Review

Regulation of ovarian function: the role of anti-Müllerian hormone

Alexandra L. L. Durlinger, Jenny A. Visser
and Axel P. N. Themmen

*Department of Internal Medicine, Erasmus MC, PO Box 1738, 3000 DR Rotterdam,
The Netherlands*

Anti-Müllerian hormone (AMH), also known as Müllerian inhibiting substance, is a member of the transforming growth factor β superfamily of growth and differentiation factors. In contrast to other members of the family, which exert a broad range of functions in multiple tissues, the principal function of AMH is to induce regression of the Müllerian ducts during male sex differentiation. However, the patterns of expression of AMH and its type II receptor in the postnatal ovary indicate that AMH may play an important role in ovarian folliculogenesis. This review describes several *in vivo* and *in vitro* studies showing that AMH participates in two critical selection points of follicle development: it inhibits the recruitment of primordial follicles into the pool of growing follicles and also decreases the responsiveness of growing follicles to FSH.

In 1947 it was shown that during development of the male fetus a testicular factor distinct from testosterone caused regression of the Müllerian ducts, which form the anlagen of the female genital tract (Jost, 1947; Munsterberg and Lovell-Badge, 1991). This factor was called anti-Müllerian hormone (AMH) and was subsequently identified as a dimeric glycoprotein related to transforming growth factor β (TGFβ) (Cate *et al.*, 1986). Together with inhibins, activins, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs), AMH is part of the TGFβ superfamily of peptide growth and differentiation factors (Massagué, 1990). Members of this family have a broad range of functions in mesenchymal–epithelial interactions, cell growth, extracellular matrix production and tissue remodelling (Massagué and Chen, 2000). In men, strong AMH expression is found in Sertoli cells from the moment of testicular differentiation during fetal development up to puberty, whereas in women AMH is produced by ovarian granulosa cells from about week 36 of gestation to menopause (Lee *et al.*, 1996; Rajpert-De Meyts *et al.*, 1999).

AMH receptors

Members of the TGFβ family signal through two related transmembrane serine–threonine kinase receptors, referred to as type I and type II receptors. Upon binding of the ligand to its type II receptor, the type I receptor is recruited to form a heterotetrameric receptor complex. Activation of the type I receptor through transphosphorylation by the type II receptor leads to subsequent downstream signalling via

Smad proteins (for a review, see Massagué and Chen, 2000).

The type II receptor for AMH (AMHRII) was cloned several years ago (rat: Baarends *et al.*, 1994; rabbit: di Clemente *et al.*, 1994a). Expression of AMHRII mRNA was found in the mesenchymal cells surrounding the Müllerian ducts (Baarends *et al.*, 1994) and, in addition, in fetal and adult gonads of both sexes. Generation of AMHRII-deficient mice proved that this type II receptor is essential for AMH signalling, as demonstrated by the lack of Müllerian duct regression in AMHRII-deficient male mice (Mishina *et al.*, 1996). In addition, the relevance of this receptor to AMH action was further demonstrated by the identification of mutations in the AMHRII gene of patients with persistent Müllerian duct syndrome (PMDS) (Imbeaud *et al.*, 1996; Josso *et al.*, 1997).

More insight into the identity of the AMH type I receptor has been gained recently. Members of the TGFβ family can use several different type I receptors to exert their effects. Two distinct sets of type I receptors can be made. Activin receptor-like kinase 1 (ALK1), ALK4 and ALK5 form the TGFβ–activin group of type I receptors, whereas ALK2, ALK3 and ALK6 form the BMP group (Nakao *et al.*, 1997; Massagué and Chen, 2000). It has been shown that AMH signals via the type I receptors of the BMP group. Experiments *in vitro* and organ culture experiments *ex vivo*, using an anti-sense oligo approach, revealed that ALK2 is involved in AMH-induced Müllerian duct regression (Visser *et al.*, 2001). ALK2-deficient mice are embryonically lethal and therefore do not provide additional information on ALK2 functioning as an AMH type I receptor. Generation of ALK3 conditional knockout mice showed that ALK3 might be involved in AMH signalling during induced Müllerian

Email: Durlinger@endov.fgg.eur.nl

duct regression (for a review, see Teixeira, 2001); however, the role of ALK6 in AMH signalling is still questionable. Studies *in vitro* showed an AMH-dependent interaction between AMHRII and ALK6 (Gouedard *et al.*, 2000). However, antisense ALK6 oligos or mutated ALK6 functioning as a dominant negative type I receptor did not disrupt AMH signalling (Clarke *et al.*, 2001; Visser *et al.*, 2001) and male ALK6-deficient mice do not retain their Müllerian ducts. These observations indicate that ALK6 is, at least during Müllerian duct regression, not crucial as an AMH type I receptor (Clarke *et al.*, 2001). Together, these results indicate that AMH, like BMPs, might signal through different type I receptors in a tissue- and cell-specific manner. Further studies are necessary to elucidate which signal transduction pathway is used by AMH in the ovary.

