

Review article

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

Salvador S. Soquila^{1,3#}, Claro N. Mingala^{2,3}

¹College of Agriculture and Veterinary Medicine, Ramon Magsaysay Technological University, San Marcelino Campus, San Marcelino 2207, Zambales, Philippines

²Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz 3120, Nueva Ecija, Philippines

³Department of Animal Science, College of Agriculture, Central Luzon State University, Science City of Muñoz 3120, Nueva Ecija, Philippines; #e-mail: buddysoq@gmail.com

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 (TGF β) [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 ($0.1 \geq$ to < 0.25 ng/ml) AMH levels had a significantly higher AFC than those with low (< 0.1 ng/ml) AMH values [9]. Stojisin-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.

REFERENCES

1. AROUCHE N., PICARD J.Y., MONNIAUX D., JAMIN S.P., VIGIER B., JOSSO N., CATE R.L., DI CLEMENTE N., TAIEB J., 2015 – The BOC ELISA, a ruminant-specific AMH immunoassay, improves the determination of plasma AMH concentration and its correlation with embryo production in cattle. *Theriogenology* 84 (8), 1397-1404.
2. BALDRIGHI J.M., SÁ FILHO M.F., BATISTA E.O.S., LOPES R.N.V.R., VISINTIN J.A., BARUSELLI P.S., ASSUMPÇÃO M.E.O.A., 2014 – Anti-Mullerian hormone concentration and antral ovarian follicle population in Murrah heifers compared to Holstein and Gyr kept under the same management. *Reproduction in Domestic Animals* 49 (6), 1015-1020.
3. BATISTA E.O.S., MACEDO G.G., SALA R.V., ORTOLAN M.D.D.V., SÁ FILHO M.F., DEL VALLE T.A., JESUS E.F., LOPES R.N.V.R., RENNÓ F.P., BARUSELLI P.S., 2014 – Plasma antimullerian hormone as a predictor of ovarian antral follicular population in *Bos indicus* (Nelore) and *Bos taurus* (Holstein) heifers. *Reproduction in Domestic Animals* 49 (3), 448-452.
4. BATISTA E.O.S., GUERREIRO B.M., FREITAS B.G., SILVA J.C.B., VIEIRA L.M., FERREIRA R.M., REZENDE R.G., BASSO A.C., LOPES R.N.V.R., RENNÓ F.P., SOUZA A.H., BARUSELLI P.S., 2016 – Plasma anti-Müllerian hormone as a predictive endocrine marker to select *Bos taurus* (Holstein) and *Bos indicus* (Nelore) calves for in vitro embryo production. *Domestic Animal Endocrinology* 54 (Jan), 1-9.
5. BÉZARD J., VIGIER B., TRAN D., MAULÉON P., JOSSO N., 1987 – Immunocytochemical study of anti-Müllerian hormone in sheep ovarian follicles during fetal and post-natal development. *Journal of Reproduction and Fertility* 80 (2), 509-516.
6. CAMPBELL B.K., CLINTON M., WEBB R., 2012 – The role of anti-Müllerian hormone (AMH) during follicle development in a monovulatory species (sheep). *Endocrinology* 153 (9), 4533-4543.
7. CRISOSTO N., SIR-PETERMANN T., GREINER M., MALIQUEO M., MORENO M., AEDO P., LARA H.E., 2009 – Testosterone-induced downregulation of anti-Müllerian hormone expression in granulosa cells from small bovine follicles. *Endocrine* 36 (2), 339-345.
8. CUSHMAN R.A., MCDANELD T.G., KUEHN L.A., SNELLING W.M., NONNEMAN D., 2014 – Incorporation of genetic technologies associated with applied reproductive technologies to enhance world food production. *Advances in Experimental Medicine and Biology* 752, 77-96.
9. GHANEM N., JIN J.I., KIM S.S., CHOI B.H., LEE K.L., HA A.N., SONG S.H., KONG I.K., 2016 – The Anti-Müllerian Hormone Profile is Linked with the In Vitro Embryo Production Capacity and Embryo Viability after Transfer but Cannot Predict Pregnancy Outcome. *Reproduction in Domestic Animals* 51 (2), 301-310.

