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Review article

Anti-Müllerian hormone as a marker of embryo production in ruminants

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This review describes the role of anti-Müllerian hormone (AMH) in embryo production for assisted reproductive technologies in ruminants. AMH is a marker of healthy follicles and oocytes, a reliable marker of gonadotropin-responsive follicles, and an indicator of longevity and productivity in dairy animals. The best times to measure AMH levels in order to select cows for embryo production is during oestrus and the period after the 12th day of the oestrous cycle. This allows animals with AMH concentrations below 87 pg/mL at oestrus or less than 74 pg/mL for multiple ovulation embryo transfer to be eliminated. Good oocyte donors, which have higher antral follicle counts, can be identified based on their higher AMH levels. In sheep and goats, the blood AMH level can serve as a marker of the animal's potential to produce high or low numbers of high-quality embryos. A plasma AMH level of 97 pg/mL in sheep has been shown to be the optimum cut-off point to predict fertility and can be useful in selecting replacement ewes.

KEY WORDS: anti-Müllerian hormone / endocrine marker / multiple ovulation embryo transfer / reproductive biotechnology

Assisted Reproductive Technologies (ARTs) serve to increase reproductive performance in animals, through the use of artificial insemination (AI) and multiple ovulation embryo transfer (MOET) to improve the genetic potential of livestock [8]. In cattle, MOET technology is widespread throughout the world, while in small ruminants embryo production is still limited [24]. Despite improvements in superovulation and MOET protocols,

there is still variation in the outcome of treatments, which has been attributed to the status of ovarian follicles at the time of gonadotropin stimulation [27]. Research has focused on analysis of the role of Anti-Müllerian hormone (AMH) in predicting the responses of animals to gonadotrophin treatments [23, 29].

Regulation of AMH in gonads

AMH belongs to the transforming growth factor beta family (TGFβ) [27], a group of proteins secreted by foetal Sertoli cells in males [17] and by the ovary in females [5]. AMH in ruminants shares the same set of identical epitopes [18]. Its concentration is highest in granulosa cells of preantral and small antral follicles, declines during terminal follicular growth, and remains at low levels when the large antral and preovulatory follicles become estrogenic [23]. The AMH pattern in growing follicles is taken into account in order to use AMH as a marker of the size of the ovarian follicular pool [27].

AMH expression is modulated by androgens/testosterone [7] and inhibited by follicle-stimulating hormone (FSH) in granulosa cells, but stimulated by bone morphogenetic proteins (BMP). BMPs enhance AMH gene expression, support follicular growth and contribute substantially to the production and/or maintenance of AMH. In the ovarian follicle, the AMH level is dependent on its stage, with the highest level detected in healthy small antral follicles, whereas at the level of the entire ovary, AMH concentration is influenced by the size of the pool of small antral growing follicles. At the endocrine level, AMH has a dynamic profile that depends on physiological conditions such as pre-puberty, the stage of the oestrous cycle, gestation and the postpartum period [23].

In sheep, AMH has no influence on the rate of primordial follicle recruitment, but controls the rate at which follicles advance into the gonadotropin-responsive phase [6]. In contrast, in goats AMH inhibits the activation of primordial follicles, and expression of AMH varies according to the stage of follicular development [30].

AMH as an endocrine marker of embryo production, longevity and productivity in large ruminants

Large individual differences in FSH-induced superovulation have been identified as a major restriction to the improvement of in vivo embryo production in cattle. In humans and in mice, AMH has been identified as the best endocrine marker of the ovarian follicular reserve [27], while in cattle it has been used as an indirect marker of the antral follicular count (AFC), which is associated with the number of morphologically healthy follicles and oocytes [12]. AMH has been also investigated as an endocrine marker of follicular populations in the ovary and as a possible predictor of the ovarian response to superovulation in cattle. AMH has been shown to be a dependable endocrine marker of small antral gonadotropin-responsive follicles [27]. In addition, AMH levels in individual animals are correlated with their ability to respond to superovulatory treatments [31]. A positive correlation has been observed between plasma AMH level and the following parameters: 1) number of embryos produced by the donor, 2) selection of donors producing

a high number of embryos [10], and 3) selection of donors with greater responses to superstimulation [31]. Because AMH concentration can be used as an indicator of longevity and productivity in dairy heifers, the AMH level is an important marker for predicting herd longevity [14]. AMH can also be used as a marker for selecting good oocyte donors, even in the case of 2-4 month old calves [4].

The plasma AMH concentration is also a marker of ovarian activity and the capacity to produce a high number of embryos in cattle [21]. Blood AMH level is of great value for ascertaining an animal's individual potential to produce transferable (viable) embryos [20] and for recognizing suboptimal fertility caused by low ovarian reserve when the AMH level is low [13]. The ideal time to measure AMH levels in order to select the best cows for embryo production is at oestrus and after the 12th day of the oestrous cycle [28]. AMH level can also be used to identify cows with good superovulatory response when used in combination with ultrasonographic evaluation of the ovaries and polymorphism analysis in the gene encoding the ionotropic glutamate receptor (alpha-amino--3-hydroxy-5-methyl-4-isoxazole propionic acid subunit 1 (AMPA1)). Single nucleotide polymorphism (substitution of adenine by guanine at amino acid residue 306 in exon 7 of the AMPA1 gene) replaces serine with asparagine. This amino acid substitution increases the number of ovulations by modulating the release of luteinizing hormone (LH), due to changes in the affinity of the receptor for glutamate. Donor cows homozygous for the guanine-containing allele at the AMPA1 locus showed high AMH level and were most responsive to superovulation [11].

