Reduced In Vitro Fertilization of Human Oocytes Correlates with Raised Circulating FSH Levels During Ovarian Stimulation in Normogonadotropic Women Downregulated with GnRH-Analogues

CLAUS YDING ANDERSEN,[1,3] LARS G. WESTERGAARD,[2] ANNE GRETE BYSKOV,[1] and KARIN ERB[2]

Submitted: August 4, 1997
Accepted: September 3, 1997

Purpose: *The possible effects of circulating FSH levels as used during IVF treatment on oocyte maturation and subsequent preembryo development were evaluated.*
Methods: *Serum levels of FSH and LH on days 1 and 8 of ovarian stimulation and on the day of oocyte retrieval (OR) were correlated with subsequent preembryo development in vitro. After pituitary downregulation, 244 normogonadotropic women followed a fixed protocol for the first 7 days of stimulation.*
Results: *The average FSH concentration on day 8 of stimulation was 11.5 IU/L and exceeded the expected midcycle surge of FSH by more than 25%. In contrast, levels of LH were below an average of 2 IU/L throughout the stimulation period. The concentration of FSH on day 8 and on the day of OR showed a significant inverse correlation with cleavage rate, whereas levels of LH, age, and body mass index showed no such correlation.*
Conclusions: *Supraphysiologic levels of FSH seems to affect oocyte maturation negatively. Premature resumption of meiosis, leading to retrieval of postmature oocytes with a reduced developmental potential, is suggested as the underlying mechanism.*

KEY WORDS: ovarian stimulation; follicle stimulating hormone levels; preembryo development; in vitro fertilization; fertilization.

[1] Laboratory of Reproductive Biology, The Juliane Marie Center for Children, Women and Reproduction, University Hospital of Copenhagen, Section 5712, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.
[2] The Fertility Clinic, Odense University Hospital, DK-5000 Odense, Denmark.
[3] To whom correspondence should be addressed.

INTRODUCTION

The precise gonadotropin requirements of the developing ovarian follicle have not yet been established, and the contribution of luteinizing hormone (LH) in this process has been controversial (1). While only basal levels of LH are required to support follicular steroid biosynthesis during the follicular phase, high tonic levels of LH negatively affect fertility (2–6). The exact mechanism by which increased levels of LH reduce fertility remains unsolved, but it is generally believed that LH induces a premature resumption of meiosis, leading to ovulation/retrieval of postmature oocytes or "aged" oocytes. Such oocytes have a reduced capacity to fertilize and to undergo normal embryo development (review in Ref. 7). Increased LH levels may also induce an abnormally high androgen production by theca cells. This may subsequently reduce the implantation potential of the possible preembryos in that cycle (7–9).

During the past decade, a number of different ovarian stimulation regimens have been used to improve the development and maturation of follicles. In the 1980s the majority of stimulation regimens did not include pituitary downregulation. This changed during the 1990s and today pituitary downregulation is effected before ovarian stimulation is commenced in the majority of IVF cycles. Consequently, the circulating levels of LH during ovarian stimulation have effectively been reduced (2,3,10), generally to levels even lower than those observed during the natural menstrual cycle.

However, in contrast to the predicted negative effect of LH, in vitro fertilization and cleavage rates of oocytes have remained remarkably constant, around

1058-0468/97/1100-0581$12.50/0

55 to 65%, independent of whether pituitary downregulation is used during ovarian stimulation or whether the circulating level of LH is not reduced. Furthermore, one recent study showed that fertilization, cleavage, and conception rates are unaffected by the use of exogenous gonadotropins containing LH (i.e., Pergonal; Serono) compared to the use of preparations with negligible LH activity [i.e., urinary derived follicle stimulating hormone (FSH)-HP] (10).

In contrast to LH, circulating levels of FSH during ovarian stimulation differ significantly from that observed during the natural menstrual cycle. Naturally FSH rises in the early follicular phase, after which a nadir is reached shortly before the midcycle surge of gonadotropins. During ovarian stimulation FSH levels exceed those of the natural menstrual cycle several times. After approximately 5 days of stimulation FSH levels reach a constant high plateau, which is maintained until the time of ovulation induction (10–12).

High circulating levels of FSH have not previously been ascribed adverse effects. But recently it has been suggested that such high FSH levels may be involved in premature luteinization (13). FSH may also promote a high local synthesis of androgens through paracrine stimulation of peptide growth factors produced by granulosa cells (14).

