

Effects of recombinant LH treatment on folliculogenesis and responsiveness to FSH stimulation

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BACKGROUND: The role of LH in sensitizing antral follicles to FSH is unclear. LH is required for normal hormone production and normal oocyte and embryo development, but follicular responses to LH may depend upon the stage of development. Potential roles at the early follicular phase were explored in a clinical setting by employing a sequential approach to stimulation by recombinant human (r-h) LH followed by r-hFSH in women who were profoundly down-regulated by depo GnRH agonist. **METHODS:** We employed a multi-centre, prospective, randomized approach. Women ($n = 146$) were treated in a long course high-dose GnRH agonist (Decapeptyl, 4.2 mg s.c.) protocol and were randomized to receive r-hLH (Luveris, 300 IU/day) for a fixed 7 days, or no r-hLH treatment. This was followed by a standard r-hFSH stimulation regime (Gonal-F, 150 IU/day). Ultrasound and hormone assessments of responses were measured at the start of r-hLH treatment, on FSH stimulation Days 0 and 8 and at the time of HCG administration. **RESULTS:** The LH treatment was associated with increased small antral follicles prior to FSH stimulation ($P = 0.007$), and an increased yield of normally fertilized (2 PN) embryos ($P = 0.03$). There was no influence of the r-hLH pretreatment upon hormone profiles or ultrasound assessments during the FSH phase. Anti-mullerian hormone increased in both groups during the week prior to FSH stimulation ($P = 0.002$). **CONCLUSIONS:** This sequential approach to the use of r-hLH in standard IVF showed a possible modest clinical benefit. The results support other recent work exploring up-regulated androgen drive upon follicular metabolism indicating that clinical benefit may be obtainable after further practical explorations of the concept.

Keywords: recombinant human LH; androgen; follicle growth; controlled ovarian stimulation

Introduction

The nature of the relationship between intraovarian follicular androgens (and/or the theca cells that produce androgens) and follicular developmental potential is not well understood. Through most of a follicle's developmental life LH responsiveness is restricted to the thecal cells that the follicle acquired in the pre-antral stages, after developing multiple layers of granulosa cells. Under normal circumstances, in antral follicles, androgens are produced in these theca cell layers, in response to LH stimulation. However, high androgen concentrations (or high androgen/estrogen ratios) have been observed in less healthy follicles (McNatty *et al.*, 1979), leading to the general concept that androgens are

associated with ill-health in small follicles. In fact, this concept is questionable, as the role of estrogen, as a metabolite of androgens, may be equally critical to the observations. The differential ratio may reflect the decline in metabolic activity of granulosa cells in follicles undergoing atresia, rather than androgens having a causative role in that atresia (Westergaard *et al.*, 1986).

Unequivocal positive effects of androgens upon aspects of follicular metabolism have been observed in cells from follicles at different stages of development (Hillier and Tetsuka, 1997; Vendola *et al.*, 1998; Weil *et al.*, 1999). In the rat model, androgens have been shown to stimulate early follicular development and reduce the incidence of apoptosis

(Beloosesky *et al.*, 2004) and androgenic stimulation of the androgen receptor (AR) is required to promote the actions of growth differentiation factor 9, to stimulate granulosa cell division and progesterone biosynthesis in the pig (Hickey *et al.*, 2005). In granulosa cells from antral follicles, androgens were shown to promote increased aromatase enzyme expression (Hillier, 2001). Since androgens are produced by the theca cells in response to LH, this may represent LH-stimulated promotion of follicular responsiveness to FSH.

Critical to these potential effects of androgens is AR, which has been demonstrated in the theca and granulosa cells of human and primate follicles (Hild-Petito *et al.*, 1991; Chadha *et al.*, 1994). The most abundant expression of AR in the developing follicle was recorded at stages immediately preceding those of FSH sensitivity—pre-antral and early antral stages. Furthermore, AR expression was associated with follicular health, raised granulosa cell mitotic index and reduced apoptosis (Hillier and Tetsuka, 1997; Weil *et al.*, 1998). How these observations relate to follicular responses to FSH is unclear, but co-expression of AR and FSH receptor mRNA has been observed (Weil *et al.*, 1999). It may or may not be a coincidence that these developmental stages are characterized by maximal expression of anti-Mullerian hormone (AMH; Weenen *et al.*, 2004).