Expression of AMH and its receptors in the ovary

In contrast to its expression in male mice and rats, AMH is not expressed in female rodents before birth (Munsterberg and Lovell-Badge, 1991; Hirobe *et al.*, 1992; Taketo *et al.*, 1993; J. A. Visser and A. P. N. Themmen, unpublished), and this lack of expression guarantees a normal differentiation of the female internal reproductive tract structures. However, a few days after birth, this sexually dimorphic pattern of expression of AMH disappears and AMH expression is easily detected in granulosa cells of growing follicles (Ueno *et al.*, 1989; Hirobe *et al.*, 1992; Durlinger *et al.*, 2002). In mice and rats, AMH displays a specific window of expression. It is first detected in the granulosa cells of early primary follicles (Munsterberg and Lovell-Badge, 1991; Taketo *et al.*, 1993; Durlinger *et al.*, 2002). Expression is highest in granulosa cells of preantral and small antral follicles (Fig. 1a). Within these follicles, AMH expression is not always evenly distributed, as in some follicles expression is highest in the granulosa cells immediately surrounding the antrum and around the oocyte (Fig. 1b) (Ueno *et al.*, 1989; Hirobe *et al.*, 1992, 1994; Baarends *et al.*, 1995; Durlinger *et al.*, 2002). This gradient of AMH expression within a follicle may reflect functional differences between the granulosa cells surrounding the oocyte and the more peripheral granulosa cells, such as differences in proliferation capacity and steroidogenic activity (Baarends *et al.*, 1995). These functional differences may arise under the influence of factors produced by the oocyte, as has been shown for oocyte factor GDF9 (Elvin *et al.*, 1999a). Some studies report that, in rats, AMH expression can be found until the preovulatory stage (Ueno *et al.*, 1989; Hirobe *et al.*, 1994), whereas other studies report that AMH is not detected from the small antral follicle stage onward (Hirobe *et al.*, 1992; Baarends *et al.*, 1995). Follicles showing signs of atresia also have decreased or no AMH expression, and expression is completely lost in corpora lutea (Fig. 1d). AMH is never found in primordial follicles, theca cells, oocytes or the interstitium (Fig. 1c,d; Ueno *et al.*, 1989; Hirobe *et al.*, 1992, 1994; Baarends *et al.*, 1995; Durlinger *et al.*, 2002). In rats, no differences occur

during the oestrous cycle in the pattern of expression in follicles of the same class, although some heterogeneity has been observed in AMH mRNA expression in preantral and small antral follicles at oestrus and dioestrus (Hirobe *et al.*, 1994; Baarends *et al.*, 1995).

In contrast to AMH, the AMH type II receptor does not show a sexually dimorphic pattern of expression. Expression is found in Müllerian duct mesenchymal cells and gonads of both sexes (Baarends *et al.*, 1994; di Clemente *et al.*, 1994b). In the postnatal ovary of mice and rats, AMHRII expression colocalizes with AMH in the granulosa cells, showing a similar expression window (Baarends *et al.*, 1995; A. L. L. Durlinger and A. P. N. Themmen, unpublished). In addition, the theca cells of preantral and small antral follicles in rat ovaries express AMHRII (Ingraham *et al.*, 2000).

The candidate AMH type I receptor ALK2 is also expressed in fetal and adult mouse ovaries (Visser *et al.*, 2001), although its exact cellular and spatial pattern of expression remains to be determined; this is also true for expression of ALK3 mRNA in the ovaries of mouse fetuses (Dewulf *et al.*, 1995). ALK6 is not expressed in the ovaries of mouse fetuses (Visser *et al.*, 2001), but in the adult ovary, ALK6 is expressed in oocytes of small antral follicles, and in both the oocytes and granulosa cells of large antral follicles (Yi *et al.*, 2001), and, therefore, it does not colocalize with ovarian AMHRII. More detailed expression studies are needed to establish the exact localization of all AMH type I receptors.

AMH action in the ovary

The specific patterns of expression of AMH and AMHRII in the ovary indicate that AMH may play a role during follicle development and function. The first report on AMH action in the ovary showed that bovine AMH inhibited meiotic maturation of both denuded and cumulus-enclosed rat oocytes *in vitro* (Takahashi *et al.*, 1986). However, Tsafriri *et al.* (1988) were not able to reproduce these results and no subsequent reports have been published on the effects of AMH on oocyte maturation. On the basis of the pattern of expression of the receptors described in this review, it is much more likely that AMH exerts its effect on ovarian follicles via the granulosa and theca cells, and not via the oocyte.

