

Concentrations of AMH and inhibin-B in relation to follicular diameter in normal human small antral follicles

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BACKGROUND: The aim of the present study was to determine the intrafollicular concentrations of anti-Müllerian hormone (AMH), inhibin-B and steroids in normal human small antral follicles and to relate them to follicular size.

METHODS: A group of 103 women having one ovary removed for fertility preservation by cryopreservation prior to gonadotoxic treatment served as a source of a total of 272 human small antral follicles. Prior to cryopreservation of the ovarian cortex, fluid from small antral follicles were collected. On the basis of the follicular volume, the diameter was calculated and follicles with diameters from 3 to 12 mm were included.

RESULTS: Concentrations of AMH decreased significantly ($P < 0.0005$) from 1124 ± 158 ng/ml (mean \pm SEM) in follicles with a diameter of 3 mm to a concentration of 392 ± 98 ng/ml in 9 mm follicles, followed by a reduction to below 100 ng/ml in 12 mm follicles. The concentrations of inhibin-B rose from 57 ± 10 ng/ml (mean \pm SEM) in 3 mm follicles to 142 ± 10 ng/ml in 12 mm follicles ($P < 0.0005$) with a peak concentration of almost 200 ng/ml in 9–10 mm follicles. Relating hormone concentrations with age showed that even follicles from girls younger than 10 years showed the same range of AMH concentrations as those from older girls or women.

CONCLUSIONS: The intrafollicular concentrations of AMH become progressively lower with increasing follicle diameters. In contrast, concentrations of inhibin-B increased with increasing follicle diameter with peak values at around 9 mm in diameter. This suggests that AMH and inhibin-B undertake important intrafollicular functions around the time of normal follicular selection in the mid-follicular phase of the menstrual cycle.

Key words: AMH / inhibin-B / human small antral follicles / follicular fluid

Introduction

In the female, synthesis of anti-Müllerian hormone (AMH) is exclusively performed by the somatic cells of the ovarian follicle (Donahoe *et al.*, 2003; Visser and Themmen, 2005; Visser *et al.*, 2006; Seifer and Maclaughlin, 2007). Granulosa cell expression of AMH takes place in a specific window of folliculogenesis that starts shortly after the primordial follicle has embarked on growth (Visser and Themmen, 2005; Seifer and Maclaughlin, 2007). Expression continues during all subsequent stages of follicular development except for pre-ovulatory follicles, where AMH synthesis becomes low or even absent (Visser and Themmen, 2005; Seifer and Maclaughlin, 2007). The AMH produced within the follicle appears to be partly

accumulated in the follicular fluid (FF) and partly released into the bloodstream. Thus, the circulatory concentration of AMH reflects the collective amount of immature granulosa cells present in the two ovaries and, as such, the collective number of growing follicles. Owing to these characteristics, the concentration of AMH measured in circulation has qualified as an excellent marker of the ovarian reserve (Coccia and Rizzello, 2008; Robertson, 2008), as a measure of the biological age of the ovaries and as a diagnostic marker to determine various endocrinological disorders of the ovaries such as polycystic ovaries (Cook *et al.*, 2002; Pigny *et al.*, 2006; van Disseldorp *et al.*, 2008; Lambalk *et al.*, 2009). Despite the increasing use of AMH in a clinical setting, the precise function of AMH has not yet been determined. Based mainly on studies in AMH knockout mice, the current

dogma suggests that FSH action is inhibited by AMH and that AMH thereby limits recruitment from the primordial follicle pool (Durlinger *et al.*, 2002).

