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Anti-Müllerian Hormone and Its Utility in Cattle Reproduction

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Abstract

Reproductive biotechnologies offer us greater possibilities to improve animal genetics. However, the success of these depends on different factors such as the proper selection of the donor female. For this reason, endocrine markers have been used to evaluate the ovarian reserve, which allow a successful selection of donors. Recent research has shown, among other things, that concentrations greater than 0.130 ng/mL of anti-Mullerian hormone (also known as Muller-inhibiting substance, which is a member of the transforming growth factor beta superfamily of growth and differentiation factors) are related to donors of more than fifteen transferable embryos. Therefore, this review describes studies showing that the measurement of anti-Müllerian hormone concentrations, before superovulation programs, reduces the costs per embryo produced.

Keywords

AMH, Anti-Müllerian Hormone, Reproductive Techniques, Follicular Population, Granulosa Cells, Superovulation

1. Introduction

Various endocrine markers have been used to evaluate ovarian reserve. Determinations of these levels are essential for the safe and successful selection of donors [1]. As a result, the expression pattern in the granulosa cells of the growing follicles and the role of anti-Mülerian hormone (AMH) in the regulation of fol-

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liculogenesis and steroidogenesis make this hormone an ideal marker for estimating the size of ovarian follicle groups. Furthermore, AMH has been shown to be a reliable endocrine marker of the quality of human oocytes and embryos [2]. The ability of donor cows to produce embryos is shown as a repeatable and hereditary trait (comparing mothers and daughters, there is a 16% variance in embryo production and a 23% variance in corpora lutea production) in protocols of multiple ovulation and embryo transfer (ET) [3] [4] [5].

AMH is well known for inhibiting the development of the Müllerian duct in male embryos during sexual differentiation. Individual gonads differentiate into testicles due to the SRY gene on chromosome Y. In the ovary, AMH expression occurs in the granulosa cells of growing small antral follicles. Its expression is higher in the granulosa cells (GC) of small pre-antral follicles and decreases during terminal follicular growth [5] [6]. AMH, or Müllerian-inhibiting substance, is a 140-kDa glycoprotein of the growth factor beta family (TGF-β, transforming growth factor β) [7].

AMH is not expressed during female sexual differentiation. However, in human fetuses after week 36 of pregnancy, it is expressed in the GC of the recruited primordial follicles that are the primary follicles observed for the first time in the ovaries [8]. It regulates follicle-stimulating hormone and thus limits follicular function [9] [10]. Moreover, AMH is a key mediator in steroidogenesis regulation because it inhibits estradiol secretion by reducing the aromatase enzyme CYP19's expression [11] and the production of progesterone in GC *in vitro*. It is also negatively associated with the expression of luteinizing hormone receptors in small human antral follicles [12].

In humans, circulating concentrations of AMH have been shown to be the most informative serum marker of the ovarian follicle reserve. The physiological function of AMH in adult women includes the regulation of early follicular growth and, as a result, the recruitment control of an excessive number of follicles in the growing follicular group [13] [14] [15].

2. Background

AMH signaling. Signaling of the TGF-β family members occurs via two transmembrane serine/threonine kinase receptors known as type I and type II receptors. After the ligand binds to its type II receptor, the type I receptor is recruited to form a hetero-tetramer receptor complex [16]. The activation of the type I receptor via transphosphorylation by the type II receptor leads to signaling via SMAD proteins. To date, the cloning of the type II receptor for AMH (AMHRII) has been achieved in rats and rabbits, which has aided in the discovery of new information about these signaling processes [17] [18].

AMHRII mRNA expression was observed in mesenchymal cells surrounding Müllerian ducts as well as in fetal and adult gonads collected from both sexes. AMHRII is essential for signaling, as evidenced by the lack of Müllerian duct in male mice with a deficiency of this receptor [19] [20].

AMH in animal production. The profitability of the cattle industry is highly

related to the output of meat and milk, genetic selection, and reproductive efficiency. Reproductive technologies, such as *in vitro* embryo production (IVP), are used worldwide to rapidly improve cattle genetics across lineages. However, the efficiency of this technique is affected by the wide range of donor responses to IVP procedures [21] [22] [23] [24].

Because the production of new calves is dependent on the female's ability to produce oocytes and later maintain the embryo, it is one of the key factors reducing its efficiency. As a result, the development of new diagnostic techniques to detect females with reduced fertility could represent a major advancement in the cattle industry. Nevertheless, establishing the factors affecting fertility becomes a complex task because of the complexity of existing interactions and the difficulty of quantifying and rating female gonads [25].

