Loss of LH-induced down-regulation of anti-Müllerian hormone receptor expression may contribute to anovulation in women with polycystic ovary syndrome

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STUDY QUESTION: Are anti-Müllerian hormone (AMH) and AMH type II receptor (AMHR-II) mRNAs similarly regulated by gonadotrophins in lutein granulosa cells (GCs) from control, normo-ovulatory and oligo/anovulatory women with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: AMH mRNA expression was induced by LH only in lutein GC of oligo/anovulatory PCOS women; down-regulation of AMHR-II, induced by LH in control and normo-ovulatory PCOS women, was absent in oligo/anovulatory women.

WHAT IS KNOWN ALREADY: It was suggested that AMH could be responsible for the blockade of follicles at the small antral stage in PCOS women. In keeping with this hypothesis, both AMH and AMHR-II are overexpressed in lutein GCs from oligo/anovulatory PCOS women.

STUDY DESIGN, SIZE, DURATION: Women undergoing IVF were included in this prospective study, either in the control group (30 women) or in the PCOS group (21 normo-ovulatory and 19 oligo/anovulatory patients) between January 2010 and July 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Human lutein GCs were isolated from follicular fluid during IVF protocols. Twenty-four hours after seeding, lutein GCs from each woman were serum starved and cultured for 48 h ± FSH, LH or cAMP. Then AMH and AMHR-II mRNAs were quantified by quantitative RT–PCR and AMH protein concentration was measured in the culture medium by ELISA. Experimental results were analyzed, within each group of women, by the non-parametric Wilcoxon test for paired comparisons between cells cultured in control medium and FSH, LH or cAMP treated cells. Clinical comparisons between the three groups of women were performed on log values using the ANOVA test with Bonferroni correction.

MAIN RESULTS AND THE ROLE OF CHANCE: FSH up-regulated both AMH expression and secretion by lutein GCs from the three groups of women (P < 0.05). LH had no effect on AMH mRNAs levels in lutein GCs from controls and normo-ovulatory PCOS women, but increased AMH expression in oligo/anovulatory PCOS women (P < 0.05). Interestingly, LH and cAMP treatments reduced AMHR-II expression by lutein GCs from controls and normo-ovulatory PCOS women (P < 0.05), but had no effect on AMHR-II mRNA levels in oligo/anovulatory PCOS women.
LIMITATIONS, REASONS FOR CAUTION: The lutein GCs are not the best model to study AMH and AMHR-II regulation by gonadotrophins. Indeed, AMH and AMHR-II are down-regulated in luteinized cells. Furthermore, these cells have been exposed to non-physiological levels of gonadotrophins and hCG. However, AMH and AMHR-II mRNAs are quantifiable by real-time RT–PCR, and the cells are still responsive to FSH and LH. The age of patients is significantly different between control and oligo/anovulatory PCOS women: this may be a bias in the interpretation of results but older women in the control group had a good ovarian reserve.

WIDER IMPLICATIONS OF THE FINDINGS: The overexpression of AMH and AMHR-II in oligo/anovulatory PCOS women could be due to increased LH levels and/or inhibition of its repressive action. The fact that this dysregulation is observed in oligo/anovulatory, but not in normo-ovulatory, PCOS women emphasizes the role of LH in the follicular arrest of PCOS women and suggests that this involves the AMH/AMHR-II system.

STUDY FUNDING/COMPETING INTEREST(S): The Assistance-Publique Hôpitaux de Paris provided a Contrat d’Interface and the Agence de Biomédecine provided a grant to Nathalie di Clemente. Schering-Plough provided an FARO grant to Alice Pierre. The authors have nothing to disclose.