The aim of the present retrospective study was to evaluate the effects of the high circulating levels of FSH during administration of exogenous gonadotropins on subsequent preembryo development and pregnancy potential. In normogonadotropic pituitary downregulated women undergoing IVF treatment, serum levels of FSH and LH were correlated with in vitro fertilization and cleavage of retrieved oocytes.

MATERIALS AND METHODS

Patients and Hormonal Treatment

A total of 244 women undergoing IVF treatment at the fertility clinic of Odense University Hospital was included in this study. The standard hormonal treatment consisted of the following elements: (i) Downregulation was induced with gonadotropin-releasing hormone agonist (GnRHa) (Suprefact, Hoechst, Copenhagen Denmark), 0.5 mg s.c. daily for a minimum of 14 days, starting in the midluteal phase of the cycle. When downregulation was confirmed by a serum estradiol concentration of less than 100 p*M* and a quiescent ovary as judged by ultrasound, the dose of GnRHa was reduced to 0.2 mg s.c. daily until the day of human chorionic gonadotropin (hCG) injection. (ii) Ovarian stimulation was achieved with a fixed dose of gonadotropin of 225 IU per day for 7 days (see below). On day 8 of stimulation the ovarian response was monitored by ultrasound and serum estradiol measurements and the daily dosage of exogenous gonadotropin was individualized accordingly. When at least four follicles with a diameter (i.e., mean of two measurements) exceeding 17 mm were observed, an ovulatory dose of 10,000 IU hCG (Profasi; Serono Nordic Solne Sweden) was given by i.m. injection.

Cleavage rate and preembryo development were defined as the proportion of oocytes which fertilized and cleaved into at least a two-cell pre-embryo. Details on oocyte retrieval, in vitro culture, preembryo transfer, and luteal support have been described elsewhere (10).

The inclusion criteria were: (i) female age younger than 40 years; (ii) a normal menstrual cycle ranging from 26 to 32 days; (iii) normal pretreatment serum concentrations of FSH and LH (i.e. less than 10 IU/L) on cycle day 2 obtained less than 6 months before the start of treatment; and (iv) a standard ovarian stimulation regimen planned and used. *The exclusion criteria* were: (i) infertility caused by an endocrine abnormality such as polycystic ovary syndrome and absence of ovarian function and (ii) cases in which intracytoplasmatic sperm injection or donor semen was used.

According to the type of gonadotropin administered the women were allocated to one of three groups: (i) the FSH-HP group, consisting of 82 women who were treated with urine-derived, highly purified FSH (Fertinorm HP, Serono Nordic); (ii) the hMG group, consisting of 93 women treated with human menopausal gonadotropin (Pergonal; Serono Nordic); and (iii) the rec-FSH group, consisting of 69 women treated with human, recombinant FSH (Gonal F; Serono Nordic).

The women in the hMG and FSH-HP groups were part of a prospective randomized study running from October 1994 to April 1995, and the results of this study have been published (10). A total of 175 women from this former study, namely, all those for whom all blood samples were taken and all data present, was included in the present study. The 69 women in the rec-FSH group were treated consecutively during the period December 1995 to January 1996. During the 16-month period of the present study there were no changes in treatment policy or in laboratory procedures.

The study was approved by the Ethical Committee of the counties of Fyn and Vejle.

Blood Sampling

In all patients blood was sampled three times during treatment: i) on the day on which pituitary downregulation was confirmed and ovarian stimulation initiated and before the first injection of exogenous gonadotropin was given (i.e., stimulation day 1); ii) on stimulation day 8 before the injection was given and 18 hr after the last injection of exogenous gonadotropin; and iii) on the day of oocyte retrieval, i.e., 36 hr after the injection of hCG and approximately 72 hr after the last injection of exogenous gonadotropin. The sera were immediately analyzed for estradiol and aliquots frozen at −20°C for later analysis of FSH and LH.

Hormone Assays

Estradiol was measured by fluoroimmunoassay (AutoDELPHIA oestradiol kit; Wallac Denmark A/S, Allerød, Denmark). Concentrations of FSH and LH were measured using an automated fluoroimmunoassay system (AutoDELPHIA hFSH and hLH sp kits; Wallac). The intra- and interassay coefficients of variation were 4.2 and 3.6% for estradiol, 2.3 and 4.5% for FSH, and 1.9 and 3.8% for LH, respectively.

