

# Suppression of LH during ovarian stimulation: effects differ in cycles stimulated with purified urinary FSH and recombinant FSH

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**There has been much debate about the role of luteinizing hormone (LH) during follicle stimulating hormone (FSH)-treated ovarian stimulation for assisted reproduction, where the endogenous LH is suppressed using a gonadotrophin-releasing hormone analogue. The requirement for LH in oestradiol biosynthesis is established, but other effects of 'insufficiency' are less clear, and little attention has been paid to the specific origin of the FSH used. The aim of this study was to examine the roles of profoundly suppressed circulating LH concentrations in cycles of ovarian stimulation for IVF, which were affected in two large separate cohorts of patients undergoing assisted reproduction. They were stimulated by either purified urinary FSH (MHP) or recombinant human FSH (rFSH). Within each dataset, outcomes were examined with respect to the circulating concentrations of LH in the mid-follicular phase, as plasma samples were stored prospectively, and assayed retrospectively. Patients with profoundly suppressed LH showed much reduced oestradiol concentrations at mid-follicular phase and at human chorionic gonadotrophin administration in cycles treated with either MHP or rFSH. However, gross ovarian response, as became evident by FSH dose demands, duration of stimulation, and also oocyte and embryo yields and embryo cryopreservation were influenced only in cycles treated with MHP. Furthermore, no effect upon pregnancy survival was observed. Thus, it is concluded that there is a demand for additional exogenous LH treatment only in cycles treated with purified urinary FSH where the LH is profoundly suppressed.**

*Key words:* follicle stimulating hormone/follicular growth/gonadotrophins/luteinising hormone/ovarian stimulation

## Introduction

There is evidence supporting roles for LH in three particular functions in the life history of human ovarian follicles prior to luteinization. The clearest evidence supports a well-defined and direct role for LH in steroid biosynthesis, but it may also be capable of influencing the dynamics of follicular growth,

as well as the development of oocyte competence, and that of the subsequent embryo.

In the maturing Graafian follicle, the role of LH is governed by the two-cell, two-gonadotrophin hypothesis, whereby LH induces androgen biosynthesis in the theca cells, which the FSH-stimulated granulosa cells convert to oestrogen. This accounts for the appearance of oestradiol in the follicular fluid and the peripheral circulation, and an absence of measurable LH leads to negligible oestradiol production, even when FSH can stimulate follicular growth (Couzinet *et al.*, 1988). In fact, it has been proposed that the latter stages of follicular growth in women, may be induced by FSH alone (Loumaye *et al.*, 1997). However, the results of a recent multicentre study with recombinant FSH (rFSH) and recombinant LH (rLH) treatment of women with hypogonadotropic hypogonadism, suggested that LH actually sensitizes follicles to FSH, and thereby encourages follicular development as well as hormone secretion (ERHLHSG, 1998). The number of follicles developing after a fixed dose of rFSH was much reduced or delayed in the group treated without rLH. The dose of 75 IU rLH per day appeared to be sufficient to facilitate most functions of follicular development.

A role of LH at other levels of ovarian function may be implied by two other lines of evidence. In an in-vitro small mammal model, the importance of the interaction between LH and the theca cell mass in physiological events as well as follicular endocrinology has been highlighted (Cortvrindt *et al.*, 1998). Mouse follicles cultured in the presence of rFSH without rLH clearly demonstrated a reduced rate of antrum formation, and oocyte nuclear maturation. In sheep follicular cells and intact follicles cultured *in vitro*, LH was seen to be obligatory for the production of specific polypeptides seen *in vivo* (Moor and Crosby, 1987).

Further evidence derives from a woman with hypogonadotropic hypogonadism undergoing IVF after treatment with purified FSH or FSH combined with additional oestradiol, in which oocytes were obtained which showed poor fertilization rates. The same individual was subsequently treated with combined FSH and LH (human menopausal gonadotrophin; HMG), and the oocytes showed normal fertilization rates. These data suggest that the LH-depleted environment may yield oocytes with a reduced potential for further development (Balasch *et al.*, 1995).