Key words: anti-Müllerian hormone / Müllerian inhibiting substance / anti-Müllerian hormone receptor / polycystic ovary syndrome / gonadotrophins

Introduction

Anti-Müllerian hormone (AMH), also called Müllerian inhibiting substance, is a member of the transforming growth factor-β family, well known for its role during male sexual differentiation (Josso et al., 2006). AMH acts through a specific type II receptor (AMHR-II) together with type I receptors and Smad molecules shared with Bone Morphogenetic Proteins (di Clemente et al., 2003). In females, AMH is secreted by granulosa cells (GCs) of growing follicles from the beginning of folliculogenesis to menopause (Weenen et al., 2004). Initiated in primary follicles, AMH expression is strongest in pre-antral and small antral follicles and decreases subsequently up to ovulation. AMHR-II is co-expressed with AMH in GCs of growing follicles (di Clemente et al., 1994b; Baarends et al., 1995). In rodents, AMH plays a retarding role upon both primordial follicle recruitment and follicle maturation (Visser et al., 2006). Recently, serum AMH levels have been recognized to be a useful diagnostic and prognostic tool in reproductive medicine, as a reliable marker of the ovarian follicular status and a predictor of the ovarian response to controlled ovarian hyperstimulation (La Marca et al., 2010). Serum AMH concentrations below 1 ng/ml are indicative of a poor ovarian response to stimulation. In contrast, women with an AMH concentration above 5 ng/ml, a situation usually observed in women with polycystic ovary syndrome (PCOS) (Dewailly et al., 2011), have a risk of hyper responsiveness to follicle stimulating hormone (FSH) leading to ovarian hyperstimulation syndrome.

PCOS is the most common cause of female infertility, affecting between 5–10% of women of reproductive age worldwide (Norman et al., 2007). It is characterized by defective follicular maturation and ovulation, hormonal dysregulation including LH, AMH and/or androgen hypersecretion. A growing body of evidence suggests that androgens are likely responsible for the excess of follicle recruitment in these women (Jonard and Dewailly, 2004). In contrast, the reason why folliculogenesis is blocked at the small follicle stage and why dominant follicle selection does not occur normally is unknown. Because serum AMH levels are 2- to 3-fold higher in women with PCOS than in ovulatory women with normal ovaries (Fallat et al., 1997), and reflect the severity of PCOS (Piouka et al., 2009), AMH might be one of the factors involved in these follicular effects. As AMH is exclusively produced by GCs, it was assumed that the rise in serum AMH in PCOS was a consequence of the increased number of small follicles in these ovaries. AMH assay of the culture medium of GCs extracted from PCOS ovaries has shown that this was also due to an increased production of AMH by PCOS GCs (Pellatt et al., 2007). We have confirmed these results at the mRNA level in lutein GCs retrieved after ovarian puncture of anovulatory PCOS women undergoing IVF, and we have shown that both AMH and AMHR-II mRNAs are overexpressed by PCOS cells compared with control (Catteau-Jonard et al., 2008). The objective of this work was to explore the origin of this dysregulation and the potential role of AMH in PCOS dysovulation. Because gonadotrophins are the main regulators of folliculogenesis, and because both reduced sensitivity and/or levels of FSH and increased levels of LH have been evoked in PCOS pathophysiology (Jonard and Dewailly, 2004; Franks et al., 2008), we studied the effects of FSH and LH on AMH and AMHR-II expression by lutein GCs from control, normo-ovulatory and oligo/anovulatory PCOS women.

Materials and Methods

Subjects

Women undergoing IVF were included in this prospective study, either in the control group or in the PCOS group. All women of the control group met the following inclusion criteria: (1) aged between 20 and 40; (2) both ovaries present, with no morphological abnormalities, adequately visualized in transvaginal ultrasound scans; (3) menstrual cycle length range between 26 and 30 days; (4) no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion; (5) no clinical signs of hyperandrogenism and (6) no polycystic ovary morphology at ultrasonography. Infertility was due either to tubal or sperm abnormalities.

Women with PCOS were selected according to the Rotterdam criteria (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2012): association of at least two of the three following criteria: (1) ovulatory disturbance, mainly oligomenorrhea and amenorrhea; (2) hyperandrogenism (clinical or biological); (3) polycystic ovary morphology at ultrasonography. Oligo/anovulatory PCOS women were defined as women with oligo- or amenorrhea (cycles over 35 days).
An informed consent was obtained from all women and this investigation received the approval of our internal institutional review board.

