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Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction

Lars G.Westergaard[1,3], Steen B.Laursen[1] and Claus Yding Andersen[2]

[1]Fertility Clinic, Odense University Hospital, Odense and [2]Laboratory of Reproductive Biology, Rigshospitalet, Copenhagen, Denmark

[3]To whom correspondence should be addressed at: Fertility Clinic, Odense University Hospital, DK-5000 Odense C., Denmark

The impact of suppressed concentrations of circulating luteinizing hormone (LH) during ovarian stimulation on the outcome of in-vitro fertilization or intracytoplasmic sperm injection treatment in 200 consecutive, normogonadotrophic women (couples) was analysed retrospectively. A standard stimulation protocol with mid-luteal gonadotrophin-releasing hormone (GnRH) agonist down-regulation and ovarian stimulation with recombinant follicle stimulating hormone (FSH) was used in all cases. Blood was sampled from each woman on stimulation days 1 and 8 for analysis of oestradiol and LH in serum. A threshold value of serum LH of 0.5 IU/l on stimulation day 8 (S8) was chosen to discriminate between women with low or 'normal' LH concentrations. Low concentrations of LH on S8 (<0.5 IU/l) were found in 49% (98/200) of the women. This group of women was comparable with the normal LH group with regard to pre-treatment clinical parameters, and to the parameters characterizing the stimulation protocol with the exception of serum oestradiol concentration, which on S8 was significantly lower than in the normal LH group ($P < 0.001$). The proportion of positive pregnancy tests was similar in the two groups (30% versus 34% per started cycle), but the final clinical treatment outcome was significantly different, with a five-fold higher risk of early pregnancy loss (45% versus 9%; $P < 0.005$) in the low LH group and consequently a significantly poorer chance of delivery than in the normal LH group. It is concluded that a substantial proportion of normogonadotrophic women treated with GnRH agonist down-regulation in combination with FSH, devoid of LH activity, experience LH suppression, which compromises the treatment outcome. Whether these women would benefit from supplementation with recombinant LH or human menopausal gonadotrophin during ovarian stimulation, remains to be proven in the future by prospective randomized trials.

Key words: assisted reproduction treatment/early pregnancy loss/luteinizing hormone/profound suppression

Introduction

Ovarian stimulation as used in women in connection with in-vitro fertilization (IVF) aims at overriding the normal monofollicular development, inducing multiple follicular growth by increasing the circulating concentrations of follicle stimulating hormone (FSH). According to the now proven two-cell, two-gonadotrophin concept, it is evident that both FSH and luteinizing hormone (LH) are required for normal follicular oestradiol biosynthesis. However, it is commonly believed that low (so-called resting) concentrations of circulating LH are sufficient to support development and maturation of follicles and oocytes in normogonadotrophic women (Chappel and Howles, 1991). During recent years it has been common practice to combine gonadotrophin-releasing hormone (GnRH) analogue-induced pituitary suppression with highly purified urinary or recombinant FSH for ovarian stimulation in IVF. This combination results in concentrations of circulating LH that are much lower than during the follicular phase of the normal menstrual cycle. The reasons for this are that the pure FSH preparations—in contrast to the classical urinary human menopausal gonadotrophins (HMG)—are devoid of LH activity, and that the GnRH analogue more or less prohibits pituitary release of endogenous LH. These low concentrations of LH lead to a reduced thecal production of androgen precursors, and consequently to a reduction of ovarian oestradiol biosynthesis, as shown in a number of clinical studies (Fleming *et al.*, 1996; Westergaard *et al.*, 1996; Agrawal *et al.*, 1998).