Women of reproductive age with increased endogenous androgen drive, such as those with polycystic ovary syndrome, show a ‘stockpiling’ of antral follicles (Maciel *et al.*, 2004). Although other factors are evident in these women, such as increased LH activity, and hyperinsulinaemia, both of which promote androgen biosynthesis, the link between androgens and follicular responses to FSH is well supported by direct and circumstantial evidence. The promotion of FSH sensitivity may express itself in a number of ways, such as promoting growth at lower concentrations of FSH or by reducing the incidence of atresia, such that more follicles maintain FSH sensitivity for a longer period of time, thus enlarging the recruitable follicle pool—or stockpile (Maciel *et al.*, 2004; Fleming *et al.*, 2006).

These observations and speculations combine to suggest that endogenously produced androgens (in response to LH stimulation) are an important component of the mechanism of acquisition of FSH sensitivity by small antral follicles.

The availability of pure gonadotrophins for therapeutic purposes facilitates the testing of this hypothesis by examining whether follicles may benefit from LH stimulation prior to exposure to FSH drive. We have tested this hypothesis by using a depo GnRH agonist formulation to induce profound suppression of endogenous gonadotrophins, and superimposing a sequence of ovarian stimulation comprising a week of recombinant human (r-h) LH treatment followed by stimulation with r-hFSH. We asked if this approach would modify the spectrum of follicles available to respond to FSH, and whether there would be a corresponding change in the profile of follicular development determined by ultrasound profiles, or oocyte yield, or an influence on oocyte maturity leading to an increased yield of viable embryos.

Materials and Methods

Patients

Women with normal menstrual rhythm (25–34 days) and both ovaries, aged between 19 and 39 years, with BMI <28 Kg/m², undergoing IVF in each of four centres (Edinburgh, Glasgow, Odense and Paris) were asked to volunteer for this randomized, prospective, open label research programme. They were block randomized by centre in blocks of 10, to receive pretreatment with r-hLH (Luveris, MerckSerono, Geneva, Switzerland; 300 IU per day for 7 days).

Exclusion criteria included ultrasound determination of polycystic ovaries, previous poor response (<5 mature sized follicles in standard IVF), and other compromising disease states, such as diabetes and kidney disease, which may affect ovarian responsiveness.

Full ethical committee approval was obtained in each centre. Full informed consent was obtained from all participants.

Treatment schedule

Patients were treated in a standard long agonist protocol with the following sequence. Down-regulation treatment was effected with the high dose (4.2 mg) GnRH agonist depo Tryptorelin (Decapeptyl[®], Ipsen Pharma, s.c.) which was administered starting in the luteal phase (Day 21). Figure 1 shows the fixed schedule of treatments starting from that time point. Fourteen days after starting the GnRH agonist (defined as day ‘L1’), patients who were randomized to the r-hLH pretreatment arm received 300 IU/day (s.c.) for 7 days, whereas those randomized to no r-hLH treatment received no treatment during that week. It has been demonstrated that a single daily application of 75 IU rLH is sufficient to elicit its biological functions in women concurrently stimulated with FSH (The European Recombinant LH Study Group, 1998), and we considered that a higher dose may be beneficial in the circumstances arising prior to FSH stimulation.

After the 7-day pretreatment period (defined as day ‘S1’), stimulation with r-hFSH (Gonal-f, MerckSerono) was started at a fixed daily dose (150 IU) for 7 days (defined as day ‘S8’), with subsequent dose adjustment according to ovarian response assessed on that day. FSH stimulation was continued until HCG administration criteria (at least 2 follicles with diameter >16 mm) were attained. Final follicular maturation was achieved using 250 µg r-HCG (Ovitrelle, MerckSerono; equivalent to 6500 IU). Luteal phase support was according to local practice, and a maximum of 2 embryos were replaced in the fresh cycle.

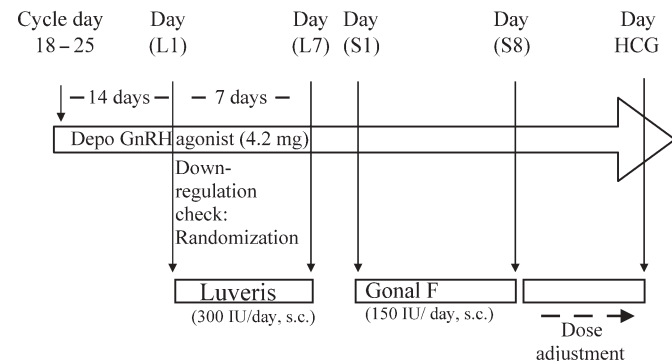


Figure 1: Plan of the experimental procedures and treatment.

