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Granulosa Cell Production of Anti-Müllerian Hormone Is Increased in Polycystic Ovaries

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Context: There has been renewed interest in anti-Müllerian hormone (AMH) because of its role in the ovary. Data on its actions are sparse, but it appears to inhibit follicle growth. Interestingly, serum AMH is two to three times higher in women with polycystic ovary (PCO) syndrome than women with normal ovaries.

Objective: We examined the production of AMH by cells from a range of follicle sizes from normal ovaries and compared this with production by ovulatory and anovulatory (anov) PCOs.

Design: Granulosa cells (GCs) and theca and follicular fluid (ff) were isolated from intact follicles. Cells were cultured for 48 h ± FSH or LH, and AMH was measured in ff and cell-conditioned media (CM).

Results: AMH levels in ff and GC-CM ranged from 42 to 2240 and 0.025 to 1.7 ng/ml, respectively, and were low or undetectable in ff and GC-CM from follicles greater than 9 mm, luteinized cells, and theca and stroma. The mean level of AMH was four times higher in GC-CM from ovulatory PCOs [mean (range) 1.56 (0.025–7)] and 75 times higher from anovPCO [21.4 (17.2–43 ng/ml)] than normal ovaries [0.37 (0.025–1.7)]. Neither LH nor FSH had an effect on AMH production by GCs from normal ovaries, but in cells from PCOs, FSH significantly decreased AMH, and in contrast, LH increased AMH.

Conclusions: The reduction of AMH in follicles greater than 9 mm from normal ovaries appears to be an important requirement for the selection of the dominant follicle. AMH production per GC was 75 times higher in anovPCOs, compared with normal ovaries. This increase in AMH may contribute to failure of follicle growth and ovulation seen in polycystic ovary syndrome. **(*J Clin Endocrinol Metab* 92: 240–245, 2007)**

ANTI-MÜLLERIAN HORMONE (AMH), also known as Müllerian-inhibiting substance, is a member of the TGFβ superfamily. Secreted by the Sertoli cells of the testis from 8 wk gestation until puberty, its classical action is to regress the Müllerian ducts during male fetal development (1). AMH is now coming under renewed scrutiny, however, because of its possible role in the ovary, in which it is expressed postnatally until menopause and is located in the granulosa cells (2). In both rodents and humans, its expression was absent from nongrowing primordial follicles, was highest in small growing follicles of the preantral and antral stages, and was again lost in luteinized and atretic follicles (3, 4). Human follicles of the size after selection of the dominant follicle have not been studied.

This renewed scrutiny of AMH is due to a series of interesting publications. Several studies have demonstrated that serum AMH levels in women decline with advancing age (5, 6), and AMH has therefore been proposed as a marker of ovarian reserve (7). Indeed, an over-the-counter kit is now available in the United Kingdom in which AMH is one of a triad of hormones used to calculate ovarian reserve index. The relationship between the number of follicles in the ovary and serum AMH has also led to the demonstration that AMH is a good predictor of poor response to *in vitro* fertilization treatment (8).

Knowledge of the actual function of AMH in the ovary is rather limited, and much of the information that we do have has been extrapolated from studies of AMH null mice (9–11). These mice had an increase in the number of follicle-initiating growth, which was reflected in an increase in the number of growing follicles of all stages. This overgrowth of follicles resulted in premature cessation of cyclicity in these animals (9). Cultured ovaries from neonatal mouse cultures with AMH contained 40% fewer growing follicles (11). Interestingly, AMH also reduced FSH-stimulated follicle growth in isolated follicle cultures (10). Taken together, these data suggest that AMH retards follicle growth and reduces sensitivity to FSH. Studies in which ovarian cells were exposed to AMH protein supported these findings in that cell proliferation, steroidogenesis, and induction of LH receptors, all required for follicle progression, were inhibited (12, 13).

A second major reason for the resurgence of interest in AMH is the fact that serum levels in women with polycystic ovary syndrome (PCOS) are 2- to 3-fold higher than in ovulatory women with normal ovaries (14–16). PCOS affects 5–20% of women of reproductive age (17) and is the primary cause of anovulatory infertility (18). It is characterized by polycystic ovaries (PCOs) with hyperandrogenism, obesity, elevated LH, and insulin resistance (19). As an exclusively granulosa cell product, it was assumed that the rise in serum AMH in PCOS was a consequence of the increased number

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Abbreviations: AMH, Anti-Müllerian hormone; anov, anovulatory; GLC, granulosa-luteal cell; M199, Medium 199; ov, ovulatory; PCO, polycystic ovary; PCOS, PCO syndrome.