Studies in GnRH antagonist down-regulated primates indicate that an intrafollicular environment depleted of LH and oestradiol negatively affects oocyte maturation, embryo development and ability to implant (Weston *et al.*, 1996). Clinical studies in women with hypogonadal hypogonadism have shown that while follicular development can be achieved by stimulation with pure FSH preparations, concentrations of circulating oestradiol and fertilization rates of retrieved oocytes are severely compromised compared to stimulation with preparations containing LH activity (Shoham *et al.*, 1991; Balasch *et al.*, 1995). Similarly, in normogonadotrophic women treated with GnRH analogues and highly purified FSH, a lower yield of oocytes, lower fertilization rates and reduced embryo quality was found in the group of women (one-third of all) with low LH (<0.5 IU/l) and low oestradiol concentrations in the mid-follicular phase as compared with the group of women with normal concentrations of LH and oestradiol (Fleming *et al.*, 1998). However, in contrast to the above-mentioned primate study, the ability of surplus embryos to form blastocysts *in vitro* was similar in the low and normal LH groups (Fleming *et al.*,

1998). Taken together, these studies suggest that profound suppression of LH during the mid-follicular phase of ovarian stimulation may have detrimental effects on the outcome of IVF treatment.

The aim of the present retrospective study was to examine the impact of circulating LH concentrations in the mid-follicular phase of ovarian stimulation on the outcome of IVF in normogonadotrophic women subjected to pituitary suppression with GnRH analogue from the mid-luteal phase, followed by ovarian stimulation with recombinant FSH.

Materials and methods

Patients and hormonal treatment

This study comprises a retrospective analysis of 200 IVF or intracytoplasmic sperm injection (ICSI) cycles in 200 women/couples, who during the period August 1997 to August 1998 consecutively fulfilled the below-mentioned inclusion criteria. These were: (i) female age below 40 years; (ii) regular menstrual cycles with pre-treatment concentrations of FSH and LH on cycle day 2 below 10 IU/l; (iii) standard hormonal treatment as follows: pituitary down-regulation with GnRH agonist (GnRHa) (Suprefact; Hoechst, 2970 Horsholm, Denmark) 0.5 mg s.c. daily for 14 days from the mid-luteal phase. After down-regulation was ascertained with ultrasound and serum oestradiol (<200 pmol), the dose of GnRHa was reduced to 0.2 mg s.c. daily and ovarian stimulation was started with recombinant FSH (Gonal-F; Serono Nordic, 2100 Copenhagen, Denmark) in a fixed dose of 225 IU s.c. per day for 7 days (stimulation days 1 to 7).

The ovarian response was monitored by ultrasound and serum oestradiol again on stimulation day 8, and the dose of FSH adjusted if necessary. When at least four follicles had reached a diameter >16 mm, an ovulatory dose of human chorionic gonadotrophin (HCG) (Profasi; Serono) 10 000 IU s.c. was given and oocyte retrieval performed 36 h later by vaginal ultrasound-guided follicle aspiration. Details on oocyte insemination, in-vitro culture and pre-embryo transfer have been described previously (Westergaard *et al*., 1996). A maximum of two pre-embryos was transferred after 3 days in culture, and surplus, transferable pre-embryos were cryopreserved. The luteal phase was supported by daily vaginal administration of 300 mg micronized progesterone (Progestan; Organon A/S, Skovlunde, Denmark) beginning 2 days before pre-embryo transfer and continuing until the day of pregnancy test. In cases of a positive test this regime was continued for another 3 weeks. In no case were HCG injections used for luteal phase support.

A positive pregnancy test was defined by >50 IU/l plasma β-HCG on day 14 after pre-embryo transfer. An ultrasound scan 3 weeks after a positive pregnancy test confirmed a clinical pregnancy.

Blood samples and hormone assays

Blood samples were taken on stimulation days 1 and 8, and on the day of oocyte retrieval. The sera were immediately analysed for oestradiol and aliquots frozen at –20°C for later analyses. Oestradiol and LH were measured by time-resolved immunofluorometric assays; oestradiol by the AutoDELFIA™ oestradiol kit and LH by the AutoDELFIA™ hLH spec. kit (Wallac Oy, Turku, Finland). The detection limit of the oestradiol assay was 50 pmol/l, and the intra- and inter-assay CV 3.9 and 3.8% respectively. The detection limit of the LH assay given by the manufacturer is 0.05 IU/l, while other studies have shown a detection limit of 0.02 IU/l (Apter *et al.*, 1989). The intra- and inter-assay coefficients of variation (CV) of this assay with varying concentrations of LH were found to be (mean concentration, intra- and inter-assay CV): (i) 0.16 IU/l, 2.2%, 2.3%; (ii) 0.13 IU/l, 1.7%, 2.2%; (iii) 0.08 IU/l, 6.9%, 11.1%; (iv) 0.01 IU/l, 12.4%, 22.2% ($n = 5$), thus confirming the above findings.