Luveris: recombinant human LH, Gonal-F: recombinant human FSH, Day L1: fourteen days after starting the GnRH agonist, Day L7: Day 7 of pretreatment period, Day S1: start of stimulation with recombinant human FSH, Day S8: Day 8 of FSH stimulation

End-points and assessments

The primary end-point was follicular development recorded both prior to and following FSH stimulation. The former assessment was effected on the day after r-hLH pretreatment, and the latter assessments were carried out after a fixed time schedule (7 days of FSH stimulation), and on the day of HCG administration. Multiple measures of follicular development were recorded, including circulating estradiol (E_2) concentrations, and ovarian ultrasound profiles. As categorization of follicles at variable stages of the stimulation phase was deemed subjective, the numbers of viable eggs and embryos obtained in each group was used as a surrogate marker for follicular development and the power calculation was based on this parameter as described below.

Secondary end-points related to events both after r-hLH (or no) pretreatment and after r-hFSH stimulation, and included the requirement for r-hFSH, the proportion of cases requiring dose adjustment (both up and down), the ratio of large follicles to small follicles at the time of HCG administration. Treatment effects upon follicular and oocyte quality were examined by comparing the proportions of eggs undergoing normal fertilization (2 PN at 16–20 h after insemination), those undergoing early cleavage (determined at 25 h post-insemination), and the yield of viable and high-quality embryos. High-quality embryos were described as those suitable for transfer and/or cryopreservation. The different centres used different embryo assessment criteria for these outcomes, so the embryologist staff were blinded to the treatment group.

Hormone measurements

Blood samples were collected for centralized assays before the treatment cycle on cycle Days 1–4 for pretreatment FSH estimations. Further blood samples were taken at L1, S1, S8 and at HCG, and were analysed centrally (University of Glasgow), in patient specific batches, after cryopreservation at -20°C .

The circulating concentrations of E_2 and LH in peripheral plasma were estimated routinely using the Immulite semi-automated assay system (DPC, Los Angeles). AMH was measured at the start of LH pretreatment (or no pretreatment) and at the end LH pretreatment: i.e. Days L1 and S1. The AMH assay was performed in patient-specific batches using the enzyme-linked immunosorbent assay (ELISA) assay (DSL Inc, TX, USA) with values presented in concentrations of pmol/l (conversion factor to pmol/l = $\text{ng/ml} \times 7.143$).

The circulating concentrations of testosterone and $17\alpha\text{OH}$ progesterone were estimated using commercial ELISA kits. Both were provided by DRG Instruments GmbH (Marburg, Germany).

Sample assays performed centrally were carried out at the Gynaecology Laboratory of the University Department of Developmental Medicine at the Royal Infirmary, Glasgow. Sample transportation and consumable costs were covered by local arrangements with MerckSerono.

Statistics and power calculation

Continuous variables were assessed for normality of distributions and where normal were examined and compared using standard Gaussian statistical methods. Where distributions were not normally distributed, they were either log transformed for comparison or compared using non-parametric tests, with the data presented as median values and centiles. The statistics package used for these analyses was Minitab (version 13, for windows; ©Minitab Ltd, UK).

Significance was determined when $P \leq 0.05$. The power calculation for the primary study end-point (follicular development) used viable embryos as a clinically relevant surrogate marker for increased follicular development. It was based on the possibility of increasing the

number of transferable grade embryos per cycle by 2, which requires 515 embryos in each arm ($\alpha = 0.05$). This equates to 60 treatment cycles cases in each arm.

Results

The 146 patients enrolled in the study were randomized to receive r-hLH pretreatment ($n = 75$) or no r-hLH pretreatment ($n = 71$; control). The four centres contributed 43, 22, 32 and 49 patients to the total (146) with no difference in the age or BMI of the patients between the centres. Figure 2 shows the progress of patients through the programme, revealing that similar numbers started treatment and progressed to HCG administration and embryo transfer. The violator patients included elevated FSH concentration ($n = 1$), multiple cysts at first ultrasound scan ($n = 1$), no response to GnRH agonist ($n = 1$) and withdrawn for personal reasons ($n = 2$).