The generation of transgenic mice over-expressing AMH revealed that the presence of AMH during the fetal period is detrimental for ovarian development (Behringer *et al.*, 1990). Female transgenic mice are infertile and most have a blind-ending vagina due to the absence of a uterus and oviducts. Although the ovaries are present at birth, in most animals the ovaries are devoid of germ cells and develop cord-like structures within 2 weeks after birth. In adult females, the ovaries have degenerated. Ovarian development appeared unaffected only in mice with the lowest serum AMH concentrations. Thus, in mice, the presence of AMH during the fetal period clearly has an impact on
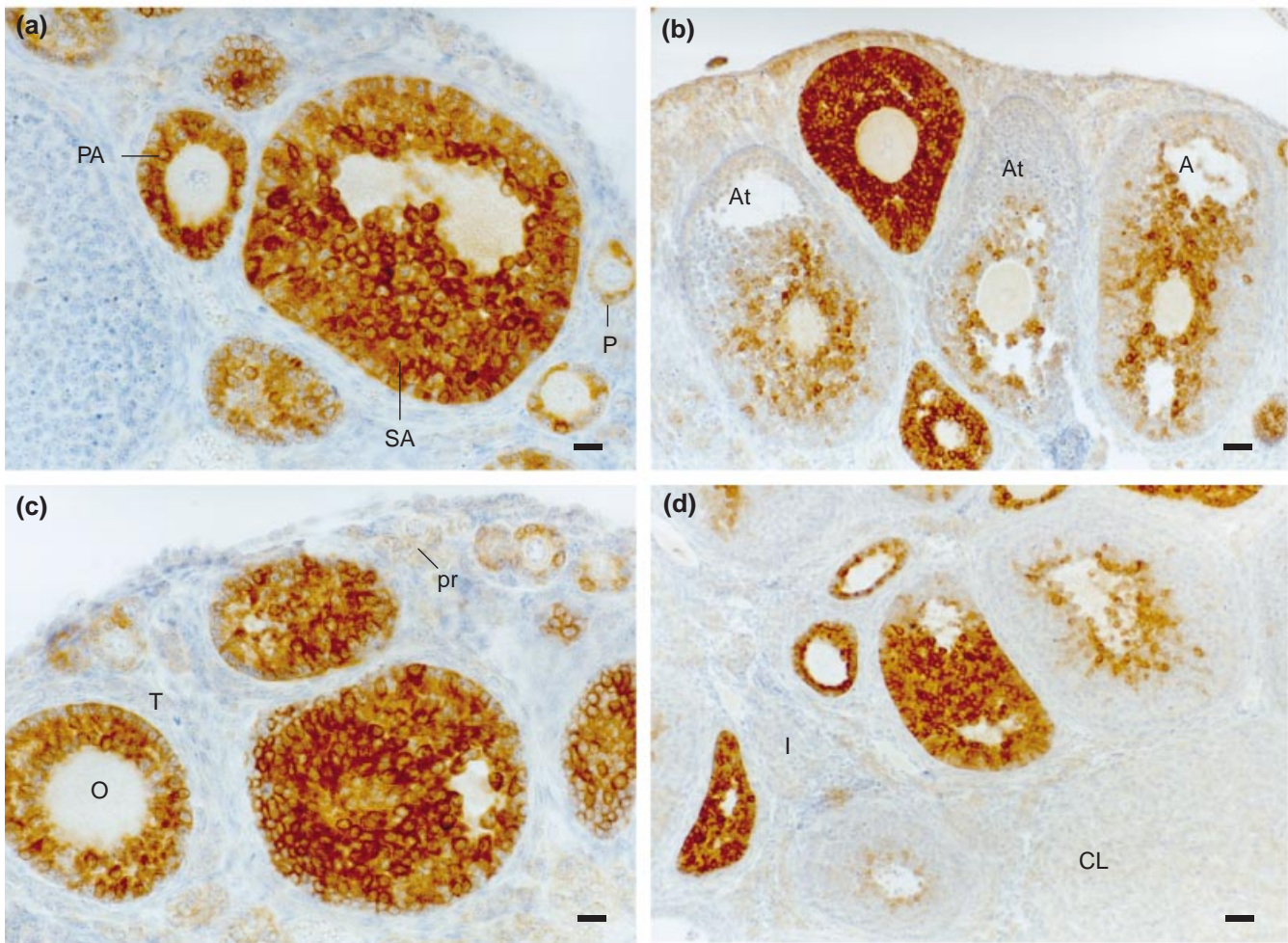
Fig. 1. Immunohistochemical localization of anti-Müllerian hormone (AMH) in a 30-day-old and a 4-month-old C57Bl/6J mouse ovary. (a) Section of an ovary of a 30-day-old mouse. AMH is found in granulosa cells from early primary follicles (P) and is highly expressed in granulosa cells of larger preantral (PA) and small antral (SA) follicles. (b) Section of an ovary of a 30-day-old mouse. AMH expression is decreased in antral (A) and atretic (At) follicles. The granulosa cells surrounding the antrum or the oocyte are the last cells to lose AMH expression. (c) Section of a 30-day-old mouse ovary. AMH expression is not present in primordial follicles (pr), oocytes (O) or theca cells (T). (d) Section of a 4-month-old mouse ovary. AMH expression is not found in the corpus luteum (CL) or the interstitium (I). Scale bars represent (a,c) 20 μm and (b,d) 40 μm. (Adapted from Durlinger, 2000.)

ovarian and female germ cell development. However, inferences cannot be drawn from this mouse model with respect to a possible role of AMH in postnatal follicle development and function.

