Effect of pituitary desensitization on the early growing follicular cohort estimated using anti-Mullerian hormone

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BACKGROUND: This study evaluated the effect of pituitary desensitization on the early growing follicle population through assessment of serum anti-Mullerian hormone (AMH) concentration. Other markers of ovarian reserve, basal follicular stimulating hormone (FSH), luteinizing hormone (LH), estradiol, inhibin-B and three-dimensional ultrasound ovarian parameters were also assessed for comparison. METHODS: One hundred and two subjects aged <40 years with FSH levels <12 IU/l underwent venepuncture and transvaginal ultrasound in the early follicular phase of the menstrual cycle and after 14 days of down-regulation using gonadotrophin releasing hormone (GnRH) agonists. Serum levels of AMH and other markers of ovarian reserve measured during the early follicular phase were compared with those measured following down-regulation. RESULTS: While AMH levels increased significantly by 1/24 - 32% (P<0.01), there was a significant decline of 1/24 - 40–50% (P<0.01) in the levels of inhibin-B, FSH, LH and estradiol. Down-regulation treatment was also associated with a decrease (P<0.01) in mean ovarian volume and in ovarian blood flow, but no difference was seen in the antral follicle count. CONCLUSIONS: Pituitary desensitization results in a significant increase in AMH levels, which implies that either the secretion of AMH by early growing follicles is enhanced or that the size of this follicle cohort is increased. The number of antral follicles visualized on ultrasound in the early follicular phase and at down-regulation appears unchanged, suggesting that any effect is restricted to the smaller selectable follicles. Our results may explain the enhanced ovarian response to conventional controlled ovarian stimulation and higher pregnancy rates when pretreatment with GnRH-agonists is employed.

Keywords: in vitro fertilization; antral follicle count; three-dimensional power Doppler ultrasound; ovarian reserve; down-regulation

Introduction

Pituitary ‘down-regulation’ using a continuous and supraphysiological dose of gonadotrophin releasing hormone (GnRH) agonist is commonly employed during assisted reproduction treatment (ART) to prevent an endogenous premature surge in luteinizing hormone (LH) and, as a consequence, premature ovulation (Janssens et al., 2000; Marcus and Ledger, 2001). The use of GnRH agonists during controlled ovarian stimulation has been shown to increase the number of oocytes retrieved, reduce the chance of treatment cancellation and improve pregnancy rates (Hughes et al., 1992).

Down-regulation is usually achieved following 14 consecutive days of treatment with a GnRH agonist commenced in mid-luteal phase of the preceding cycle. The pituitary desensitization induced by GnRH agonists results in profound suppression of follicular stimulating hormone (FSH), regardless of whether short acting or depot preparations are used (Sonntag et al., 2005). FSH has a regulatory role in the cyclical recruitment of growing follicles (Gougeon, 1998; Durlinger et al., 2001) and in their rescue from the process of atresia that otherwise occurs (McGee and Hsueh, 2000). This decrease in FSH, secondary to the short-term administration of GnRH agonist, may be expected to affect the growing follicle population but recent data suggest that the number of ultrasonographically detected antral follicles, a cohort of larger growing follicles measuring 2–10 mm, does not change following 2 weeks of down-regulation (Yu Ng et al., 2004). However, no studies have evaluated the effect of pituitary desensitization on the early growing follicle population, which are not evident even with high-resolution ultrasound.

AMH is expressed exclusively in granulosa cells of growing follicles in the ovary (Themmen, 2005). In rodents, AMH expression starts in the columnar granulosa cells of primary follicles, is highest in granulosa cells of pre-antral and small...
antral follicles and gradually diminishes in the subsequent stages of follicle development, so that AMH is no longer expressed during the gonadotrophin-dependent terminal stages of follicle development. In addition, AMH expression disappears when follicles become atretic. In AMH null mice, effects are observed during both follicle initiation and selection (Durlinger et al., 2002b). Although limited, the results of observational studies in women have broadly supported the rodent studies. Immunohistochemical analysis has shown that AMH expression can first be observed in granulosa cells of primary follicles; expression is strongest in pre-antral and small antral follicles (4 mm) and then declines with increasing size and is almost lost in follicles $>$8 mm (Weenen et al., 2004). More recently, these results have been confirmed by a more quantitative analysis of antral follicle concentrations utilizing a specific immunoassay for human 25 kDa AMH dimer (Andersen and Byskov, 2006).

