Study of anti-Müllerian hormone in predicting embryo productivity of donor cows

Anna Kozitsyna[1], and *George* Nikitin[1*]

[1] Federal State Budgetary Educational Institution of Higher Education "Saint Petersburg State University of Veterinary Medicine", 5, st. Chernigovskaya, Saint Petersburg, 196084, Russia

Abstract. The study included a study of biochemical parameters at different stages of the productive cycle. Ultrasound images at different stages of the cycle, and during superovulation were analyzed. A high positive correlation was found between AMH, urea, and creatinine levels. A correlation was also found between AMH and calcium, and phosphorus levels. The effect of domestic follicle-stimulating medicine for inducing superovulation was investigated. Criteria for determining cow embryo donors by ultrasound and biochemical analysis were identified. The AMH level did not depend on the phase of the reproductive cycle. This allows using AMH as a universal marker without taking into account the phase. During superovulation, the maximum number of corpora lutea oincided with the AMH level peaks. Disadvantages of domestic follicle-stimulating medicine in comparison with foreign analogue were discovered.

1 Introduction

Anti-Müllerian hormone (AMH) is a glycoprotein that plays a key role in the regulation of the reproductive system in animals, including mammals. Its level in blood serum directly correlates with the development of the ovaries and testicles, which, in turn, affects the productivity of animals. Research shows that high levels of AMH in females are associated with increased fertility and the number of eggs available, which can significantly improve herd productivity.

In males, AMH helps suppress the formation of fallopian tubes and the development of secondary sexual characteristics. However, its role is not limited only to reproduction. AMH also influences metabolic processes and general health, which can affect the growth and development of animals. For example, cows with high levels of AMH not only have better fertility but also higher milk production.

Thus, the relationship between anti-Mullerian hormone and performance in animals is a complex mechanism in which hormones, ecology, and genetic factors interact.

Studying AMH is an important aspect in predicting the embryo productivity of donor cows [1-3]. AMH, produced by ovarian granulosa cells, is considered a reliable marker of ovarian reserve. High levels of this hormone may indicate a large number of follicles and, as a result, the potential to create more embryos.

* Corresponding author: nir@spbguvm.ru

© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

In this context, AMH testing allows not only to assess the reproductive potential of cows but also to optimize the selection and artificial insemination processes [1, 3]. Understanding the relationship between AMH levels and embryo productivity opens up new horizons in the fields of reproductive biology and veterinary medicine.

Clinical trials show that analyzing AMH levels in combination with other reproductive indicators, such as ovulation rate and embryo quality, can significantly improve the efficiency of embryo technology programs. As a result, the practical application of AMH knowledge can contribute to improving the overall productivity of the herd, which is important for animal husbandry as a whole [4-5].

The purpose of this study was to gain an in-depth understanding of the factors influencing fertility, which can help optimize reproductive management practices in livestock breeding.

2 Materials and methods

For the study, 12 cows were selected, whose anamnesis was studied, an ultrasonographic examination of the reproductive system was performed, and blood was collected for clinical and biochemical studies. During the diagnostic process, special attention was paid to identifying abnormalities in the functional state of the ovaries and uterus, as well as possible signs of infectious diseases. Ultrasonography allowed not only to accurately assess the morphological state of the reproductive organs but also to determine the presence of ovulatory follicles and corpora lutea [6-7].

For the clinical study, standard tests were performed: assessment of general condition, measurement of body temperature, and examination of the skin. Biochemical blood tests focused on the hormone levels and major metabolites such as glucose, total protein, and liver enzyme activity [8].

Biochemical parameters of blood serum in the luteal and follicular phases of the reproductive cycle were studied in selected experimental cows. The concentration of the following reproductive hormones was also determined in the blood serum of the experimental animals: estradiol, testosterone, progesterone, and anti-Müllerian hormone (AMH). To induce the reproductive cycle, all experimental animals were previously injected with 2.0 ml of the medicine “Magestrophan” 14 days before the study. As a result of studying the biochemical profile of experimental animals in the luteal and follicular phases of the reproductive cycle, correlations were calculated using the method of determining the linear Pearson correlation between the concentration of AMH, other biochemical parameters, and reproductive hormones.