In addition, all sera were assayed for LH using the AxSYM system based on the microparticle enzyme immunoassay (MEIA) technology. The detection limit of this assay was 0.5 IU/l, defined as the concentration at two SD from the AxSYM LH calibrator A (0 IU/l) (Abbott Laboratories, 100 Abbott Park Road, IL, USA). Using a serum with a mean LH concentration of 0.66 IU/l, intra- and inter-assay CV of this assay were found to be 8.5% and 11.6% respectively ($n = 5$).

The correlation coefficient between the two LH assays used was 0.92 ($P < 0.0001$), when analysing all samples. Analysing samples with LH concentration below 0.5 IU/l showed a correlation coefficient of 0.46 ($P < 0.01$), indicating a relatively poor but significant correlation.

Based on this validation of LH assays and on evidence from the literature (Fleming *et al.*, 1998), a serum LH concentration of 0.5 IU/l on stimulation day 8 was chosen, as assayed by the AutoDELPHIA assay, as the threshold value to discriminate between the group of patients with low LH concentrations from those with 'normal' LH concentrations and compare treatment outcome between the two groups.

Statistical methods

Differences between the two groups of patients were tested statistically by use of the χ^2 test and the Wilcoxon rank sum test.

Results

On stimulation day 8 (S8), serum concentrations of LH were <0.5 IU/l in 98 cycles (women) and >0.5 IU/l in the remaining 102 cycles (women), with a median concentration of 0.52 IU/l for the whole patient cohort.

The demographic characteristics of all the women/couples and grouped according to S8 LH concentrations above or below 0.5 IU/l are shown in Table I. Female age, number of previous IVF attempts, body mass index and pre-treatment concentrations of FSH and LH were similar between the low and normal LH groups. The most common infertility diagnosis, tubal and male infertility were also similar, but significantly more couples in the low LH group were diagnosed with idiopathic infertility. The similarity between the two groups with regard to cancellation rate, duration of ovarian stimulation, consumption of gonadotrophins, mean number of retrieved, fertilized and cleaved oocytes, and rate of transferable pre-embryos and mean number of transferred pre-embryos are shown in Table II. The serum concentration of oestradiol on S8 was significantly lower in the low LH group compared to the normal LH group ($P < 0.001$). A similar proportion of positive pregnancy tests in the two groups of women is shown in Table III, but those with a low LH concentration on S8 had a poorer outcome due to a significantly increased risk of early pregnancy loss ($P < 0.005$), and consequently a significantly decreased chance of delivering a live baby ($P < 0.05$). The number of ectopic pregnancies was higher in the normal than in the low LH group, but the difference was not statistically significant.

The relationship between outcome of IVF and ICSI and the serum concentration of LH when down-regulation was

Table I. Demographic and pre-treatment clinical characteristics of the couples included in the study related to the concentration of serum luteinizing hormone (LH) below or above 0.5 IU/l on stimulation day 8

	Concentration of LH (IU/l) on stimulation day 8		
	<0.5	>0.5	Total
No. of cycles (%)	98 (49)	102 (51)	200 (100)
Female age, mean (range) (years)	32.9 (24–40)	32.2 (24–39)	32.5 (24–40)
Infertility diagnosis			
Tubal factor, *n* (%)	48 (49)	49 (49)	97 (49)
Mixed tubal-male factor, *n* (%)	11 (11)	16 (15)	27 (14)
Male factor, *n* (%)	20 (20)	26 (25)	46 (23)
Idiopathic infertility, *n* (%)	17 (17)[a]	8 (8)[a]	25 (13)
Endometriosis, *n* (%)	2 (2)	3 (3)	5 (3)
No. of previous IVF attempts			
None, *n* (%)	40 (40)	42 (42)	82 (41)
One, *n* (%)	35 (36)	37 (36)	72 (36)
Two or more, *n* (%)	23 (23)	23 (23)	46 (23)
Body mass index[b]	23.1 ± 0.4	22.7 ± 0.3	23.1 ± 0.4
Pre-treatment concentrations on cycle day 2			
LH (IU/l)[b]	5.5 ± 1.0	5.5 ± 1.5	5.5 ± 1.3
FSH (IU/l)[b]	6.7 ± 1.3	6.8 ± 1.4	6.7 ± 1.3

