Biological Functions and Clinical Applications of Anti-Müllerian Hormone in Stallions and Mares

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KEYWORDS

• Anti-Müllerian hormone • Equine • Mare • Stallion • Cryptorchidism
• Sertoli cell tumor • Ovarian reserve • Equine granulosa cell tumor

KEY POINTS

- Anti-Müllerian hormone (AMH) can serve as an endocrine marker for equine cryptorchidism and as an immunohistochemical marker for Sertoli cell tumors.
- AMH can be useful in the assessment of ovarian reserve and reproductive life-span of aged mares.
- AMH can serve as a diagnostic marker for equine granulosa cell tumors.

BASIC ASPECTS OF ANTI-MÜLLERIAN HORMONE

The Discovery of Anti-Müllerian Hormone

The discovery of anti-Müllerian hormone (AMH) dates back to the middle of last century when Alfred Jost, a French physiologist, was interested in the process of sexual differentiation. Initially, he showed that when fetal gonads were removed in utero (**Fig. 1**, top right), the Müllerian or paramesonephric ducts developed and the Wolffian of mesonephric ducts regressed.[1] Consequently, it was assumed that testosterone plays a key role in sexual differentiation; this was rejected because administration of androgens to female fetuses (see **Fig. 1**, bottom right) resulted in differentiation of the Wolffian ducts while the Müllerian ducts failed to regress. However, when small pieces of testicular tissue were grafted in close proximity to the ovary (see **Fig. 1**, bottom left), regression of Müllerian ducts was observed, indicating that the fetal testis must have an important role in sexual differentiation. Therefore, Alfred Jost concluded that the fetal testis does not only produce androgens, but also secretes another

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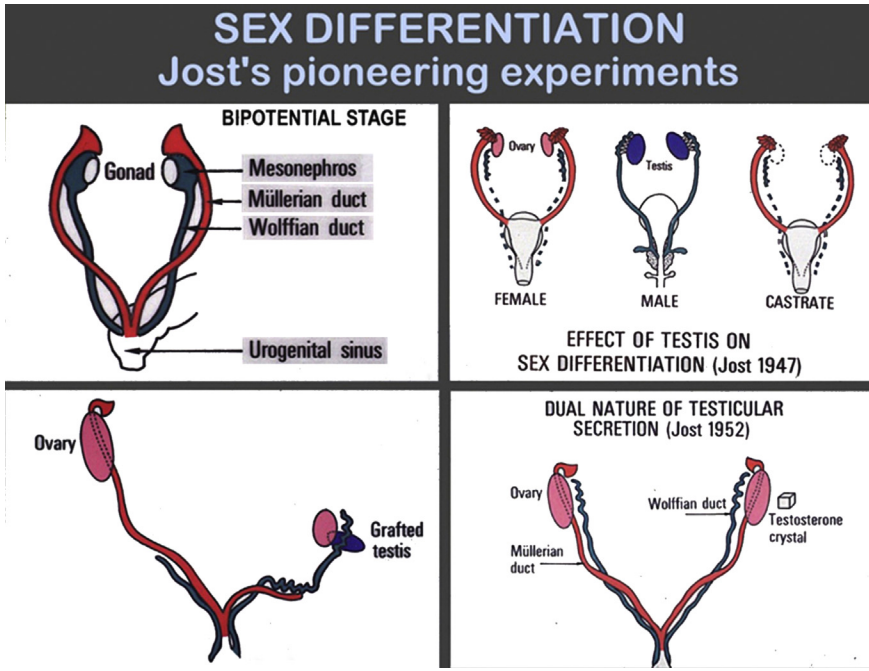

Fig. 1. Pioneering experiments conducted by Alfred Jost who investigated the underlying mechanism of sexual differentiation. (*Top left*) Internal reproductive organs prior to sexual differentiation (bipotential stage). (*Top right*) Removal of the fetal gonads in utero resulted in regression of the Wolffian of mesonephric ducts while the Müllerian or paramesonephric ducts developed. (*Bottom right*) Administration of androgens to female fetuses resulted in differentiation of the Wolffian ducts while the Müllerian ducts failed to regress. (*Bottom left*) Grafting small pieces of testicular tissue in close proximity to the ovary resulted in regression of Müllerian ducts indicating that the fetal testis must have an important role in sexual differentiation. (*From* Rey R, Josso N. Sexual differentiation. In: de Groot LJ, Beck-Peccoz P, Chrousos G, et al, editors. Endotext. South Dartmouth (MA): MDText.com, Inc; 2000; with permission from MDText.com, Inc.)