AMH inhibits initiation of primordial follicle growth

More insight into the role of AMH in the ovary was obtained from our studies of AMH-deficient (AMHKO) mice. Male AMHKO mice retain their Müllerian duct derivatives and in some male mice Leydig cell hyperplasia is also observed. The AMHKO males are fertile, although their fertility is impaired for anatomical reasons. In female AMHKO mice, the lack of AMH does not affect fertility and initially no obvious ovarian abnormalities were observed (Behringer *et al.*, 1994).

Despite this lack of an ovarian phenotype in AMHKO females, it is possible that AMH might be involved in subtle and long-term aspects of control of follicle development (Durlinger *et al.*, 1999). This contention was based on the prominent and specific patterns of expression of AMH and AMHRII in the postnatal ovary and on the marked changes in expression of AMH and AMHRII mRNA during the oestrous cycle in rats (Baarends *et al.*, 1995). A study of the entire follicle population in AMHKO females was performed to test this hypothesis (Durlinger *et al.*, 1999) and revealed that, in the absence of AMH, ovaries are depleted of their primordial follicles earlier than they are in control mice, as ovaries of adult and aged AMHKO females contain fewer primordial follicles than do ovaries of their wild-type littermates (Fig. 2). This decrease is caused by increased recruitment of primordial follicles in AMHKO females, as
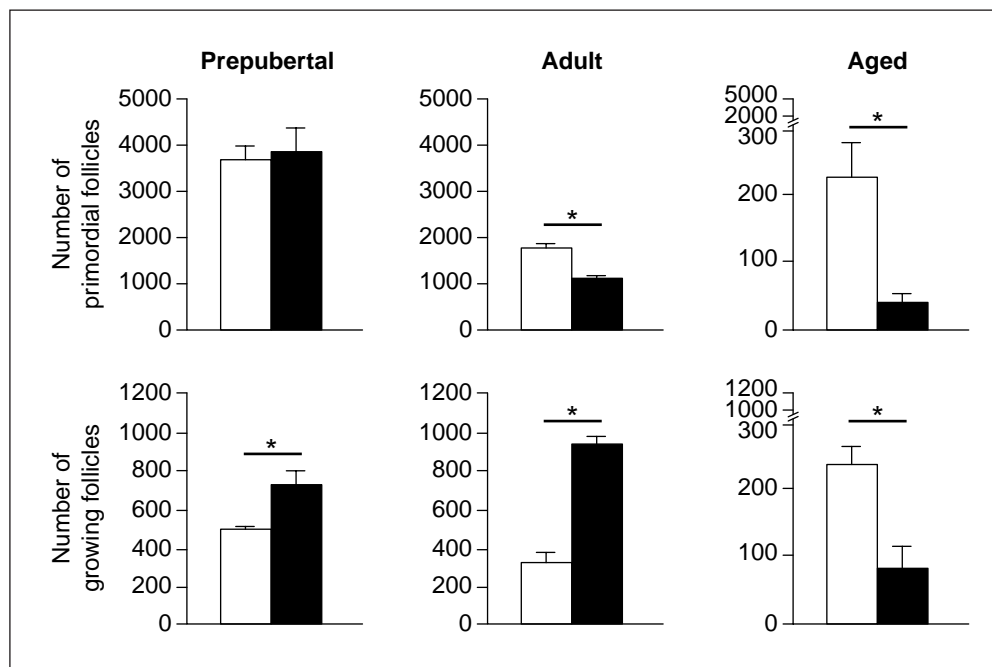
Fig. 2. Follicle population in prepubertal, adult and aged wild-type (□) and anti-Müllerian hormone knockout (AMHKO; ■) female mice. Mice were killed at oestrus. Follicles were counted in both ovaries. Data represent the mean number of follicles per two ovaries ± SEM (4–5 animals per genotype). The number of primordial follicles was significantly lower in adult (4 months of age) and aged (13 months of age) AMHKO female mice compared with the number in their wild-type littermates. Significantly more growing follicles were detected in prepubertal (25 days of age) and adult AMHKO female mice, whereas in aged AMHKO female mice the number of growing follicles was significantly lower. Asterisks indicate a significant difference ($P \leqslant 0.05$). (Adapted from Durlinger *et al.*, 1999.)

more preantral and small antral follicles (together referred to as small follicles) are found in prepubertal and adult AMHKO mice (Fig. 2). This increase in the number of small follicles is reflected by a twofold increase in total ovarian mass of adult AMHKO females relative to wild-type controls (Durlinger *et al.*, 1999). Mice heterozygous for the *amh* mutation have an intermediate phenotype (not shown). This AMH gene dose-dependency indicates that ovarian AMH production or secretion may not be under stringent feedback control, but instead depends on the intrinsic activity of the gene itself.