The enzyme-linked immunosorbent assay (ELISA) has been shown to accurately measure AMH levels and potential AMH cut-off values for cows that respond poorly to hormonal stimulation. Gonadotrophin-treated cows producing fewer than 15 large follicles with an AMH level below 87 pg/mL at oestrus, as well as those with less than 74 pg/mL AMH and less than 10 embryos in MOET, can be excluded [29]. In contrast, Vernunft et al. [34] do not recommend a boundary value for the AMH level to identify animals suitable for retrieval of good quality oocytes, but suggest that plasma AMH levels can be used to identify animals as good oocyte and embryo donors. Furthermore, the development of a BOC (bovine-ovine-caprine) ELISA protocol paved the way to better efficiency than the Active Mullerian-inhibiting substance/AMH ELISA in determining AMH levels in ruminants [1].

The amounts of AMH in natural and synchronized oestrous cycles have been found to be highly correlated within individual heifers. AMH levels varied among beef heifers, exhibiting high concentrations of AMH compared with dairy females [25]. Moreover, *Bos indicus* breeds have been found to have higher plasma AMH levels than *Bos taurus* heifers [3]. Jerseys have higher AMH concentrations than Holstein-Jersey crossbreds, and the crossbreds have higher AMH levels than Holsteins [26]. Both Murrah buffaloes and Holsteins have lower AMH concentrations than Gyr cattle [2]. Furthermore, the AMH concentration in the follicular fluid of buffaloes (*Bubalus bubalis*) has been shown to be positively correlated with the antral follicular count (AFC). Good

donors (≥12 follicles) can be identified by having higher AMH levels than poor donors (<12 follicles) [19]. Korean Hanwoo cows with high (≥0.25 ng/ml) and intermediate (0.1≥ to <0.25 ng/ml) AMH levels had a significantly higher AFC than those with low (<0.1 ng/ml) AMH values [9]. Stojsin-Carter et al. [32] recommend taking into account the genetic background of animals when evaluating AMH values in terms of reproductive performance.

AMH as an endocrine marker of embryo production in sheep and goats

In sheep, AMH does not affect the rate of primordial follicle recruitment but regulates the rate at which follicles progress through the gonadotropin-responsive phase. AMH expression is negatively influenced by aromatase, which exerts an inhibitory effect on AMH by modulating the response of the theca to luteinizing hormone (LH) and of granulosa cells to FSH [6]. In goats, plasma AMH levels are highly correlated with the number of collected, transferable, and freezable embryos. Follicles 1-5 mm in diameter are the major contributors of circulating AMH and can be used to predict the donor's ability to produce high numbers of high-quality embryos. Accurate predictions can be made from a single AMH measurement during either the breeding or anoestrus season [22].

In young lambs, AMH levels were about 3 to 4 times higher in ovulating females than in non-ovulating ones. A value of 97 pg/mL AMH has been suggested as the best cut-off point to predict sheep fertility. Female lambs have the ability to respond to equine chorionic gonadotropin (eCG) stimulation, and a single AMH measurement as early as 3.6 months of age may be helpful in selecting replacement ewes with the best predicted fertility [15]. In 40-day-old lambs, there was a positive correlation between AFC, plasma AMH levels, total follicle number and the number of large follicles (≥3 mm) obtained after exogenous FSH administration. A high AFC can also predict oocyte quality, which reflects the ovarian potential for follicular development [33]. In adult sheep, AMH measurements provide a good estimate of the ovarian response to FSH stimulation. It has been demonstrated that for each 100 pg/mL increase in AMH concentration, an average of 5.1 extra follicles and 2.7 extra cumulus-oocyte complexes (COC) can be expected [16].

Conclusions

In cattle, blood AMH levels can be used as a reliable endocrine marker of gonadotropin--responsive follicles and is of great value in ascertaining an animal's individual potential to produce transferable (viable) embryos. The AMH concentration can also be utilized as a good marker for choosing oocyte donors, as early as 2-4 months of age. The optimal time to measure AMH levels in order to select the best cows for embryo production is at oestrus and after the 12th day of the oestrous cycle, while the identification of animals with good superovulatory response can be performed in combination with USG examination of ovaries and by animal genotyping at the AMPA1 locus (homozygous females are preferred). BOC ELISA is more efficient than Active Mullerian-inhibiting substance/

AMH ELISA in determining AMH levels in ruminants. Good oocyte donors can be identified by higher AMH levels and by higher AFC. In sheep and goats, a single blood measurement of AMH during either the breeding or anoestrus season can predict an animal's capacity to produce high or low numbers of high-quality embryos.

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