[a]$P < 0.05$.
[b]Values are mean ± SEM.
FSH = follicle stimulating hormone; IVF = in-vitro fertilization.

Table II. Outcome of ovarian stimulation, oocyte retrieval, in-vitro fertilization (intracytoplasmic sperm injection included) and pre-embryo development related to serum luteinizing hormone (LH) concentrations below or above 0.5 IU/l on stimulation day 8

	Concentration of LH (IU/l) on stimulation day 8		
	<0.5	>0.5	Total
No. of cycles started (%)	98 (49)	102 (51)	200 (100)
No. of cycles with oocyte retrieval (%)	98 (49)	102 (51)	200 (100)
No. of cycles with pre-embryo transfer (%)	85 (87)	91 (89)	176 (88)
No. of days of stimulation[b]	9.9 ± 0.4	9.9 ± 0.4	9.9 ± 0.4
No. of ampoules (75 IU) used[b]	30.0 ± 0.8	30.1 ± 0.8	30.1 ± 0.8
Serum oestradiol (pmol/l), stimulation day 8[b]	1349 ± 101[a]	2908 ± 225[a]	2117 ± 136
No. of oocytes retrieved per cycle[b]	12.7 ± 1.6	14.0 ± 1.7	13.4 ± 1.7
Mean fertilization rate (%)	62.1	64.2	63.2
Oocyte cleavage rate (%)[b]	57.6 ± 4.2	59.5 ± 3.2	58.6 ± 3.7
Transferable pre-embryos/cycle (%)[b]	27.1 ± 3.9	27.4 ± 3.5	27.3 ± 3.7
No. of pre-embryos transferred[b]	1.6 ± 0.6	1.7 ± 0.5	1.7 ± 0.5

[a]$P < 0.0001$.
[b]Values are mean ± SEM.

completed and at the start of ovarian stimulation (stimulation day 1) is shown in Table IV. None of the differences was statistically significant.

Discussion

This study showed that possible negative consequences of profound suppression of mid-follicular LH became manifest only after conception was established. Although the chance of a positive pregnancy test was independent of circulating concentrations of LH, women with low LH concentrations had a significantly increased risk of an early termination of the pregnancy and consequently a poorer chance of delivery. Collectively, the present results suggest that low concentrations of LH have a detrimental effect in the outcome of IVF, and that circulating LH concentrations above a certain critical level are required for optimal oocyte maturation. Thus, the present study argues against previous studies (Chappel and Howles, 1991; Loumaye *et al.*, 1997) that advocate the insignificance of LH during folliculogenesis. In addition, recent studies in women with hypogonadotrophic hypogonadism support the importance of LH for normal follicular development and oocyte maturation. Although follicular development, oocyte retrieval and pre-embryo development may be obtained using pure FSH, no pregnancies have so far been reported (Gordon, 1999), whereas a number of pregnancies have been achieved in such women when stimulated with preparations containing both FSH and LH-like activity (Shoham, 1999).