The above-mentioned results indicate that AMH may be able to inhibit the initiation of follicle growth, a process also known as follicle activation or initial recruitment (McGee and Hsueh, 2000). Durlinger *et al.* (2002) confirmed this conclusion by a study in which neonatal mouse ovaries were cultured *in vitro* for 2 or 4 days in the absence or presence of AMH. Ovaries of 2-day-old mice contain predominantly primordial follicles and some naked oocytes, but no growing follicles and, therefore, they provide an excellent model to investigate the influence of AMH on the recruitment of primordial follicles. AMH caused a 40–50% decrease in the number of growing follicles after 2 and 4 days of culture (Table 1). Consistent with these findings, a decreased expression of inhibin α-subunit mRNA was found in AMH-treated ovaries

compared with control ovaries. In contrast, expression of AMHRII and the oocyte markers GDF9 and zona pellucida protein 3 (ZP3) were not influenced by AMH. These results show that AMH is able to inhibit the initiation of primordial follicle growth and therefore functions as an inhibitory growth factor in the ovary during the early stages of folliculogenesis. This inhibitory effect is probably the result of a paracrine, (pre)granulosa cell-derived effect of AMH on the primordial follicle. An RNase protection assay showed that expression of AMHRII mRNA, which is essential for an effect of AMH, is present in ovaries of 2-day-old mice (Durlinger *et al.*, 2002). In addition, *in situ* hybridization studies demonstrated ovary-specific AMHRII expression in neonatal ovaries, although exact localization of the AMHRII expression to the pregranulosa cells was not possible because the radioactive AMHRII probe is best visualized using a microscope with dark field, making it difficult to discern specific structures or types of cell in neonatal ovaries (J. A. Visser, A. L. L. Durlinger and A. P. N. Themmen, unpublished).

The inhibitory effect of AMH on primordial follicle recruitment is consistent with our previous observation that the absence of, or reduced, AMH production in females results in an earlier depletion of the stock of primordial follicles. Therefore, the formation of preovulatory follicles would be expected to cease at a younger age in AMHKO

Table 1. Inhibitory effect of anti-Müllerian hormone (AMH) on the number of growing follicles in an *in vitro* 2-day-old mouse ovarian culture

Day of culture	Control	+ AMH
2	81 (100%) ($n = 9$)	50 (59%)* ($n = 9$)
4	153 (100%) ($n = 11$)	97 (66%)* ($n = 11$)

n = number of ovaries examined.
%: the number of growing follicles of the control was set at 100%.
*Indicates a significant difference from control (no AMH; $P \leqslant 0.05$).

females than in wild-type females, as the size of the primordial follicle pool is positively correlated with the duration of the reproductive lifespan of the female. This contention is clearly supported by, for example, the Bax-deficient female mouse model. Bax is involved in the execution of apoptosis, or programmed cell death, in many types of cell. In Bax-deficient females, the stock of primordial follicles shrinks much more slowly than it does in wild-type females, resulting in sustained ovarian function in aged Bax-deficient mice (20–22 months) (Perez *et al.*, 1999).

A premature exhaustion of the follicle pool predicts an earlier cessation of ovulation in ageing animals. Indeed, when the presence of corpora lutea was used as an indication of ovulatory cycles, it was found that 56% of AMHKO females compared with only 18% of the aged wild-type females had stopped ovulating at 16–17 months of age (Durlinger, 2000).

Studies on the use of AMH as a marker for ovarian ageing in women have recently started. A study in young normo-ovulatory women showed that serum concentrations of AMH decrease over time and with advancing age before changes occur in other currently known ageing-related variables, such as serum concentrations of FSH and inhibin B, and the number of antral follicles (de Vet *et al.*, 2002). Furthermore, a strong correlation was found between serum concentrations of AMH and the number of antral follicles, meaning that low serum AMH concentrations coincide with low numbers of antral follicles. A similar correlation was found on day 3 of the menstrual cycle in women undergoing *in vitro* fertilization (IVF) treatment (I. van Rooij, A. P. N. Themmen and E. R. te Velde, unpublished). As the age-related decrease in number of antral follicles corresponds to the age-related decrease in the number of primordial follicles (Scheffer *et al.*, 1999), it can be postulated that serum AMH concentration is a good indication of the size of the ovarian follicle pool and, thus, of ovarian ageing. Support for the use of serum AMH concentration as a marker for ovarian response in women undergoing controlled ovarian stimulation for IVF is provided by the demonstration of an association between higher serum AMH concentration and a larger number of retrieved oocytes (I. van Rooij, A. P. N. Themmen and E. R. te Velde, unpublished). A similar observation has been made in another study in women undergoing IVF treatment (Seifer *et al.*, 2002).

AMH is not the only regulator of initial recruitment. In addition to the inhibitory effect of AMH, initiation of follicle growth can also be positively influenced by stem cell factor (SCF; Parrott and Skinner, 1999), GDF9 (Vitt *et al.*, 2000), basic fibroblast growth factor (bFGF) (Nilsson *et al.*, 2001) and nerve growth factor (NGF) (Dissen *et al.*, 2001). It would be interesting to study the possible functional interactions of these different factors in initial recruitment. Indications for such an interaction have already been detected for SCF and GDF9. Expression of SCF mRNA is greatly increased in mice lacking GDF9 (GDF9KO), indicating that GDF9 may be one of the oocyte-secreted factors that negatively regulates SCF expression (Elvin *et al.*, 1999b), although it seems more likely that this difference in expression of SCF mRNA in intact ovaries is caused by the radical change in composition of the follicle population in GDF9KO mice (Dong *et al.*, 1996).