Collection of human lutein GCs and culture
In our study, we included 30 controls and 40 women with PCOS (21 normo-ovulatory and 19 oligo/anovulatory patients). Only the follicles >12 mm were retrieved and after oocyte isolation, follicular fluids from each patient were pooled and centrifuged through a one-step density Percoll gradient (vol/vol, phosphate-buffered saline (PBS)/Percoll) at 3300 g for 15 min to remove red blood cells. Lutein GCs were collected at the interface, washed with PBS and seeded at 3 × 10^3 cells per well in six-well plates in 2 ml of Dulbecco’s modified Eagle’s medium (DMEM)/F-12 (Invitrogen, Carlsbad, CA, USA) containing 10% fetal calf serum (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen).

Twenty-four hours after seeding, human lutein GCs from each patient were serum starved for 1 h then cultured for 48 h without serum, in control medium and, depending on the number of cells, in the presence of recombinant human FSH (Gonal-F 1 IU/ml, Merck Serono, Lyon, France), LH (Luveris 1 IU/ml, Merck Serono, Lyon, France), control medium and, depending on the number of cells, in the presence of cAMP (Sigma, Saint Quentin Fallavier, France).

RNA extraction and reverse transcription
Total RNAs were extracted from lutein GCs using the RNA Plus extraction kit (QIAGEN, Valencia, CA, USA). Reverse transcription (RT) was performed in a total of 20 μl with the Omniscript Reverse Transcription Kit (QIAGEN) using 1 μg RNA, Omniscript reverse transcriptase and oligo-dT primers and random hexamers, as recommended by the manufacturer.

Quantitative real-time PCR
Quantification of AMH, AMHR-II, StAR (Steroidogenic Acute Regulatory) and housekeeping genes [RPL13a (Ribosomal protein L13a) and SDHA (Succinate dehydrogenase complex subunit A)] mRNAs was performed by real-time PCR using the TaqMan PCR method. The primers and the UPL probes (Roche Diagnostics, Indianapolis, IN, USA) used to amplify these genes are indicated in Table I. Real-time PCR was performed in duplicates with one-fifth dilution of the cDNAs using the Lightcycler 480 Probes Master kit (Roche Diagnostics). The PCR protocol used an initial denaturing step at 95°C for 10 min followed by 45 cycles of 95°C for 10 s, 60°C for 30 s, 72°C for 1 s. To generate standard curves, different concentrations of the purified and quantified PCR products were amplified. The amplification efficiency was 99, 97, 95, and 96% for AMH, AMHR-II, StAR, SDHA and RPL13a genes, respectively. Copy numbers were normalized by two reference genes, RPL13a and SDHA, and expressed per microgram of RNA.

ELISA test
AMH was measured in each cell-conditioned medium using an ELISA test (Elisa Immunotech Beckman-Coulter Laboratories kit, ref A 16507, Villepinte, France). Samples were tested in duplicates.

Statistics
Experimental results (in relative copy number per microgram of RNA for quantitative RT–PCR experiments and in ng/ml for ELISA experiments) were analyzed by the non-parametric Wilcoxon test for paired comparisons between cells cultured in control medium and FSH, LH or cAMP treated cells, within each group of women. Comparisons for a given treatment (control medium, FSH, LH or cAMP) between groups were performed using the Kruskal–Wallis test. Because values of most parameters were not normally distributed, all clinical comparisons between the three groups of women were performed on log values using the ANOVA test with Bonferroni correction. Statistical procedures were run on SPSS 15.0 (IBM, Armonk, NY, USA).

Results
Population
The main clinical parameters in control, normo-ovulatory and oligo/anovulatory PCOS women are summarized in Table II. No difference between the three populations was found for the body mass index (BMI), the FSH and estradiol (E2) serum levels on Day 3 of the menstrual cycle. In PCOS groups, AMH and antral follicular count on Day 3 of the menstrual cycle were higher than in the control group. Serum AMH level was found to be higher than 5 ng/ml in 93% of PCOS patients, consistent with our previous results (Dewailly et al., 2011). Oligo/anovulatory PCOS women were younger and had higher LH serum levels on Day 3 of the menstrual cycle than controls.