Therefore, ovarian reserve has been defined as the number of viable oocytes in an ovary. A decrease in these oocytes has a direct effect on ovarian function and the concentration of gonadotropins and progesterone, which can directly affect fertility. Although the follicular population of the ovary can be quantified using ultrasound, the presence of high concentrations of AMH is positively related to the ovarian reserve, making the reproductive assessment of the animal easier. The prenatal period is a critical moment in the development of this ovarian reserve; it has been observed that the sub nutritional status of pregnant females can increase maternal testosterone and, as a result, produce altered off-spring. These offspring have a lower ovarian reserve, fewer antral follicles in the ovaries, and a lower concentration of AMH [26].

To use AMH in a practical way for assessing reproductive females in cattle operations, it is necessary to understand which other factors can affect the aforementioned hormonal concentration. The breed and age of animals have been identified as factors related to AMH concentration. However, there have been concerns raised about the interference of synchronization protocols (routinely applied in many operations) in the aforementioned concentration.

3. Purpose

Use of reproductive techniques. In the case of reproductive techniques such as ET, the development of a method to determine the potential of a donor cow to produce an expected number of embryos could be based on the measurement of circulating AMH concentrations [27].

The variability in the super-ovulatory response and the number of good quality embryos among donors is an issue in cattle ET programs [28] [29] [30]. Over the last 30 years, commercial cattle ETs have grown into a large international business in genetic improvement programs. "Multiple ovulation and ET" technology is now widely used in cattle breeding worldwide to increase the number of offspring produced by genetically superior females [31].

Large-scale commercial programs for *in vitro* embryos of beef and dairy cattle are ongoing and expanding worldwide. Ovum pick-up (OPU) followed by *in vitro* production (OPU-IVP) of cattle embryos has enabled the production of large

quantities of transferable products from embryos of selected females [31].

Many factors influence OPU-IVP efficiency. However, key factors generally include the donor status, the intervals between OPU sessions, the quality of oocytes, and the techniques used to grow embryos from the zygote to the blastocyst stage. The quality of oocytes and embryos produced by OPU-IVP is a crucial element in determining the efficiency of this program. Furthermore, the number of blastocysts produced by OPU-IVP is proportional to the number of retrieved oocytes [31] [32].

Therefore, a substantial improvement in OPU-IVP efficiency can be attained by selecting donors with a high antral follicle count [33] [34]. It is also possible to improve the conditions of cumulus-oocyte complexes and their *in vitro* maturation as well as the growth of embryos [35].

The number of antral follicles is mainly determined by ovarian reserve, which is defined as an estimate of the ovarian follicles capable of being ovulated and producing oocytes that can be fertilized and, thus, result in a healthy, successful pregnancy [36].

The AMH is a reliable endocrine marker of ovarian reserve. It is produced by the GC of pre-antral follicles. Although the number of ovarian antral follicles varies among females, it is highly repeatable among individuals [37]. Circulating AMH concentrations before a superovulation treatment are strongly associated with the superovulatory response [38] (**Table 1**).

The AMH assessment could be used to identify cows with a higher response to superovulation, improving the efficiency of superovulation programs. Thus, measuring AMH concentrations before registering cows in superovulation programs would increase the number of produced embryos, reducing costs per produced embryo [39].

Several authors mention that AMH concentrations show little variation during estrous cycles and that measuring AMH concentration only once during the estrous cycle or measuring it on multiple occasions or during different estrous cycles does not show a significant difference [40], indicating that these values are highly constant in either naturally or artificially induced estrous cycles by the synchronization [41]. This indicates that AMH concentrations are reliable when analyzed through a blood sample on any day of the adult cow's estrous cycle.

Table 1. Superovulatory response of unweaned Holstein calves divided by quartiles of circulating anti-Müllerian hormone (adapted from Souza *et al.*, 2015).

	AMH1 quartiles			
	C1	C2	C3	C4
Animals	18	18	18	18
Circulating AMH (pg/mL)	44.9 ± 6.9	114.1 ± 3.3	155.6 ± 3.8	243.1± 14.3
Total oocytes/embryos retrieved	5.0 ± 1.2	5.5 ± 1.3	7.2 ± 1.2	14.0 ± 2.3

AMH quartiles 1: C1 = 0.01 at 82.6 pg/mL; C2 = 91.1 at 132.5 pg/mL; C3 = 135.3 at 183.8 pg/mL; C4 = 184.4 at 374.3 pg/mL.

These data are important from a zootechnical point of view given that some researchers have demonstrated the reliability of AMH concentrations and antral follicle count as excellent predictors of ovarian reserve, in cows of the same age, with no differences between European and Zebu breeds [42] [43] [44]. On the other hand, in heifers it has been observed that low AMH concentrations are manifested as a fertility rate below the expected after the first calving, with low conception rates, days open, a lengthening of the interval between calving's, and a greater number of semen doses per conception, Likewise, several investigations postulate AMH as a possible predictor of heifer herd retention, since heifers with low concentrations of this hormone were discarded due to reproductive problems compared to heifers with higher levels of AMH [45].

