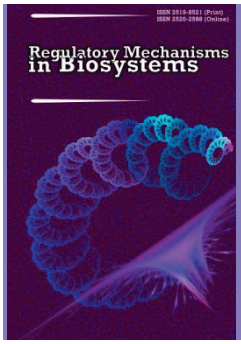

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Ovarian response to different doses of follicle-stimulating hormone in donor cows with different levels of anti-Müllerian hormone

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Variability of response to stimulation of superovulation is one of the biggest problems of industrial production of bovine embryos. Given the importance of the effect of the hormonal stimulation scheme and the role of anti-Müllerian hormone as a predictor of ovarian response to stimulation, the aim of the study was to determine the effect of the dose of follicle-stimulating hormone on stimulation of superovulation in donor cows with different concentrations of anti-Müllerian hormone in the blood serum. All animals admitted to the experiment were randomly divided into 3 groups based on the concentration of anti-Müllerian hormone in the blood serum: the first group – < 0.1 ng/cm^3 (low level); the second group – 0.1–0.25 ng/cm^3 (medium level); the third group – > 0.25 ng/cm^3 (high level) and their estrus cycles were synchronized. Before stimulation of superovulation, each group of donor cows, formed on the basis of the level of anti-Müllerian hormone in blood serum, was again divided into 3 groups depending on the dose of follicle-stimulating hormone administered to the cows: 800 IU (reduced dose of follicle-stimulating hormone); 1000 IU (medium dose recommended by the manufacturer); 1500 MO (increased). The control group consisted of animals with a medium level of anti-Müllerian hormone with a dose of follicle-stimulating hormone of 1000 IU. Differences between groups were evaluated by counting the corpora lutea, the total number of flushed embryos and the quality assessment of the latter. It was found that the number of corpora lutea and flushed embryos increased proportionally with the concentration of anti-Müllerian hormone in the studied animals, regardless of the dose of follicle-stimulating hormone. A decrease in the dose of follicle-stimulating hormone led to a decrease in the studied indicators in all groups. While increasing the dose of follicle-stimulating hormone, some differences were noted. Thus, in groups of animals with a low and medium level of anti-Müllerian hormone, a decrease in all studied indicators was observed, while with a high level, a significant increase in the number of corpora lutea and the total number of flushed embryos was observed against the background of a sharp decrease in the quality of the latter. In summary, measuring the concentration of anti-Müllerian hormone in the blood serum of donor cows allows one to predict the number of embryos obtained after stimulation. A decrease in the dose of follicle-stimulating hormone led to a decrease in the studied indicators in all the studied groups, while an increase led to a significant decrease in the quality of the obtained embryos, compared to the control group.

Keywords: AMH; FSH; reproductive biotechnologies; synchronization; fertility; cattle; hormonal stimulation.

Introduction

Embryo transplantation in cattle breeding has been used in breeding programs on an international scale for more than four decades (Kanitz et al., 2002; Moore & Hasler, 2017) and is one of the most effective approaches for obtaining genetically valuable calves (Kovpak et al., 2023; Santos et al., 2023). The use of this biotechnological method opens up opportunities for genetic selection of livestock, diversification of crossbreeding schemes and an increase in the percentage of fleshiness (Dochi, 2019; Valchuk et al., 2023). The effectiveness of this technology largely depends on the results of multiple ovulation stimulation. However, there is still a high degree of variability in the reaction of cows to superovulation, and as a result, it is impossible to predict the number of embryos obtained (Jaton et al., 2016; Alward et al., 2023).

In human medicine, an individual approach to the patient is used (Iliodromiti et al., 2015) and the method for assessing ovarian reserve and reproductive aging by determining the concentration of anti-Müllerian hormone in the blood serum has been successfully used for more than 20 years as an accurate and non-invasive technique (Kwee et al., 2008; Wang et al., 2021; Liu et al., 2023). Currently, this method is considered the most convenient and reliable, since the AMH level remains stable during the menstrual cycle (Hehenkamp et al., 2006; Stalzer et al., 2023). The correlation between the level of anti-Müllerian hormone and the ovarian response in women who underwent hormonal stimulation has also been confirmed (Hazout et al., 2004; Tolikas et al., 2011).