In the present study, all clinical parameters characterizing the stimulation protocol were similar between the low and

Table III. Clinical outcome of in-vitro fertilization and intracytoplasmic sperm injection related to serum concentrations of luteinizing hormone (LH) below or above 0.5 IU/l on stimulation day 8

	Concentration of LH (IU/l) on stimulation day 8		
	<0.5	>0.5	Total
No. of started cycles	98	102	200
No. of cycles with pre-embryo transfer	85	91	176
Positive pregnancy test (*n*)	29	35	64
% per started cycle	30	34	32
% per transfer	34	38	36
Biochemical pregnancies (*n*)	4	2	6
Clinical pregnancies (*n*)	25	33	58
Ectopic pregnancy (*n*)	1	5	6
% of positive tests	3	14	9
Early pregnancy loss[a] (*n*)	13	3	16
% of positive tests	45[c]	9[c]	25
% of clinical pregnancies[b]	36[c]	3[c]	17
Deliveries (*n*)	15	27	42
% of positive tests	52[d]	77[d]	66
% of clinical pregnancies	60	82	72

[a]Including biochemical pregnancies and clinical spontaneous abortions, but excluding ectopic pregnancies.
[b]Including clinical spontaneous abortions but excluding biochemical and ectopic pregnancies.
[c]$P < 0.005$.
[d]$P < 0.05$.

Table IV. Clinical outcome of in-vitro fertilization and intracytoplasmic sperm injection related to serum concentrations of luteinizing hormone (LH) on stimulation day 1

	Concentration of serum LH (IU/l) on stimulation day 1				
	<0.5	0.5–1.0	1.1–1.5	>1.5	Total
No. of started cycles	35	65	56	44	200
No. of cycles with pre-embryo transfer	31	56	53	36	176
Positive pregnancy test (*n*)	9	22	20	13	64
% per started cycle	26	34	36	30	32
% per transfer	29	39	38	36	36
Biochemical pregnancies (*n*)	2	1	3	0	6
Clinical pregnancies (*n*)	7	21	17	13	58
Ectopic pregnancy (*n*)	1	1	2	2	6
Clinical spontaneous abortion (*n*)	1	4	2	3	10
Early pregnancy loss[a] (*n*)	3	5	5	3	16
% of positive tests	33	23	25	23	25
% of clinical pregnancies[b]	14	19	12	23	17
Deliveries (*n*)	5	16	13	8	42
% of positive tests	56	73	65	62	66
% of clinical pregnancies	71	76	76	62	72

[a]Including biochemical pregnancies and clinical spontaneous abortions, but excluding ectopic pregnancies.
[b]Including clinical spontaneous abortions, but excluding biochemical and ectopic pregnancies.

normal LH groups, with the important exception of circulating concentrations of oestradiol. In accordance with the two-cell, two gonadotrophin concept of oestradiol biosynthesis, significantly reduced concentrations of serum oestradiol were found in the group of women with low LH concentrations compared to those with LH concentrations >0.5 IU/l. Whether such reduced oestradiol concentrations may be linked to an underlying mechanism for the observed effects is uncertain, but it may be speculated that in women with low LH concentrations an intrafollicular environment with reduced oestradiol concentration is created. This oestradiol-depleted intrafollicular environment may be less than optimal for normal oocyte maturation during the follicular phase, which later on is expressed as a reduced ability to form a viable conceptus. Previously published clinical and experimental data give some credit to this assumption. Studies in women have shown that a low oestrogen to androgen ratio in the follicular fluid is negatively correlated to a successful outcome of IVF (Yding Andersen, 1993), that oestrogen receptors are identifiable within the human oocyte (Wu *et al*., 1993), and that oestrogens apparently exert their actions mostly–if not exclusively–on the oocyte cytoplasm and cellular membrane and not on the nuclear, meiotic maturation (Yoshimura and Wallach, 1987; Tesarik and Mendoza, 1995). Actual studies on oocytes and

pre-embryos from women with low and normal LH concentrations are needed to evaluate whether the nuclear maturation is insufficient, or whether the intrafollicular cytoplasmic maturation is suboptimal.