substance that induces the regression of the Müllerian ducts.[2] Although the nature of this substance remained unidentified, he defined it as 'hormone inhibitrice,' which later became better known as the Müllerian-inhibiting substance, Müllerian-inhibiting factor, or AMH.[3]

Anti-Müllerian Hormone: The Protein

Although the existence of a substance 'hormone inhibitrice' was already implied by Alfred Jost in 1953,[2] specific information about this substance remained unknown for some time. One of the first studies examining AMH indicated that it was a large molecule, such as a polypeptide, because it was not able pass through a membrane that allowed the passage of androgens.[4] Subsequent experiments demonstrated that AMH was a glycoprotein,[5,6] which contained disulfide bridges as smaller fragments were obtained when preparations of fetal testes were examined using sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions.[6] As the methods of AMH purification improved, more detailed information was obtained about the physical properties of this glycoprotein[7] as well as its biochemical composition with respect to amino acids and carbohydrates.[8,9] The protein AMH is derived from

a precursor that varies slightly in length among different species, ranging from 553 (rat) to 575 (bovine) amino acids.[3] The equine precursor is similar to the length of the bovine precursor with 573 amino acids (unpublished data, Drs Ball and Conley; National Center for Biotechnology Information: GenBank AEA11205.1, 2012). The amino acid sequence of the carboxyl terminal portion of the precursor is highly conserved across different species and among other members of the transforming growth factor-β family. In contrast, the amino acid sequence in the amino-terminal region is less conserved between different species.[3] Furthermore, the bovine AMH precursor starts with a signal sequence (16–17 amino acids) followed by a prosequence (7–8 amino acids). However, both sequences are cleaved from the precursor before secretion.[9] Finally, dimerization of the remaining monomer (70 kDa) results in the formation of the glycoprotein AMH, which is approximately 140 kDa.[10] Another interesting feature of this 140 kDa AMH glycoprotein is that cleavage is not required for it to be biologically active. Nonetheless, in vitro cleavage of AMH using plasmin also results in a biologically active product, which contains a 25-kDa and 110-kDa fragment representing the carboxyl and amino terminal dimer, respectively. Furthermore, sequencing of those fragments revealed that cleavage occurs at the monobasic cleavage site between the amino acid arginine and serine; 109 amino acids upstream of the carboxyl terminus.[11] Interestingly, the biological activity of AMH is localized in carboxyl terminal fragment while the amino terminal fragment is biologically inactive.[12] However, even though this amino terminal fragment is biologically inactive by itself, it supports the activity of the carboxyl terminal fragment because more complete regression of Müllerian ducts is observed when both fragments are included in organ culture media compared with only the carboxyl terminal fragment.[13]

Anti-Müllerian Hormone: The Gene

In 1986, Cate and colleagues[9] cloned the bovine and human AMH gene. The human AMH gene is 2.75 kbp in size, has a guanine-cytosine content of approximately 70% and contains 5 exons. The biological activity of the protein is encoded by the fifth and last exon of the gene.[10] The location of the AMH gene is different between species. The AMH gene is located on chromosome 19 in humans[14] and on chromosome 7 in cattle[15] and horses (National Center for Biotechnology Information: gene ID 102148318). Mutations in the AMH gene can result in persistent Müllerian duct syndrome (PMDS), an autosomal-recessive condition in males in which the Müllerian ducts fail to regress.[16] However, not all patients with PMDS have a mutation in the AMH gene; this condition can also arise from a mutation in the AMH receptor gene. Differentiating a mutation in the gene or gene receptor can be accomplished by measuring circulating AMH concentrations. Patients with mutations in receptor gene are characterized by normal circulating AMH concentrations, whereas patients with mutations in the AMH gene usually have undetectable AMH concentrations. Although the majority of the patients with PMDS have mutations in either the AMH gene or receptor, a small percentage of patients (15%) have another underlying cause that has not yet been identified.[17] The relationship between AMH and its receptor to PMDS in the horse has yet to be established.