AMH inhibits FSH-stimulated follicle growth

Studies of female AMHKO mice have revealed that AMH, in addition to its role in primordial follicle recruitment, plays a role in fine-tuning the sensitivity of growing follicles to FSH. Despite a lower serum FSH concentration, ovaries of 4-month-old AMHKO mice contain more growing follicles than do ovaries of their wild-type littermates (Durlinger *et al.*, 1999), indicating that AMH may inhibit FSH-induced follicle growth. This hypothesis was confirmed by culture of mouse preantral follicles *in vitro*. Addition of AMH to the culture inhibited FSH-induced follicle growth in a time-dependent manner, as follicles cultured in the presence of AMH (at days 4 and 5 of culture) had a significantly smaller diameter. This AMH-induced inhibition in follicle growth was mainly the result of reduced granulosa cell proliferation, compared with FSH stimulation alone (Durlinger *et al.*, 2001). In contrast, McGee *et al.* (2001) described a stimulatory effect of AMH on FSH-induced growth of rat follicles. Although both the above studies used preantral follicles, species differences and absence or presence of serum (that is, other growth factors) in the culture medium may account for the opposing results (E. A. McGee, personal communication). The results of two *in vitro* studies in which exogenous AMH reduced expression of aromatase mRNA and decreased the number of LH receptors in cultured granulosa cells are in agreement with an inhibitory effect of AMH on follicle growth (di Clemente *et al.*, 1994a). In addition, AMH opposes EGF-induced proliferation of cultured granulosa-luteal cells (Kim *et al.*, 1992).

The inhibitory effect of AMH on FSH-stimulated follicle growth was also confirmed in an *in vivo* model in which follicle growth in the presence of high or low serum FSH concentrations was compared between AMHKO females and their wild-type littermates (Durlinger *et al.*, 2001). This study showed that, in the presence of low serum FSH concentrations, more growing follicles were found in
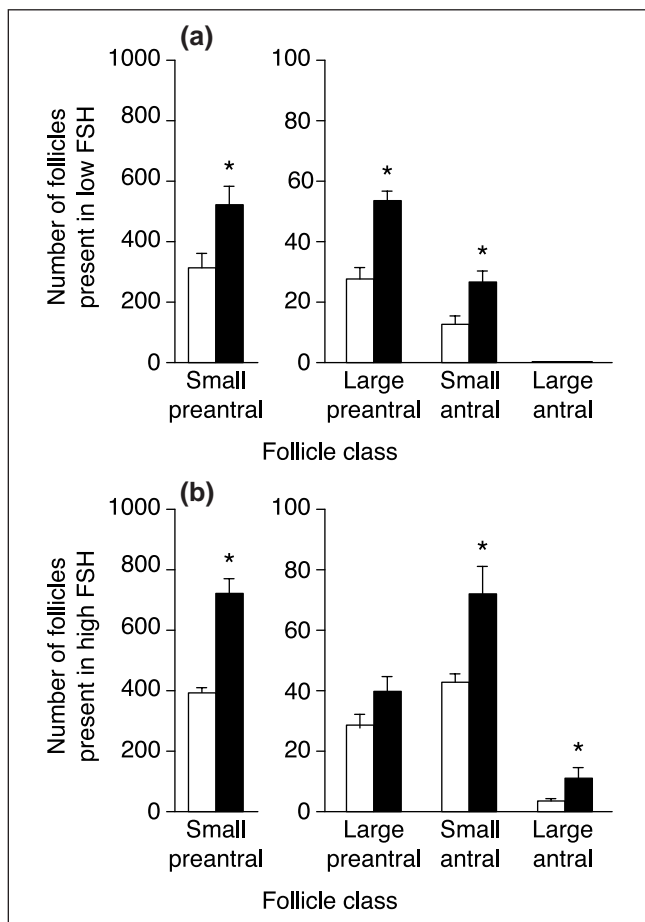
Fig. 3. Number of growing follicles in the presence of (a) low or (b) high serum FSH concentrations in prepubertal wild-type (□) and anti-Müllerian hormone knockout (AMHKO; ■) female mice. Data represent the mean number of follicles per two ovaries ± SEM (n = 6 animals per group). In the presence of both low and high serum FSH concentrations, more growing follicles are found in AMHKO females compared with wild-type females. Asterisks indicate a significant difference from wild-type females within the same treatment group (P ≤ 0.05). (Adapted from Durlinger et al., 2001.)