However, it also appears that AMH affects ovarian steroidogenesis (Kim *et al.*, 1992; Seifer *et al.*, 1993; Knight and Glistler, 2006). Recent studies in humans have found concentrations of AMH in fluid from small human antral follicles to be very high compared with that in circulation, reaching levels of several hundred ng/ml when compared with just a few ng/ml in circulation (Yding Andersen and Byskov, 2006; Yding Andersen *et al.*, 2008). The high intrafollicular concentrations of AMH were found to inversely correlate with the follicular concentrations of estradiol (Yding Andersen and Byskov, 2006; Yding Andersen *et al.*, 2008). On the basis of these and earlier studies, it was suggested that one additional function of AMH was to reduce aromatase activity in the granulosa cells of immature follicles (Vigier *et al.*, 1989; Grossman *et al.*, 2008; Yding Andersen *et al.*, 2008; Lutterodt *et al.*, 2009). In addition, concentrations of AMH in the fluid from such small follicles exceed the concentration of inhibin-B approximately by an order of magnitude, suggesting that, alongside inhibin-B, AMH plays an important function in follicular development (Yding Andersen and Byskov, 2006).

In order to determine a physiological function of AMH in women, quantitative data on AMH concentrations in FF in relation to the follicular diameter from normal small antral follicles are of interest but currently unavailable. An immunohistochemical study of human antral follicles semi-quantified AMH expression and found the highest expression in granulosa cells of secondary, pre-antral and small antral follicles <4 mm in diameter with gradual disappearance thereafter (Veenen *et al.*, 2004).

Previous studies have shown that the average FF concentration of inhibin-B in fluid from small human antral follicles is twice as high as that observed in the fluid from pre-ovulatory follicles (Yding Andersen and Byskov, 2006; Yding Andersen *et al.*, 2008). How the intrafollicular concentration of inhibin-B develops in relation to the follicular diameter is, however, not yet known.

The present study was based on the collection of fluid from a large number of normal small antral human follicles obtained in connection with a program of fertility preservation by cryopreservation of ovarian tissue. The aim was to determine the intrafollicular concentration of AMH and inhibin-B in relation to the follicular diameter and to age of the girl/woman. Further, these concentrations were related to those of estradiol, progesterone, androstenedione and testosterone in the same follicles.

Materials and Methods

Patients and collection of FF from small antral follicles

FF samples of individual small antral follicles were obtained by aspiration from ovaries surgically removed for fertility preservation. Isolation and cryopreservation of the ovarian cortex was offered to women with a disease requiring treatment with a high risk of inducing ovarian failure.

A total of 272 follicles were obtained from 103 girls/women aged 1–38 years (median 27 years). For women old enough to have menstrual cycles, collection was performed at various times during their menstrual cycle. Diagnoses for ovarian cryopreservation included breast cancer (38),

Hodgkin's and non-Hodgkin's disease (19), Ewing's and other sarcomas (7), lymphoma (4), leukemia (9) and various other cancer forms (26), not related to any endocrine disorder (e.g. polycystic ovarian syndrome) or ovarian disease. All ovaries appeared normal by (i) visual inspection in connection with the cryopreservation procedure and (ii) subsequent microscopically evaluation of histological sections from a small piece of the ovarian cortex.

After surgical recovery of the ovary, it was transported to the laboratory where the follicles were collected in connection with the procedure to isolate the ovarian cortex. Each antral follicle was aspirated directly from the ovary prior to preparation of the tissue for cryopreservation using a 1 ml syringe with a 26 G needle (Becton Dickinson, Brøndby, Denmark). FF was isolated from granulosa cells by centrifugation (2000 g, 2 min) and subsequently snap-frozen in liquid nitrogen and stored at -80°C until measurement of hormones.

FF samples were collected from 1 to 10 antral follicles per patient (1 from 37 patients; 2 from 20 patients; 3 from 23 patients; 4 from 10 patients; 5 from 4 patients; 6 from 2 patients; 7 from 4 patients; 8 from 2 patients; 10 from 1 patient). The volume of each follicle was estimated in steps of 10 μl as indicated on the syringe. The diameter of the follicle was calculated based on this volume assuming a spherical follicle. The ethical committee of the municipalities of Copenhagen and Frederiksberg approved the project.

Part of the material used in this study has been used in previous studies (Yding Andersen and Byskov, 2006; Yding Andersen *et al.*, 2008).