The present study demonstrates that nearly half of normogonadotrophic women subjected to ovarian stimulation with a GnRH analogue combined with recombinant FSH experienced a profound suppression of circulating concentrations of LH, below 0.5 IU/l, during the mid-follicular phase of ovarian stimulation. Measured on stimulation day 1 (data not shown), this study found that 21% (41/200) of the women had LH concentrations below 0.5 IU/l. This was in contrast to the results of a previous study which showed a much lower frequency of women with low LH concentrations during ovarian stimulation: 6% with LH <0.5 IU/l on stimulation day 1, and 18% on the day of HCG administration (Loumaye *et al.*, 1997). The reason for this discrepancy is not clear, but it may be ascribed to differences in the method and efficiency of GnRHa down-regulation, the type of gonadotrophin preparation, or the sensitivity of the LH assays used. Furthermore, the earlier study (Loumaye *et al.*, 1997) consisted of combined data from three prospective multicentre studies, including a total of 323 women subjected to GnRHa down-regulation and stimulation with recombinant or urinary FSH. As pituitary gonadotrophin suppression is less efficient on stimulation day 1 than on day 8, it may be expected that LH measurements on day 8 more accurately predict deficiency of endogenous LH stimulation. Actually, the data in this study support this view, as LH concentrations below 0.5, 1.0 or 1.5 IU/l on stimulation day 1 did not predict an increased risk of early pregnancy loss (Table IV). This finding agrees with preliminary findings of an American prospective multicentre study using a serum LH concentration of 1.5 IU/l on stimulation day 1 in down-regulated women to discriminate between the group of women with low and normal LH concentrations (Kelly and Nebiolo, 1999). However, it raises the question of what threshold value should be used to discriminate between LH concentrations which may be considered sufficient and those which may be too low. Recent studies on the use of GnRH antagonist in ovarian stimulation in IVF may actually provide some information in answering this question. The immediate and dose-dependent reduction of endogenous pituitary LH secretion resulting from administration of GnRH antagonist in combination with recombinant FSH and the possible consequences for the outcome of IVF was studied in a large European multicentre dose-finding study (The Ganirelix dose-finding study group, 1998). In this study, which included 333 women receiving six different doses of the GnRH antagonist, ganirelix, administration of the antagonist was started on day 6 of stimulation to prevent premature LH surge during ovarian stimulation with recombinant FSH. Only in the two highest dose groups, i.e. 1 mg and 2 mg per day, was serum LH concentration suppressed well below 1 IU/l on the day of HCG injection, 0.6 and 0.4 IU/l respectively. Interestingly, in spite of the fact that the number of retrieved oocytes and the number of good quality embryos was similar to that found in the lower dose groups, implantation rates were lower and early miscarriage rates higher in these two groups, with no ongoing pregnancies in the 2 mg per day group (The Ganirelix dose-finding study group, 1998).

In the present study, unwanted effects were observed when LH concentrations on stimulation day 8 were below 0.5 IU/l, whereas a threshold value of 1.0 or 1.5 IU/l on day 8 or on day 1 of stimulation did not show similar detrimental effects on pregnancy outcome. Therefore, this study suggests that when LH concentrations fall below a limit of around 0.5 IU/l, clinical effects become manifest. To make this a suitable threshold value, on the other hand, requires assays which monitor LH concentrations in this range with a reasonable accuracy. In this study, two commercial assays were evaluated, and it was found that one assay measured LH concentrations in the 0.5 IU/l range with good precision. Taken together, a threshold value of 0.5 IU/l LH on stimulation day 8 seems to reflect possible unwanted effects, which can be measured reliably using the presently available assay.

Although the present study does not offer an explanation for the detrimental effects of low LH concentrations, the results clearly raise the question of whether women with low mid-follicular LH concentrations would benefit from supplementation with exogenous LH-like activity during ovarian stimulation. To answer this question however, large prospective clinical trials comparing the use of recombinant FSH only with preparations containing some LH activity or LH given separately are needed. Such a study including 400 cycles is currently under way in our clinic.

References

Agrawal, R., Conway, G.S. and Engmann, L. (1998) Implications of using follicle-stimulating hormone preparations depleted of luteinizing hormone to achieve follicular growth in *in vitro* fertilization. *Gynecol. Endocrinol.*, **12**, 9–15.

Apter, D., Cacciatore, B., Alfthan, H. *et al.* (1989) Serum luteinizing hormone concentrations increase 100-fold in females from 7 years of age to adulthood, as measured by time-resolved immunofluorometric assay. *J. Clin. Endocrinol. Metab.*, **68**, 53–57.