In this prospective study, we aim to evaluate the effects of pituitary down-regulation, using GnRH agonists as part of a conventional ART long protocol, on the early growing follicle population through assessment of serum AMH concentration. Other indirect hormonal markers of ovarian reserve including basal FSH, LH, estradiol and inhibin-B and three-dimensional ultrasound ovarian parameters have also been assessed as part of a comparative analysis.

Materials and Methods

Experimental design

We prospectively recruited 114 consecutive subjects aged $\leq$ 40 years with regular menstrual cycles of 21–35 days duration. All subjects had an early follicular phase FSH level of $<12$ IU/l and were undergoing their first cycle of ART. They underwent venepuncture and a baseline pretreatment three-dimensional ultrasound assessment in the early follicular phase (Day 2–4) of the spontaneous menstrual cycle before commencing treatment with a GnRH agonist in the luteal phase of the same cycle. Venepuncture and the ultrasound examination were repeated after 14 consecutive days of treatment. Subjects were excluded if they had a history of ovarian surgery or were found to have either polycystic ovaries, as defined by Rotterdam PCOS consensus workshop group (Balen et al., 2003), or had an ovarian cyst or follicle measuring 20 mm or more in diameter during any of the two ultrasound assessments or if down-regulation was not achieved with 14 days of GnRH agonist treatment. In accordance with the guidelines of the Declaration of Helsinki (1996), the principles of Good Clinical Practice and the Department of Health Research Governance Framework for Health and Social Care (2005), the study was approved by the National Health Service (NHS) research ethics committee and informed, written consent was obtained prior to the enrolment of each subject.

Hormonal analysis

Blood samples were collected into two plain 6 ml tubes. One tube was immediately centrifuged at 4000 r.p.m. (2522 g) for 20 min and the supernatant serum was stored at $-20^\circ$C until the AMH and inhibin-B assays were performed. The other tube was used for the FSH, LH and estradiol assays which were performed within 2 h of venepuncture, or the next day but within 24 h in which case the serum was stored at 2$^\circ$C. All measurements were performed in duplicate and the mean value was used for analysis.

Measurement of serum AMH was performed using the MIS/AMH enzyme-linked immunosorbent assay kit (Diagnostic System Lab, Webster, TX, USA). The lowest detection limit and the intra- and inter-assay coefficients of variation were 0.006 ng/ml, $<5\%$ and $<8\%$, respectively. Inhibin-B was measured using the Inhibin B enzyme-linked immunosorbent assay kit (Diagnostic System Lab). The lowest detection limit and the intra- and inter-assay coefficients of variation were 7 pg/ml, $<6\%$ and $<8\%$, respectively. The AMH and inhibin-B assays were performed in a single run.

FSH, LH and estradiol levels were measured using Microparticle Enzyme Immunoassay (MEIA) method on an AxSYM auto-analyser (AxSYM; Abbott Laboratories, Abbott Park, IL, USA). The lowest detection limit and the intra- and inter-assay coefficients of variation for FSH were 0.37 IU/l, $<5\%$ and $<5\%$, respectively. The lowest detection limit and the intra- and inter-assay coefficients of variation for LH were 0.5 IU/l, $<7\%$ and $<8\%$, respectively. The lowest detection limit and the intra- and inter-assay coefficients of variation for estradiol were 8 pmol/l, 2.9–11% and 4.8–15.2%, respectively.