Hormone measurements

Estradiol and progesterone were measured using commercially available RIA kits (DSL-43 100 and DSL-3400; Diagnostic System Laboratories, Webster, TX, USA). Samples for both assays were diluted 1:50 in steroid-free serum just prior to measurement. Androstenedione was measured using RIA kit (DSL-3800, Diagnostic System Laboratories) with samples being diluted 1:200 in steroid-free serum and testosterone was measured using an RIA kit (DSL-4000, Diagnostic System Laboratories) after dilution of 1:100 in steroid-free serum.

AMH was measured using a specific ELISA kit according to the manufacturer's instructions (DSL-10-14400; Diagnostic System Laboratories). FF samples from small antral follicles were diluted either 1:500 or 1:3000 in the zero standard provided by the manufacturer. Inter-assay variation of a sample containing 7.6 ng AMH/ml was 4.4% ($n = 12$) and intra-assay variation was 3.3% ($n = 5$) for a sample containing 0.45 ng/ml. Dilution curves of FF samples proved to be parallel to the standard curve.

Inhibin-B was measured using a specific ELISA kit according to the manufacturer's instructions (The Oxford Bio-innovation kit; Biotech-IgG, Copenhagen, Denmark). Prior to measurement, all FF samples, irrespective of whether they derived from small antral or pre-ovulatory follicles, were diluted 1:100 or 1:500 in serum obtained from a pool of five post-menopausal women (who showed no inhibin-B activity). The FF samples were pre-treated with SDS, heated and exposed to hydrogen peroxide before they were applied to the wells of the plate and incubated overnight at room temperature. Subsequently, the plates were washed and incubated with detection antibody for 3 h at room temperature. Substrate solution was applied and incubated for 1 h. The amplifier solution was added, and the plates were read with an ELISA reader at 490 nm with its reference at 620 nm (CV <7%).

In some follicles, the isolated volume of FF was not sufficient to measure all six hormones as mentioned above, in which case priority was given to measurements of AMH and inhibin-B. Thus, the total number of follicles in which estradiol was not measured was 54 follicles, while for progesterone it was 30 follicles, for testosterone 26 follicles and for androstenedione 25 follicles.

Statistics

For comparison of levels of the measured substances, ANOVA was applied when in subgroups $n > 2$. *Post hoc* comparison and comparison of two independent groups were done using Student's *t*-test.

The intrafollicular concentration of AMH and inhibin-B varied from one follicle to the next independent of whether it originated in the same woman or not, as previously shown (Yding Andersen et al., 2008). On the basis of this observation, each follicle was considered as an independent observation. A *P*-value of <0.05 was accepted as statistically significant.

Results

The intrafollicular concentrations of AMH and inhibin-B in relation to the follicular diameter are given in Figs 1 and 2. Follicles allocated to the 5 mm group, for instance, were follicles in which the calculated diameter based on the aspirated volume varied from 4.5 to 5.5 mm. The concentration of AMH starts at an average concentration of 1124 ng/ml in follicles with a diameter of 3 mm and then gradually declined until a diameter of 9 mm. As the follicular diameter increased

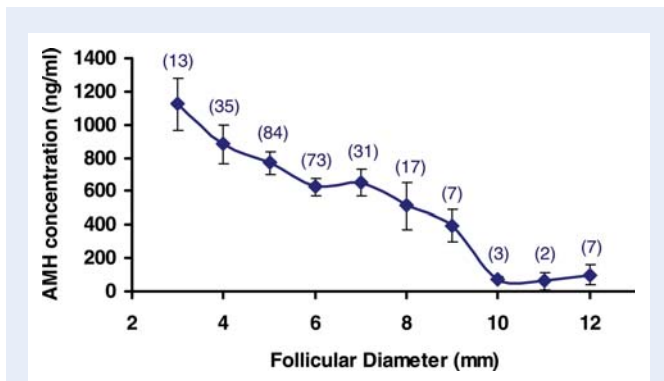


Figure 1 FF concentration of AMH in human antral follicles with an estimated diameter from 3 to 12 mm.

Numbers in parentheses indicate the number of individual observations (ANOVA: $P < 0.0005$).