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of follicles in these ovaries. Given that AMH appears to be an inhibitor of follicle growth, however, we were interested to see whether AMH production per cell was raised in PCOS.

We examined the production of AMH by cells from the normal ovary and compared this with AMH production by ovulatory and anovulatory PCOs. Given the likelihood of AMH production being modulated by gonadotropins, we also examined the effect of LH and FSH on granulosa cell AMH production.

Patients and Methods

Ovarian dissection

Informed written consent was obtained from women undergoing transabdominal hysterectomy with bilateral oophorectomy for benign gynecological conditions at St. George's Hospital (London, UK), Hope Hospital (Manchester, UK), and St. Luke's Hospital (Malta). Ethical approval was granted by the relevant committee in each institution. Details of menstrual history (cycle length and duration) and medication were obtained before surgery. The ovaries removed from each patient were seen by a pathologist before a portion of each was taken to the laboratory for dissection. Morphology and ovulatory status were assigned as previously published and were based on ovarian size, follicle sizes and numbers, the presence of a dominant follicle or corpus luteum, and the amount and density of stroma as determined by dissection in conjunction with patient history (20). The timing of surgery was random. Patient details and follicle numbers isolated and used in each experiment are shown in Table 1.

Follicles were isolated from the surrounding stroma under sterile conditions using binocular microscopy as previously described (21). Accurate measurement of follicle diameter was determined with calipers before the follicular fluid was aspirated. A cross-wise incision into the follicle was then made to expose the fragile granulosa cell layer, which was flushed away from the theca with a stream of Medium 199 (M199; Invitrogen Ltd., Paisley, Scotland, UK) and collected. Finally the theca cell layer was carefully peeled off and digested in an enzyme cocktail for 30 min at 37 C with gentle agitation (22). The granulosa or theca cells were cultured in M199 supplemented with penicillin/streptomycin, L-glutamine, and amphotericin B (all purchased from Invitrogen) pooled from either a number of follicles from a single patient or individual follicles, based on the number of follicles and cells and the experimental protocol. Only cells from those follicles visually designated healthy were used. A healthy follicle is well vascularized and contains the expected number of granulosa cells on subsequent counting (23).

Granulosa cells were plated at 5×10^4 and theca cells at 2.5×10^5 cells/well. Pieces of stroma were removed from normal ovaries and 10- to 50-mg pieces cultured in 24-well plates with 1 ml M199. Cells and tissue were incubated at 37 C in 95% air-5% CO_2 in a humidified incubator.

Granulosa-luteal cell (GLC) preparation

GLCs were isolated and prepared from follicular aspirates collected from women undergoing *in vitro* fertilization treatment, with ethical approval and informed written consent. Isolation of GLCs was performed as previously described (22). Cells were plated out at 5×10^4 cells/well in a 96-well plate with 200 μl of serum (5%) supplemented medium for 48 h, after which the medium was removed and replaced with experimental medium as detailed below.