The basic anthropometric data and the diagnosis distributions of the two groups, 'no r-hLH' and 'LH pretreatment' are shown in Table I, revealing that there was no difference between them.

Changes during the week of r-hLH pretreatment (samples L1 and S1)

Table II shows the hormone concentrations during the week of \pm LH pretreatment. Significant changes were recorded in the circulating LH in both groups. The no r-hLH group showed a statistically significant decline, whereas the r-hLH treated showed increases into the low-normal range. The blood samples were taken on the day after the previous injection, but with different time gaps depending upon local practice.

There was neither a difference between groups nor a change in the circulating E_2 , testosterone or $17\alpha\text{OH}$ progesterone during the week of LH treatment. The concentrations of both E_2 and testosterone were close to the limits of sensitivity of the assays throughout the period, but the concentrations of $17\alpha\text{OH}$ progesterone were in the recoverable range. The products with normal distributions (AMH and $17\alpha\text{OH}$ progesterone) are presented as mean and SEM.

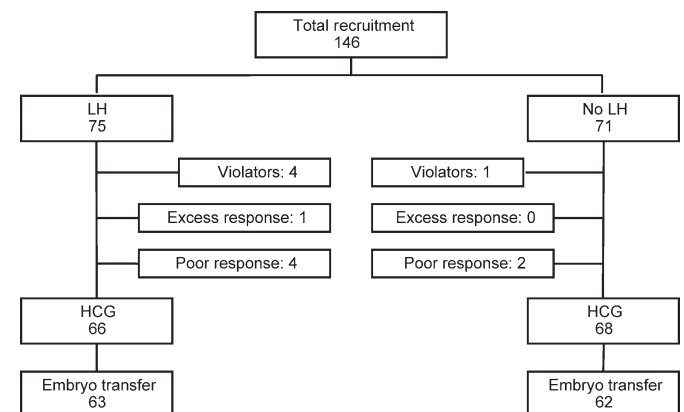


Figure 2: Chart of patient processing through the programme

Table I. The distribution of anthropometric and diagnostic criteria in the two groups after randomization.

	<i>n</i>	Mean age years (C.L.)	Mean BMI kg/m ² (C.L.)	Infertility diagnosis (%)		
				Tubal	Un-explained	Male factor
No r-hLH	71	32.6 (31.5, 33.4)	23.9 (23.0, 24.8)	42	37	11
r-hLH pretreatment	75	33.3 (31.0, 35.7)	23.6 (22.8, 24.4)	49	36	11

BMI, body mass index; CL, confidence limits; r-hLH, recombinant human LH.

Table II. Changes in hormone concentrations during the week defined by L1 to S1 where patients were randomized to receive r-hLH (300 IU/d) or no r-hLH treatment.

Variable	No r-hLH (<i>n</i> = 71)			r-hLH (<i>n</i> = 75)			<i>P</i> -value ^a Between groups
	Mean	C.L. (95%)	P L1 to S1 (paired)	Mean	C.L. (95%)	P L1 to S1 (paired)	
LH at L1 (IU/l)	1.4	1.24, 1.57		1.5	1.31, 1.76		0.58
LH at S1 (IU/l)	0.97	0.80, 1.18	0.0006	2.8	2.35, 3.30	<0.001	<0.001
E ₂ at L1 (pmol/l)	79	70, 91		73	66, 79		0.21
E ₂ at S1 (pmol/l)	76	68, 84	0.33 ^a	76	68, 84	0.34	0.75
Testosterone at L1 (pmol/l)	0.41	0.37, 0.46		0.37	0.34, 0.41		0.62
Testosterone at S1 (pmol/l)	0.40	0.36, 0.44	0.91	0.36	0.33, 0.40	0.16	0.44
	Mean	SEM		Mean	SEM		
17αOH progesterone at L1 (pmol/l)	0.74	0.05		0.73	0.06		0.94
17αOH progesterone at S1 (pmol/l)	0.69	0.05	0.41	0.58	0.03	0.15	0.63
AMH at L1 (pmol/l)	16.8	2.3		20.2	2.2		0.38
AMH at S1 (pmol/l)	20.0	2.9	0.001	23.3	2.7	0.002	0.40

^a*P* Mann–Whitney *U* test was used to compare the differences between the groups with non-Gaussian distributions, and *t* tests were used for those with normal distributions. Day L1, 14 days after starting the GnRH agonist; Day S1, start of stimulation with recombinant human FSH; AMH, anti-Mullerian hormone; E₂, estradiol.