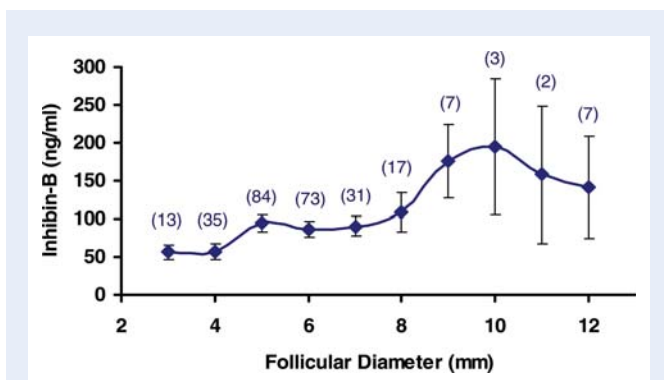


Figure 2 FF concentration of inhibin-B in human antral follicles with an estimated diameter from 3 to 12 mm.

Numbers in parentheses indicate the number of individual observations (ANOVA: $P < 0.0005$).

from 9 to 10 mm, there was a sharp reduction in the AMH concentration from >300 to <100 ng/ml. From a follicular diameter of 10 mm or more, the intrafollicular concentration of AMH remained low. The decline of AMH in relation to the diameter was highly statistically significant (ANOVA: $P < 0.0005$).

In contrast, the intrafollicular concentrations of inhibin-B gradually increased from around 60 ng/ml to a little <200 ng/ml at a follicular diameter of 9–10 mm (Fig. 2). Further follicular growth appeared to be accompanied by a reduction in the intrafollicular levels of inhibin-B as seen for follicular diameters of 11–12 mm. The increased inhibin-B levels in relation to diameter were highly statistically significant (ANOVA: $P < 0.0005$).

The intrafollicular concentrations of estradiol, progesterone, androstenedione and testosterone in relation to follicular diameter are given in Fig. 3. Owing to greater variability and relatively few observations in the flanking groups (i.e. follicular diameters of 3–4 and 11–12 mm), the data have been presented in 2 mm intervals (e.g. 2.5–4.5 mm). Estradiol exhibited low average levels in small follicles and only showed an increasing concentration with follicular diameters exceeding around 6–8 mm (Fig. 3A). Concentrations of progesterone showed a moderate increase with follicular diameter, nonetheless average concentrations increased from the smallest to the largest follicles by a factor of around 3 (Fig. 3B).

Concentrations of androstenedione and testosterone remained relatively constant of the examined span of follicular diameters with an approximate ratio of 1–10 between testosterone and androstenedione (Fig. 3C and D).

The measured hormone levels in relation to the age of the girl/woman at the time of tissue collection are shown in Table 1. Intrafollicular levels of AMH remained relatively constant even in young girls, whereas inhibin-B and the steroids tended to be lower in FF from girls younger than 10 years of age, although the differences were not significant (ANOVA: $P > 0.10$).

Discussion

This study demonstrates for the first time the intrafollicular concentrations of AMH in normal human follicles with diameters from around 3 to 12 mm. AMH concentrations exhibited a highly significant inverse relationship with follicular size. Further, at a follicular diameter of around 9 mm, a sharp decline in AMH levels was observed, and after this, FF concentrations remained low during the final stages of follicular development. Thereby the present study, with quantitative data, expands and confirms a previous study in which AMH expression in human follicles was detected by immunohistochemistry (Weenen et al., 2004) and strengthens the concept of AMH as an important intrafollicular mediator of follicular development prior to the stage of follicular selection.