Further evidence for LH activity during the follicular phase having an impact upon oocyte/embryo competence was provided by studies in macaque monkeys in which LH was suppressed using a gonadotrophin-releasing hormone antagonist, and follicular growth stimulated with rFSH or rFSH with rLH. Reasonable follicular growth and oocyte yields were

obtained after stimulation with rFSH alone, and higher fertilization rates were obtained with rFSH compared with the combination of rFSH and rLH (Zelinski-Wooten *et al.*, 1996). However, the absence of rLH had a detrimental effect upon embryo cryosurvival, development and implantation after cryopreservation and thawing (Weston *et al.*, 1996). These data imply that there may be an undefined physiological role of LH with implications for oocyte maturation and embryo developmental potential.

In the clinical situation of assisted reproduction, the three most important criteria are numbers of oocytes obtained after a course of ovarian stimulation, their developmental competence, and the ability of implanted embryos to sustain viable fetal development.

It is clear that the widespread use of urinary FSH or purified urinary FSH, without LH supplementation in ovarian stimulation, has not led to a drop in overall programme success rates, and it has been suggested that there is no need to explore the requirement for LH supplementation further (Hull *et al.*, 1994). However, different hormone profiles have been recorded according to LH activity in the stimulant and/or in the circulation (Fleming *et al.*, 1996; Fried *et al.*, 1996), and the exploration of other possible effects of LH depletion after gonadotrophin-releasing hormone analogue (GnRHa) co-treatment have been rare. Two prospective studies were performed to examine the effects of profoundly suppressed LH during the follicular phase of COS cycles. The results indicated that when purified, urinary FSH was used, profound suppression of LH had no effect upon oocyte or embryo developmental potential, but there were implications with respect to ovarian steroid and inhibin biosynthesis, and also a reduced oocyte and embryo yield (Fleming *et al.*, 1998). One of the aims of this study was to determine if effects seen in a relatively small and controlled prospective study could be detected in an unselected evaluation within a treatment programme, where the population shows much greater diversity.

Recently, rFSH has also enjoyed widespread use in ovarian stimulation programmes with considerable success. It has different isoform profiles from urinary-derived FSH and is probably, thereby, more potent (De Leeuw *et al.*, 1996) and appears to show increased efficacy and improved pregnancy rates by comparison with purified FSH treatment (Out *et al.*, 1995). However, the role of profoundly suppressed follicular phase LH during COS has not been determined with rFSH as the ovarian stimulant. Differences in responses with respect to LH in the circulation which are influenced by FSH source would indicate that in this clinical situation, there is a balance of importance between FSH potency and the biochemical roles of LH.

In response to these observations an investigation was undertaken exploring the effects of profound LH suppression in two complete databases of patients in the assisted conception programme treated with purified, urinary FSH or with rFSH. The two databases represented separate cohorts of patients with no selectivity bias. Chronological differences dictated that data could only be compared within databases, not between them.

## Materials and methods

### *Patients and treatment*

All patients were nulliparous with a minimum duration of infertility of 3 years, and they underwent IVF in the assisted conception clinic at the Royal Infirmary, Glasgow.

All were treated with the standard long course GnRHa treatment starting on cycle day 21, or, in women with oligomenorrhoea, at least 14 days prior to initiation of gonadotrophin stimulation. The normal starting dose of FSH for ovarian stimulation was 225 IU daily, unless otherwise indicated by previous experience, and response assessment effected on stimulation day 7 (S7), when dose titration was effected. If the oestradiol concentration was <200 pg/ml the dose was increased, whereas if the oestradiol value was >1000 pg/ml the dose was reduced to 150 IU. The luteinizing signal of human chorionic gonadotrophin (HCG, Profasi; Serono UK Ltd, Welwyn Garden City, UK: 10 000 IU) was administered only when there were more than two mature sized follicles (diameter >16 mm) observed by ultrasonography. Oocyte retrieval was effected 37–40 h after HCG administration. Cycles were cancelled for 'poor response' after 5 days continued treatment with the increased dose without attaining, or the imminent prospect of attaining, the criteria for HCG administration.