10. GUERREIRO B.M., BATISTA E.O.S., VIEIRA L.M., SÁ FILHO M.F., RODRIGUES C.A., CASTRO NETTO A., SILVEIRA C.R.A., BAYEUX B.M., DIAS E.A.R., MONTEIRO F.M., ACCORSI M., LOPES R.N.V.R., BARUSELLI P.S., 2014 – Plasma anti-Müllerian hormone: an endocrine marker for in vitro embryo production from *Bos taurus* and *Bos indicus* donors. *Domestic Animal Endocrinology* 49, 96-104.
11. HIRAYAMA H., KAGEYAMA S., NAITO A., FUKUDA S., FUJII T., MINAMIHASHI A., 2012 – Prediction of superovulatory response in Japanese Black cattle using ultrasound, plasma anti-Müllerian hormone concentrations and polymorphism in the ionotropic glutamate receptor AMPA1/GRIA1. *The Journal of Reproduction and Development* 58 (3), 380-383.
12. IRELAND J.L.H., SCHEETZ D., JIMENEZ-KRASSEL F., THEMME A.P.N., WARD F., LONERGAN P., SMITH G.W., PEREZ G.I., EVANS A.C.D., IRELAND J.J., 2008 – Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biology of Reproduction* 79 (6), 1219-1225.
13. IRELAND J.J., SMITH G.W., SCHEETZ D., JIMENEZ-KRASSEL F., FOLGER J.K., IRELAND J.L.H., MOSSA F., LONERGAN P., EVANS A.C.O., 2011 – Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reproduction, Fertility, and Development* 23 (1), 1-14.
14. JIMENEZ-KRASSEL F., SCHEETZ D.M., NEUDER L.M., IRELAND J.L.H., PURSLEY J.R., SMITH G.W., TEMPELMAN R.J., FERRIS T., ROUDEBUSH W.E., MOSSA F., LONERGAN P., EVANS A.C.O., IRELAND J.J., 2015 – Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life. *Journal of Dairy Science* 98 (5), 3036-3045.
15. LAHOZ B., ALABART J.L., MONNIAUX D., MERMILLOD P., FOLCH J., 2012 – Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. *BMC Veterinary Research* 23; 8, 118.
16. LAHOZ B., ALABART J.L., COCERO M.J., MONNIAUX D., ECHEGOYEN E., SÁNCHEZ P., FOLCH J., 2014 – Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of BMP15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. *Theriogenology* 81 (2), 347-357.
17. LANE A.H., DONAHOE P.K., 1998 – New insights into mullerian inhibiting substance and its mechanism of action. *The Journal of Endocrinology* 158 (1), 1-6.
18. LEGEAI L., VIGIER B., TRAN D., PICARD J.Y., JOSSO N., 1986 – Monoclonal antibodies raised against bovine anti-Müllerian hormone: bovine, ovine, and caprine hormones share a set of identical epitopes. *Biology of Reproduction* 35 (5), 1217-1225.
19. LIANG A., SALZANO A., D'ESPOSITO M., COMIN A., MONTILLO M., YANG L., CAMPANILE G., GASPARRINI B., 2016 – Anti-Müllerian hormone (AMH) concentration in follicular fluid and mRNA expression of AMH receptor type II and LH receptor in granulosa cells as predictive markers of good buffalo (*Bubalus bubalis*) donors. *Theriogenology* 86 (4), 963-970.
20. MONNIAUX D., BARBEY S., RICO C., FABRE S., GALLARD Y., LARROQUE H., 2010 – Anti-Müllerian hormone: a predictive marker of embryo production in cattle? *Reproduction, Fertility, and Development* 22 (7), 1083-1091.