Although reproductive parameters in bovine females are known to be poorly heritable AMH may be a suitable candidate to correlate fertility in dairy cattle which could contribute to improve genetic and reproductive performance in cattle, as shown by some researchers, who through genomic heritability estimates of this hormone (AMH) in 2905 dairy Holstein females estimated a heritability of 0.36 ± 0.03 through SNP's by collecting their pedigree information from 4 generations [46].

On the other hand Gobikrushanth *et al.*; also performed this same AMH estimation in 198 Canadian Holstein cows obtaining a heritability index of 0.46 ± 0.07, *i.e.* these estimates both genomic and pedigree based are high compared to some other genetic trait associated to reproductive parameters in bovine females, in turn these heritability traits were contrasted with the heritability indices of antral follicle count which showed a 0.31 ± 0.14 which is moderately heritable [47].

These parameters together can be useful to determine the size of the ovarian reserve which can be an important factor in the selection of cattle to improve fertility in the herd, however it is advisable to consider that these heritability indices at least in the case of AMH was not observed a positive correlation with important economic parameters such as milk production [45], and in the case of heritability for antral follicle count (AFC) a negative correlation was observed with respect to milk fat concentration [10].

These findings suggest that circulating AMH levels are good indicators of ovarian reserve, can be considered as a biomarker of fertility, and in turn improve reproductive schemes in female cattle. As long as factors such as the environment, the presence of infections such as mastitis, and feeding are taken into account [25] [48].

It is currently known that approximately 30% of bovine females are responsible for the majority of embryos (70%) produced through superovulation programs, therefore having the ability to select a greater number of cows with AMH concentrations could improve embryo production from superior genetics [49] [50].

In turn Souza *et al*, suggest a concentration of 0.130 ng/ml of circulating AMH as a cut-off point to consider the cow as a possible embryo donor with

more than 15 of them transferable [10], however, this does not guarantee the success of fertilization since the morphological quality of the embryo must be considered as this can vary depending on the seminal quality and concentration, the insemination technique, the moment of insemination and the heritability index of the sire's fertility. In the case of the female, superovulation programs can alter follicular development, ovulation, oocyte maturation, and sperm transport [51].

Since AMH is produced by the granulosa cells of antral follicles, it has been theorized that circulating AMH levels may indicate AFP and thus the animal's hyper stimulatory response. This theory has been proven correct, showing that AMH can replace AFP as an accurate and easy-to-use technique for measuring hyper stimulus responses in cattle [52].

4. Conclusions

Currently, AMH has become an important topic among cattlemen due to its ability to make predictions about ovarian reserve pool. Due to its static nature, single-dose blood collection is becoming increasingly used in the assisted reproduction techniques field. In contrast to count follicles antral, the strong genomic and pedigree-based heritability of AMH supports its use as a reproductive biomarker of fertility. The AMH levels in the periphery have become brochures representative of the ovarian reserve and are now a promising marker of fertility in companion animals and a diagnostic marker of ovarian disease. Relations with fertility parameters, as it is Breed, age, lifespan, fertility and ovarian reserve (for heifers), need larger confidence intervals in larger herds. The effects of control factors such as disease and diet, hormones, and reduced responses of count follicles antral on bovine and goat granulosa cells open new avenues for future research. In many livestock species, the association of AMH with superovulation and infertility remains not fully established.

The development of diagnostic techniques that allow for the assessment of the ovarian reserve and knowledge of reproductive potential in animals of high genetic value could make a huge difference in animal selection. AMH can be suggested as a reliable marker of ovarian activity and a potential predictor of the response to a superovulation treatment because there is a strong relationship between AMH endocrine levels and the number of follicles responding to gonadotropin in cow ovaries.

AMH concentrations in plasma may be able to predict the number of follicles because cows with higher plasma concentrations have a higher follicular count and better responses to super-ovulatory treatments than those with low levels. Using this tool, we can identify animals with reproductive potential at an early age. Furthermore, it is a noninvasive method for quantifying the number of follicles and oocytes. Reproductive programs, such as ETs, could benefit from measuring AMH in plasma before beginning treatment, allowing animals with low response to be ruled out.

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Author Contributions

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, GAAJ; MDG, UCV; SDA, methodology, UCV; SDA.; validation, GAAJ; MDG, UCV; SDA; investigation, MAAJ; UCV, SDA.; resources, MDG; UCV writing original draft preparation GAAJ; MDG, UCV; SDA.; writing review and editing, UCV; SDA. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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