Ultrasound data acquisition

All subjects had a transvaginal scan performed by a single investigator (K.J.) using a Voluson Expert 730TM (GE Medical Systems, Zipf, Austria) and a four-dimensional 5–9 MHz transvaginal probe. Subjects were scanned with their legs supported by stirrups in a modified Lloyd Davies position to limit discomfort and ensure free manipulation of the transducer. Our technique for the acquisition of three-dimensional volumetric and power Doppler data has been described in detail (Raine-Fenning et al., 2003a,b) but, in brief, this included an initial two-dimensional ultrasound assessment of the pelvis to exclude any obvious pathology before the application of a region of interest over the ovary which defined the volume to be acquired. Three-dimensional volumetric data were then acquired using the automated programme and the data set was assessed to ensure that the entire ovary had been captured. Power Doppler was then applied using predefined settings, which offer the best compromise between small ovarian vessel detection and artefact, and these were kept constant for each subject (Raine-Fenning et al., 2002). Three-dimensional power Doppler data were then acquired and assessed in a similar fashion to that described above. Two volume acquisitions for each ovary, one with grey scale and the other with power Doppler information, were therefore obtained. The data were subsequently transferred to a personal computer via a digital video disk (DVD) without any data compression.

Ultrasound data measurement

All measurements were made on a personal computer using 4D View (version 7.0; GE Healthcare, Zipf, Austria) by a single investigator (K.J.). The three-dimensional grey-scale ovarian volume data set was initially displayed in the multiplanar view, and the total number of antral follicles measuring 2–10 mm in diameter was counted as previously described (Jayaprakasan et al., 2007a). Briefly, this involved measurement of largest follicles in two planes to determine which were above and below the cut-off level of 10 mm and all of the latter follicles were counted thereafter. All follicles measuring $>10$ mm were excluded. Virtual Organ Computer-aided AnLysis (VOCAL®; GE Medical Systems, Zipf, Austria) was used to measure ovarian volume through the delineation of the ovarian cortex in the B (transverse image) plane as the data set was rotated 180$^\circ$ through 9$^\circ$ rotation steps (Raine-Fenning et al., 2003a). Quantification of power Doppler information within the resultant three-dimensional ovarian model was performed using the ‘histogram facility’ which generates three indices of vascularity through the application of various algorithms: the vascular index (VI) represents the
ratio of power Doppler information within the total data set relative to both colour and grey information, the flow index (FI) is proportional to the power Doppler signal intensity and the vascularization FI (VFI) reflects a combination of the two (Raine-Fenning et al., 2004b).

The reproducibility of volume acquisition and data measurement, including determination of the antral follicle count and calculation of ovarian volume and vascularity, has been established (Raine-Fenning et al., 2003a,b, 2004a; Jayaprakasan et al., 2007b). In this study, two measurements of each variable were made for each data set and the mean value was used for the analysis. The mean intraclass correlation coefficient (ICC) and 95% confidence interval (CI) for measurement of the number of antral follicles and ovarian volume was 0.983 (0.968–0.992) and 0.989 (0.960–0.997), respectively, which are suggestive of a high degree of intraobserver reliability for these measures. Measurement of the three-dimensional vascular indices also showed a high level of intraobserver agreement with mean ICCs (95% CI) of 0.982 (0.974–0.991), 0.985 (0.977–0.993) and 0.983 (0.976–0.990) for the VI, FI and VFI, respectively.

Down-regulation protocol

The down-regulation protocol involved treatment with GnRH agonists (500 µg/day of buserelin; Suprefact®, Aventis Pharma, Kent, UK or 800 µg/day of Nafarelin; Synarel®, Pharmacia, Milton Keynes, UK) started in the mid-luteal phase of the menstrual cycle 7 days prior to the earliest expected date of menstruation. Two weeks later, ovarian suppression was confirmed by the finding of an endometrial thickness of <5 mm and absent ovarian activity, as indicated by the absence of a follicle measuring 20 mm or more, on ultrasound scan in association with an estradiol level below 200 pmol/l.