Effect of gonadotrophins on StAR protein expression by lutein GCs from control and PCOS women
Lutein GCs from each woman were treated independently. Depending on the number of cells obtained for each woman, we performed a single treatment (control medium and FSH or LH) or two treatments (control medium, FSH and LH). To validate our culture model of

| Table I Sequence of the primers and probes used for real-time RT–PCR experiments. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Genes | Nucleotides sequence written 5’ to 3’ sense | Nucleotides sequence written 5’ to 3’ antisense | UPL probes |
| AMH | CGCCCTGGTGTTGCTCTACAC | GAACCTCAAGCAGGGTGT | 69 |
| AMHR-II | CCCTACATCCCATCCACCT | GGTCTGACATCACAAGCT | 30 |
| StAR | GGCCATCTAGCAACAAAGA | TCATTTTGGGCTCTT | 11 |
| RPL13a | CTGGACCAGTCTCAAGGGTT | GCCCGAATAGGCAAA | 74 |
| SDHA | GGACCTGTGGTTGTCCTT | CCAACATTGTTTAT | 80 |

AMH, anti-Mullerian hormone; AMHR-II, anti-Mullerian hormone receptor type II; RPL13a, ribosomal protein L13a; SDHA, succinate dehydrogenase complex subunit A; StAR, steroidogenic acute regulatory protein.
human lutein GCs, we first studied their ability to respond to FSH and LH. That is why, using quantitative RT–PCR, we analyzed the regulation by gonadotrophins of one of their target genes, the StAR protein, responsible for the translocation of cholesterol across the mitochondrial membrane (Lin et al., 1995). As expected, both FSH (Fig. 1A) and LH (Fig. 1B) significantly up-regulated StAR mRNAs in most preparations of lutein GCs, from control, normo- or oligo/anovulatory PCOS women.

Either because AMH copy number was too low (>32 Ct; two women in the control group, one in the oligo/anovulatory PCOS group and one in the anovulatory PCOS group, respectively), or because we did not have enough RNAs to re-do the experiment when the duplicates were not validated (Std Ct > 0.5) or for other quantitative RT–PCR technical problems, both AMH and AMHR-II expression could not be analyzed for all lutein GCs preparations.

### Effect of FSH and LH on AMH expression and production by lutein GCs from control and PCOS women

FSH significantly increased AMH mRNA levels in lutein GCs from control, normo-ovulatory and oligo/anovulatory PCOS women (1.5-, 1.3- and 1.5-fold increases, respectively, Fig. 2A). LH had no effect on AMH expression by lutein GCs from control and normo-ovulatory PCOS women (Fig. 2B, left and middle). In contrast, LH treatment of lutein GCs from oligo/anovulatory PCOS women significantly up-regulated AMH mRNAs (2.1-fold increase; Fig. 2B, right).

AMPH production in the culture medium was determined in parallel by ELISA. However, given the volume of culture medium required to culture enough cells for RNA preparation, and the detection limit of the ELISA assay (0.14 ng/ml), AMH could not be measured for a large number of lutein GCs preparations, preventing us from distinguishing the results obtained for normo- and oligo/anovulatory PCOS women. FSH treatment significantly stimulated AMH production by control and PCOS cells (1.5- and 1.4-fold increases, respectively; Fig. 3). In contrast, LH treatment had no effect on AMH production by lutein GCs from control women but increased AMH production by lutein GCs from PCOS patients (1.6-fold increase; Fig. 3).

### Effect of FSH, LH and cAMP on AMHR-II expression by lutein GCs from control and PCOS women

We used the same gonadotrophin responsive preparations of lutein GCs to investigate the regulation of AMHR-II mRNAs by FSH and LH. FSH had no effect on AMHR-II expression by lutein GCs from control, normo-ovulatory or oligo/anovulatory PCOS women (Fig. 4A). Interestingly, LH treatment significantly reduced AMHR-II mRNA levels in lutein GCs from control and normo-ovulatory PCOS women (1.6- and 2.2-fold decreases, respectively; Fig. 4B). On the contrary, LH had no effect on AMHR-II expression by lutein GCs from oligo/anovulatory PCOS patients (Fig. 4B right). To confirm these results, we then treated new preparations of lutein GCs with LH second messenger, cAMP. cAMP significantly reduced AMHR-II mRNA levels in lutein GCs from control and normo-ovulatory PCOS women (1.6- and 1.9-fold decreases, respectively; Fig. 4C) but had no effect on AMHR-II expression by lutein GCs from oligo/anovulatory PCOS patients.