Balasch, J., Miro, F. and Burzaco, I. (1995) The role of luteinizing hormone in human follicle development and oocyte fertility: evidence from in-vitro fertilization in a woman with long-standing hypogonadotrophic hypogonadism and using recombinant human follicle stimulating hormone. *Hum. Reprod.*, **10**, 1678–1683.

Chappel, S. and Howles, C. (1991) Reevaluation of the roles of luteinizing hormone and follicle stimulating hormone in the ovulatory process. *Hum. Reprod.*, **6**, 1206–1212.

Fleming, R., Chung, C.C., Yates, R.W.S. *et al.* (1996) 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.*, **11**, 1854–1858.

Fleming, R., Lloyd, F., Herbert, M. *et al.* (1998) Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. *Hum. Reprod.*, **13**, 1788–1792.

Gordon, U.D. (1999) Effects of different menotropin preparations for assisted reproduction in hypogonadotropic hypogonadism. In Filicori, M. (ed.), *The Role of Luteinizing Hormone in Folliculogenesis and Ovulation Induction*. Monduzzi Editore, Bologna, 1999, pp. 135–143.

Kelly, E.E. and Nebiolo, L. (1999) Recombinant FSH therapy alone (Gonal-F) versus combination therapy (Gonal-F + Lhadi, R-HLH) in patients down-regulated with low dose luteal GnRH agonist protocol. Preliminary results. *11th World Congress on In vitro Fertilization and Human Reproductive Genetics*. Abstract S-003.

Loumaye, E., Engrand, P., Howles, C. *et al.* (1997) Assessment of the role of serum luteinizing hormone and estradiol response to follicle-stimulating hormone on in vitro fertilization treatment outcome. *Fertil. Steril.*, **67**, 889–899.

The Ganirelix dose-finding study group (1998) 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.*, **13**, 3023–3031.

Shoham, Z. (1999) Effects of different menotropin preparations for ovulation induction in hypogonadotropic women. In Filicori, M. (ed.), *The Role of Luteinizing in Folliculogenesis and Ovulation Induction*. Monduzzi Editore, Bologna, 1999, pp. 105–114.

Shoham, Z., Balen, A. and Patel, A. (1991) Results of ovulation induction using human menopausal gonadotropin or purified follicle-stimulating hormone in hypogonadotropic hypogonadism patients. *Fertil. Steril.*, **56**, 1048–1053.

Tesarik, J. and Mendoza, C. (1995) Nongenomic effects of 17 beta-estradiol on maturing human oocytes: relationship to oocyte developmental potential. *J. Clin. Endocrinol. Metab.*, **80**, 1438–1443.

Westergaard, L.G., Erb, K., Laursen, S.B. *et al.* (1996) The effect of human menopausal gonadotrophin and purified, urine-derived follicle stimulating hormone on the outcome of in-vitro fertilization in down-regulated normogonadotrophic women. *Hum. Reprod.*, **11**, 1209–1213.

Weston, A.M., Zelinski-Wooten, M.B., Hutchison, J.S. *et al.* (1996) Developmental potential of embryos produced by in-vitro fertilization from gonadotrophin-releasing hormone antagonist-treated macaques stimulated with recombinant human follicle stimulating hormone alone or in combination with luteinizing hormone. *Hum. Reprod.*, **11**, 608–613.

Wu, T.C., Wang, L. and Wan, Y.J. (1993) Detection of estrogen receptor messenger ribonucleic acid in human oocytes and cumulus-oocyte complexes using reverse transcriptase-polymerase chain reaction. *Fertil. Steril.*, **59**, 54–59.

Yding Andersen, C. (1993) Characteristics of human follicular fluid associated with successful conception after *in vitro* fertilization. *J. Clin. Endocrinol. Metab.*, **77**, 1227–1234.

Yoshimura, Y. and Wallach, E.E. (1987) Studies on the mechanism(s) of mammalian ovulation. *Fertil. Steril.*, **47**, 22–34.

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