BIOLOGICAL FUNCTIONS OF ANTI-MÜLLERIAN HORMONE

Sexual Differentiation: Regression of the Müllerian Ducts

Without any doubt, the most important biological function of AMH is to induce regression of the Müllerian ducts in the male fetus. Before sexual differentiation, a fetus contains an undifferentiated gonad along with a pair of Wolffian and Müllerian ducts. In the

presence of the SRY gene, the undifferentiated gonad develops into a testis, which produces AMH and testosterone.[18] The production of AMH by the fetal Sertoli cells is of crucial importance in sexual differentiation; the interaction of AMH with the AMH receptor type II induces a cascade of events that result in the regression of the Müllerian ducts.[19] Meanwhile, the production of testosterone by the fetal Leydig cells causes the Wolffian ducts to differentiate into the epididymis, vas deferens, and seminal vesicles. In the absence of the SRY gene, the undifferentiated gonad develops into a fetal ovary.[18] In contrast with the testis, the fetal ovary does not produce AMH or testosterone. As a result, the Müllerian ducts develop into the fallopian tube, uterus, cervix, and cranial end of the vagina and the Wolffian ducts regress. Because the duration of gestation varies between species, there are differences in the period during gestation when the regression of the Müllerian ducts is initiated and completed. The regression of the Müllerian ducts is completed by gestational day 46 in dogs[20] and by day 64 in humans.[21] To date, it is unknown when regression of the Müllerian ducts is initiated or completed in the equine male fetus.

Inhibition of Leydig Cell Differentiation

Although regression of the Müllerian ducts during fetal development is the most important function of AMH, the secretion of AMH in the circulation of males continues after birth until puberty.[22] These relative high circulating AMH concentrations before puberty suggest that AMH also has an important role in males during the postnatal period. As a matter of fact, Behringer and colleagues[23] provided the first evidence that AMH has an influence on Leydig cell differentiation; testicular tissue in AMH deficient mice is characterized by Leydig cell hyperplasia. Moreover, AMH acting through the AMH receptor on the Leydig cells does not only inhibit the differentiation of Leydig cells, but also decreases the steroidogenic activity of Leydig cells. In fact, AMH seems to have a downregulatory effect on the messenger RNA expression of cytochrome P450 17α-hydroxylase/C17 to 20 lyase in Leydig cells.[24] In support of this finding, Rouiller-Fabre and associates[25] demonstrated that testicular steroidogenesis is inhibited by AMH. Based on these studies, it seems that the relatively high concentrations of AMH have a downregulatory role on differentiation and steroidogenic activity of Leydig cells, which might suggest that the quiescent state of the testis before pubertal development is maintained by AMH.

Folliculogenesis

AMH also seems to have an important role in females after birth. Through the use of AMH knockout mice, it became clear that AMH is actively involved in folliculogenesis.[26] Initially, it was shown that the follicular pool is depleted earlier in AMH knockout mice than in wild-type mice. This rapid decline in primordial follicles in AMH knockout mice was attributed to an increased rate of follicular recruitment. Based on these results, it was concluded that the recruitment of primordial follicles is inhibited by AMH.[26,27] The negative influence of AMH on the recruitment of primordial follicles was also examined by assessing follicular growth in the ovary of newborn mice after ovaries were cultured in vitro with or without AMH. Indeed, the number of growing follicles was significantly lower in ovaries cultured with AMH than without AMH.[28] Furthermore, as the number of growing follicles was increased in AMH knockout mice, the possibility exists that the growth of follicle-stimulating hormone (FSH)-sensitive follicles was also reduced by AMH.[26,27] A subsequent study clearly showed that the growth of FSH-sensitive preantral follicles in mice is inhibited by AMH.[29] Likewise, Pellatt and colleagues[30] showed that granulosa cells of growing follicles become less sensitive to FSH in the presence of AMH.