In contrast to the steady decline of AMH, estradiol levels remained almost constant in follicles from 3 to 7 mm in diameter and only in larger follicles were the average concentrations of estradiol increased significantly. In connection with results from previous studies that have shown a strong negative relationship between intrafollicular concentrations of AMH and estradiol (Yding Andersen and Byskov, 2006; Yding Andersen et al., 2008), the present results confirm that this relationship is not a function of follicular size and normal follicular

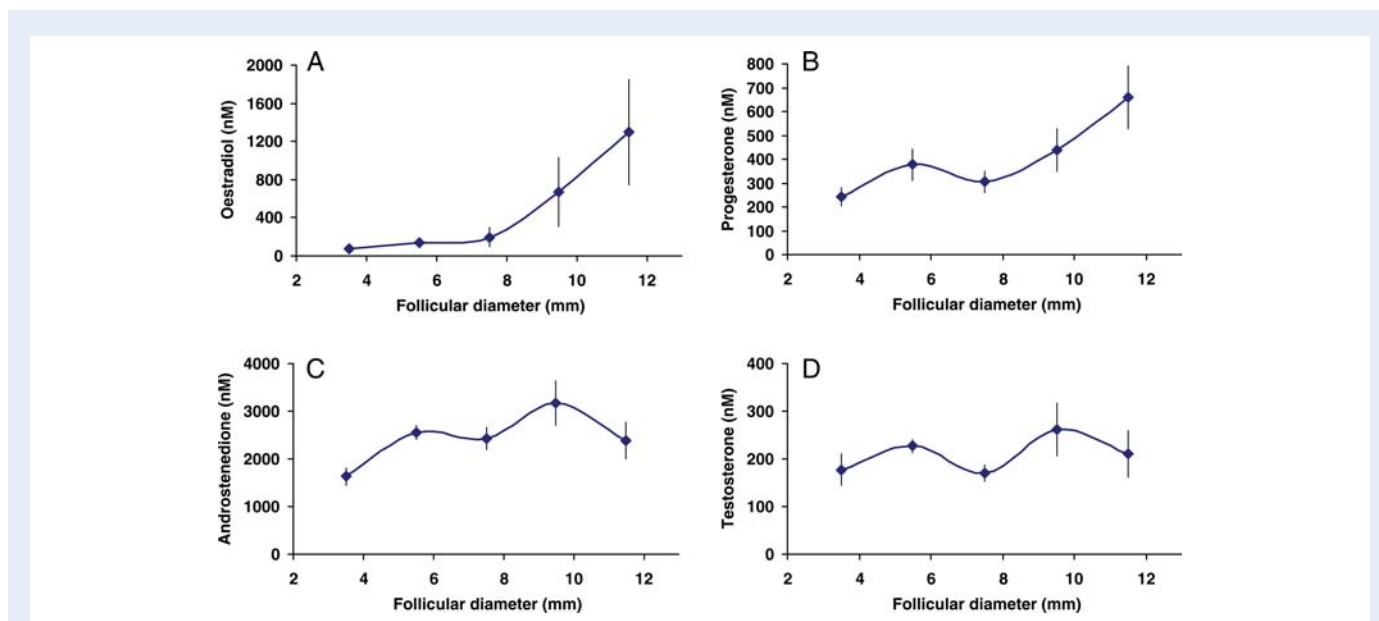


Figure 3 Intrafollicular concentrations of steroids in relation to follicular diameter (mean \pm SEM).

Table 1 Mean FF concentration of AMH, inhibin-B and steroids in relation to age (mean \pm SEM).

	Age (years)						
	<10	$\geq 10-15$	$\geq 15-20$	$\geq 20-25$	$\geq 25-30$	$\geq 30-35$	$\geq 35-40$
No. of patients	8	8	11	19	19	23	7
No. of FF samples	11	27	33	35	63	74	23
AMH (ng/ml)	775 \pm 146	485 \pm 77	762 \pm 94	780 \pm 93	720 \pm 68	796 \pm 74	468 \pm 63
Inhibin-B (ng/ml)	51 \pm 15	106 \pm 23	71 \pm 13	79 \pm 14	104 \pm 13	80 \pm 10	115 \pm 20
Estradiol (nmol/l)	32 \pm 12	216 \pm 93	60 \pm 21	226 \pm 135	267 \pm 78	259 \pm 129	449 \pm 220
Progesterone (nmol/l)	83 \pm 19	196 \pm 28	168 \pm 23	297 \pm 40	602 \pm 280	470 \pm 101	594 \pm 89

ANOVA: AMH, $P = 0.07$; inhibin-B, estradiol and progesterone, $P > 0.10$.

development, but may represent a true negative effect of AMH on follicular estradiol production.