Statistical Methods

Results are expressed as the mean ± SE unless otherwise stated. Differences between two groups were tested using Student's *t* test and chi-square test. Correlation between variables was established using regression analysis.

RESULTS

The study comprised a total of 244 women, who underwent one treatment cycle each. Characteristics of ovarian stimulation and IVF outcome are given in Table I. Women receiving different gonadotropin preparations were comparable with regard to all parameters except for the number of retrieved oocytes in the group receiving HMG, which was significantly higher than in the group receiving rec-FSH. The frequency with which pregnancy was achieved was also similar in the three groups.

Concentrations of FSH and LH on the day ovarian stimulation was initiated (i.e., day 1), on day 8 of ovarian stimulation, and on the day of oocyte retrieval are shown in Table II. After 7 days of stimulation, during which all women received 225 IU FSH/day, concentrations of FSH rose to an overall average of 11.5 IU/L. There was a significant difference between the three groups, the level being highest in the urinary FSH-HP group and lowest in the rec-FSH group.

Table I. Patient Characteristics, Outcome of Ovarian Stimulation, Ooocyte Retrieval, In Vitro Fertilization, and Preembryo Development[a]

Number	244
Age (years)	33 (23–40)
BMI [height (cm)/(weight (kg))^2]	22.8 ± 0.3
Duration of stimulation (days)	9.5 ± 0.1
No. of ampoules used	29.2 ± 0.4
No. of oocytes retrieved	13.1 ± 0.5
No. of preembryos	7.1 ± 0.4
Clinical pregnancy rate	
Per cycle	83/244 (34%)
Per preembryo transfer	83/219 (38%)
Pretreatment basal levels (IU/L) on cycle day 2	
FSH	7.9 ± 0.3
LH	5.6 ± 0.2

[a] Values are the mean ± SE per cycle.

Table III shows the correlation between the measured FSH concentrations and the mean frequency at which the retrieved oocytes subsequently fertilize and cleave in vitro and the number of obtained pregnancies. On day 1, when pituitary downregulation is achieved and before ovarian stimulation with exogenous gonadotropins is initiated, there is no significant correlation between levels of FSH and subsequent formation of preembryos. On day 8 of ovarian stimulation there is a significant negative correlation between FSH levels and the subsequent frequency at which preembryos form; concentrations in the interval of 4–7 IU FSH/L result in the formation of preembryos in 64% of the retrieved oocytes, which gradually declines to only 30% in those women who have an FSH level above 20 IU FSH/L ($r = 0.36$, $P < 0.0001$). On the day of oocyte retrieval a similar correlation is observed ($r = 0.42$, $P < 0.0001$). Each of the three gonadotropin preparations separately shows a picture similar to all measurements taken together (data not shown). The actual numbers of preembryos developed in vitro show a similar inverse significant correlation of the levels of FSH on day 8 ($r = 0.18$, $P < 0.01$) and on the day of oocyte retrieval ($r = 0.27$, $P < 0.0001$). No pregnancies are observed in the group of women with the highest levels of FSH on day 8.

Table IV shows the correlation between the circulating concentrations of LH and the mean frequency at which the retrieved oocytes subsequent fertilize and cleave in vitro and the number of pregnancies achieved. On day 1 a significantly higher cleavage rate

Table II. Concentrations of Circulating FSH and LH Correlated with the Gonadotropin Preparation and the Subsequent Mean Oocyte Cleavage In Vitro (Mean ± SE)*

	Uniary FSH-HP (Metrodin-HP)	hMG (Pergonal)	Rec-FSH (Gonal-F)	Total
No. of cycles	82	93	69	244
% oocyte cleavage	44 ± 3.2[a,b]	53 ± 3.0[a,c]	67 ± 2.7[b,c]	54 ± 2.0
Day 1 of stimulation				
FSH	3.2 ± 0.1	3.1 ± 0.1	2.5 ± 0.2	3.1 ± 0.1
LH	1.6 ± 0.1	1.8 ± 0.1	1.3 ± 0.1	1.7 ± 0.1
Day 8 of stimulation				
FSH	13.7 ± 0.4[a,b]	11.4 ± 0.3[a,c]	9.1 ± 0.3[b,c]	11.5 ± 0.2
LH	0.9 ± 0.1	1.2 ± 0.1	0.3 ± 0.1	1.0 ± 0.1
Day of oocyte retrieval				
FSH	8.0 ± 0.2[a,b]	5.9 ± 0.2[a,c]	4.0 ± 0.1[b,c]	6.0 ± 0.2
LH	0.2 ± 0.02	0.2 ± 0.02	0.3 ± 0.02	0.2 ± 0.01