TABLE 1. Details of patients whose follicles were included in the study

Patient no.	Age (yr)	Indication for surgery	Ovarian morphology	Cycle history Day of cycle (length of bleeding/ length of cycle)[a]	No. of follicles dissected (size range)	Follicle sizes pooled in experiment [no. (size range)]
1	46	Fibroid	Normal	17 (5/28)	3 (4–11)	1 (11)
2	47	Menorrhagia	Normal	21 (5/28)	1 (11)	1 (11)
3	42	nk	Normal	nk	1 (5)	1 (5)
4	43	Fibroid	Normal	7 (14/28)	3 (4–6)	2 (4, 6)
5	39	Pelvic pain	Normal	nk	1 (10)	1 (10)
6	44	Menorrhagia	Normal	nk (14/28)	2 (13, 15)	2 (13, 15)
7	39	Fibroid	Normal	11 (5/28)	8 (2–15)	1 (15)
8	48	Menorrhagia, pelvic pain	Normal	nk (8/30)	2 (7, 17)	1 (17)
9	41	Dysmenorrhea menorrhagia	Normal	14 (5/28)	8 (3–9)	1 (5)
10	49	Fibroid	Normal	nk	3 (5–10)	1 (10), 1 (5)
11	38	Fibroid	Normal	14 (5/28)	2 (4, 10)	1 (10)
12	48	Fibroid	Normal	2 (5/28–30)	7 (3–12)	1 (12)
13	51	Fibroid	Normal	20 (4/28)	5 (4–19)	1 (9), 1 (19)
14	45	Dysmenorrhea, menorrhagia	OvPCO	22 (5/28)	14 (2–9.5)	14 (2–9.5)
15	46	Fibroid	OvPCO	27 (5/28)	6 (3–12)	5 (3–9), 1 (12)
16	40	Pelvic pain, dyspareunia dysmenorrhoea	OvPCO	9 (5/28–52)	18 (3–19)	17 (3–9)
17	29	Pelvic pain, menorrhagia	OvPCO	nk	11 (2–4)	11 (2–4)
18	48	Menorrhagia	OvPCO	32 (3–4/28–38)	6 (3–8)	4 (7–8)
19	42	Menorrhagia	OvPCO	11 (5/28)	18 (3–5)	18 (3–5)
20	38	Menorrhagia, pelvic pain	OvPCO	21 (2–5/28–30)	4 (4–12)	3 (7–12)
21	34	Pelvic pain	OvPCO	nk	20 (2–15)	1 (15), 19 (2–6)
22	30	Pelvic pain	OvPCO	25 (5/28)	20 (2–7)	20 (2–7)
23	36	Pelvic pain	OvPCO	27 (5/28)	15 (3–8)	15 (3–8)
24	63	Menorrhagia, fibroid	AnovPCO	Continual	11 (2–4)	11 (2–4)
25	37	Dysfunctional bleeding, severe hirsutism	AnovPCO	10 (irreg)	33 (2–7)	33 (2–7)
26	39	Severe hirsutism	AnovPCO	14 (irreg)	21 (3–15)	21 (3–15)
27	34	Pelvic pain	AnovPCO		40 (2–9)	40 (2–9)
28	30	Menorrhagia, PMS	AnovPCO	29 (irreg)	40 (3–7.5)	40 (3–7.5)
29	37	Pelvic pain	AnovPCO	47 (irreg)	18 (2–10)	9 (4–10)

nk, Not known; PMS, premenstrual syndrome; irreg, irregular cycle.
[a] All patients with normal ovaries had regular cycles.

Protocol

Experimentation on nonluteinized cells was performed on the day of dissection. To assess AMH production with increasing follicle size in normal ovaries, granulosa cells were counted using Trypan blue exclusion and plated out at a density of 5×10^4 in 96-well plates in 200 μl of supplemented M199 for 48 h. The cells from each follicle were cultured separately except those obtained from patients 4 and 6 in which cells from two follicles of similar size were pooled (4 + 6 mm and 13 + 15 mm). After removal of serum-supplemented medium, GLCs were also incubated in serum-free medium for 48 h.

For studies of comparative AMH production among normal, ovulatory (ov) PCOs, or anovulatory (anov) PCOs, cells were pooled from follicles sized 2–10 mm from a single patient and incubated as above. Cells from eight normal, nine ovPCOs, and six anovPCOs were cultured. To investigate the effect of FSH, cells from normal ovaries or PCOs were pooled from 2- to 10-mm follicles and incubated with or without purified human FSH (5 ng/ml; Endocrine Services, Bideford on Avon, UK) with testosterone (5×10^{-7} M) added as an aromatase substrate for 48 h. Estradiol was measured in the same medium as a marker of response to FSH. Each experiment was performed in a minimum of triplicate wells, and four experiments were performed in normal ovaries and eight in PCOs. To investigate the effects of LH, cells from individual or pooled follicles greater than 10 mm (*i.e.* after acquisition of LH receptors) from five separate patients with normal ovaries and four with ovPCOs were incubated with 5 ng/ml LH for 48 h. Theca cells and pieces of stroma were incubated in serum-free media without treatment for 48 h. All reagents used were purchased from Sigma (Poole, Dorset, UK) unless otherwise stated. AMH was measured in the cell-conditioned media using a serum-based ELISA (Diagnostic Systems Laboratories, Oxon, UK) (23). Initially we validated this ELISA for measurement of AMH in cell-conditioned medium, samples diluted in parallel with serum-based standards; there was good recovery and there was no effect of repeat freeze/thaw cycles.