Embryo cryopreservation was effected when there were more than one 'good quality' embryos available after the fresh embryo transfer. In order to reduce the risks and consequences of ovarian hyperstimulation syndrome, cases with >16 oocytes recovered did not receive a fresh embryo transfer, as all embryos were cryopreserved for transfer in a subsequent cycle.

### *Treatment and hormone assays*

All cycles of ovarian stimulation where the ovarian stimulant was confirmed by examination of the records were included in two separate databases where the gonadotrophin was either purified, urinary FSH (Metrodin-HP, Serono Laboratories UK: 'MHP'), or rFSH (Gonal-F, Serono Laboratories, UK: 'GOF'). The MHP cycles were treated between January and December 1996, and the GOF cycles were treated between February 1997 and February 1998. The LH status of each cycle was determined in a plasma sample taken in the mid-follicular phase: stimulation day 7 (S7), when the first ovarian ultrasound scan was effected. The samples were stored for batch analyses of oestradiol and LH.

Hormone assays were performed using a semi-automated fluoro-immunometric system (Delfia; Wallace, Milton Keynes, UK) for LH in both databases, and oestradiol in the MHP database, while oestradiol was estimated using the Immulite chemiluminescent immunoassay system (Diagnostic Products Corporation, Caerphilly, UK) in the rFSH database.

The circulating LH concentration used to identify those cycles with profoundly suppressed LH was taken as  $\leq 0.7$  IU/l (Fleming *et al.*, 1998), as the distribution of values indicated that this value represented the lowest quartile (Fleming, 1999).

### *Retrospective analyses of the treatment databases*

The functions of ovarian stimulation cycles that were deemed likely to be influenced by the circulating LH were examined for both databases. These were: the oestradiol concentrations after a fixed period of stimulation (on S7), and on the day of HCG administration; the 'poor response' cancellation rates, the proportion of cases where the gonadotrophin dose was changed, and also the duration of stimulation. The other factors examined were oocyte yields, fertilization rates, embryo yields, the proportions of cases with embryo cryopreservation and finally the rates of pregnancy loss. These factors were examined for each of the complete treatment databases.

**Table I.** The stimulation and response details examined for complete data sets of patients treated with either Metrodin high purity (MHP) or Gonal-F (GOF) divided into groups dictated by the circulating mid-follicular phase LH concentration

	MHP			GOF		
	<LH	nLH	<i>P</i>	<LH	nLH	<i>P</i>
No. of cycles	139	290		160	536	
Age (years) <sup>a</sup>	34.2 (3.4)	34.0 (3.8)	NS	34.1 (4.1)	33.4 (4.2)	NS
Poor response (%)	10.1	5.2	0.09	12.2	7.2	NS
HCG ( <i>n</i> )	125	271	NS	139	493	NS
Duration of stimulation (days) <sup>a</sup>	12.8 (2.2)	11.9 (2.1)	0.0001	12.3 (1.8)	12.1 (2.4)	NS
Dose increase (%)	15.8	7.2	0.009	11.9	11.6	NS
Dose decrease (%)	7.9	15.2	NS	5.6	12.5	0.02
Oestradiol at S7 (pg/ml) <sup>a</sup>	260 (259)	577 (669)	0.001	248 (224)	524 (566)	0.001
Oestradiol at HCG administration (pg/ml) <sup>a</sup>	1695 (1091)	2304 (1527)	0.001	1477 (877)	1972 (1179)	0.001

<sup>a</sup>Mean (SD).

&lt;LH = suppressed follicular phase LH; nLH = normal LH; HCG = human chorionic gonadotrophin; S7 = stimulation day 7; NS = not significant.

Furthermore, all first treatment cycles were examined in isolation, as they represented an unbiased cohort and had a common starting dose (universally 225 IU FSH per day, for at least 6 days) and response assessment.

### Statistical analyses

Due to changes in practice, and the non-randomized treatment schedules, the data could only be compared within databases based upon the discriminating LH value. Hormone concentrations were compared using *t*-tests, and group comparisons were compared using contingency table analyses. Data were deemed to be significantly different when  $P \leq 0.05$ , and 'marginally' significant when  $P \leq 0.08$  and  $P > 0.05$ .