After clinical examination the experimental group animals were subjected to hormonal treatment to achieve polyovulation. The drug administration regimen was as follows: day 0: intravaginal administration of progesterone with an Controlled Internal Drug Release (CIDR) device; 4 days: 2 ml of a medicine based on GnRH was administered. From the 6th to the 8th day, follicle-stimulating and luteinizing hormone was administered as part of the FSH-super medicine in a volume of 3.0 ml, 2.5 ml, 2.0 ml, and 1.5 ml twice a day. The total dose was 1000 IU. On the 9th day (the last day of FSH administration), a prostaglandin analogue was administered and the progesterone administration device was removed. On the 11th day, all experimental animals came into heat and were inseminated with one dose twice to fertilize the oocytes produced during superovulation.

During the period of superovulation, morphometry of the ovaries was performed, and the dynamics of folliculogenesis was monitored.

The collected data was subjected to statistical analysis to identify patterns and relationships between the health of cows and their reproductive function.

3 Results

3.1 Biochemical correlations

The results of the clinical blood test are presented in Table 1.

Table 1. Results of the clinical blood test in cows.

No / Parameters	WBC, 10^9/L	RBC, 10^{12}/L	Haemoglobin, g/L	Platelets, 10^9/L	Leukogram, %								ESR, mm/h
					Basophils	Eosinophils	Neutrophils				Lymphocytes	Monocytes	
							Myelocytes	Young	Band	Segmented			
1/174867	4.5	3.9	93	295	0	1	0	0	5	44	45	5	0.5
2/475470	6.6	5.2	104	170	0	3	0	0	2	48	45	2	0.5
3/97218	7.0	5.7	99	210	0	5	0	0	1	37	54	4	0.7
4/31315	7.6	3.9	79	110	0	3	0	0	1	35	60	2	0.3
5/435012	7.4	5.4	120	180	0	5	0	0	1	35	57	2	0.3
6/391156	6.5	5.4	110	175	0	2	0	0	3	50	43	2	0.5
7/474572	10.4	5.2	99	150	0	1	0	0	1	60	37	1	0.5
8/456149	19.2	4.5	97	280	0	2	0	0	4	58	33	3	1.0
9/6840047	8.4	4.7	116	170	0	7	0	0	1	44	44	4	0.5
10/198171	8.9	4.8	109	160	0	4	0	0	4	49	42	1	0.5
11/946197	10.3	4.3	104	210	0	1	0	0	2	59	35	3	0.4
12/140223	6.8	5.1	109	240	0	4	0	0	1	46	46	3	0.5

According to the results of a clinical blood test in all experimental animals, the indicators were within the reference values, with the exception of a slight excess of the total number of leukocytes in animal No. 456149, which is probably associated with a local inflammatory process in the thigh area, namely: a localized abscess of medium size, which is not a strict criterion for exclusion from the experiment.

In the course of assessing the correlation parameters, the following dependencies were identified. A high direct correlation was found between the AMH level and the urea and urea nitrogen levels (0.89 and 0.89, respectively). A direct, significant correlation (0.63) was found between the AMH level and the serum creatinine level. Strong correlation dependencies were also found during the evaluation of mineral metabolism parameters: negative when comparing the AMH level with the calcium level (-0.71) and positive when comparing the AMH level with the phosphorus level (0.78). Based on the data obtained to date, the main correlation patterns have been identified between indicators of protein (urea, urea nitrogen) and mineral metabolism (calcium and phosphorus).