Analyses

Mean AMH levels in follicles from normal ovaries sized 2–10 mm were compared with levels in follicles greater than 10 mm using unpaired Student's *t* test. Comparisons among normal ovaries, ovPCOs, and anovPCOs were performed using Kruskal-Wallis with Tukey-Kramer *post hoc* test. Only follicles sized 2–10 mm from normal ovaries were included in this latter comparison to avoid bias caused by the increased number of small follicles from PCOs. Due to the wide variation in absolute AMH levels between experiments, the results from the LH- and FSH-treated granulosa cells were calculated as a percentage of the control with the control set at 100%. Results were compared with Mann-Whitney test; $P \leq 0.05$ was considered statistically significant.

Results

AMH production and levels in granulosa cells, theca, stroma, follicular fluid, and GLCs from normal ovaries

AMH was measured in follicular fluid from 14 follicles between 3 and 12 mm in diameter. The concentration ranged from 42 to 2240 ng/ml (Fig. 1) with levels being highest in fluid from small follicles and being present in very low amounts in those follicles above 9 mm. Granulosa cells were isolated from 17 follicles ranging in size from 4 to 19 mm from 13 women with normal ovaries (Fig. 1). The concentration of AMH produced by the cultured cells ranged from 0.025 to 1.7 ng/ml, with the highest level again being made by cells from the smallest follicles. Cells from follicles of greater than 10 mm produced AMH at or below the detection limit of the assay (0.025 ng/ml) as did GLCs, theca, and stroma (data not shown).

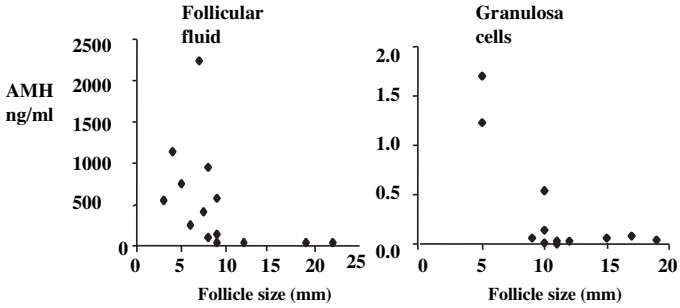

FIG. 1. *Left*, AMH concentration in follicular fluid from a range of individual follicles isolated from women with regular ovulatory cycles and normal ovaries. Note the loss of AMH in fluid from follicles greater than 10 mm. *Right*, AMH concentration (nanograms per milliliter per 50,000 cells) in granulosa cell-conditioned medium from a range of follicle sizes from normal ovaries. AMH fell exponentially as the follicle size increased and again granulosa cells from follicles greater than 10 mm produced low or undetectable levels of AMH.

Comparison of AMH production in granulosa cells from normal ovaries, ovPCOs, and anovPCOs

The mean (range) number of follicles isolated from portions of ovPCOs and anovPCOs was 13 (6–20) and 29 (18–40), respectively, and cells from all follicles from 2 to 10 mm were pooled. Follicle size distribution among the three groups did not differ significantly. Levels of AMH produced by granulosa cells from 2- to 10-mm follicles from normal ovaries ranged from undetectable to 1.7 ng/ml, with a mean of 0.37 ng/ml. There was a greater range of AMH production by cultured cells from ovPCOs, with levels ranging up to 7 ng/ml (mean 1.56), with the highest concentration again being in medium from cells from 3- to 5-mm follicles. Medium conditioned by cells from anovPCOs contained remarkably high levels of AMH with a range of 17.22 to 42.96 ng/ml and a mean of 27.4. This mean is 16 times higher than the highest level from normal ovaries and 75 times higher than the mean normal level. AMH production by granulosa cells from normal ovaries, ovPCOs, and anovPCOs were significantly different from each other ($P = 0.0005$, Kruskal-Wallis) (Fig. 2).