CLINICAL APPLICATIONS OF ANTI-MÜLLERIAN HORMONE IN STALLIONS

Biomarker for Equine Cryptorchidism

Cryptorchidism is a condition in which 1 or both testes are retained within the inguinal canal and/or abdominal cavity. Cryptorchid horses without a scrotal testis are usually presented as geldings with the complaint of displaying stallionlike behavior. Transrectal[31] and transcutaneous (inguinal and abdominal)[32] ultrasound imaging are valuable methods to detect and localize retained testicular tissue. Nonetheless, equine field practitioners routinely use an endocrine test to diagnose cryptorchidism in horses without a scrotal testis because it requires less experience and time compared with ultrasonography. For more than 3 decades, peripheral testosterone and estrogen concentrations have been used by veterinary practitioners as endocrine markers for equine cryptorchidism.[33,34] A recent study showed that AMH can also be used as biomarker for equine cryptorchidism because circulating AMH concentrations are significantly higher in cryptorchid and intact stallions than in geldings. More precisely, circulating AMH concentrations in geldings are either undetectable or approach the lower detection limit of the AMH assay whereas cryptorchid stallions without a descended testis have significantly higher AMH concentrations than intact stallions (**Fig. 2**).[35] This is in contrast to testosterone[36] and estrone sulfate concentrations,[37] which are either significantly lower or not different between cryptorchid stallions and intact stallions. Furthermore, cryptorchid stallions without a descended testis tend to have higher AMH concentrations than cryptorchid stallions with a descended testis, whereas no differences in AMH concentrations were observed between cryptorchid stallions with a descended testis and intact stallions (see **Fig. 2**).[35] Differences in circulating AMH concentrations between cryptorchid stallions without a scrotal testis and intact stallions could be the result of a variety of factors. In human males, the formation of the blood testis barrier at puberty seems to be associated with a decrease in circulating AMH concentrations owing to a redirection in the secretion of AMH from the peripheral circulation into seminal plasma.[22] Considering that a cryptorchid equine testis largely resembles a prepubertal testis,[38] it is plausible that the

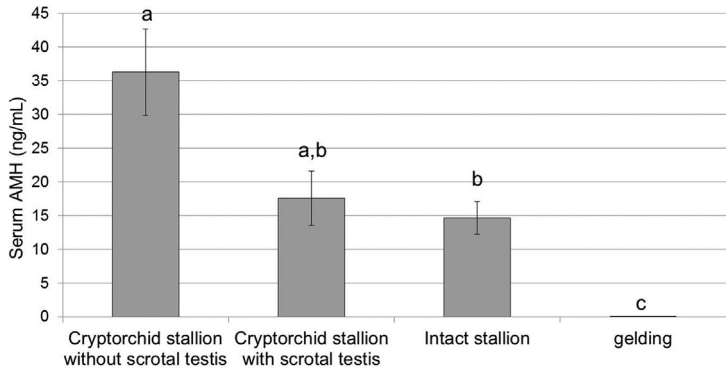

Fig. 2. Circulating anti-Müllerian hormone (AMH) concentrations in cryptorchid stallions with and without a scrotal testis, intact stallions and geldings. Data are presented as mean values ± standard error of the mean. Different letters indicate significant statistical significance ($P<.05$). (*Adapted from* Claes A, Ball BA, Almeida J, et al. Serum anti-Müllerian hormone concentrations in stallions: Developmental changes, seasonal variation, and differences between intact stallions, cryptorchid stallions, and geldings. Theriogenology 2013;79(9):1232–33; with permission from Elsevier.)

secretion of AMH in cryptorchid stallions persists into the peripheral circulation instead of being redirected into the seminal plasma. Second, increasing concentrations of testosterone[39] as well as initiation of meiosis within the seminiferous tubules[40] during pubertal development concurs with a decrease in AMH expression and circulating AMH concentrations in other species. Therefore, arrested spermatogenesis and a reduced expression of androgen receptors[38] in the equine cryptorchid testis could contribute to the higher AMH concentrations observed in cryptorchid stallions compared with intact stallions. Nevertheless, more research is required to elucidate further the mechanism behind the higher AMH concentrations in cryptorchid stallions.