It is interesting to record that during the week of FSH deprivation (Days L1 to S1), the circulating AMH values showed significant increases in both groups. There was no difference between the groups, at either the starting value (L1) or the values at S1, but the paired comparison assessing the effect of FSH deprivation, both with and without LH stimulation showed significantly increased AMH in the circulation.

Small follicle (follicle diameter <10 mm) development was ascertained in an incomplete dataset (*n* = 49 and 53 for the treatment and no treatment groups, respectively) as one centre did not collect these data. The profiles revealed some interesting changes shown in Table III. During the week of r-hLH pretreatment, those treated with r-hLH showed a significant (*P* = 0.007) increase (ca 20%) in the number of small antral follicles, whereas there was no change in the untreated group. However, the numbers of small follicles at the end of pretreatment did not differ between the groups.

Changes during the first week of FSH stimulation (S1–S8)

Although the LH pretreated group showed higher concentrations of LH at the start of r-hFSH stimulation (S1), they had declined to identically low concentrations by Day 8 of r-hFSH stimulation (S8), as both groups showed values generally <1.0 IU/l (Table IV).

Table IV also shows the circulating concentrations of other reproductive hormones and profiles of follicular development. Evaluation of markers of follicular development at S8 revealed that there was no difference in either the concentrations of E₂ or the numbers of small or medium size follicles recorded.

Demand for FSH

The need for FSH dose adjustment was equivalent in the two groups as 55% of cases had a dose increase at S8, in both groups, while only 3 and 4% underwent dose reductions. The duration of treatment (12.1 and 12.5 days, control and LH pretreated, respectively; *P* = 0.37) and the total doses of r-hFSH

Table III. Small Follicle (follicle diameter = 6–9 mm) development observed on Days L1 and S1 in both groups.

Evaluation point	No r-hLH (<i>n</i> = 49)		r-hLH treated (<i>n</i> = 53)		<i>P</i> -value 2-tailed <i>t</i> -test
	Mean	C.L. (95%)	Mean	C.L. (95%)	
L1	8.2	6.7, 9.6	7.3	5.9, 8.6	0.36
S1	8.5	7.0, 10.1	8.8	7.4, 10.2	0.77
<i>P</i> -value (paired <i>t</i> -test)	0.447		0.007		

A paired *t*-test was used to compare the number of follicles within each group.

Table IV. Evaluation of hormones and follicular development on stimulation S8 with FD ratios on the day of HCG in both groups of patients.

	No r-hLH (<i>n</i> = 71)		r-hLH-pretreated (<i>n</i> = 75)		<i>P</i> -value
	Median	Centiles (25, 75)	Median	Centiles (25, 75)	Mann–Whitney <i>U</i> test
LH (IU/l)	0.56	0.37, 0.40	0.57	0.39, 0.82	0.85
E ₂ (pmol/l)	624	165, 1890	505	185, 1974	0.81
Testosterone (pmol/l)	0.42	0.33, 0.56	0.44	0.31, 0.53	0.78
	Mean	C.L.s	Mean	C.L.s	<i>t</i> -test
17 α OH progesterone (pmol/l)	1.1	0.87, 1.36	0.86	0.77, 0.95	0.24
FDs 6–11 (mm)	9.6	8.4, 10.8	10.8	9.6, 11.9	0.17
FDs 12–14 (mm)	2.5	1.8, 3.3	2.1	1.5, 2.8	0.43
FDs >14 (mm)	0.67	0.3, 1.1	0.37	0.14, 0.59	0.20
	Median	Centiles (25, 75)	Median	Centiles (25, 75)	
Ratio of large to small FDs (S8)	0.15	0.0, 0.54	0.13	0.0, 0.38	0.87
Ratio of large to small FDs	1.71	1.0, 3.5	1.78	1.1, 3.0	0.87

Day S8, Day 8 of FSH stimulation; FD, follicle diameter.

(2221 and 2282 IU, respectively) also showed no differences between the \pm r-hLH treated groups.