Fig. 4. Follicle population in adult wild-type (□), anti-Müllerian hormone knockout (AMHKO; ■), FSHβ knockout (FSHβKO; ▨), and FSHβ and AMH knockout (FAKO; ▧) female mice. Wild-type and AMHKO females were killed at oestrus. Follicles were counted in one ovary. Data represent the mean number of follicles per ovary ± SEM (n = 4 animals per genotype). There is no significant difference for both the number of (a) primordial and (b) growing follicles between wild-type and FSHβKO females. In both AMHKO and FAKO females, the number of primordial follicles is lower, whereas the number of growing follicles is higher compared with wild-type females. [a]Indicates a significant difference compared with the wild type; [b]indicates a significant difference from AMHKO mice; [c]indicates a significant difference from FSHβKO mice; and [d]indicates a significant difference from FAKO females (P ≤ 0.05). (Adapted from Durlinger et al., 2001.)

AMHKO females, whereas in the presence of high serum FSH concentrations, stimulation of follicle growth was more pronounced in AMHKO females than in wild-type females, both in terms of numbers and developmental stage (Fig. 3). These findings also indicate that, in the absence of AMH, follicles are more responsive to FSH.

This modulation of responsiveness to FSH by AMH could be very important during the process of cyclic recruitment. During cyclic recruitment in mice and rats, a group of follicles is recruited from the pool of large preantral and small antral follicles to continue growth to the preovulatory stage. Cyclic recruitment (McGee and Hsueh, 2000) takes place at oestrus, as a result of the secondary surge of FSH. This FSH peak is important for the number of follicles that are recruited (Hirshfield and De Paolo, 1981; van Cappellen et al., 1993). It is thought that, depending on the stage of development, each follicle requires a certain
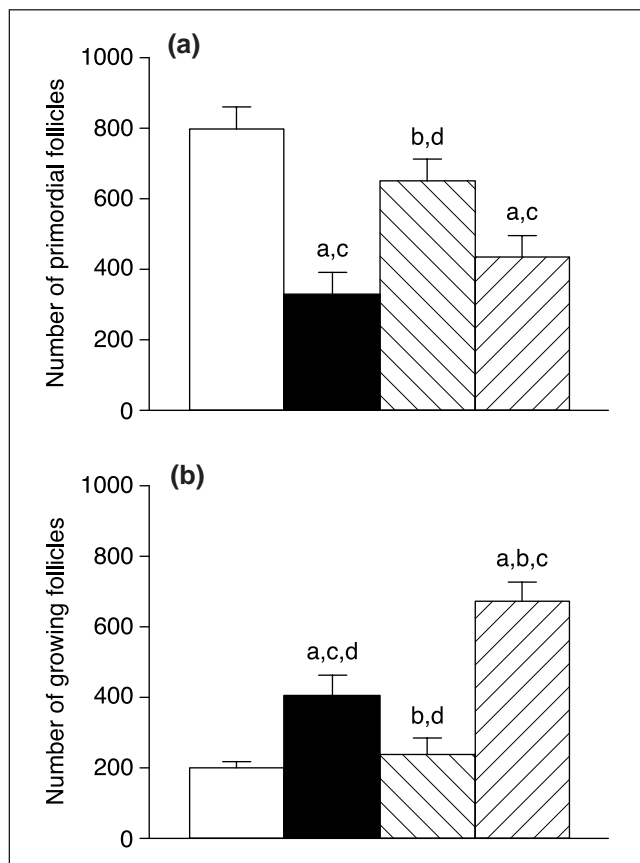
concentration of FSH to continue growth; that is, each follicle displays its own FSH threshold concentration and this threshold concentration has to be exceeded to ensure growth to the preovulatory stage. This concept of an FSH threshold was developed after studies in humans (Brown, 1978). AMH may be one of the factors involved in determining the responsiveness of the follicle to FSH during cyclic recruitment. A role for AMH in cyclic recruitment is supported by the differential pattern of expression of AMH observed at oestrus in non-atretic large preantral and small antral follicles (Baarends et al., 1995). Although these follicles are morphologically indistinguishable, some follicles show high AMH expression whereas others have a much lower
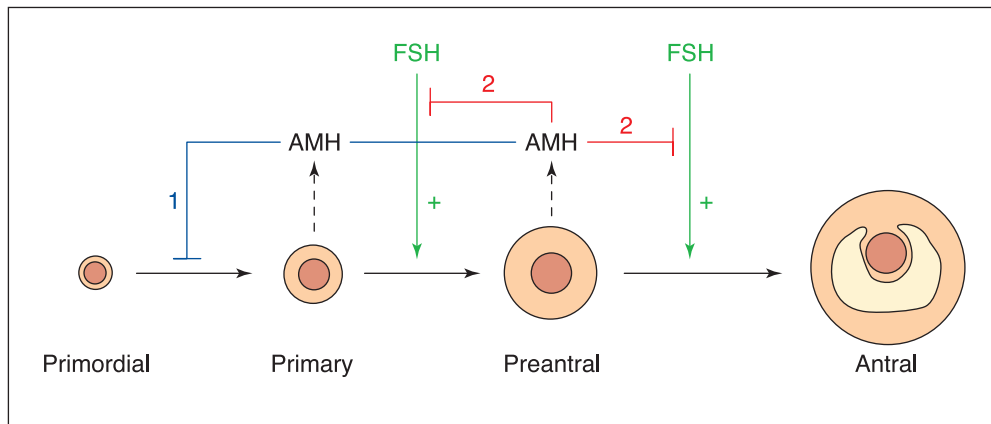
Fig. 5. Action of anti-Müllerian hormone (AMH) in the postnatal ovary. AMH produced by preantral and small antral follicles in the postnatal ovary has two sites of action in the postnatal ovary. (1) It inhibits initial recruitment and (2) it inhibits the stimulatory effect of FSH on the growth of preantral and small antral follicles.