The diagnostic value of AMH to distinguish between geldings and cryptorchid stallions depends on the sensitivity and specificity of the AMH assay to detect retained testicular tissue. Even though this remains to be determined, some data suggest that AMH is more suitable to detect retained testicular tissue than testosterone or estrone sulfate. The specificity of AMH in distinguishing cryptorchid stallions from geldings might be higher than specificity of testosterone[41] because the Sertoli cells of the testis are the only source of AMH in male species, whereas testosterone can be either of testicular or, to a lesser extent, of adrenal origin.[42,43] This could explain the 11% to 14% of inconclusive test results when baseline testosterone concentration was solely used to diagnose cryptorchidism.[36,44] The usefulness of AMH in diagnosing equine cryptorchidism when baseline testosterone concentration failed to do so was also demonstrated in a small number of challenging cases.[41] Despite the low or inconclusive concentration of testosterone, horses with retained testicular tissue could easily be distinguished from geldings, and vice versa, by determining the concentration of AMH in a single blood sample. The results of the AMH assay were confirmed either by an extended human chorionic gonadotropin (hCG) stimulation test in which samples were collected 1 and 24 hours after administration of hCG to detect the biphasic response in testosterone concentrations or by ultrasonography or cryptorchidectomy.[41] Although these preliminary results are promising, more cases are required to confirm this diagnostic advantage of AMH over testosterone.

Among all biomarkers, AMH might be the only endocrine marker that is applicable in all ages. The reliability of baseline or hCG-induced testosterone in identifying cryptorchid stallions is limited in horses younger than 18 months of age[45] and the accuracy of estrone sulfate in detecting retained testicular tissue is considerably reduced in horses before 3 years of age.[44] In contrast, circulating AMH concentrations are high in neonates and prepubertal colts, and even though a decrease in AMH concentrations is observed during puberty, concentrations of AMH remain high in postpubertal stallions.[35] The ability of AMH to detect cryptorchid males during the prepubertal and peripubertal period is clearly demonstrated in cattle[46] and humans.[47] Measuring circulating AMH concentrations is more useful in detecting unilateral cryptorchid calves than baseline or hCG-stimulated testosterone concentrations.[46] In accordance, measurable concentrations of AMH in newborn humans without scrotal testes are indicative of retained testicular tissue.[48] Thus, AMH seems to be the biomarker of choice to detect retained testicular tissue in horses younger than 18 months of age.

As for any other diagnostic markers, it is imperative to be aware of factors that could influence circulating AMH concentrations. First, season has a significant impact on serum AMH concentrations in intact stallions with higher AMH concentrations during the breeding season than during the nonbreeding season.[35] However, it is unclear whether those seasonal variations in AMH concentrations in intact stallions can be extrapolated to cryptorchid stallions. In comparison with testosterone, AMH concentrations are less subjected to diurnal variations as the biological half-life of AMH ($t_{1/2}$ = 1.5 days)[35] is considerably longer than the biological half-life of

testosterone ($t_{1/2}$ = 1.1 hour; unpublished data from Dr A. Esteller-Vico, 2014). In conjunction, circulating AMH concentrations decrease rather slow after castration,[35] which is also attributed to the relatively long biological half-life of AMH. Depending on the concentration of AMH before castration, it might take several days to a week before AMH concentrations are reached that are consistent with concentrations in geldings. Therefore, measurement of serum testosterone concentrations might be a better approach than measuring AMH concentrations if testing is warranted in the immediate period (24–48 hours) after castration to confirm that all retained testicular tissue is removed, such as after a questionable cryptorchidectomy. To conclude, AMH has some additional value in the endocrine diagnosis of equine cryptorchidism and, therefore, should be included in the diagnostic workup of cryptorchid horses because it could increase the likelihood of detecting retained testicular tissue, particularly in difficult cases.