Statistical analysis

Statistical Package for the Social Sciences (version 14.0; SPSS, Chicago, IL, USA) was used for statistical analysis. The distribution of the data was checked for normality using a normal probability plot. Student’s paired t-test or Wilcoxon signed rank test were used to examine for significant differences in each variable, between the assessment made during the early follicular phase and the one made following down-regulation, for normally distributed and skewed data, respectively, and a P-value of <0.05 considered statistically significant. Correlation between FSH and other variables were assessed using the Spearman correlation to exclude the possibility of a direct relationship between FSH and other markers.

Results

A total of 114 subjects were recruited. There were seven subjects excluded as they had ovarian follicles or cysts measuring >20 mm in diameter during one of the two ultrasound assessments. Additionally, two subjects were not adequately down-regulated with 14 days of standard GnRH agonist treatment and data were incomplete in three subjects, resulting in a final study group of 102 subjects therefore. These subjects had a variety of causative factors for their subfertility including tubal disease (24 subjects; 24.5%), endometriosis (6 subjects; 5.9%), male factor (39 subjects; 38.2%), combined factors (3 subjects; 2.9%) and unexplained subfertility (30 subjects; 29.4%). Of the subjects, 75 (73.5%) had primary subfertility and the mean ± SD (range) duration of infertility was 44.4 ± 27.3 (6–156) months. The mean ± SD (range) age of the subjects studied was 33.7 ± 3.5 (24–40) years and the mean ± SD (range) BMI was 24.2 ± 3.4 (20–35 kg/m²).

The effects of down-regulation on the endocrine and ultrasonographic parameters are summarized in Table I and Fig. 1. Although there was a significant decline of ~40–50% (P < 0.01) in serum levels of inhibin-B, FSH, LH and estradiol, serum AMH levels increased by ~32% (P < 0.01) following treatment with GnRH agonists for 2 weeks. Figure 2 depicts the change in AMH in individual subjects. The increase in AMH levels did not appear to relate to the subject’s age which had little if any relationship to AMH (r = 0.02; P = 0.85) and this is supported by a similar percentage change in AMH levels (19.1%, 42.9%, 42.9% and 38.5%; P = 0.60) across four different age groups (24–30, 31–34, 35–36 and 37–40 years, respectively) when subjects are stratified into groups divided by each quartile of age. Down-regulation treatment was also associated with a significant decrease (P < 0.01) in mean ovarian volume and in ovarian blood flow, as measured by quantitative three-dimensional power Doppler angiography, but no differences were seen in the total number of antral follicles over the same time period.

Table I. Effect of pituitary down-regulation with GnRH analogues on endocrine and three-dimensional ultrasound markers of ovarian reserve in women undergoing ART.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretreatment (Early follicular phase)</th>
<th>After down-regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/l)</td>
<td>7.05 ± 1.85</td>
<td>4.19 ± 1.50**</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>5.28 ± 2.99</td>
<td>2.58 ± 1.55**</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>156.50 ± 66.31</td>
<td>64.29 ± 45.31**</td>
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<tr>
<td>AMH (ng/ml)</td>
<td>1.29 ± 0.73</td>
<td>1.55 ± 0.91**</td>
</tr>
<tr>
<td>Inhibin-B (pg/ml)</td>
<td>47.87 ± 26.48</td>
<td>15.01 ± 15.98**</td>
</tr>
<tr>
<td>Total AFC</td>
<td>14.88 ± 4.36</td>
<td>14.61 ± 6.00</td>
</tr>
<tr>
<td>Mean ovarian volume</td>
<td>6.45 ± 1.95</td>
<td>5.60 ± 2.19**</td>
</tr>
<tr>
<td>Mean VI (%)</td>
<td>7.48 ± 4.34</td>
<td>6.05 ± 5.04**</td>
</tr>
<tr>
<td>Mean FI (0–100)</td>
<td>36.30 ± 4.70</td>
<td>34.09 ± 7.85**</td>
</tr>
<tr>
<td>Mean VFI (0–100)</td>
<td>2.87 ± 1.75</td>
<td>2.33 ± 1.78**</td>
</tr>
<tr>
<td>Mean echogenicity (0–100)</td>
<td>37.76 ± 6.08</td>
<td>36.98 ± 5.26</td>
</tr>
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</table>