* $P < 0.05$; groups with the same superscript letters are significantly different.

is found in the group of women with LH levels between 0 and 1 IU/L compared to those with LH levels in the range of 4–7 IU/L, reflecting a significant correlation between LH day 1 levels and the cleavage rate ($r = 0.24$, $P < 0.001$) and the number of preembryos ($r = 0.24$, $P < 0.001$). On day 8 a significantly lower cleavage rate is found in the group of women who have a LH level of more than 3 IU/L, representing, however, only seven cycles. The level of LH during ovarian stimulation does not seem to correlate with the achievement of pregnancy. Of the total 244 cycles, 6 samples showed a higher LH concentration than the mean pretreatment value of 5.8 IU LH/L (5 samples on day 1, 1 sample on day 8, and none on the day of aspiration). Pregnancy was achieved in two of these six cycles.

A high or a low FSH concentration during the course of ovarian stimulation was not correlated with a concomitant high or low LH concentration, i.e., FSH levels on day 8 did not correlate with LH levels on day 8 ($P > 0.10$), nor did FSH and LH levels on the day of oocyte retrieval correlate ($P > 0.10$).

Table III. Concentration of FSH During Ovarian Stimulation for IVF-ET Treatment in Relation to Subsequent Oocyte Cleavage In Vitro[a,*]

FSH level (IU/L)	No. of cycles	No. of oocytes	No. of preembryos	Oocyte cleavage rate (mean% ± SE)	No. of pregnancies
FSH level on day 1 of stimulation (range, 1–8 IU/L)					
1–2	70	1112	596	50 ± 3.1	20
3–4	143	1860	1019	54 ± 2.5	53
5–8	18	226	90	47 ± 6.5	7
FSH level on day 8 of stimulation (range, 4–23 IU/L)					
4–7	27	388	232	64 ± 4.9[a,b,c]	7
8–11	107	1331	767	56 ± 2.6[d,e]	33
12–15	79	1094	530	50 ± 3.4[a,f]	33
16–19	22	306	148	47 ± 5.6[b,d]	10
20–23	7	79	28	30 ± 10.0[c,d,f]	0
FSH level on day of oocyte retrieval (range, 2–20 IU/L)					
2–3	41	520	310	62 ± 3.6	12
4–5	72	1009	574	57 ± 3.1	23
6–7	78	1064	578	56 ± 3.6	29
8–9	35	426	173	38 ± 4.2	11
10–13	12	113	54	41 ± 8.2	7
14–20	5	66	16	24 ± 6.8	1

[a] The table includes data for all three types of gonadotropin preparations.
* $P < 0.05$; groups with the same superscript letters are significantly different.

Table IV. Concentration of Circulating LH During Ovarian Stimulation for IVF-ET Treatment in Relation to Subsequent Oocyte Cleavage In Vitro[a,*]

LH level (IU/L)	No. of cycles	No. of oocytes	No. of preembryos	Oocyte cleavage rate (mean% ± SE)	No. of pregnancies
			LH level on day 1 of stimulation (range, 0–7 IU/L)		
0–1	145	1887	1075	56 ± 2.3[a]	46
2–3	82	1066	536	50 ± 3.2	29
4–7	17	245	94	42 ± 6.1[a]	5
			LH level on day 8 of stimulation (range, 0–7 IU/L)		
0–1	200	2617	1448	55 ± 2.0[b]	64
2–3	37	481	210	46 ± 5.2	15
4–7	7	100	47	43 ± 9.5[b]	4
			LH level on day of oocyte retrieval (range, 0–2 IU/L)		
0	218	2863	1513	53 ± 1.9	77
1	23	300	186	61 ± 5.0	6
2	3	35	6	22 ± 10.9	0

[a] The table includes data for all three types of gonadotropin preparations.
* $P < 0.05$; groups with the same superscript letter are significantly different.

The mean concentrations of FSH and LH on each of the 3 monitored days were similar between those who did and those who did not become pregnant (data not shown).

The age of the woman showed no correlation with the oocyte cleavage rate or with the FSH level on day 1, day 8, and the day of oocyte retrieval (data not shown).