Effect of FSH and LH on AMH production

FSH had no effect on AMH production by granulosa cells from normal ovaries (Fig. 3). In contrast, FSH treatment of

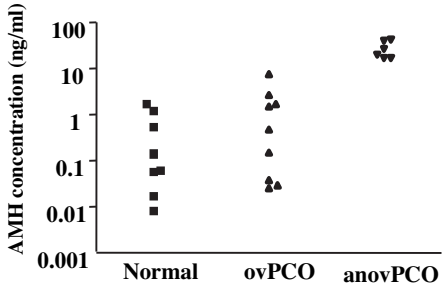

FIG. 2. AMH concentration in granulosa cell-conditioned medium from normal ovaries (n = 8), ovulatory (n = 9), and anovulatory (n = 6) PCOs. AMH production was significantly different between normal ovaries, ovPCOs, and anovPCOs ($P < 0.001$). The mean concentration in cells from anovPCOs was 75 times higher than the mean for normal ovaries.

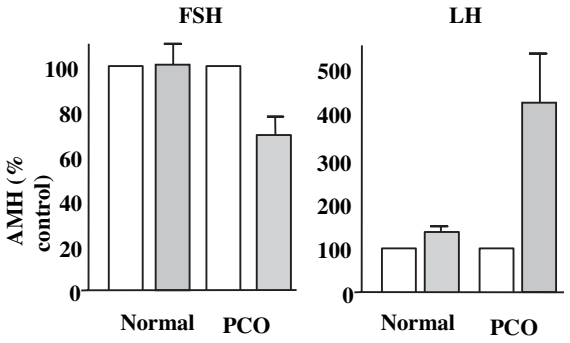

FIG. 3. AMH production in response to gonadotropin: *white bars*, control; *gray bars*, FSH or LH (5 ng/ml). There was no significant difference in production of AMH after FSH treatment in granulosa cells from normal ovaries (n = 4); however, FSH significantly reduced AMH production by granulosa cells from PCOs (n = 8), compared with controls ($P = 0.008$). There was a slight but not significant increase in AMH in response to LH ($P = 0.06$) in cells from follicles greater than 10 mm from normal ovaries (n = 5), but a mean 4-fold increase in cells from PCOs (n = 4, $P = 0.06$).

granulosa cells from PCOs significantly reduced AMH with the mean percentage production falling by 30% ($P = 0.008$, Mann-Whitney). Estradiol production in response to FSH was significantly increased in the same experiments in each case. Incubation of cells from dominant follicles with LH increased AMH by a modest 28% but caused levels to rise by 4-fold in cells from polycystic ovaries (Fig. 3).

Discussion

This is the first report of AMH concentrations in a large size range of follicles from normal unstimulated human ovaries; it demonstrates an exponential fall as the follicle size increased. This was seen in both follicular fluid and in granulosa cell-conditioned medium and was particularly apparent once the follicle reached 10 mm in diameter, *i.e.* the size at which follicle selection occurs. Production by cells collected after human chorionic gonadotropin (hCG) was largely undetectable. This suggests that the decrease in AMH is important for the dominant follicle to be selected and reinforces the suggestion that AMH inhibits follicle growth. Serum AMH levels in women with normal ovaries range between 1.2 and 2.4 ng/ml (25–27). AMH levels in follicular fluid in this study were between 100 and 1000 times higher, emphasizing local granulosa cell production and follicular fluid accumulation.

We have also demonstrated that the levels of AMH in media conditioned by granulosa cells from anovPCOs are on average 75 times higher than in media of cells from normal ovaries. Because cells from sized-matched follicles in all three groups were plated at the same density, the AMH production in anovPCOs is significantly higher per granulosa cell. Serum levels of AMH have been shown to be 2- to 3-fold higher in women with PCOS, compared with ovulatory women with normal ovaries (14, 15), and there are more follicles in anovPCOs, compared with normal ovaries (28). The raised serum AMH in PCOS is therefore a reflection of both an increase in production per cell and the increase in follicle number. Although the levels produced by cells from both ovPCOs and anovPCOs were higher than normal, the latter were considerably higher than the former. We have shown that AMH has virtually disappeared from follicles proceeding beyond selection in normal ovaries, signifying that the high levels in anovPCOs may indeed be sufficient to inhibit folliculogenesis. One possible criticism of our data is that the mean age of the patients in our PCOS group was lower than that of our controls, and it has clearly been shown that serum AMH declines with age. The latter, however, gives an integrated measure that reflects the total number of antral follicles in both ovaries. In contrast, this study was performed to compare production per granulosa cell, *i.e.* to determine production independently of ovarian reserve. We did not find any relationship between age and AMH production per cell; however, this relationship would be masked by differences in follicle size.