## Results

### The complete data sets

Table I shows the numbers of cycles and the ages of the patients as well as the stimulation data for the patient cycles in each database. The cycles with suppressed follicular phase LH (MHP < LH, and GOF < LH) showed the same age profiles as their counterparts with normal LH values (nLH), and they had much reduced circulating oestradiol concentrations at S7 and at HCG administration in both groups of patients, compared with those whose LH was > 0.7 IU/l. With respect to the proportions of cycles cancelled due to poor response, there was no difference according to LH status within either database or between the two databases, with respect to the circulating LH concentration. However, the proportions of cases showing dose increases were higher in the MHP < LH patients compared with the MHP–nLH group. The duration of stimulation also was significantly increased in the MHP < LH group. Neither of these variables showed differences when the stimulant was Gonal-F.

Table II shows the ovarian and embryological details in the two groups of patients. Only the MHP < LH group showed an effect of suppressed LH upon gross ovarian yield. The data indicate a clear effect upon the yield of oocytes, which showed consequences upon the numbers of embryos and cases with embryo cryopreservation. The policies for embryo cryopreservation were not the same during the periods constituting the two databases, so results cannot be compared between the datasets —

only within the groups. There was no difference in fertilization rates, nor in the proportion of cycles in which all embryos were cryopreserved in order to reduce the risk of ovarian hyperstimulation syndrome.

### First treatment cycles only

Tables III and IV examine the extracted data for first treatment cycles only from the same datasets as those above. This avoids the bias of individuals being represented more than once, and a consistent and universal starting dose of FSH (225 IU/l) was used in these cases. Unlike the complete data sets, the MHP-treated cycles with reduced circulating LH concentrations showed significantly higher rates of cycle cancellation due to poor response. They also showed higher rates of increased daily doses, and a greater duration of treatment, as in the complete data sets. As previously, these differences were not observed in the Gonal-F-treated cycles. This contrasted with the differences in circulating oestradiol at S7 and at HCG administration, where significant differences were observed in both treatment groups. The effects of profoundly suppressed LH upon gross ovarian yield were similar to those of the complete data sets, although the tendency towards a reduced yield of oocytes (Table IV) was not significant. In both complete data sets and first treatment cycles only, there was a profound reduction in cases with cryopreserved embryos which were only detected in the group treated with MHP.

### Early pregnancy loss

Table V shows that there was no difference in the rate of early pregnancy loss in the patients, irrespective of ovarian stimulant used or the circulating LH. Combining the two databases revealed no effect of suppressed follicular phase LH at this level of embryo development. Cases were included where conception occurred after fresh embryo transfer during the cycle of stimulation and ectopic pregnancies were excluded.

## Discussion

The results confirmed that reduced circulating LH concentrations are associated with lower oestradiol concentrations in

**Table II.** Gross ovarian yields in the two complete data sets of patients treated with Metrodin high purity (MHP) or Gonal-F (GOF) divided into groups dictated by the circulating mid-follicular phase LH concentration

	MHP			GOF		
	<LH	nLH	<i>P</i>	<LH	nLH	<i>P</i>
<i>n</i>	125	271		160	536	
Oocyte yield <sup>a</sup>	7.42 (4.6)	8.9 (6.0)	0.015	8.26 (5.0)	8.1 (5.6)	NS
Fertilization rates (%)	75.8	76.0	NS	78.3	75.0	NS
Embryo yield <sup>a</sup>	5.6 (4.0)	6.8 (5.2)	0.023	6.5 (4.6)	6.1 (4.6)	NS
Cryopreserved embryos: no. of cases (%)	32 (25.6)	112 (41.3)	0.004	45 (28.1)	136 (25.4)	NS
All embryos cryopreserved: no. of cases (%)	10 (8)	33 (12.2)	NS	22 (13.8)	62 (11.6)	NS

<sup>a</sup>Mean (SD).

For definition of groups see Table I.

