220.
33. Filicori M, Cognigni GE, Pocognoli P, Tabarelli C, Spettoli D, Taraborrelli S, Ciampaglia W. Modulation of folliculogenesis and steroidogenesis in women by graded menotrophin administration. *Hum Reprod* 2002;17:2009–15.