Similarly, correlations between biochemical parameters of blood serum, reproductive hormones, and AMH concentration in the follicular phase of the reproductive cycle were calculated. As a result, similar results were obtained, namely: a pronounced relationship was noted between the protein metabolism parameters: urea and urea nitrogen to AMH, which amounted to 0.73 units, respectively, and the mineral metabolism parameters: calcium and phosphorus to AMH, which amounted to -0.33 and 0.81 units, respectively. There was a pronounced correlation between creatinine and AMH in the follicular phase of

the reproductive cycle, which amounted to 0.85 units, and a less pronounced correlation of this indicator in the luteal phase of the reproductive cycle, which amounted to 0.63 units. Among other indicators, the concentrations of estradiol, progesterone, and testosterone did not confirm their significance, showing low correlation values in the range of -0.06 to -0.35 units in both study periods, and in all cases the relationship was negative. At the same time, the concentration of AMH itself in both the follicular and luteal phases of the reproductive cycle did not correlate with the period of its measurement and, conversely, did not allow for a reliable assessment of its average concentration in these groups due to a significant deviation from the average. Thus, the maximum AMH value in the luteal phase was 5766.00 ng/ml, and the minimum was 929.9 ng/ml. During the follicular phase, the maximum value reached 6432.00 ng/ml, and the minimum value was 838.9 ng/ml. Moreover, taking into account that the maximum AMH values were recorded in the same individuals both in the luteal and follicular phases, we can conclude that there is no dependence of the concentration of this hormone on the stage of folliculogenesis and the functioning of the ovaries. However, in animals in which the AMH concentration was close to the average values and lower, such conclusions are more difficult to draw, and the distribution of AMH across the group in the phases of the cycle showed itself to be less predictable.

3.2 Sonographic examination

Sonography, as an imaging method, plays a key role in veterinary obstetrics and gynecology, providing unique opportunities for diagnosing and monitoring the condition of the reproductive system of animals. It allows you to assess the condition of the fetuses, identify possible anomalies, and assess the health of the pregnant female. The use of ultrasound technology significantly simplifies the process of determining the stages of pregnancy and diagnosing various diseases, such as tumors or infectious processes.

In veterinary practice, sonography makes it possible to minimize the invasiveness of the study, which is especially important for pregnant animals. This not only reduces the risk to the mother and fetus but also allows the doctor to get a complete picture of the state of health without the need to resort to surgical interventions. Thanks to developed algorithms for analyzing ultrasound images, veterinary specialists are able to more accurately assess the age of the fetus, its development, and identify pathologies in the early stages.

Thus, the role of sonography in veterinary obstetrics and gynecology cannot be overestimated: it helps to improve diagnosis, increase the effectiveness of treatment, and, ultimately, protect the health of animals.

During a sonographic examination of the ovaries in cows, a classic picture of their functioning was observed. At the beginning of the study, the majority of animals were in the luteal phase of the reproductive cycle, which is confirmed by elevated values of progesterone in the blood serum, which ranged from 2.47 ng/ml to 19.68 ng/ml. The number of animals with the luteal phase of the reproductive cycle amounted to 83.33% of the sample, which corresponds to the physiological norm due to the duration of the luteal phase being about 80% of the entire reproductive cycle. In these animals, the sonogram recorded the presence of a corpus luteum on one of the ovaries.

Also during the study, the longitudinal section area of the ovaries of the experimental animals was recorded, which varied from 621 mm2 to 1120 mm2. The diameter of the dominant follicle in the current follicular wave ranged from 9.9 to 23.7 mm. It was not possible to establish a pronounced diagnostic value of the ovarian examination in determining the relationship with the concentration of reproductive hormones, AMH, and other parameters at the beginning of the experiment. However, subsequent data during the period of treatment of animals with follicle-stimulating hormone and at the time of embryo

washout (the results of measuring folliculogenesis are confirmed by the number of corpus luteum obtained during superovulation) show a significant trend. When calculating the total number of corpora lutea on both ovaries, it was found that the maximum result in the sample was 10 and 14 pieces, which corresponds to the maximum concentration of AMH in the sample, 1657 ng/ml and 6432 ng/ml, respectively. In other experimental animals, the average number of corpora lutea was 3-4, and the AMH concentration was in the range from 838.9 ng/ml to 1064 ng/ml.