These results appear to be at variance with those studies comparing the relationship between the number of small follicles and serum AMH in normal ovaries and PCOs in which it was shown that the slope of the regression line did not differ between the two morphologies (16). The conclusion reached by these authors was that each follicle from a PCO was secreting the same amount of AMH as one from a normal ovary. Closer analysis of the data obtained by ultrasound does, however, suggest some possible reasons for the difference. The relationship between follicle number and AMH only held for 2- to 5-mm follicles and was lost in those of 6–9 mm, and although size matched between groups, our pools do contain significant numbers of follicles of 6–9 mm. Second, the patients in the ultrasonographic studies were a mixed group with patients with amenorrhea, oligomenorrhea, and regular cycles. It is stated that the patients with anovulation did have higher levels, but the correlation with AMH was not analyzed separately. One other possibility is that size-matched follicles from polycystic ovaries contain significantly fewer granulosa cells than those from normal ovaries, and so the increase per cell is partly negated. This has not been our experience; however, we have not made a systematic analysis of granulosa cell number in these follicles. Given our finding that AMH was so greatly increased per cell in PCOs, these differences are still difficult to reconcile and remain largely unexplained.

The cause of raised AMH production in PCOS is unknown; however, clinical studies point to androgens as a likely candidate. There was a correlation between serum androgen and AMH in patients with PCOS (16, 29, 30), and in particular, levels in women undergoing *in vitro* fertilization were higher when the patient had hyperandrogenism and PCOs than in women with PCOs and normal androgens (31). Interestingly, androgens tend to be higher in anovulatory women with PCOS than in their ovulatory counterparts (32), and androgen secretion is higher per theca cell in anovulatory women than ovulatory (33). Raised androgens could therefore not only explain the increased AMH production in PCOs but could also explain why we found higher levels in anovPCOs than ovPCOs.

We were rather surprised by the findings of our experiment with FSH. Although FSH has been shown to inhibit AMH production in rat fetal Sertoli cells (34), it had no effect in the equivalent human cells (35). To our knowledge there has been only one study investigating the effects of FSH on granulosa cell AMH production. In this study, FSH had no

effect on AMH mRNA expression in luteinized cells, and the protein was not measured (35). We also found no effect on AMH production in cells from normal ovaries, which seems surprising, given the apparent ability of AMH to inhibit FSH responsiveness. In contrast, AMH was reduced by FSH in cells from PCOs. The difference in response is hard to explain, although cells from PCOS have been shown to be hypersensitive to FSH in other regards, *i.e.* in terms of estradiol production (20) and in response to induction of ovulation and controlled ovarian hyperstimulation (36). It is tempting to speculate that the FSH given as a treatment for induction of ovulation might inhibit local production of AMH as one of its mechanisms of action. Cells from normal ovaries also produced very little response to LH, whereas this gonadotropin stimulated AMH production 4-fold in cells from PCOs. The differential response is again hard to explain, although it has previously been suggested that granulosa cells from PCOS acquire LH responsiveness at a smaller size than those from normal ovaries (37). The cells in this study, however, were all from follicles that were already greater than 10 mm, *i.e.* they all should have had LH receptors. Many women with PCOS have raised serum levels of LH, and these data indicate that this may further increase ovarian AMH production.

To conclude, this report is the first to show that levels of AMH are on average 75 times higher in granulosa cells from anovPCOs, compared with levels in normal ovaries, and that this increase is per cell and not simply due to an increase in the number of follicles. It is important to bear in mind that these raised levels could be misleading if used as a marker of ovarian reserve. The overproduction of AMH could have important implications for the mechanism of anovulation in these women. The finding of AMH type II receptor (AMHRII) on granulosa and theca cells (38) makes the determination of the actions of AMH in the human ovary an important next step.