Embryological parameters

Table V shows the embryological assessments in the two groups which reveal an interesting profile of responses. The yield of total oocytes was not significantly different between the two groups. However, in contrast, the r-hLH-treated group produced significantly more normally fertilized oocytes ($P = 0.03$). The proportion of embryos undergoing early cleavage did not differ between the groups, and neither did the numbers of embryos described as ‘high grade’ (sufficient quality to be transferred or cryopreserved). There were 62 fresh embryo transfers (mean of 1.86 embryos per transfer) in the control group and 63 (mean of 1.93 embryos per transfer) in the LH pretreated group, which resulted in 23 (37%) ongoing pregnancies in the control and 24 (38%) in the LH pretreated group.

Discussion

We show that a sequential approach to follicular stimulation through recruitment and growth, employing r-hLH prior to r-hFSH, resulted in a modestly increased yield of normally fertilized embryos. Other beneficial observations of the r-hLH pretreatment from assessments of follicular development and endocrinology during the later FSH treatment phase were absent. However, significant short-term effects of the r-hLH treatment upon follicular dynamics were observed in the pretreatment phase. As a proof of principle, the results are encouraging but not conclusive. The changes induced by r-LH were modest,

and down-stream effects upon later follicular profiles or hormone output were not detected. However, this does not rule out a promotion of the cohort available to recruitment by FSH, and suggests that any such quantitative benefit might have been overwhelmed by the subsequently recruited cohort. This in turn suggests that any beneficial influence of LH promoted thecal androgen activity on follicular FSH sensitivity might have been short-lived within the current treatment paradigm.

In respect of these primary observations, a recent study, exploring the same hypothesis (Lossl *et al.*, 2006), showed that elevation of intrafollicular androgen concentrations using an aromatase inhibitor in the early follicular phase resulted in a modest increase in the number of good-quality embryos.

We observed no measurable effect of r-hLH treatment upon circulating androgen concentrations or upon precursor 17 α OH progesterone concentrations, both of which were low at the start and remained so. LH-stimulated thecal cells may yet have influenced follicular development through local paracrine stimulation involving androgens or other factors, whose presence went undetected in the circulating plasma. This is supported by the study of Lossl *et al.* (2006) where the aromatase inhibitor treatment resulted in raised follicular concentrations of androgens that were not observable in the circulation, but remained detectable in the follicular fluid 10 days after discontinuation of treatment.

It is also possible that increases in circulating ovarian androgens in response to LH, also require significant FSH stimulated follicular growth. This concept has been proposed previously following studies in rats (Smyth *et al.*, 1993, 1995). Thus, we may speculate that LH-promoted androgen biosynthesis may also require FSH activity, and the profound suppression of FSH by the depo GnRH agonist inhibited that process during this experiment.

The observation of an effect of LH treatment upon small follicle development prior to FSH treatment may be independent of its possible androgen promoting effects.

During the period of r-hLH pretreatment, circulating AMH concentrations increased significantly. This is an interesting observation in itself, but the effect appeared to be related more to FSH deprivation than r-hLH treatment, as there was no difference between the groups. It is known that AMH is down-regulated in the granulosa cells of growing antral follicles

Table V. Embryology data in the No r-hLH and r-hLH pretreated groups of patients.

	No r-hLH		r-hLH-pretreated		<i>P</i> -value
	Mean	C.L.s	Mean	C.L.s	<i>t</i> -test
Oocytes	9.2	8.0, 10.3	10.8	9.3, 12.4	0.09
Embryos (2 PN)	5.5	4.6, 6.3	7.0	5.8, 8.2	0.03
Early cleavage	1.3	0.8, 1.8	1.1	0.7, 1.6	0.64
High-grade embryos	3.2	2.5, 3.9	3.9	3.0, 4.8	0.19

(Weenen *et al.*, 2005) which may be an effect of FSH stimulation.

The absence of an impact of r-hLH treatment upon circulating E₂ concentrations is not surprising after examination of the LH concentrations in both groups. The circulating LH declined to minimal levels in both groups during this stage of the programme, and it is acknowledged that this leads to lower circulating E₂ concentrations during multiple follicular development than when LH is co-administered (Tarlantzis *et al.*, 2006).

In summary, this multi-centre, randomized study exploring the effects of pretreatment of small antral follicles with r-hLH in the absence of FSH, demonstrated increased development of small antral follicles in the immediate term and a small increase in the number of normally fertilized embryos after subsequent r-hFSH treatment. However, there was little supporting evidence of a carry over effect during the period of r-hFSH treatment.

This suggestive, rather than conclusive, evidence, along with other recent work exploring possible beneficial effects of up-regulated androgen drive upon follicular metabolism, suggests that clinical benefit may be obtainable with judicious application of the concept. The results, along with other recent studies indicate that the concept should be explored further, perhaps exploring different doses or timing of r-hLH administration.