In contrast to AMH, intrafollicular levels of inhibin-B showed a highly significant positive relationship with follicular size until a diameter of around 9–10 mm where concentrations peak and thereafter levels appeared to decline slowly. This is to our knowledge the first time an intrafollicular peak of inhibin-B has been localized in relation to follicular growth and development. In circulation, the concentrations of inhibin-B have been shown to peak in the mid-follicular phase around 7 days prior to the mid-cycle surge of gonadotrophins (Groome *et al.*, 1996), which corresponds to a follicular diameter of 8–10 mm. Thus, conditions in circulation show a good correspondence with those observed in fluid of follicles present in the ovary at the mid-follicular phase as observed in the present study. The intrafollicular peak of inhibin-B in follicles just around the time of selection is based on a relatively small number of observations in the present study and collection of more data is warranted. However, the circulatory concentrations of inhibin-B corroborate the present findings and enforce the concept that the calculated diameter based on the

aspirated volume represents the true follicular size. The increased intrafollicular production of inhibin-B in follicles just around the time of selection in combination with the concomitant increased production of estradiol is probably because that the newly selected follicle utilizes both substances in order to achieve a reduced pituitary FSH release, with a resulting demise of subordinate follicles.

In the present study, we have not been able to distinguish between healthy and atretic follicles. On the basis of studies of granulosa cells in the S-phase of the cell cycle, performed around 25 years ago, it was shown that the majority of normal human follicles, with diameters similar to those of the present study, were to some degree atretic (Westergaard *et al.*, 1986). However, it is now well accepted that most follicles with diameter of around a few millimeters and onwards can be rescued and provide oocytes with a good pregnancy potential in connection with administration of exogenous FSH and assisted reproduction. We therefore anticipate that the present data reflect normal follicular development with good accuracy, which is confirmed by similar relationships between intrafollicular steroid concentrations and follicular diameter in both a former study

(Westergaard et al., 1986) and the present study. However, immunohistochemical studies have suggested that AMH expression in atretic follicles are lower than in healthy follicles (Visser et al., 2006) and we plan to perform a study in which the health status of the follicle is related to the FF concentrations of AMH and inhibin-B.

This study also demonstrated that the intrafollicular concentrations of AMH were unrelated to the age of the woman confirming earlier studies (Yding Andersen et al., 2008). It is new and interesting that even in young girls under the age of 10 years, in which follicles have not yet been exposed to circulating concentration of gonadotrophins, the concentration of AMH is similar to that in older age groups. This indicates that synthesis of AMH is independent of gonadotrophins and confirms studies in pregnant women with very high concentrations of gonadotrophins (Lutterodt et al., 2009) where serum levels of AMH were similar to non-pregnant women. These observations further enforce that notion that AMH in particular exerts intrafollicular effects.

The diameter of the individual follicles was calculated based on the aspirated volume. This potentially introduces a bias since the formula assumes the follicle to be spherical, which may not always be the case and the entire volume of the follicle may not always be aspirated. Further, in the follicles with a small diameter, the volume is modest (e.g. 20–50 μ l) and the precise volume can be difficult to record by just using the indications given on the syringe. However, we do not consider this potential inaccuracy to be of any real significance to the results, because of the relative high number of observations for most of the diameters.

The diameter at which the granulosa cells alter their AMH production may not correlate directly to the figures given in this study; it may be that follicles at a diameter of 9 mm have reduced AMH production earlier on in development, and dilution and release from the follicle only at this stage is visualized as a steep decline in intrafollicular levels of AMH. However, since the effect of AMH appears to closely related to its concentration, these considerations do not distract from the importance of AMH in particular around follicular selection.