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References

1. **Josso N, Lamarre L, Picard JY, Berta P, Davies N, Morichon N, Peschanski M, Jeny R** 1993 Anti-Mullerian hormone in early human development. Early Hum Dev 33:91–99
2. **Rajperts-de Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE** 1999 Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 84:3836–3844
3. **Baarends WM, Uilenbroek JTJ, Kramer P, Hoogerbrugge JW, van Leeuwen ECM, Themmen APN, Grootegoed JA** 2005 Anti-Müllerian hormone and anti-Müllerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle and gonadotropin-induced follicle growth. Endocrinology 136:4951–4962
4. **Weenen C, Laven JSE, von Bergh ARM, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BCJM, Themmen APN** 2004 Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 10:77–83
5. **te Velde ER, Pearson PL** 2002 The variability of female reproductive aging. Hum Reprod Update 8:141–154
6. **de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC** 2002 Anti-Mullerian hormone levels: a putative marker for ovarian ageing. Fertil Stertil 77:357–362
7. **Van Rooij IAJ, Broekmans FJM, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP** Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 17:3065–3071
8. **Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P** 2005 Antral follicle count, anti-Mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? Br J Obstet Gynaecol 112:1384–1390
9. **Durlinger ALL, Kramer P, Karels B, de Jong FH, Uilenbroek JTJ, Grootegoed JA, Themmen JPN** 1999 Control of primordial follicle recruitment by anti-Mullerian hormone in mouse ovary. Endocrinology 12:5789–5796
10. **Durlinger ALL, Gruijter MJG, Kramer P, Kavels B, Kumar TR, Matzuk MM, Rose UM, De Jong FH, Uilenbroek JTJ, Grootegoed VA, Themmen APN** 2001 Anti-Mullerian hormone attenuates the effect of follicle-stimulating hormone on follicle development in the mouse ovary. Endocrinology 142:4890–4899
11. **Durlinger ALL, Gruijters MJG, Kramer P, Karels B, Ingraham HA, Machtigal MW, Uilenbroek JTJ, Grootegoed JA, Themmen APN** 2002 Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 143:1076–1084
12. **Kim JH, Seibel MM, MacLaughlin DT, Donahoe PK, Ransil BJ, Hametz PA, Richards CJ** 1992 The inhibitory effects of mullerian-inhibiting substance on epidermal growth factor induced proliferation and progesterone production of human granulosa-luteal cells. J Clin Endocrinol Metab 75:911–917
13. **Di Clemente N, Goxe B, Remy J-J, Cate R, Josso N, Vigier B, Salesse R** 1994 Inhibitory effect of AMH upon the expression of aromatase and LH receptors by cultured granulosa cells of rat and porcine immature ovaries. Endocrine 2:553–558
14. **Cook CL, Siow Y, Brenner AG, Fallatt ME** 2002 Relationship between serum Mullerian inhibiting substance and other reproductive hormones in untreated women in PCOS and normal women. Fertil Steril 77:141–146
15. **Laven JSE, Mulders AGMGJ, Visser JA, Themmen AP, De Jong FH, Frauser BCJM** 2004 Anti-Mullerian hormone serum concentrations in normovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab 89: 318–323
16. **Pigny P, Jonard S, Robert Y, Dewailley D** 2006 Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 91:941–945
17. **Polson DW, Wadsworth J, Adams J, Franks S** 1988 Polycystic ovaries a common finding in normal women. Lancet 1:870–872
18. **Franks S, Mason H, Willis D** 2000 Follicular dynamics in the polycystic ovary syndrome. Mol Cell Endocrinol 163:49–52
19. **ESHRE/ASRM** 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome Hum Reprod 19:41–47
20. **Mason HD Willis DS, Beard RW, Winston RML, Margara R, Franks S** 1994 Estradiol production by granulosa cells of normal and polycystic ovaries: relationship to menstrual cycle history and concentrations of gonadotrophins and sex steroids in follicular fluid. J Clin Endocrinol Metab 79:1355–1360
21. **Mason HD, Margara R, Winston RML, Beard RW, Reed MJ, Franks S** 1990 Inhibition of oestradiol production by epidermal growth factor in human granulosa cells of normal and polycystic ovaries. Clin Endocrinol (Oxf) 33: 511–517
22. **Wright R, Willis DS, Watson H, Galea R, Brincat M, Holly JMP, Mason HD** 2002 The inhibitory effects of IGFBP-4 on ovarian steroidogenesis are independent of its ability to sequestrate endogenous IGF. Biol Reprod 6:776–781
23. **McNatty KP** 1981 Hormonal correlates of follicular development in the human ovary. Aust J Biol Sci 34:249–268
24. **Al Qahtani A, Muttukrishna S, Appasamy M, Johns J, Cranfield M, Visser JA, Themmen AP, Groome NP** 2005 Development of a sensitive enzyme immunoassay for anti-Mullerian hormone and the evaluation of potential clinical applications in male and females. Clin Endocrinol (Oxf) 63:267–273
25. **Fleming R, Harborne L, MacLaughlin DT, Ling D, Norman J, Sattar N, Seifer DB** 2005 Metformin reduces serum Mullerian-inhibiting substance levels in women with polycystic ovary syndrome after protracted treatment. Fertil Steril 83:130–136
26. **Piltonen T, Morin-Papunen L, Koivunen R, Perhenntupa A, Ruokonen A, Tapanainen JS** 2005 Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with PCOS. Hum Reprod 20:1820–1826
27. **Lazzarini V, Quirici B, Parrini D, Fruzzetti F** 2006 Anti-Mullerian hormone serum concentrations in PCOS women and its changes during metformin treatment. Gynecol Endocrinol 22(Suppl 1):251–252 (Abstract only)
28. **Adams J, Polson DW, Franks S** 1986 Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J (Clin Res Ed) 293:355–359
29. **Fallat ME, Siow Y, Marra M, Cook C, Carrillo A** 1997 Mullerian-inhibiting