The derivation of patient variation in FSH levels under GnRHa suppression was not predicted by the FSH level on day 1; there was no significant correlation of the FSH levels on day 1 and on day 8 or on the day of oocyte retrieval (i.e., high levels of FSH on day 1 are not correlated with high levels on day 8 or on the day of oocyte retrieval; data not shown). Each of the three gonadotropin preparations showed a similar picture.

The body mass index (BMI) correlates inversely with the concentration of FSH on day 8 of ovarian stimulation for each of the three gonadotropin preparations separately and for all the measurements taken together. No such correlation was seen between BMI and the pretreatment values of FSH and LH (data not shown). The range of BMI was from 17 to 39 kg/m^2. When the BMI was divided into four groups (i.e., 17–20, 21–24, 25–28, and 29 or more kg/m^2), there were no significant differences among the four groups with regard to the frequency at which the oocytes fertilized and cleaved in vitro.

DISCUSSION

During the natural midcycle surge of gonadotropins the concentration of LH exceeds that of FSH. While concentrations of LH are in the range of 50–90 IU/L, the mean concentration of FSH reaches 9.3 IU/L (range, 3.9–13.3 IU/L) during the midcycle surge measured with the assay used in this study (kit instructions, AutoDELPHIA). The mean concentration of FSH in women undergoing ovarian stimulation on day 8 was 11.5 IU/L in this study and exceeded the expected midcycle surge of gonadotropins by almost 25%. Nearly 75% of all the measured FSH concentrations on day 8 were above the mean peak FSH level. Furthermore, in the original work by Ross *et al.* (15) the midcycle FSH peak is defined to "exceed the mean FSH value of the second half of the follicular phase be at least 40%." In the present study, the concentration of FSH on stimulation day 8 exceeded the mean FSH value of the second half of a normal follicular phase (approximately 4 IU/L) by more than 250%. Therefore, this study shows that levels of FSH during ovarian stimulation reach and exceed ovulatory heights of FSH. This observation was most clearly expressed in the group of women who received urinary FSH-HP, with the mean value on day 8 of stimulation being almost 50% higher and above the range of the normal midcycle FSH surge. Therefore, these data show that levels of FSH are truly supraphysiological and indicate that some of the physiological actions that FSH under-

takes during the natural midcycle surge of gonadotropins may be expressed during ovarian stimulation with exogenous FSH preparations several days before ovulation is induced with hCG.

Pituitary downregulation was effected and the ovaries were quiescent in all women before ovarian stimulation was commenced, and levels of LH consequently remained at a very low level throughout the stimulation period in the majority of cycles. Concentrations of LH showed no correlation with the frequency at which the retrieved oocytes underwent preembryo development in vitro or with the achievement of conception. This indicates that concentrations of LH are too low to affect fertility negatively. In contrast, oocytes from women with high concentrations of FSH showed a poor cleavage rate and developed a reduced number of preembryos, whereas low concentrations of FSH correlated with a subsequent high cleavage rate. Furthermore, clinical pregnancies were not observed in the group of women with high FSH levels. Quite unexpectedly, therefore, this study suggests that FSH levels during the follicular phase exceeding ovulatory heights have negative effects on oocyte maturation and fertilizability. This study does not reveal the cause of this correlation. However, several lines of evidence seem to suggest that the two key events in the ovulatory process, the resumption of meiosis in the follicle enclosed oocyte and the actual ovulatory process itself, resulting in expulsion of the oocyte from the follicle to the oviduct, exhibit different sensitivities to the gonadotropins. In vitro and in vivo studies in rats have shown that resumption of meiosis is induced in oocytes of all preovulatory follicles by exposing the ovaries to only 5 to 30% of the preovulatory surge without eliciting the ovulatory process (16,17). Furthermore, it is well known that the oocyte is fertilizable for only a limited period after ovulation, maybe only around 12 hr. After this period, fertilization does not result in a viable conception. The decline in fertilizablity with aging may be an intrinsic property of unfertilized oocytes, and it has been suggested that this aging is related to meiotic resumption (18). It may therefore be speculated that the high FSH levels during ovarian stimulation induce resumption of meiosis without inducing the ovulatory process itself, i.e., a premature resumption of meiosis, leading to ovulation/retrieval of "aged" oocytes with a reduced potential for preembryo development and conception.