Data are presented as mean ± SD. **P < 0.01
Although we used two different types of GnRH agonists, buserelin \((n = 55)\) and Nafarelin \((n = 47)\), for pituitary desensitization, the degree of suppression was similar for both agonists as indicated by similar percentage changes in FSH concentration \((–40.2\% \text{ versus } –36.1\%; \text{ } P = 0.37)\), LH levels \((–45.7\% \text{ versus } –46.8\%; \text{ } P = 0.86)\) and estradiol levels \((–47.3\% \text{ versus } –57.4\%; \text{ } P = 0.21)\). The increase in mean \((\pm SD)\) AMH levels after 2 weeks of down-regulation treatment persisted regardless of the type of GnRH agonists used \((1.30 \pm 0.08 \text{ versus } 1.54 \pm 0.11 \text{ ng/ml for buserelin and } 1.28 \pm 0.12 \text{ versus } 1.56 \pm 0.15 \text{ ng/ml for Nafarelin})\).

Although AMH and AFC showed a significant negative correlation \((P < 0.01)\) with early follicular phase FSH, this relationship was no longer evident after down-regulation (Table II). In contrast, inhibin-B was positively correlated with FSH levels at down-regulation but not prior to the treatment. As expected, the positive relationship between FSH and LH levels remained significant even after down-regulation. There was a significant relationship \((P < 0.01)\) between the ultrasound markers, antral follicle count and ovarian volume, and basal FSH with the assessments made in the early follicular phase before commencing the down-regulation but not when the measurements were made after down-regulation (Table II). This indicates that the significant correlation between these markers before down-regulation reflects only an indirect relationship because of their association, although of varying degree, with ovarian reserve.

### Discussion

This is the first study to examine the effect of pituitary desensitization, induced by GnRH agonists as a part of conventional IVF treatment, on the growing follicle population as assessed by AMH levels. The data in this study indicate that pituitary down-regulation is associated with a significant increase in AMH levels, but has no effect on the total number of antral follicles visible by ultrasound. This finding was unexpected but is unlikely to be spurious as it was seen in association with expected significant falls in serum FSH, LH, inhibin-B, estradiol, ovarian volume and blood flow.

The initial recruitment of follicles, when a proportion of primordial follicles leave the resting follicular pool and start to undergo growth and differentiation, is a continuous process occurring throughout reproductive life that is independent of the circulating levels of gonadotrophins (Gougeon, 1998; McGee and Hsueh, 2000). Once follicles have been initiated to grow, the granulosa cells proliferate to form multilaminar structures (pre-antral follicles) which subsequently form a fluid-filled space (antrum), a well differentiated theca layer, and they gain the ability to respond to the pituitary gonadotrophins (FSH and LH) at a diameter of around 250 \(\mu m\) (Picton and Gosden, 2000; Campbell et al., 2004). Further development of these so-called ‘gonadotrophin-responsive’ or ‘selectable follicles’ beyond a diameter of 2–4 mm, in most species, relies on the provision of threshold levels of FSH and these larger antral follicles are termed ‘gonadotrophin-dependent’ follicles (Baird, 1987; Hillier, 1994). The transition from the gonadotrophin-responsive to the gonadotrophin-dependent phase is associated with widespread atresia of the growing follicle population (Turnbull et al., 1977). The observed increase in AMH concentrations, following suppression of FSH concentrations by down-regulation with GnRH agonist, seen in this study could be attributed, therefore, to either a direct effect of FSH on AMH expression by the follicular granulosa cells or an increase in their number, or improved quality of the gonadotrophin-responsive AMH-secreting follicles that lie below the resolution limit of conventional ultrasound.