4 Discussion

Anti-Müllerian hormone (AMH) is an important protein that plays a key role in the development of the reproductive system in animals [4, 6]. The first mention of its existence dates back to studies conducted in the mid-20th century, when scientists began to study its effects on sexual differentiation. AMH is produced by the Sertoli cells in the male testes and is responsible for the regression of the Müllerian ducts, which can develop into the uterus in embryos and the fallopian tubes in females [1-3, 9].

Over time, research on AMH has expanded to include different animal species. In mammals such as pigs, cows, and primates, AMH levels have been shown to correlate with reproductive status and health [10]. In the 1990s, scientists began using AMH as a biomarker to assess fertility in animals, which opened up new horizons in veterinary medicine and animal husbandry [9-10]. These studies demonstrated the potential application of AMH in selective breeding and conservation of rare species, which emphasizes the importance of this hormone in modern zoology and medicine.

AMH and urea levels in cows may correlate due to their connection with ovarian function. AMH is produced by the ovaries and plays a role in regulating follicular growth [11]. Its level may be associated with the reproductive function of cows and their ability to produce eggs, characterizing the ovarian reserve. Urea, on the other hand, is an end product of nitrogen metabolism and also an important indicator of protein metabolism in ruminants [2]. Thus, if AMH and urea levels correlate, this may indicate an increase in metabolic processes associated with protein metabolism and amino acid conversion reactions with a more significant degree of ovulation. Calcium and phosphorus are essential macronutrients necessary for the growth, development, and maintenance of healthy bones, muscles, the nervous system, and other organs [8, 10].

The correlation between the level of AMH and the content of calcium and phosphorus may indicate a relationship between the activation of active follicular growth processes associated with phosphorylation reactions and the general state of mineral metabolism, since ovulation and subsequent cascades of physiological reactions are an energy-consuming process involving, among other things, electrolyte body composition [12, 13]. The regulation of the synthesis of AMH itself and its metabolic effects have been little studied; there is only a limited amount of data on the relationship of the hormone with other factors of autocrine and paracrine regulation. Additional research is needed to better understand this correlation and its significance.

Thus, we can conclude that, on average, in comparison with classical indicators and results of superovulation in cows, when using domestically produced follicle-stimulating hormone, the results of superovulation are somewhat lower than world practice, since on average it is necessary to obtain 5-6 embryos and, as a consequence, fix at least 5-6 corpora lutea on the ovaries. When comparing the number of antral follicles during the period of FSH administration and the number of corpora lutea, no correlation was also established, and animals with a significant number of follicles in the middle of the treatment regimen (the maximum number of follicles on two ovaries was 21) did not show a comparable number of corpora lutea during the washout period. However, as a result of comparing the

number of follicles during the period of FSH administration and the total number of antral follicles and corpus luteum during the washout period, similar values are observed, which is probably due to the stimulation of follicles by the FSH hormone but incomplete ovulation in some of the stimulated follicles. In view of this hypothesis, it can be assumed that this was influenced by both the individual characteristics of the endocrine background of animals in a particular population in view of their feeding and maintenance and the characteristics of the study medicine, in which the ratio of FSH and LH is 1000-1500 IU of follicle-stimulating hormone to 1 IU of luteinizing hormone. For example, in the Pluset medicine, the ratio of FSH and LH is 500 IU to 500 IU, respectively [14]. Considering this fact and the possible deficiency of endogenous LH in the donor population under study, it can be assumed that this was the decisive factor that reduced the effectiveness of the superovulation regimen. At the same time, it is possible that the negative effect can be compensated by introducing an additional dose of medicines based on gonadotropin-releasing hormone on the last day of administration of the FSH medicine, which will provoke the release of endogenous LH and form a pre-ovulatory complex of gonadotropins, an LH peak, and ensure ovulation of the remaining follicles.

5 Conclusion

The relevance of studying anti-Mullerian hormone (AMH) in animals can hardly be overestimated, since this marker is an important indicator of reproductive health and the functional state of the gonads. AMH, produced by granulosa cells of the ovaries in females and Sertoli cells of the testes in males, serves as an important tool in veterinary endocrinology and zoology for assessing the fertility and reproductive prospects of animals.