There is actually also good evidence from in vitro studies to support a central role for FSH in regulating gonadotropin-induced resumption of meiosis in vivo. Recent studies have shown that only 8 IU/L of FSH is capable of inducing resumption of meiosis in cultured mouse oocytes, whereas recombinant LH and hCG at concentrations up to 1500 IU/L are without any such effect (19). These studies are in agreement with earlier studies which showed that cumulus cells possess receptors for FSH but not for LH/hCG (20,21).

Animal studies seem to support the idea that FSH in vivo may facilitate a premature resumption of meiosis. A study (18) showed that subovulatory doses of pure FSH given to rats in proestrus were able to induce asynchrony in resumption of meiosis of follicle-enclosed oocytes in vivo and ovulation. The follicle-enclosed oocytes resume meiosis before the midcycle surge of gonadotropins. When mated, these rats showed a pronounced reduction in fertility.

Taken together and based on observations in animal studies, the present study may suggest that the FSH levels observed during ovarian stimulation reach a magnitude that is sufficient to induce resumption of meiosis in the follicle-enclosed oocyte without triggering the actual ovulatory signal. This hypothesis parallels the suggested detrimental effect on fertility of elevated levels of LH during the follicular phase.

The present observations indicate that future ovarian stimulation regimens may profit from mimicking the natural follicular phase more closely. After an initial recruitment phase in which the FSH levels are raised above the threshold and follicles are stimulated to grow, FSH concentrations should not continue to rise but should be kept at a lower level, possibly around the threshold level. This could potentially lead to sufficient follicular growth without inducing negative effects by overexposure to gonadotropins.

The present retrospective study does not provide sufficient information for a comparison of the clinical outcome among the three types of gonadotropin preparations. Rather, we focused on the marked difference in the frequency at which the retrieved oocytes subsequently cleave in vitro. This may be a result of the absolute concentrations of FSH or, more likely, represent differences in the biological activity of the FSH preparations possibly represented by the isohormone profile (22,23). From the present study the inverse relationship between the observed FSH concentrations on day 8, when the amount of administered exogenous FSH was identical in all three groups, and the cleavage rate may indicate that the biopotency of rec-FSH is higher than that of the other two types of gonadotropin preparations. The rec-FSH preparation also seems to have a more basic FSH isohormone profile compared to the other two FSH preparations (22,23). This is, however, flawed by the difficulty of measuring differ-

ent FSH isoforms immunologically (24), and further prospective studies are needed to clarify this issue. It will also be interesting to evaluate whether the different FSH isoforms affect resumption of meiosis differently.

In this study the level of FSH during ovarian stimulation did not correlate with the age of the woman. Furthermore, there is no correlation with the FSH concentration on day 1, i.e., before ovarian stimulation is commenced. There was, however, an inverse correlation between FSH and BMI, expressed in each of the three groups receiving different gonadotropin preparations and in the all the samples taken together. A similar correlation has been found in women with a BMI above 25 kg/m^2 (25). Therefore, some of the great variability in FSH levels on day 8 may be explained by differences in BMI between the women. Because no significant correlation was found between the BMI and the cleavage rate of the retrieved oocytes, obesity of the women cannot explain the observed correlation between levels of FSH and the oocyte cleavage rate.

In conclusion, the present study suggests that elevated concentrations of FSH as a result of exogenous administration of gonadotropins negatively affect oocyte maturation and fertilizability. The mechanism behind this negative effect may be caused by a premature resumption of meiosis, leading to retrieval of "aged" or postmature oocytes.