Immunohistochemical Biomarker for a Sertoli Cell Tumor

Testicular neoplasia is a rare condition in stallions, which is usually characterized by unilateral or bilateral testicular enlargement.[49] Nonetheless, histopathology is generally required to distinguish between the different types of equine testicular tumors. In humans, it is well-established that AMH can be used to differentiate between Sertoli cell tumors and other types of tumors because AMH is expressed exclusively in sex-cord stromal tumors.[50] The immunoexpression of AMH has also been examined in testicular tumors of stallions including Sertoli cell tumors, seminomas and teratomas. Among all examined tumor types, AMH immunolabeling is only present in equine Sertoli cell tumors and localized either to only a small number of neoplastic cells or, to a moderate extent, to the tubular component of the equine Sertoli cell tumor (**Fig. 3**).[51] Despite the heterogeneous expression of AMH in Sertoli cell tumors in different species, detectable AMH immunolabeling is indicative of a Sertoli cell tumor or a mixed tumor containing a Sertoli cell tumor component.[50,51] Along with being an immunohistochemical biomarker, AMH can also serve as a serologic biomarker for Sertoli cell tumors in dogs; dogs with a Sertoli cell tumor had significantly higher circulating AMH concentrations than healthy dogs with no palpable testicular enlargement.[52] Nonetheless, whether the same is true in stallions remains to be determined.

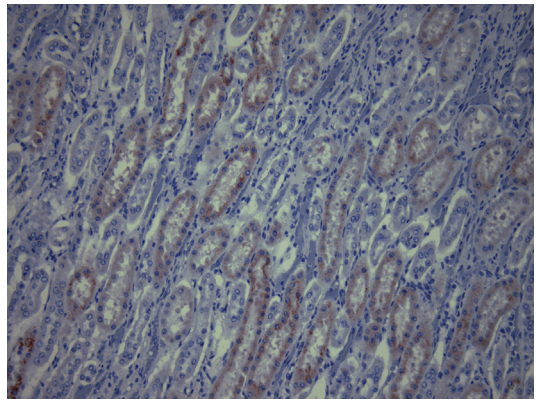

Fig. 3. The immunoexpression of anti-Müllerian hormone (AMH) in an equine Sertoli cell tumor. AMH labeling is localized to the tubular component of the Sertoli cell tumor (H&E).

CLINICAL APPLICATIONS OF ANTI-MÜLLERIAN HORMONE IN MARES

Biomarker for Ovarian Tissue

Besides being synthesized by the Sertoli cells, AMH can also originate from the granulosa cells of the ovarian follicles.[53] Because AMH is an ovarian-specific protein, peripheral AMH concentrations can be used to differentiate between ovariectomized and intact females.[54] In contrast with intact mares, circulating AMH concentrations are undetectable in ovariectomized mares.[55] Nonetheless, undetectable AMH concentrations in mares must be interpreted with some caution in the absence of transrectal examination findings because undetectable AMH concentrations have also been observed in intact older mares with a low number of antral follicles.[56] Notwithstanding this shortcoming, the usefulness of this application in mares is limited at best. In contrast, detection of ovarian tissue is of great importance in small animals especially in case of ovarian remnant syndrome. Intact dogs can be differentiated from spayed dogs using peripheral AMH concentrations with a sensitivity and specificity of approximately 94%. However, the sensitivity of AMH to detect ovarian tissue in dogs is influenced by either pubertal status or age, because it decreases to 50% during the first 6 months of life.[54] Although AMH has some value in differentiating intact from ovariectomized mares, AMH is more widely used in small animals suspected of ovarian remnant syndrome.

Biomarker for Ovarian Reserve and Function

As reported, the ovarian granulosa cells are the only source of AMH in females.[53] The expression of AMH is confined to the cytoplasm of equine granulosa cells and changes during follicular development (**Fig. 4**). The primary follicle is the first type of ovarian follicle displaying AMH expression and as the number of granulosa cells layers increases so does the expression of AMH.[57] Moreover, the expression of AMH is strong in small antral follicles (15–20 mm) but decreases around follicle selection resulting in only faint AMH labeling in dominant and preovulatory follicles.[58] In addition to follicle type, follicular viability is another factor that has an influence on the expression of AMH in equine ovarian follicles. As opposed to viable follicles, only weak AMH expression is detected in granulosa cells of atretic follicles.[57] Thus, the expression of AMH in equine granulosa cells is influenced by follicular development and viability.