A cross-sectional study examining the predictive value of antral follicles according to their absolute size suggested that the smaller follicles measuring 2–6 mm represent the functional ovarian reserve, rather than the larger follicles measuring between 7 and 10 mm, as a steady decline in the number of these smaller follicles with age was demonstrated, whereas the larger follicle cohort remained constant across different age groups (Haadsma et al., 2007). Although we considered looking at the effect of down-regulation on these different antral follicle populations, we were unable to measure, and therefore define, these two groups reliably. We chose to use the 2–10 mm measurement criteria therefore which is in...
agreement with the literature, but further research is warranted to qualify the importance of antral follicle size.

Most in vivo and in vitro studies suggest that AMH has an inhibitory effect on the initiation of follicular growth and on the sensitivity of the follicles to FSH (Durlinger et al., 1999, 2001, 2002a,b; Visser and Themmen, 2005; Carlsson et al., 2006), although other in vitro studies indicate enhanced growth of primordial and pre-antral follicles (McGee et al., 2001; Schmidt et al., 2005). A direct physiological interaction between AMH expression and FSH is therefore consistent with AMH being an inhibitor of FSH secretion. The negative correlation between early follicular AMH and FSH levels ($r = -0.315$; $P < 0.01$) reported in this study supports this interpretation. Conversely, however, we found no correlation between AMH and FSH levels measured at down-regulation, a time when the ovaries are relatively quiescent, which suggests that FSH has little role in the regulation of AMH secretion by the early growing follicles. Furthermore, there is minimal variation in AMH levels throughout the normal menstrual cycle in both fertile and subfertile women (Hehenkamp et al., 2006; La Marca et al., 2006). In men, exogenous FSH administration has been shown to affect serum AMH levels and while the initial increase in FSH seen following a single dose of GnRH agonist is not associated with an increase in AMH (van Rooij et al., 2002), the levels do increase when patients with hypogonadotropic hypogonadism are treated with FSH directly (Young et al., 2005). AMH levels have also been shown to fall when supraphysiological doses of FSH are used for controlled ovarian stimulation, although this may relate to a relative reduction in the number of smaller follicles, the predominant source of AMH secretion, with increasing follicular recruitment and maturation, rather than a direct influence of FSH (Fanchin et al., 2003; La Marca et al., 2004). The relationship between AMH and FSH is clearly complex and likely to be influenced by other factors.

An increase in AMH concentration through an increase in the size of the gonadotrophin-responsive pool, either through a direct effect or indirectly by slowing the rate of follicular atresia, is supported by the well-established observations that the response to ovarian stimulation is enhanced by pretreatment with GnRH agonists (Hughes et al., 1992; Smitz et al., 1992; Tan et al., 2005). This indicates that raised AMH levels are associated with an increased number of recruitable follicles, most of which will be stimulated to grow by the supraphysiological doses of gonadotrophin used during controlled ovarian stimulation as part of ART. Serum AMH levels at down-regulation, and at other stages of treatment, have been shown to be predictive of the quantitative response to controlled ovarian stimulation as measured by the number of oocytes retrieved (van Rooij et al., 2002; Eldar-Geva et al., 2005; Muttukrishna et al., 2005; Penarrubia et al., 2005; La Marca et al., 2007) and qualitative response in terms of oocyte quality and treatment outcome (Penarrubia et al., 2005; Ebner et al., 2006). This may explain why treatment using the standard long protocol is frequently associated with higher numbers of oocytes and better pregnancy rates than treatment using GnRH antagonists to prevent premature oocyte maturation and ovulation (Tan et al., 2005; Al-Imany et al., 2007). The present study found no effect of GnRH-agonist down-regulation on the number of antral follicles visible by ultrasound and is therefore in agreement with a number of other studies both in humans (Yu Ng et al., 2004) and in monovulatory animals (Picton et al., 1990). There appears to be little data in humans or animal species, however, on the effect of down-regulation on the quality of gonadotrophin-responsive follicles or the number of antral follicles below the resolution of conventional ultrasound. Campbell et al. (2004) reported that profound FSH suppression with GnRH agonist in combination with estradiol in animals, resulted in an inhibition in the rate of both pre-antral and antral follicle development and a fall in proliferative markers (proliferative cell nuclear antigen; PCNA), indicators of follicular functional status, in this hypogonadotropic state when compared with pretreatment state (Campbell et al., 2004). However, in the same study, high FSH was observed to stimulate both follicle development and the expression of proliferative markers in both pre-antral and small antral follicles and numerous other studies have shown similar effects of FSH on pre-antral follicle development in vitro (Newton et al., 1999; Mitchell et al., 2002). Although it appears unlikely, therefore, that suppression of FSH per se would stimulate gonadotrophin-responsive follicle development following down-regulation, it is possible that the loss of the growing cohort of gonadotrophin-dependent follicles may have this effect. It has long been hypothesized that growing follicles release factors which have an intra-ovarian inhibitory feedback effect on less developed follicles (diZerega et al., 1982; Adashi, 1992) and there is some evidence that factors such as activin may exert this effect (Mizunuma et al., 1999). Thus, loss of inhibitory feedback may result in higher rates of proliferation and/or lower rates of atresia and the release of more AMH. Clearly, much more work is required to define the effect of down-regulation on the overall population of gonadotrophin-responsive follicles and on subgroups of follicles at different stages of development and therefore degree of FSH-responsiveness.