substance in follicular fluid and serum: a comparison of patients with tubal factor infertility, polycystic ovary syndrome, and endometriosis. Fertil Steril 67:962–965
30. **La Marca A, Orvieto R, Giulini S, Jasonni VM, Volpe A, De Leo V** 2004 Mullerian-inhibiting substance in women with polycystic ovary syndrome: relationship with hormonal and metabolic characteristics. Fertil Steril 82:970–972
31. **Eldar-Geva T, Margalioth EJ, Gai M, Ben-Chetrit A, Algur N, Zylber-Haran E, Brooks B, Huerta M, Spitz IM** 2005 Serum anti-Mullerian hormone levels during controlled ovarian hyperstimulation in women in polycystic ovaries with and without hyperandrogenism. Hum Reprod 20:1814–1819
32. **Franks S** 1995 Polycystic ovary syndrome. N Engl J Med 28:333):853–861
33. **Franks S, Willis D, Mason HD, Gilling-Smith C** 1995 Comparative androgen production from theca cells of normal women and women with polycystic ovaries. In: Chang RJ, ed. Polycystic ovary syndrome. Serono Symposia Reviews. Proc of Serono International Symposium on polycystic ovary syndrome. New York: Springer-Verlag
34. **Bercu BB, Morikawa Y, Jackson I, Donahoe PK** 1979 Inhibition of Mullerian inhibiting substance secretion by FSH. Pediatr Res 13:246–249
35. **Voultilainen R, Miller W** 1987 Human Mullerian inhibitory factor messenger ribonucleic acid is hormonally regulated in the fetal testis and in adult granulosa cells. Mol Endocrinol 1:604–608
36. **Smitz J, Camus M, Devroey P** 1990 Incidence of severe ovarian hyperstimulation syndrome after gonadotrophin releasing hormone agonist/hMG superovulation for *in vitro* fertilisation. Hum Reprod 5:933–937
37. **Willis DS, Mason HD, Watson H, Brincat M, Galea R, Franks S** 1998 Premature response to LH of granulosa cells from anovulatory women with PCOS: relevance to mechanism of anovulation. J Clin Endocrinol Metab 83: 3984–3991
38. **Hanna L, Pellatt L, Rice S, Whitehead S, Mason HD,** Anti-Müllerian hormone (AMH) production by and AMH type II receptor in normal human ovaries. Proc 8th European Congress of Endocrinology, Glasgow, Scotland, 2006 (Endocrine Abstract 11:P687)

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