A different approach is used in cattle breeding – standardization of donor cow stimulation schemes (Deng et al., 2015; Hayden et al., 2023). Protocols are constantly being improved, increasing the number of embryos obtained per unit of time and simplifying the application of embryo transplantation programs (Irshad et al., 2015; López-Gatius et al., 2015; Kovpak et al., 2022c). However, the number of transferable embryos per donor cow does not increase (Bó & Mapletoft, 2014). With this in mind, scientists began searching for methods of predicting superovulation in cows by examining the genetic profile (Cory et al., 2013; Hirayama et al., 2019), counting the number of antral follicles (Santos et al., 2016; Zangirolamo et al., 2018), etc. However, in recent years, the number of publications is increasing arguing that the most effective method of predicting the response of animals to stimulation, similar to humans, is the determination of the AMH level in the blood serum of donor cows (Rico et al., 2009; Umer et al., 2019; Fushimi et al., 2020).

Anti-Müllerian hormone (AMH), also known as Mullerian inhibitory substance (MIS), was discovered by Alfred Yost in three original experi-

ments conducted between 1947 and 1952 (Hugon et al., 2010). This hormone is a member of the transforming growth factor beta (TGF-β) superfamily (Visser & Themmen, 2005) named after its first described function in fetal sex differentiation (Rey et al., 2003). AMH in the early stages of embryonic development is produced by Sertoli cells and interacts with cell surface receptors, causing the regression of the precursor of the female reproductive tract – the Müllerian ducts and the development of a male fetus, while the absence of the hormone leads to the development of female genital organs (MacLaughlin et al., 2001; Cate, 2022). In the perinatal period of development (Bezard et al., 1987; Rajpert-De Meyts et al., 1999), the specified model of sexual dimorphism is lost, and AMH is localized to the ovarian stromal cells surrounding germ cell nests and newly formed primordial follicles (Nilsson et al., 2011). AMH expression begins in the columnar granulosa cells of primordial follicles, reaches its maximum level in the granulosa cells of preantral and small antral follicles, and then gradually decreases in later stages of follicle development (Rajpert-De Meyts et al., 1999), until the hormone is no longer expressed during the gonadotropin-dependent terminal stages of follicle development (Campbell et al., 2012). Granulosa cells of atretic follicles and early corpus luteum are AMH-negative (Rajpert-De Meyts et al., 1999).

AMH has effect only on the reproductive organs (La Marca & Volpe, 2006). At the initial stages of folliculogenesis, it induces changes in oocyte apoptosis, which, in turn, regulates the speed of assembly of oocytes into the primordial follicle (Nilsson et al., 2011). Subsequently, this hormone is produced by small follicles and exerts a paracrine effect on primordial follicles, preventing their recruitment to the pool of growing follicles, thus maintaining primordial follicles in their arrested state (Durlinger et al., 2002; Visser & Themmen, 2005). Durlinger et al. (1999) found that AMH null mice had almost three times more growing follicles, which in turn caused premature depletion of the primary follicle pool. Gigli et al. (2005) in their studies confirm the inhibitory effect of the hormone on the activation of follicles in both mice and cattle. However, it is worth noting that enhanced recruitment of primordial follicles in AMH null mice is counteracted by increased oocyte degeneration and follicular atresia (Visser et al., 2007). The level of AMH is regulated by estradiol through ERα and ERβ in a receptor-specific manner, inhibiting its production through ERβ and stimulating it through ERα (Grynberg et al., 2012). Convissar et al. (2017), in turn, found that factors secreted by oocytes (growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15) are able to change the level of AMH, which also plays a role in the control of follicle recruitment and the selection of dominant follicles.

AMH acts autocrinely by influencing the sensitivity of follicles to FSH (Grossman et al., 2008; Zhou et al., 2022). Both *in vitro* and *in vivo* studies have shown that follicles are more sensitive to FSH in the absence of AMH (Durlinger et al., 2001). This effect of the hormone results from a reduction in granulosa cell proliferation and is consistent with another in vitro study in which exogenous AMH was shown to reduce aromatase expression and the number of LH receptors in cultured granulosa cells (di Clemente et al., 1994). In their research, Sacchi et al. (2016) confirm these data, because when the hormone is added to the culture of granulosa cells, aromatase induction and p450scc expression caused by FSH and luteinizing hormone (LH) are inhibited. Thus, AMH is one of the factors that indicate the size of the growing follicular pool and is able to control it by reducing the recruitment and slowing down the growth of follicles.