21. MONNIAUX D., RICO C., LARROQUE H., DALBIÈS-TRAN R., MÉDIGUE C., CLÉMENT F., FABRE S., 2010 – Anti-Müllerian hormone, an endocrine predictor of the response to ovarian stimulation in the bovine species. [Article in French]. *Gynécologie, Obstétrique & Fertilité* 38 (7-8), 465-470.
22. MONNIAUX D., BARIL G., LAINE A.L., JARRIER P., POULIN N., COGNIÉ J., FABRE S., 2011 – Anti-Mullerian hormone as a predictive endocrine marker for embryo production in the goat. *Reproduction* 142 (6), 845-854.
23. MONNIAUX D., DROUILHET L., RICO C., ESTIENNE A., JARRIER P., TOUZÉ J.L., SAPA J., PHOCAS F., DUPONT J., DALBIÈS-TRAN R., FABRE S., 2012 – Regulation of anti-Müllerian hormone production in domestic animals. *Reproduction, Fertility, and Development* 25 (1), 1-16.
24. PARAMIO M.T., IZQUIERDO D., 2014 – Current status of in vitro embryo production in sheep and goats. *Reproduction in Domestic Animals* 49, Suppl. 4, 37-48.
25. PFEIFFER K.E., JURY L.J., LARSON J.E., 2014 – Determination of anti-Müllerian hormone at estrus during a synchronized and a natural bovine estrous cycle. *Domestic Animal Endocrinology* 46, 58-64.
26. RIBEIRO E.S., BISINOTTO R.S., LIMA F.S., GRECO L.F., MORRISON A., KUMAR A., THATCHER W.W., SANTOS J.E.P., 2014 – Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. *Journal of Dairy Science* 97 (11), 6888-6900.
27. RICO C., FABRE S., MÉDIGUE C., DI CLEMENTE N., CLÉMENT F., BONTOUX M., TOUZÉ J.L., DUPONT M., BRIANT E., RÉMY B., BECKERS J.F., MONNIAUX D., 2009 – Anti-Mullerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. *Biology of Reproduction* 80 (1), 50-59.
28. RICO C., MÉDIGUE C., FABRE S., JARRIER P., BONTOUX M., CLÉMENT F., MONNIAUX D., 2011 – Regulation of anti-Müllerian hormone production in the cow: a multiscale study at endocrine, ovarian, follicular, and granulosa cell levels. *Biology of Reproduction* 84 (3), 560-571.
29. RICO C., DROUILHET L., SALVETTI P., DALBIÈS-TRAN R., JARRIER P., TOUZÉ J.L., PILLET E., PONSART C., FABRE S., MONNIAUX D., 2012 – Determination of anti-Müllerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: from the laboratory to the farm. *Reproduction, Fertility, and Development* 24 (7), 932-944.
30. ROCHA R.M.P., LIMA L.F., CARVALHO A.A., CHAVES R.N., BERNUCI M.P., ROSA-E-SILVA A.C.J.S., RODRIGUES A.P.R., CAMPELLO C.C., FIGUEIREDO J.R., 2016 – Immunolocalization of the Anti-Müllerian Hormone (AMH) in Caprine Follicles and the Effects of AMH on In Vitro Culture of Caprine Pre-antral Follicles Enclosed in Ovarian Tissue. *Reproduction in Domestic Animals* 51 (2), 212-219.
31. SOUZA A.H., CARVALHO P.D., ROZNER A.E., VIEIRA L.M., HACKBART K.S., BENDER R.W., DRESCH A.R., VERSTEGEN J.P., SHAVER R.D., WILTBANK M.C., 2015 – Relationship between circulating anti-Müllerian hormone (AMH) and superovulatory response of high-producing dairy cows. *Journal of Dairy Science* 98 (1), 169-178.
32. STOJSIN-CARTER A., MAHBOUBI K., COSTA N.N., GILLIS D.J., CARTER T.F., NEAL M.S., MIRANDA M.S., OHASHI O.M., FAVETTA L.A., KING W.A., 2016 – Systemic and local anti-Mullerian hormone reflects differences in the reproduction potential of Zebu and European type cattle. *Animal Reproduction Science* 167, 51-58.

33. TORRES-ROVIRA L., GONZALEZ-BULNES A., SUCCU S., SPEZZIGU A., MANCA M.E., LEONI G.G., SANNA M., PIRINO S., GALLUS M., NAITANA S., BERLINGUER F., 2014 – Predictive value of antral follicle count and anti-Müllerian hormone for follicle and oocyte developmental competence during the early prepubertal period in a sheep model. *Reproduction, Fertility, and Development* 26 (8), 1094-1106.
34. VERNUNFT A., SCHWERHOFF M., VIERGUTZ T., DIEDERICH M., KUWER A., 2015 – Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. *The Journal of Reproduction and Development* 61 (1), 74-79.