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References

Beloosesky R, Gold R, Almog B, Sasson R, Dantes A, Land-Bracha A, Hirsh L, Itskovitz-Eldor J, Lessing JB, Homburg R. Amsterdam A Induction of polycystic ovary by testosterone in immature female rats: modulation of apoptosis and attenuation of glucose/insulin ratio. *Int J Mol Med* 2004;**14**:207–215.

Chadha S, Pache TD, Huikeshoven JM, Brinkmann AO, van der Kwast TH. Androgen receptor expression in human ovarian and uterine tissue of long-term androgen-treated transsexual women. *Hum Pathol* 1994;**25**: 1198–1204.

Fleming R, Deshpande N, Traynor I, Yates RW. Dynamics of FSH-induced follicular growth in subfertile women: relationship with age, insulin

resistance, oocyte yield and anti-Mullerian hormone. *Hum Reprod* 2006;**21**:1436–1441.

Hickey TE, Marrocco DL, Amato F, Ritter LJ, Norman RJ, Gilchrist RB, Armstrong DT. Androgens augment the mitogenic effects of oocyte-secreted factors and growth differentiation factor 9 on porcine granulosa cells. *Biol Reprod* 2005;**73**:825–832.

Hild-Petito S, West NB, Brenner RM, Stouffer RL. Localization of androgen receptor in the follicle and corpus luteum of the primate ovary during the menstrual cycle. *Biol Reprod* 1991;**44**:561–568.

Hillier SG. Gonadotropic control of ovarian follicular growth and development. *Mol Cell Endocrinol* 2001;**179**:39–46.

Hillier SG, Tetsuka M. Role of androgens in follicle maturation and atresia. *Baillieres Clin Obstet Gynaecol* 1997;**11**:249–260.

Lossl K, Andersen AN, Loft A, Freiesleben NL, Bangsboll S, Andersen CY. Androgen priming using aromatase inhibitor and hCG during early-follicular-phase GnRH antagonist down-regulation in modified antagonist protocols. *Hum Reprod* 2006;**21**:2593–2600.

Maciel GA, Baracat EC, Benda JA, Markham SM, Hensinger K, Chang RJ, Erickson GF. Stockpiling of transitional and classic primary follicles in ovaries of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2004;**89**:5321–5327.

McNatty KP, Smith DM, Makris A, Osathanondh R, Ryan KJ. The Microenvironment of the human antral follicle: interrelationships among 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.

Smyth CD, Miro F, Whitelaw PF, Howles CM, Hillier SG. Ovarian thecal/interstitial androgen synthesis is enhanced by a follicle-stimulating hormone-stimulated paracrine mechanism. *Endocrinology* 1993;**133**: 1532–1538.

Smyth CD, Miro F, Howles CM, Hillier SG. Effect of luteinizing hormone on follicle stimulating hormone-activated paracrine signalling in rat ovary. *Hum Reprod* 1995;**10**:33–39.

Tarlantzis B, Tavmergen E, Szamatowicz M, Barash A, Amit A, Levitas E, Shoham Z. The use of recombinant human LH (lutropin alfa) in the late stimulation phase of assisted reproduction cycles: a double-blind, randomized, prospective study. *Hum Reprod* 2006;**21**: 90–94.

The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient ovulatory women: a dose-finding study. *J Clin Endocrinol Metab* 1998;**83**:1507–1514.

Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. *J Clin Invest* 1998;**101**:2622–2629.

Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Viss JA, Kramer P, Fauser BC, Themmen AP. Anti-mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004;**10**:77–83.

Weil SJ, Vendola K, Zhou J, Adesanya OO, Wang J, Okafor J, Bondy CA. Androgen receptor gene expression in the primate ovary: cellular localization, regulation, and functional correlations. *J Clin Endocrinol Metab* 1998;**83**:2479–2485.

Weil S, Vendola K, Zhou J, Bondy CA. Androgen and follicle-stimulating hormone interactions in primate ovarian follicle development. *J Clin Endocrinol Metab* 1999;**84**:2951.

Westergaard L, Christensen IJ, McNatty KP. Steroid levels in ovarian follicular fluid related to follicle size and health status during the normal menstrual cycle in women. *Hum Reprod* 1986;**1**:27–232.

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