AMH gene expression in cattle is restricted to one type of cells – granulosa cells of growing follicles (Rico et al., 2011). There is an increasing number of scientific publications on the possibility of this hormone being a predictor of the ovarian response to superovulation in cattle, similar to humans. Souza et al. (2015) indicate that it is possible to predict superovulation in high-yielding dairy cows by the concentration of circulating AMH. According to Rico et al. (2009) concentration of anti-Müllerian hormone in the blood plasma of cows is strongly correlated with the number of antral follicles from 3 to 7 mm in size and the number of ovulations after treatment. Similar results were obtained by Hirayama et al. (2012), during superovulated treatment of Japanese Black cattle, the concentration of AMH in blood plasma was positively correlated with the number of all follicles and small (<5 mm) follicles, oocytes/embryos obtained and transplantable embryos. Rico et al. (2012) found that in gonadotropin-stimulated Holstein cows producing less than 15 large follicles during estrus and less than 10 embryos according to MOET protocols, plasma AMH concentrations were below 0.087 and 0.074 ng/cm^3, respectively. Ghanem et al. (2016) reported a significantly higher number of oocyte cumulus complexes per donor in cows in groups with high (AMH ≥ 0.25 ng/cm^3) and medium (0.1 ≥ AMH < 0.25 ng/cm^3) AMH levels, which is higher than in the low AMH group.

However, despite extensive data on the correlation of serum AMH concentration in cows and response to superovulation, there are still no publications on the differential response of donor animals to different doses of FSH based on AMH (Hayden et al., 2023). Taking this into account, the aim of this study was to study the influence of different schemes of superovulation stimulation on the production of transplantable embryos by donor cows with different levels of AMH.

Materials and methods

The research was carried out in the period from 2022 to 2024. The experiment was carried out on 45 cows of the Ukrainian black-and-white dairy breed after 1–2 lactations, which were kept on the farm of LLC "Golden meadows" Ukraine, Vinnytsia region, Illinetskyi district, village of Pariivka, 18 Sadova street. Experiments on animals were performed in accordance with the requirements of the Law of Ukraine "On Protection of Animals from Cruel Treatment" (Article 230 of 2006), "General ethical principles of experiments on animals", approved by the National Congress on Bioethics and coordinated with the provisions of the "European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes" (Strasbourg, 1986). The conditions for keeping cows met the established standards of the Order of the Ministry of Economic Development, Trade and Agriculture of Ukraine "On Approval of Requirements for the Welfare of Farm Animals During Their Keeping" (No. 224 of 2021). Before the start of the experiment, a positive conclusion was received from the local commission on bioethics of the National University of Bioresources and Nature Management of Ukraine of Ukraine regarding the use of experimental cows (protocol No. 026 dated April 10, 2022).

Cattle were kept loose, providing comfortable conditions for the animals. They were looked after by one or two qualified workers. The conditions of existence of cows took into account their basic, psychological, social and physiological needs, bringing them as close as possible to natural ones. The microclimate in the stalls (relative humidity, temperature, degree of dustiness and concentration of gases in the air) corresponded to the parameters that minimize stress and discomfort for the animals without harming their health. To ensure optimal conditions in the cowsheds, air exchange was organized: in winter – 10 times a day, in summer – 40–60, and in autumn and spring – 15–20. The diet of the cows met the standards and requirements, the animals have free access to food and clean water. Experimental animals were examined daily by a veterinarian, and if signs of disease were detected, the cows were immediately given qualified treatment to avoid suffering and pain. The principles of humaneness were observed when putting the cows to the experiment: during the manipulations, pain and stress were minimized without harming their health. Donor animals had at least one normal estrus cycle before being put into the experiment, and the interval from calving to the start of superovulation stimulation was at least 3 months.