### Discussion

The objective of this work was to compare FSH and LH regulation of AMH and AMHR-II expression by lutein GCs from control, normo-ovulatory and oligo/anovulatory PCOS women. We demonstrate that LH could be one of the factors responsible for the overexpression of AMH and AMHR-II in lutein GCs from anovulatory PCOS women (Pellatt et al., 2007; Catteau-Jonard et al., 2008).

We used human lutein GCs isolated from follicular fluid after an IVF protocol. Obviously, it is not the best GCs model to study the regulation of the AMH/AMHR-II system by gonadotrophins, because AMH and AMHR-II are down-regulated in luteinized cells and because these cells have been exposed to non-physiological levels of gonadotrophins and hCG. However, AMH and AMHR-II mRNAs are quantifiable by real-time PCR (Catteau-Jonard et al., 2008; Shi

### Table II Main clinical parameters in control, normo-ovulatory and oligo/anovulatory PCOS women.

<table>
<thead>
<tr>
<th></th>
<th>Control women (n = 30)</th>
<th>Normo-ovulatory PCOS women (n = 21)</th>
<th>Oligo/anovulatory PCOS women (n = 19)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.0 (30.8–38.3)</td>
<td>32.0 (25.0–35.0)</td>
<td>32.0 (28.0–34.0)</td>
<td>a, b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 (19.8–26.5)</td>
<td>22.0 (19.8–24.9)</td>
<td>22.3 (19.9–25.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 FSH (IU/l)</td>
<td>6.55 (5.6–8.33)</td>
<td>5.7 (4.83–6.05)</td>
<td>6.5 (5.2–7.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 E2 (pg/ml)</td>
<td>40.0 (25.0–53.0)</td>
<td>29.0 (18.0–47.3)</td>
<td>40.5 (33.0–65.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 LH (IU/l)</td>
<td>4.0 (2.78–4.93)</td>
<td>4.25 (3.48–6.65)</td>
<td>6.8 (4.8–11.5)</td>
<td>b, c</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.1 (1.5–3.6)</td>
<td>7.6 (5.4–10.3)</td>
<td>8.3 (7.2–11.3)</td>
<td>a, b</td>
</tr>
<tr>
<td>Day 3 AFC</td>
<td>15.0 (12.0–18.0)</td>
<td>33.0 (29.0–43.0)</td>
<td>32.5 (25.8–45.5)</td>
<td>a, b</td>
</tr>
</tbody>
</table>

PCOS, polycystic ovary syndrome; BMI, body mass index; Day 3, hormonal dosage on Day 3 of the menstrual cycle; FSH, follicle stimulating hormone; E2, estradiol; LH, luteinizing hormone; AMH, anti-Müllerian hormone; AFC, antral follicular count; NS, non-significant; Med, Median (25–75th percentile).

*By ANOVA with Bonferroni correction on log values: a: normo-ovulatory PCOS women compared with control women, P < 0.05; b: oligo/anovulatory PCOS women compared with control women, P < 0.05; c: oligo/anovulatory PCOS women compared with normo-ovulatory PCOS women, P < 0.05.
et al., 2009; Winkler et al., 2010; Taieb et al., 2011), and the cells are still responsive to FSH and LH as shown by the ability of these hormones to stimulate StAR expression (Fig. 1). In addition, IVF protocols are the only way to get enough cells to perform RNA expression studies and to compare cells from control and PCOS women.

We recently showed that both FSH and cAMP up-regulated AMH mRNAs in human lutein GCs and activated a 3 kb human AMH reporter gene in a murine granulosa cell line (Taieb et al., 2011). We confirm in the present study the stimulatory effect of FSH on both AMH expression and secretion and we show that it is also observed in lutein GCs from normo-ovulatory and oligo/anovulatory PCOS women.