Considering that AMH is synthesized exclusively by the ovarian follicles, it seems likely that circulating AMH concentrations are a reflection of the size of the follicular pool. Indeed, a strong mutual relationship exists between circulating AMH concentrations, antral follicle count (AFC) and the number of primordial follicles in women and mice.[59,60] Consequently, circulating AMH concentrations as well as AFC are commonly used to assess ovarian reserve in women. In addition, the onset of menopause in women can be predicted reasonably well using circulating AMH concentrations.[61] In contrast with women, aged mares do not go through menopause but can experience ovarian senescence, a condition somewhat similar to menopause. Early signs of ovarian senescence are prolonged interovulatory intervals, smaller preovulatory follicles, and a low number of antral follicles. Eventually, the follicular pool becomes depleted, resulting in ovulation failure and cessation of estrous cycles.[62] As in humans, AMH could have some usefulness in assessing the size of the follicular pool in aged mares. In fact, circulating AMH concentrations are correlated positively with AFC in middle-aged and aged mares but not in young mares, and the correlation between AMH and AFC is moderate in middle-aged mares and strong in aged mares. Also, aged mares have significantly lower AMH concentrations and AFC compared with middle-aged mares, indicating that the size of the follicular pool in mares declines

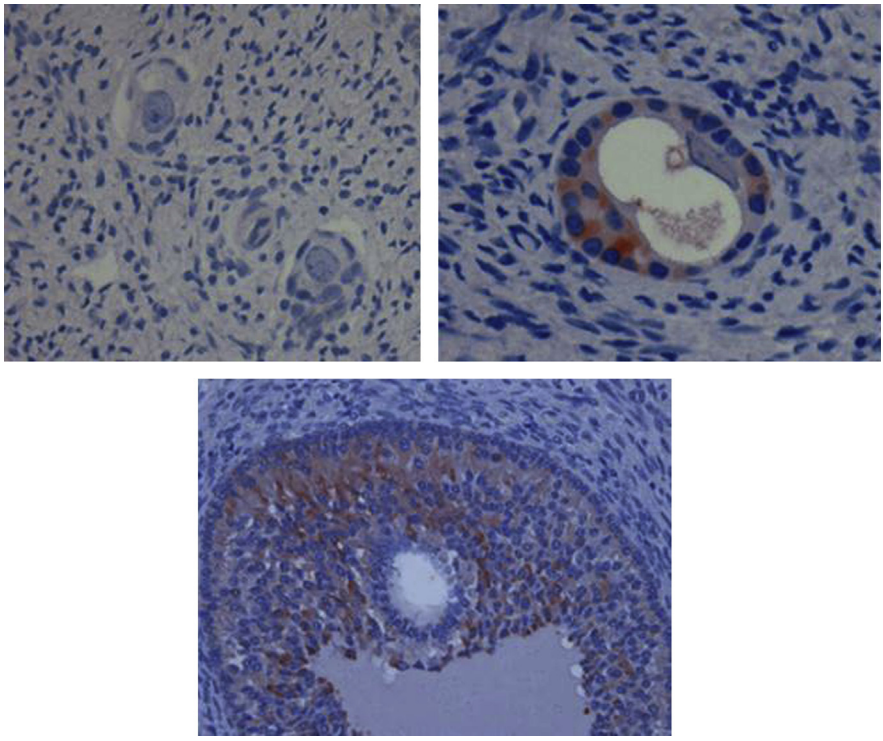

Fig. 4. The expression of anti-Müllerian hormone (AMH) in equine primordial, preantral, and antral follicles (from *left to right*) using immunohistochemistry. No AMH expression is present within primordial follicles, whereas mild expression of AMH can be detected within the granulosa cells of a primary follicle. The expression of AMH increases with follicular development; granulosa cells of antral follicles display stronger AMH labeling (H&E, 200×). (*Adapted from* Ball BA, Conley AJ, MacLaughlin DT, et al. Expression of anti-Müllerian hormone (AMH) in equine granulosa-cell tumors and in normal equine ovaries. Theriogenology 2008;70(6):974; with permission from Elsevier.)

with age. Irrespective of these age-related decreases in AMH and AFC, distinct differences in circulating AMH concentrations and AFC have been observed among young, middle-aged, and aged mares. This, in turn, might indicate that the size of the follicular pool is inherently different between mares of the same age and, therefore, the reproductive age of a mare must be distinguished from the calendar age.[56] Finally, AMH is particularly suitable as biomarker for ovarian reserve in mares because concentrations of AMH are relatively stable during and between different estrous cycles,[56] and this stability is likely owing to the long biological half-life of AMH (1.9 days).[55] Thus, AMH seems to be a reflection of population of follicles in mares and could be valuable in assessing the reproductive life-span of aged mares.