Determination of anti-Müllerian hormone concentration in blood serum of cows. To select the necessary number of animals for the experiment, the concentration of anti-Müllerian hormone in the blood serum of 45 animals was determined. The day of the cycle was not taken into account, considering the lack of fluctuations in the level of this hormone during the estrus cycle. Before the material was collected, the injection site was subjected to sanitary treatment. Blood was collected from experimental cows from the tail vein. 4 cm^3 of blood was collected from each animal by a standard method, using sterile vacuum tubes "Vacutainer Premium" with silicon dioxide (SiO_2) (Greiner Bio-One, Austria). The material was delivered to the laboratory at a temperature of +4 °C no more than 2 hours after selection. The samples were sent to the medical laboratory of the Diagnostic Center "CSD LAB" LLC (Ukraine, city of Kyiv, 45 Vasylkivska st.) in cold conditions, where the concentration of anti-Müllerian

hormone was determined by the chemiluminescent immunoassay method (CLIA).

Depending on the concentration of anti-Müllerian hormone in the blood serum, we divided the donor cows into three groups:

– group 1 – low hormone level, its concentration was lower than 0.10 ng/cm^3;

– group 2 – medium level – the concentration of anti-Müllerian hormone varied between 0.10–0.25 ng/cm^3;

– group 3 – high level – the concentration was higher than 0.25 ng/cm^3.

Superovulation stimulation protocol. Before setting up a group of donor cows for stimulation of superovulation, their estrus cycles were synchronized. Synchronization was carried out according to the scheme below (Fig. 1). Estrofan (Bioveta a.s., Czech Republic) was used as a synthetic analogue of Prostaglandin F2α (PGF2α), while Surfaron (Reagent PrAT, Ukraine) was used as a synthetic analogue of Gonadotropin releasing hormone (GnRH) (Valchuk et al., 2023).

After synchronization, heat was clinically confirmed in the donor animals. Before placing the donor cows on the experiment, each of the groups of animals formed on the basis of the level of AMH in the blood serum was again divided into three more groups depending on the dose of the follicle-stimulating hormone administered to the cows to stimulate superovulation.

– 1st group – reduced dose of FSH – 800 IU;

– 2nd group – standard – 1000 IU;

– 3rd group – advanced – 1500 IU.

"Pluset" (Laboratorios Calier, Spain) was used as follicle stimulating hormone (FSH). Stimulation of superovulation in donor cows of the control group (2nd group – standard dose of FSH) was carried out according to the scheme below (Fig. 2). In the first group of donor cows, the FSH dose of each injection was reduced by an index of 0.8, and in the third group, it was increased by 1.5, compared to the control. During stimulation, constant ultrasound control of follicle growth and ovulation was carried out.