**Table III.** Ovarian responses in all first treatment cycles only. In these cycles there was a common starting dose of 225 IU day. The patients were treated with Metrodin high purity (MHP) or Gonal-F (GOF) and divided into groups dictated by the circulating mid-follicular phase LH concentration

	MHP			GOF		
	<LH	nLH	<i>P</i>	<LH	nLH	<i>P</i>
<i>n</i>	48	108		61	251	
Age	34.4 (3.6)	33.3 (3.7)	NS	33.4 (4.4)	32.6 (4.5)	NS
Poor response (%)	12.5	2.8	0.04	13.1	10.0	NS
HCG ( <i>n</i> )	42	102	NS	52	224	NS
Duration of stimulation <sup>a</sup>	13.4 (2.7)	11.9 (2.15)	0.001	12.4 (1.9)	11.8 (2.3)	NS
Dose increase: no. of cases (%)	18 (37.5)	11 (10.2)	0.001	12 (19.7)	33 (13.1)	NS
Dose decrease: no. of cases (%)	3 (6.3)	16 (14.8)	NS	3 (4.9)	30 (12.0)	NS
Oestradiol at S7 (pg/ml) <sup>a</sup>	210 (186)	599 (766)	0.001	248 (246)	582 (665)	0.001
Oestradiol at HCG (pg/ml) <sup>a</sup>	1701 (1568)	2358 (1535)	0.02	1521 (933)	2132 (1346)	0.002

<sup>a</sup>Mean (SD).

For definition of groups see Table I.

**Table IV.** Gross ovarian yields in the first treatment cycles in the two groups of patients in the two databases. The patients were treated with Metrodin High Purity (MHP) or Gonal-F (GOF) and divided into groups dictated by the circulating mid-follicular phase LH concentration

	MHP			GOF		
	<LH	nLH	<i>P</i>	<LH	nLH	<i>P</i>
<i>n</i>	42	102		52	224	
Oocyte yield <sup>a</sup>	7.7 (5.6)	9.7 (5.9)	NS	8.2 (4.9)	8.6 (6.3)	NS
Fertilization rate (%)	78.2	75.8	NS	80.3	73.0	NS
Embryo yield <sup>a</sup>	6.0 (4.8)	7.3 (5.1)	NS	6.6 (4.1)	6.3 (5.1)	NS
Cryopreserved embryos: no. of cases (%)	15 (36)	65 (63)	0.005	22 (40)	71 (32)	NS
All embryos cryopreserved: no. of cases (%)	4 (10)	16 (16)	NS	10 (20)	36 (16.1)	NS

<sup>a</sup>Mean (SD).

For definition of groups see Table I.

ovarian stimulation cycles for IVF, irrespective of whether the FSH derives from purified urinary or recombinant sources. The observation of a longer treatment duration combined with a reduced oocyte yield in the MHP < LH group indicated a clear impact of profound suppression of LH upon ovarian responses in women treated with ovarian stimulation where the stimulant is purified, urinary FSH. This confirms that the

detrimental effect of suppressed LH leading to a reduced oocyte yield previously recorded in a prospective study with selective patient recruitment (Fleming *et al.*, 1998) was also observed in a complete treatment programme. This effect had further consequences upon the treatment cycle, as fewer embryos were available for cryopreservation for the group with suppressed LH. However, no effect of profound LH

**Table V.** Details of early pregnancy failure in all cycles in the two datasets after fresh embryo transfer (excluding ectopic pregnancies)

	Fresh cycle pregnancy <i>n</i>	<LH		nLH	
		<i>n</i>	Early loss <i>n</i> (%)	<i>n</i>	Early loss <i>n</i> (%)
MHP	71	25	5 (20)	46	14 (30)
GOF	140	35	10 (28.6)	105	19 (18.1)
Combined databases	211	60	15 (25)	151	33 (22)

There were no significant differences between the two groups. For definition of groups see Table I.

suppression upon gross ovarian yields was determined in cycles treated with rFSH.

The effect of a reduced yield of oocytes in the MHP < LH cases was reflected in the lower proportion of cycles in the MHP < LH group with embryo cryopreservation. This clearly impacts upon the pregnancy potential of a treatment cycle. But again, there was no such effect in cycles treated with rFSH. To some degree the local treatment protocol influenced these results, since embryo cryopreservation was only affected when there was more than one quality of embryo available (data not shown). Thus the relatively small effect upon oocyte yield had a more profound effect upon embryo cryopreservation in the MHP < LH group. Although there was no effect of the LH suppression upon embryo scores (data not shown) the frequency of sufficient embryos of sufficient quality was critical to the cryopreservation data.