Figure 1 Human lutein granulosa cell (GC) sensitivity to gonadotrophins. Real-time RT–PCR analysis of StAR expression in lutein GCs from control (left), normo-ovulatory PCOS (middle) and oligo/anovulatory PCOS (right) women. Lutein GCs from each woman were treated for 48 h in control medium alone or with FSH 1 UI/ml (A) or LH 1 UI/ml (B), and StAR expression was analyzed by real-time RT–PCR. Results are expressed in relative copy number per microgram of total RNAs and analyzed by the Wilcoxon paired test (\(^*\)P < 0.05; \(**\)P < 0.01 versus control medium). The log values of the results were used for graphic representation. Data are represented by box-and-whisker plots. Vertical bars represent the minimum and the maximum and the boxes indicate the 25–75th range. The horizontal line in each box corresponds to the median. \(n\) represents the number of women. StAR, steroidogenic acute regulatory protein.

Figure 2 Effect of gonadotrophins on AMH mRNAs. Real-time RT–PCR analysis of AMH mRNAs in lutein GCs from control (left), normo-ovulatory PCOS (middle) or oligo/anovulatory PCOS (right) women. Lutein GCs from each woman were treated for 48 h in control medium alone or with FSH 1 UI/ml (A) or LH 1 UI/ml (B). AMH expression was analyzed by real-time RT–PCR. Results were expressed in relative copy number per microgram of total RNAs. The effect of treatment within groups of women was analyzed by the Wilcoxon paired test (\(^*\)P < 0.05 versus control medium). No difference for a given treatment between groups was found with the Kruskal–Wallis test. The log values of the results were used for graphic representation. Data are represented by box-and-whisker plots. Vertical bars represent the minimum and the maximum and the boxes indicate the 25–75th range. The horizontal line in each box corresponds to the median. \(n\) represents the number of women. AMH, anti-Mullerian hormone.
women (Figs 2A and 3). In contrast, Rico et al. (2011), using bovine GCs from growing follicles showed that FSH inhibited AMH secretion. Pellatt et al. (2007), using human GCs from 2–10 mm follicles, did not detect any effect of FSH on cells extracted from control women and observed an inhibitory effect of FSH on GCs from anovulatory PCOS women. However, these two groups added to the culture medium androstenedione or testosterone, respectively, which has been shown to inhibit AMH expression (Crisosto et al., 2009). The intermediate action of estradiol, absent in our model but present in others after aromatization of androgens, could also explain the discrepancy between the results. Indeed, we have shown recently that estradiol represses AMH transcription through its nuclear receptor beta (Grynberg et al., 2012). Voutilainen and Miller (1987) also failed to detect an effect of FSH on AMH expression by human lutein GCs, but they pooled the cells from different women which, as demonstrated in the present study (Fig. 1), are not all sensitive to FSH. However, they observed that cAMP, which mediates gonadotrophin effects, stimulated AMH mRNAs. Conversely, little is known on AMHR-II regulation by FSH. Only one study reported a down-regulation of AMHR-II in pre-pubertal rats treated with GnRH antagonist and FSH (Baarends et al., 1995). In contrast, we did not detect any effect of FSH on AMHR-II expression in the three groups of women (Fig. 4A). This discrepancy could be due to the fact that quantitative RT–PCR is not sensitive enough or that in vivo local changes in the environment surrounding the follicle may facilitate or mask an effect of FSH.