In the only other human study to evaluate the relationship between AMH levels and pituitary down-regulation using GnRH agonists, Mohamed et al. (2006) examined the effect of prolonged down-regulation induced by long-acting depot preparations used in subjects with endometriosis. They reported similar AMH levels at 4 and 8 weeks after the depot injection but did not measure the baseline, pretreatment AMH level and the true effect of down-regulation on AMH and the early growing follicles therefore cannot be ascertained from their work. Our findings are also not in agreement with the work of Arbo et al. (2007) who reported a significant fall in AMH levels when pituitary suppression was induced with combined oral contraceptive (COC) pills (Arbo et al., 2007). Although the degree of FSH suppression was much more profound in their subjects (change in the median values: 5.47 to 1.99 IU/l; % change: 68.3%) than in our subjects (change in the median values: 6.59 to 3.98 IU/l; % change: 37.9%), the timing and duration of suppression, 14 days treatment commenced in the mid-luteal phase of the preceding cycle, was identical. It is unlikely that the relatively higher suppression of FSH led to a reduction in AMH through a direct effect,
however, as there was no significant correlation between these variables. The reduction in AMH may relate to the progestogen component in the COC pill as progesterone has been shown to correlate with AMH in follicular fluid. Fanchin et al. (2005) demonstrated a significant negative correlation between the levels of progesterone and AMH in the follicular fluid of subjects undergoing IVF treatment with lower AMH levels and higher progesterone levels seen in the fluid from the larger follicles than in the aspirates from the smaller follicles.

Down-regulation was also associated with significant falls in serum FSH, LH, inhibin-B and estradiol and both ovarian volume and blood flow as expected. The fall in inhibin-B levels with pituitary desensitization has been described previously and confirms that the ovarian production of inhibin-B is gonadotrophin-dependent and that inhibin-B levels at down-regulation are not an appropriate marker of size of the follicular cohort (Lockwood et al., 1996). The fall in ovarian volume and ovarian vascularity indices, as measured using three-dimensional ultrasound, is most likely due to the hypoestrogenic state which is correlated with ovarian stromal volume (Mango et al., 1988) and ovarian stromal blood flow (Pellizzari et al., 2002; Carmina et al., 2005).

Conclusion

Pituitary desensitization with GnRH agonists in an ART programme results in a significant increase in AMH levels but has no effect on the total number of antral follicles visualized on ultrasound. This implies that either the secretion of AMH by early growing follicles is enhanced or that the size of this follicle cohort, but not of the larger gonadotrophin-dependent follicles, is increased. This may be through a direct or indirect effect of short-term GnRH-agonist treatment on the follicular functional status or the number and/or rate of atresia in the gonadotrophin-responsive pool of follicles. This may explain the well-established observations of an enhanced ovarian response during conventional controlled ovarian stimulation and higher pregnancy rates when pretreatment with GnRH agonists is employed.

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