Modern research shows that AMH levels directly correlate with the number of follicles and sperm, which allows for more accurate diagnosis of infertility conditions and predicting the results of artificial insemination. The study of AMF also contributes to a better understanding of the processes of sexual differentiation and can serve as the basis for the development of new methods for controlling reproduction in farm animals.

Another significant reason for its relevance is the need to monitor the reproductive state of populations in the context of species conservation and restoration, which becomes especially important against the background of changes in ecosystems and threats associated with the loss of biodiversity. Thus, the study of anti-Müllerian hormone in animals opens up new horizons for veterinary medicine.

Thus, the following conclusions can be drawn:

- The concentration of AMH in the blood serum of embryo donor cows does not correlate with the phase of the reproductive cycle due to the absence of significant differences in experimental animals in the luteal and follicular phases. In view of this, it is possible to focus on the AMH concentration without taking into account the stage of the reproductive cycle.
- The AMH concentration can serve as a prognostic criterion for the selection of embryo donors, as indicated by the coincidence of AMH peaks and the maximum number of corpus luteum formed on the ovaries during superovulation with a domestic follicle-stimulating medicine.
- The number of antral follicles during the follicular wave during the period of administration of an exogenous follicle-stimulating medicine is not a reliable criterion for assessing the future embryonic productivity of donors.
- During the assessment of the effectiveness of the domestic follicle-stimulating medicine, a combination of the formation of corpora lutea with a significant number of large antral follicles, the diameter of which exceeded 5 mm, was established, which is

likely due to the ratio of FSH and LH in this medicine, which is 1000-1500 IU FSH/1IU LH.

Acknowledgments

The study was supported by the Russian Science Foundation grant No. 23-76-01089

References

1. Yu.E. Kuznetsov, A.M. Lunegov, V.S. Ponamarev, E.B. Romashova, Agricultural biology, **57 (6)**, 1217 (2022)
2. A.V. Prusakova, N.V. Zelenevskiy, A.V. Prusakov, A.V. Yashin, V.S. Ponamarev, Veterinarski Glasnik, **77(2)**, 176-187 (2023)
3. L.D. Belotserkovtseva, L.V. Kovalenko, V.V. Danilogorskaya, E.V. Bondareva, Bulletin of Surgut State University. Medicine, **2(12)**, 20-23 (2012)
4. I.D. Gyulmamedova, O.S. Dotsenko, Reproductive medicine, **1-2(14-15),** 31-35 (2013)
5. A.A. Skriganyuk, Student Bulletin, **29-1(49)**, 95-97 (2018)
6. E. I. Baranovskaya, Russian Bulletin of Obstetrician-Gynecologist, **22(2),** 65-70 (2022)
7. J.F. Gurbanova, N.K. Kaziyeva, R.M. Mammadova, Q. Salakhova, Actual Issues in Modern Gynecology and Perinatology, **8(4),** 31-34 (2023)
8. R. Almasud, E.E. Tituh, G.A. Yunsi, S.A. Vasilenko, International Student Scientific Bulletin, **6**, 58 (2018)
9. A.Ya. Rak, A.V. Trofimov, A.V. Petrov, Cytokines and inflammation, **15(3-4)**, 256-264 (2016)
10. T.I. Ustinkina, S.B. Shustov, Problems of Endocrinology, **57(6)**, 39-44 (2011)
11. N.V. Aleksandrova, Gynecology, **22(6)**, 21-26 (2020)
12. T.V. Strizhova, I.V. Sergeeva, I.V. Besman, E.B. Rudakova, Clinical analysis in obstetrics, gynecology and reproductive science, **1(1)**, 36-39 (2021)
13. E.V. Kazantseva, G.Kh. Tolibova, T.G. Tral, I.Yu. Kogan, Journal of Obstetrics and Women's Diseases, **70(6),** 33-40 (2021)
14. L.V. Tkachenko, I.A. Gritsenko, K.Yu. Tikhaeva, N.I. Sviridova, I.S. Gavrilova, V.A. Dolgova, Obstetrics, Gynecology and Reproduction, **16(1)**, 73-80 (2022)