REFERENCES

1. Chappel SC, Howles C: Reevaluation of the roles of luteinizing hormone and follicle-stimulating hormone in the ovulatory process. Hum Reprod 1991;6:1206–1212
2. Howles CM, Macnamee MC, Edwards RG, Goswamy R, Steptoe PC: Effect of high tonic levels of luteinizing hormone on outcome of in vitro fertilisation. Lancet 1986;2:521–522.
3. Howles CM, Macnamee MC, Edwards RG: Follicular development and early luteal function of conception and nonconception cycles after human in-vitro fertilization: Endocrine correlates. Hum Reprod 1987;2:17
4. Macnamee MC, Howles CM, Edwards RG: Pregnancies after IVF when high tonic LH is reduced by long-term treament with GnRH agonists. Hum Reprod 1987;2:569–571
5. Regan L, Owen EJ, Jacobs HS: Hypersecretion of luteinizing hormone, infertility, and miscarriage. Lancet 1990;336:1141–1144
6. Conway GS, Honour JW, Jacobs HS: Heterogeneity of the polycystic ovary syndrome: clinical, endocrine and ultrasound features in 556 pateints. Clin Endocrinol 1989;30:459–470
7. Shoham Z, Jacobs HS, Insler V: Luteinizing hormone: Its role, mechanism of action, and detrimental effects when hypersecreted during the follicular phase. Fertil Steril 1993;59:1153–1161
8. McNatty KP, Smith DM, Makris A, Osathanondh R, Ryan KJ: The microenvironment of the human antral follicle: Interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. J Clin Endocrinol Metab 1979;49:851–860
9. Yding Andersen C: Characteristics of human follicular fluid associated with successful conception after *in vitro* fertilization. J Clin Endocrinol Metab 1993;77:1227–1234
10. Westergaard LG, Erb K, Laursen S, Rex S, Rasmussen P: The effect of human menopausal gonadotrophin and highly purified, urine-derived follicle stimulating hormone on the outcome of in-vitro fertilization in down-regulated normogonadotrophic women. Hum Reprod 1996;11:1209–1213
11. Ben-Rafael Z, Strauss JF, Mastroianni L, Flickinger GL: Differences in ovarian stimulation in human menopausal gonadotropin treated women may be related to follicle-stimulating hormone accumulation. Fertil Steril 1986;46:586–592
12. Howles CM, Loumaye E, Giroud D, Luyet G: Multiple follicular development and ovarian steroidogenesis following subcutaneous administration of a highly purified urinary FSH preparation in pituitary desensitized women undergoing IVF: A multicentre European phase III study. Hum Reprod 1994; 9:424–430
13. Ubaldi F, Camus M, Smitz J, Bennink HC, Van Steirteghem A, Devroey P: Premature luteinization in in vitro fertilization using gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle-stimulating hormone (FSH) and GnRH-a and urinary FSH. Fertil Steril 1996;66:275–280
14. Yding Andersen C: Regulation of ovarian androgen production: a key parameter to succes during ovarian stimulation? Hum Reprod 1995;10:2227–2233
15. Ross GT, Cargille CM, Lipsett MB, Rayford PL, Marshall JR, Strott CA: Pituitary and gonadal hormones during spontaneous and induced menstrual cycles. Recent Prog Horm Res 1970; 26:1–62
16. Dekel N, Ayolon D, Lewysohn, O, Nemo N, Kaplan-Kraices R, Shalgi R: Experimental extension of the time interval between oocyte maturation and ovulation: Effect on fertilization and first cleavage. Fertil Steril 1995;64:1023–1028
17. Peluso A: Role of the amplitude of the goandotropin surge in the rat. Fertil Steril 1990;53:150–154
18. Mattheij JAM, Swarts JJM, van der Heijden AJH, van Helvoort ALB, Kusters IC: Advancement of meiotic resumption in graafian follicles reduces fertility in the rat. Gynecol Obstet Invest 1993;36:129–135
19. Byskov AG, Yding Andersen C, Hossaini A, Guoliang X: Cumulus cells of oocyte-cumulus complexes secrete a meiosis activating substance when stimulated with FSH. Mol Reprod Dev 1997;46:296–305
20. Lawrence TS, Dekel N, Beers WH: Binding of human chorionic gonadotropin by rat cumuli oophori and granulosa cells: A comparative study. Endocrinology 1980;106:1114–1118
21. Oxberry BA, Greenwald GS: An autoradiographic study of the binding of 125 I-labelled follicle stimulating hormone, human chorion gonadotropin and prolactin to the hamster ovary throughout the astrous cycle. Biol Reprod 1982;27:505–516
22. Lambert A, Rodgers M, Mitchell R, Wood AM, Wardle C, Hilton B, Robertson WR: In-vitro biopotency and glycoform

distribution of recombinant human follicle stimulating hormone (Org 32489), Metrodin and Metrodin-HP. Hum Reprod 1995;10:1928–1935

23. Robertson WR: Gonadotrophin isoform pattern in different pharmaceutical preparations. *In* Gonadotrophin Isoforms; Facts and Future, J Kahn (ed). Serono Fertility Series Vol 2, 1997, pp 53–61
24. Chappel SC: Heterogeneity of follicle stimulating hormone: Control and physiological function. Hum Reprod Update 1995;1:479–487
25. Kiddy DS, Sharp PS, White DM: Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: An analysis of 263 consecutive cases. Clin Endocrinol 1990;32:213–220