More in-depth research suggests that variations in circulating AMH concentrations are also a reflection of follicular function. Distinct molecular differences have been detected between granulosa cells of growing follicles of mares with high and low AMH concentrations. Low circulating AMH concentrations are associated with a low expression of AMH, AMH receptor type 2, estrogen receptor 1, estrogen receptor 2, inhibin alpha, and follitropin receptor in granulosa cells of growing follicles.[58] It is

well-established that these genes play a crucial role in folliculogenesis and a reduced expression in other species seems to be linked to reduced oocyte quality,[63] responsiveness to FSH,[64] and granulosa cell proliferation.

Biomarker for Equine Granulosa Cell Tumors

The equine granulosa cell tumor (GCT), a sex-cord stromal tumor, is the most common tumor of the equine ovary. Although rarely metastatic, equine GCTs are usually associated with changes in behavior such as failure to cycle, stallionlike behavior, or persistent estrus (nymphomania). Other diagnostic features of a classical GCT in mares are a unilaterally enlarged ovary with a honeycomb appearance on ultrasonography and a contralateral inactive ovary. Similar findings can occasionally be observed in mares with a hemorrhagic anovulatory follicle or ovarian hematoma but, in contrast with those ovarian conditions, the equine GCT is hormonally active. Therefore, a crucial step in the diagnostic workup of a mare with a GCT is an endocrine analysis, which includes the measurement of progesterone, testosterone, and inhibin. Low progesterone concentrations in conjunction with increased concentrations of inhibin and/or testosterone are indicative of a GCT. Inhibin concentrations are increased in nearly 90% of GCT mares and testosterone concentrations are increased in approximately 50% to 60% of the GCT cases.[65] Nonetheless, concentrations of testosterone[66] and inhibin[67] are also increased during pregnancy, whereas slightly increased testosterone concentrations in nonpregnant mares could be attributed to increased adrenal gland activity.[42] In contrast, a small percentage of mares with a GCT do not have increased concentrations of inhibin or testosterone. In such cases, it can be challenging to diagnose a GCT using the conventional endocrine markers, namely, inhibin and testosterone.

Besides inhibin and testosterone, AMH also proves to be important in the diagnosis of equine GCTs.[55,57,68] AMH is heterogeneously expressed in equine GCTs and present in sera of GCT mares in a bioactive form because it is able to induce regression of the Müllerian ducts in an in vitro assay.[57] More important, at least clinically, mares with a GCT have higher circulating AMH concentrations than nonpregnant and pregnant mares without a GCT indicating that AMH can serve as an endocrine marker for equine GCTs. Interestingly, AMH turns out to be the most important endocrine marker to identify mares with a GCT because it has a higher sensitivity (98%) than testosterone (48%), inhibin (80%), or testosterone and inhibin combined (84%).[68] Another important of advantage is that AMH can be used to detect GCTs in pregnant mares because circulating AMH concentrations are not influenced by gestation,[55] whereas testosterone[66] and inhibin[67] concentrations are increased during gestation. Furthermore, circulating AMH concentrations in nonpregnant mares are more stable throughout the cycle[55] than testosterone or inhibin concentrations, which are influenced by the stage of the cycle.[69,70] Finally, data in other species indicate that AMH has an inhibitory effect on the growth of antral follicles.[68] Hence, AMH might be involved in suppressing follicular development on the contralateral inactive ovary but more research is required to confirm this hypothesis.[57] To conclude, it is apparent that AMH is not only more widely applicable but also improves the diagnostic accuracy of the current equine GCT panel.

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