In conclusion, in normal human follicles, this study found a highly significant inverse relationship between intrafollicular concentrations of AMH and follicular diameter and a significant positive relationship between concentrations of inhibin-B and follicular diameter. In follicles around the time of follicular selection, AMH is greatly reduced and levels of inhibin-B exhibit a peak. This study suggests that both AMH and inhibin-B are engaged in intrafollicular events taking place during follicular selection in the mid-follicular phase of the menstrual cycle.

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References

- Coccia ME, Rizzello F. Ovarian reserve. *Ann N Y Acad Sci* 2008; **1127**:27–30.
- Cook CL, Siow Y, Brenner AG, Fallat ME. Relationship between serum Mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 2002; **77**:141–146.
- Donahoe PK, Clarke T, Teixeira J, Maheswaran S, MacLaughlin DT. Enhanced purification and production of Müllerian inhibiting substance for therapeutic applications. *Mol Cell Endocrinol* 2003; **211**:37–42.
- Durlinger ALL, Gruijters MJG, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology* 2002; **143**:1076–1084.
- Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996; **81**:1401–1405.
- Grossman MP, Nakajima ST, Fallat ME, Siow Y. Müllerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertil Steril* 2008; **89**(suppl. 5): 1364–1370.
- Kim JH, Seibel MM, MacLaughlin DT, Donahoe PK, Ransil BJ, Hametz PA, Richards CJ. 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* 1992; **75**:911–917.
- Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. *Reproduction* 2006; **132**:191–206.
- Lambalk CB, van Disseldorp J, de Koning CH, Broekmans FJ. Testing ovarian reserve to predict age at menopause. *Maturitas* 2009; **63**:280–291.
- Lutterodt M, Byskov AG, Skouby SO, Tabor A, Yding Andersen C. Anti-Müllerian hormone in pregnant women in relation to other hormones, fetal sex and in circulation of second trimester fetuses. *Reprod Biomed Online* 2009; **18**:694–699.
- Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006; **91**:941–945.
- Robertson DM. Anti-Müllerian hormone as a marker of ovarian reserve: an update. *Womens Health* 2008; **4**:137–141.
- Seifer DB, MacLaughlin DT, Penzias AS, Behrman HR, Asmundson L, Donahoe PK, Haning RV Jr, Flynn SD. Gonadotropin-releasing hormone agonist-induced differences in granulosa cell cycle kinetics are associated with alterations in follicular fluid Mullerian-inhibiting substance and androgen content. *J Clin Endocrinol Metab* 1993; **76**:711–714.
- Seifer DB, MacLaughlin DT. Mullerian inhibiting substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril* 2007; **88**:539–546.
- van Disseldorp J, Faddy MJ, Themmen AP, de Jong FH, Peeters PH, van der Schouw YT, Broekmans FJ. Relationship of serum anti-Müllerian hormone concentration to age at menopause. *J Clin Endocrinol Metab* 2008; **93**:2129–2134.
- Vigier B, Forest MG, Eychenne B, Bézard J, Garrigou O, Robel P, Josso N. Anti-Müllerian hormone produces endocrine sex reversal of fetal ovaries. *Proc Natl Acad Sci USA* 1989; **86**:3684–3688.
- Visser JA, Themmen AP. Anti-Müllerian hormone and folliculogenesis. *Mol Cell Endocrinol* 2005; **234**:81–86.
- Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction* 2006; **131**:1–9.
- Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod* 2004; **10**: 77–83.

- Westergaard L, Christensen IJ, McNatty KP. Steroid levels in ovarian follicular fluid related to follicle size and health status during the normal menstrual cycle in women. *Hum Reprod* 1986;**1**: 227–232.
- Yding Andersen C, Byskov AG. Estradiol and regulation of anti-Müllerian hormone, inhibin-A, and inhibin-B secretion: analysis of small antral and preovulatory human follicles' fluid. *J Clin Endocrinol Metab* 2006;**91**:4064–4069.
- Yding Andersen C, Rosendahl M, Byskov AG. Concentration of anti-Müllerian hormone and inhibin-B in relation to steroids and age in follicular fluid from small antral human follicles. *J Clin Endocrinol Metab* 2008;**93**:2344–2349.