Our present model of primary culture of luteal GCs is probably more appropriate for studying AMH and AMHR-II regulation by LH, especially
as LH effects are predominant at this stage of the ovarian cycle. In this study, we confirm the results of Pellatt et al. (2007), showing that LH has no effect on AMH expression by lutein GCs from control and normo-ovulatory PCOS women, whereas it has a stimulating effect in lutein GCs from oligo/anovulatory PCOS patients (Fig. 2B). In addition, we show for the first time that LH represses AMHR-II mRNA in both control and normo-ovulatory PCOS cells (Fig. 4). This result is in agreement with a role of LH in promoting the differentiation of GCs. Indeed, by inhibiting AMHR-II expression, LH may counteract all the repressive effects of AMH on GCs differentiation previously described, namely progesterone production (Kim et al., 1992), aromatase and LH receptor expression (di Clemente et al., 1994a) and FSH responsiveness (Durlinger et al., 2001). In contrast, LH does not repress AMHR-II expression by lutein GCs from oligo/anovulatory PCOS women (Fig. 4) providing an explanation for the overexpression of AMHR-II in these cells. The stimulatory effect of both FSH and LH on AMH expression (Fig. 2), and the LH:FSH ratio > 1 often encountered in PCOS women could contribute to AMH overexpression by their GCs. Indeed, we have shown recently that a combination of both gonadotrophins corresponding to an LH:FSH ratio > 1 enhanced AMH transcription more effectively than an LH:FSH ratio < 1 (Taieb et al., 2011). In line with the involvement of LH in AMH overexpression, serum LH and AMH levels are higher in oligo/anovulatory PCOS than in control women (Table II). Moreover, a positive correlation between LH and AMH serum levels was reported by several authors in oligo/anovulatory women (Willis receptors than GCs from follicles of the same size from control women, thus not seeming to be a prerequisite to anovulation. An advanced differentiation is always observed in patients with oligo/anovulatory PCOS and does not involve LH in AMH overexpression, serum LH and AMH levels are higher in oligo/anovulatory PCOS than in control women (Table II). Moreover, a positive correlation between LH and AMH serum levels was reported by several authors in oligo/anovulatory PCOS women exclusively (Laven et al., 2004; Catteau-Jonard et al., 2007; Piouka et al., 2009). However, excessive serum LH level is not always observed in patients with oligo/anovulatory PCOS and does not seem to be a prerequisite to anovulation. An advanced differentiation of PCOS GCs, which have been reported to express more LH receptors than GCs from follicles of the same size from control women (Wills et al., 1998; Jakimiuk et al., 2001), could favor the stimulating effect of LH on AMH expression in vivo. An alternative explanation, suggested by the fact that LH up-regulates AMH expression but does not repress AMHR-II mRNA in the same cells, is that the repressive LH action on the AMH/AMHR-II system is inhibited in lutein GCs from oligo/anovulatory PCOS women. An alteration of the LH signaling pathway is unlikely because GCs from anovulatory PCOS women have been shown to produce more estradiol and progesterone than GCs from control women in vitro (Wills et al., 1998). The down-regulating effect of cAMP in lutein GCs from control and normo-ovulatory PCOS women but not in lutein GCs from oligo/anovulatory PCOS women (Fig. 4C) suggest that LH action is inhibited downstream of cAMP. The activation of a molecule or a signaling pathway that could counteract LH/cAMP action is under investigation.

Our data about LH may help understanding the follicular arrest of PCOS. It has been postulated that it is the follicle which contains more GCs and FSH receptors, and is thus more sensitive to FSH, that emerges as dominant at the time of the inter-cycle FSH rise. Once selected, the dominant follicle becomes less dependant on FSH and more responsive to LH, but a balance between FSH and LH is required for GC proliferation and differentiation. AMH, reducing FSH responsiveness (Durlinger et al., 2001) and inhibiting aromatase activity and LH receptor expression (di Clemente et al., 1994a), could repress follicular maturation. At later stages of follicular differentiation, LH, repressing AMHR-II expression, lessens AMH sensitivity, allowing follicle terminal maturation and ovulation. In PCOS women, lower or similar serum FSH levels than those seen in control ovulatory women, together with a higher number of follicles could contribute to the absence of follicle selection. The effect of LH on the overexpression of the AMH/AMHR-II system could amplify the defect in follicle terminal maturation and ovulation, making an explanation for the link between serum AMH levels and the severity of PCOS.

In conclusion, we have shown in the present work that the overexpression of AMH and AMHR-II mRNAs in lutein GCs from oligo/anovulatory PCOS women could be due to both rising LH levels and/or inhibition of its repressive action. The fact that this dysregulation is observed in oligo/anovulatory, but not in ovulatory, PCOS women emphasizes the role of LH in the follicular arrest of PCOS and suggests that this role involves the AMH/AMHR-II system.

Acknowledgements

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Authors’ roles

A.P. and M.P. performed the experiments, analyses and data interpretation. M.G., L.H. and R.F. were involved in sample recruitment. N.A. participated in some experiments. J.T., J.G., J.Y.P. and D.D. revised the manuscript and approved the final version. S. C.-J. and N.D.C. contributed equally to the management of the work. N.D.C. wrote the paper.

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Conflict of interest

None declared.

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