Ovarian hyperstimulation syndrome is a suitable candidate for analyses in database examinations such as these, especially as effects upon oestradiol concentrations have been observed, but the data were deemed unsuitable for comparisons within this context. This is because of the intrinsic bias incurred by the unit policy to cryopreserve all embryos when excessive numbers are retrieved, and also patients often refer themselves to other hospitals when in distress, such that reliable diagnoses and recording are not available.

The carbohydrate composition of rFSH, and consequently the isoform profile of rFSH, is different from that of highly purified FSH, and studies *in vivo* (Out *et al.*, 1995) and *in vitro* (De Leeuw *et al.*, 1996) have suggested that rFSH is more potent than urinary-derived FSH in this clinical situation. In the data presented above, the oestradiol responses to stimulation were affected equally by the LH designation in both treatment databases, reflecting the direct role of LH in oestradiol biosynthesis. However, with rFSH treatment, suppression of LH failed to show the impact upon gross ovarian responses that were evident with purified urinary FSH (MHP).

Both FSH and LH induce increases in intracellular cyclic AMP (cAMP) activity in unluteinized follicular granulosa cells, and both are capable of stimulating aromatase activity. In fact, in mature granulosa cells, LH is more potent in the role of stimulating the cAMP internal messenger (Yong *et al.*,

1994). Thus, low amounts of LH activity may contribute to the overall potency of an FSH preparation, as well as helping provide more androgen precursor for oestradiol biosynthesis. Therefore it is possible that differences in the effects of profound suppression of LH on ovarian responses in the two treatment schedules may be explained by differences in potency alone. The urinary FSH (MHP) may be less potent than the rFSH, but the low concentrations of LH remaining in the circulation may compensate for the ‘shortfall’, while the rFSH is sufficiently potent to obviate the need for LH, except in its function of providing precursors for oestradiol biosynthesis.

Pregnancy rates with different drug treatment regimes have been addressed in other studies, with larger scope and formal randomization, with the general indication that treatment with FSH alone does not reduce pregnancy rates (Out *et al.*, 1995) in IVF programmes where rFSH is used as the ovarian stimulant. However, pregnancy loss has not been explored with respect to suppressed follicular phase LH. Despite much interest in the role of excessive LH in early pregnancy loss (Homburg *et al.*, 1988), there has been no investigation into the effects of profound LH suppression into the viability of pregnancies achieved under these controlled conditions. If excessive activation of the LH receptor has implications for pregnancy viability, then it is possible that insufficient activation may be damaging also.

It has already been reported that the implantation potential of cryopreserved embryos was not affected by profound follicular phase LH suppression (Rehka *et al.*, 1998) and furthermore that fresh blastocyst development *in vitro* was also unaffected (Fleming *et al.*, 1998). Pregnancy survival also appears to be unaffected by LH suppression, at least in the results from these two datasets (Table V).

In conclusion, the data demonstrate that the impact of profound LH suppression depends upon the origin of the FSH, and that clinical implications may only apply when the ovarian stimulant is purified urinary FSH. In women with hypogonadotrophic hypogonadism, LH can be seen to sensitize follicles to FSH for recruitment and growth, as well as oestradiol secretion (ERHLHSG, 1998). However, although the degree of LH suppression achieved in ovarian stimulation cycles does not normally reach that of true hypogonadotrophic hypogonadism, similar effects based on potency can be detected

with purified urinary-derived FSH. Subsequent potential effects of LH suppression upon oocyte fertilization rates, embryo developmental capacity and pregnancy survival were not identified in these analyses.

enzyme mRNA expression by gonadotrophins and cyclic AMP in human granulosa cells. *J. Mol. Endocrinol.*, **12**, 239–249.  
Zelinski-Wooten, M.B., Hutchison, J.S., Hess, D.L. *et al.* (1996) Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. *Hum. Reprod.*, **10**, 1658–1666.