AMH expression. Low AMH expression would correlate with increased sensitivity to FSH, allowing these follicles to be selected for continued growth and ovulation in the next oestrous cycle. Indeed, in a preliminary study, using 5-bromodeoxyuridine (BrDU) as a cell proliferation marker, in rats, more proliferating granulosa cells were found in follicles that expressed less AMH, and vice versa (Durlinger, 2000).

The inhibitory action of AMH on growth stimulation by FSH can also be relevant for small preantral follicles. Although these follicles do not depend on FSH for their growth (Halpin and Charlton, 1988; Kumar *et al.*, 1997), they are sensitive to FSH (van Cappellen *et al.*, 1989; McGee *et al.*, 1997). Therefore, complete loss of FSH production in mice would be expected to change preantral follicle growth. However, a study of the follicle population of 4-month-old FSHβ-deficient (FSHβKO) and wild-type female mice revealed that the absence of FSH had no impact on the number of primordial and preantral–small antral follicles (Fig. 4) (Durlinger *et al.*, 2001). The same study revealed that, in female mice lacking both *fshβ* and *amh* expression (FAKO), the ovarian phenotype caused by AMH-deficiency is even more pronounced (Fig. 4), indicating that AMH may be a much more dominant regulator of early follicle growth than FSH.

Thus, in addition to factors that stimulate preantral follicle growth, such as FSH (Halpin and Charlton, 1988; van Cappellen *et al.*, 1989), stem cell factor (SCF; Parrott and Skinner, 1997) and GDF9 (Hayashi *et al.*, 1999), inhibitory factors such as AMH are important in making preantral follicle growth a well-balanced process.

AMH and cancer in women

As AMH expression is restricted to ovarian granulosa cells in women, AMH may prove a useful serum marker of granulosa cell tumours (GTCs). Indeed, several studies showed an increased serum AMH concentration in women with both primary and recurrent GTCs (Gustafson *et al.*, 1992; Rey *et al.*, 1996; Silverman and Gitelman, 1996; Lane *et al.*, 1999; Rey *et al.*, 2000). An ultrasensitive enzyme-linked immunosorbent assay (ELISA) has been developed using immunopurified monoclonal AMH antibodies to enhance the availability and sensitivity of serum AMH determination (Long *et al.*, 2000), enabling researchers to detect the recurrences of GTCs at an early, preclinical stage.

AMH is not only a useful GCT marker but may also be applicable as a chemotherapeutic agent for ovarian epithelial tumours, as AMH causes regression of the Müllerian ducts, which arise from a common embryological precursor with the surface epithelium of the ovary, from which epithelial ovarian tumours originate (Scully, 1995). Several studies by P. Donahoe and co-workers have shown that AMH inhibits proliferation of cells derived from non-ovarian tumours (Fuller *et al.*, 1985; Chin *et al.*, 1991; Parry *et al.*, 1992). More recent studies have demonstrated that growth of ovarian tumours of epithelial origin is also inhibited by AMH (Masiakos *et al.*, 1999; Ha *et al.*, 2000), causing the cells to accumulate in the G1 phase of the cell cycle and subsequently to undergo apoptosis (Ha *et al.*, 2000). The inhibitory effect of AMH on the growth of epithelial ovarian tumour cells is probably mediated via AMHRII, as these tumour cells express AMHRII (Masiakos *et al.*, 1999). AMH may also be able to act as a therapeutic agent for ovarian cancer *in vivo*, as was demonstrated in a study in which growth of human ovarian cancer cell line (IGROV-1) implanted under the renal capsule of the kidney of immuno-deficient mice was inhibited in animals 'treated' with an AMH-expressing cell line (Stephen *et al.*, 2001).

Conclusion

In conclusion, several studies on the function of AMH in the postnatal ovary indicate that AMH has at least two functions

during follicular development (Fig. 5). First, AMH plays an inhibitory role during initial recruitment, when resting primordial follicles are initiated to grow, and second, it may modify preantral and small antral follicle growth by decreasing the FSH-responsiveness of the follicle. The second effect may be important during cyclic recruitment, when certain large preantral and small antral follicles are recruited to grow on to the preovulatory follicle stage.

Furthermore, in women, there are indications that AMH can be used as a marker for ovarian ageing and as a marker for ovarian response in women undergoing IVF treatment. AMH has also proved to be a marker for granulosa cell tumours and has potential to be used as a therapeutic agent for ovarian epithelial tumours.

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