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## References

- Balasz, J., Miro, F., Burzaco, I. *et al.* (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 FSH. *Hum. Reprod.*, **10**, 1678–1683.
- Cortvriendt, R., Hu, Y. and Smitz, J. (1998) Recombinant luteinizing hormone as a survival and differentiation factor increases oocyte maturation in recombinant follicle stimulating hormone-supplemented mouse pre-antral follicle culture. *Hum. Reprod.*, **14**, 1292.
- Couzinet, B., Lestrat, N., Brailly, S. *et al.* (1988) Stimulation of ovarian follicular maturation with pure follicle stimulating hormone in women with gonadotropin deficiency. *J. Clin. Endocrinol. Metab.*, **66**, 552–556.
- De Leeuw, R., Mulders, J., Voortman, G. *et al.* (1996) Structure–function relationship of recombinant follicle stimulating hormone (Puregon). *Hum. Reprod.*, **2**, 361–369.
- ERHLHSG: The European Recombinant Human LH Study Group (1998) Recombinant human luteinizing hormone (LH) to support recombinant human follicular stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose finding study. *J. Clin. Endocrinol. Metab.*, **83**, 1507–1514.
- Fleming, R. (1999) Follicular phase serum LH levels and assisted reproduction outcome. In Filicori, M. (ed.), *The Role of Luteinizing Hormone in Folliculogenesis and Ovulation Induction*. Monduzzi Editori, Bologna, Italy, pp. 189–200.
- Fleming, R., Chung, C.C., Yates, R.W.S. and Coutts, J.R.T. (1996) Purified urinary follicle stimulating hormone induces different hormone profiles compared with menotrophins, dependent upon route of administration and endogenous luteinizing hormone activity. *Hum. Reprod.*, **11**, 1854–1858.
- Fleming, R., Lloyd, F. and Herbert, M. *et al.* (1998) Effect 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.
- Fried, G., Harlin, J., Csemiczky, G. and Wramsby, H. (1996) Controlled ovarian stimulation using highly purified FSH results in a lower serum oestradiol profile in the follicular phase as compared with HMG. *Hum. Reprod.*, **11**, 474–477.
- Homburg, R., Armar, N.A., Eshel, A. *et al.* (1988) Influence of serum LH concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. *Br. Med. J.*, **297**, 1024–1026.
- Hull, M.G.R., Armatage, R.J. and McDermott, A. (1994) Use of follicle stimulating hormone alone (urofollitropin) to stimulate the ovaries for assisted conception after pituitary desensitization. *Fertil. Steril.*, **62**, 997–1003.
- Loumaye, E., Engrand, P., Howles, C.M. and O’Dea, L. (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.
- Moor, R.M. and Crosby, I.M. (1987) Cellular origin, hormonal regulation and biochemical characteristics of polypeptides secreted by Graafian follicles of sheep. *J. Reprod. Fertil.*, **79**, 469–483.
- Out, H.J., Mannaerts, B.M.J., Drieeson, S.G.A.J. and Coelingh Bennink, H.J.T. (1995) A prospective randomized, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon versus Metrodin) in in-vitro fertilization. *Hum. Reprod.*, **10**, 2534–2540.
- Rehka, P., Mowat, L., Jamieson, M.E. *et al.* (1998) Effect of profound suppression of luteinizing hormone during treatment with gonadotrophin releasing hormone analogue and purified follicle stimulating hormone upon development of cryopreserved embryos. *Hum. Reprod.*, **13**, 696–698.
- Weston, A.M., Zelinsky-Wooten, M.B. and Hutchison, J.S. (1996) Developmental potential of embryos produced by in-vitro fertilization from gonadotrophin releasing hormone antagonist treated macaques stimulated with recombinant human follicle stimulating hormone in combination with LH. *Hum. Reprod.*, **11**, 608–613.
- Yong, E.L., Hillier, S.G., Turner, M. *et al.* (1994) Differential regulation of cholesterol side-chain cleavage (P450<sub>scc</sub>) and aromatase (P450<sub>arom</sub>)