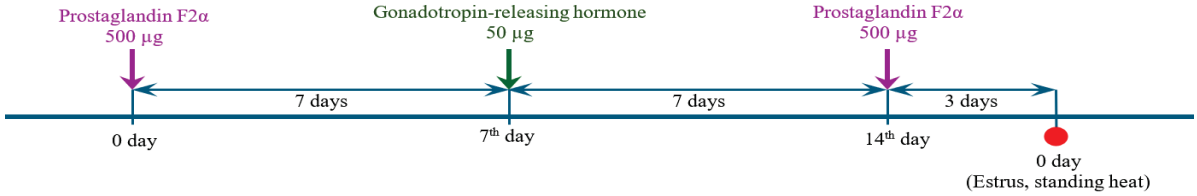

Fig. 1. Scheme of hormonal synchronization of estrus cycles in cows

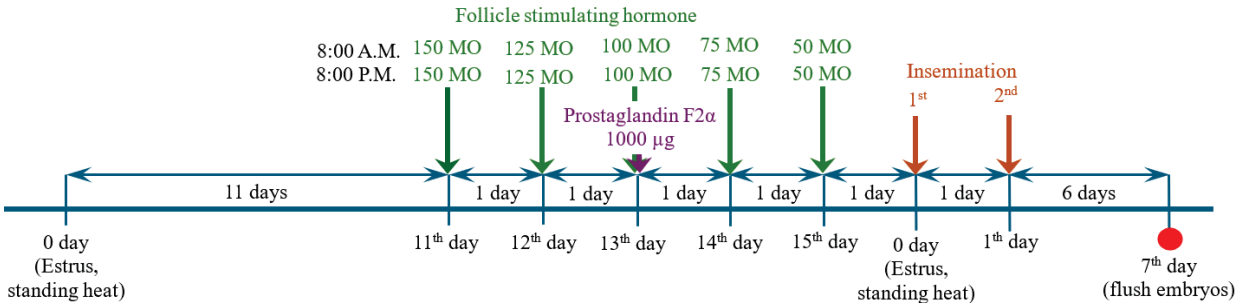

Fig. 2. Scheme of hormonal stimulation of superovulation of donor cows (average dose of FSH recommended by the manufacturer)

Insemination was carried out twice. The first insemination of the donor cow was carried out 70–72 hours after the introduction of Prostaglandin F2α with a double dose of the sperm of the bull L.P. Alfonso et CA 105585553, regardless of the manifestation of heat, repeated insemination was carried out after 10–12 hours with a single dose. Embryo flushing was performed on the 7th day from the beginning of the heat (23 days from the introduction of the animals into the experiment).

In connection with removing some cows at the stages of their synchronization and hormonal stimulation, 27 animals were used in the main experiment, that is, three animals were selected for each experimental group, according to the specified characteristics, in which the number of corpora lutea in the ovary, the total number of embryos obtained were counted and their quality was determined.

Counting the number of corpora lutea in donor cows after flushing out embryos. In order to identify animals that did not respond well to treatment and to predict the number of embryos obtained on the 5th day after the first insemination, hormonally superstimulated donors underwent clinical and transrectal ultrasound examination to count corpora lutea in the ovaries (Fig. 3). Their detection and counting were carried out by changing the position of the sensor. The examination was performed using ultrasound diagnostics in mode B (Kaixin KX5200, China). According to the results of the study, animals with follicles that did not ovulate, with cysts, were withdrawn from the experiment because this indicates a lack of response to hormonal stimulation.

Flushing and counting the number of embryos. Embryos were flushed out by a non-surgical method on the 7th day after the first insemination. Manipulations were carried out under epidural anesthesia, directly in the cowshed, without fixing the animals. For flushing, a closed system consisting of Y-Junction tubing Luer for Miniflush (Minitube, Germany) and 2-way Silicone ET catheter CH 12 Foley (Minitube, Germany) was assembled. The solution for flushing embryos consisted of 90% Dulbecco's Modified Eagle Medium (DMEM) (Sigma, USA) and 10% inactivated bovine blood serum with the addition of heparin (Novofarm-Biosyntez, Ukraine) at the rate of 10 IU/cm^3. Flushing was carried out alternately from each horn of the uterus by portioned injection of flushing solution, at the rate of 500 cm^3 per horn. Filtering of the medium obtained from the uterine horns was carried out in the EmSafe system for embryo collection in bovines and equines (Minitube, Germany).

Counting and preliminary evaluation of embryos was performed under a stereomicroscope SZ51 (Olympus, Japan). High-quality embryos were repeatedly flushed in embryo flushing medium and more precisely evaluated under a Zeiss Axio Observer A inverted microscope (Carl Zeiss, Germany) (Fig. 4). Embryos with Stage Code – 5, 6 and 7, Quality Code – 1 (Kovpak et al., 2022a), according to the classification proposed by Bó & Mapletoft (2013), recommended by the International Embryo Transfer Society (IETS), were considered high-quality embryos (Rocha et al., 2016).

High-quality embryos were transferred to a drop system (at the rate of 6 embryos per 1 microdrop of 100 mm^3) with an embryo culture medium consisting of 5 cm^3 culture medium with pyruvate (Minitube, Germany), 0.5 cm^3 bovine estrous serum, 200 mm^3 essential amino acids (Sigma, USA), 50 mm^3 of substitute amino acids and 50 mm3 of antibiotic-antimycotic (Sigma, USA) under a layer of mineral oil (Origio, Denmark). After evaluation, the embryos were vitrified and placed in liquid nitrogen for further use or storage.

Statistical data processing. Results were presented as x ± SE (main ± standard error). $P < 0.05$ were considered significant. Values were compared using one-way ANOVA with Bonferroni post hoc test.

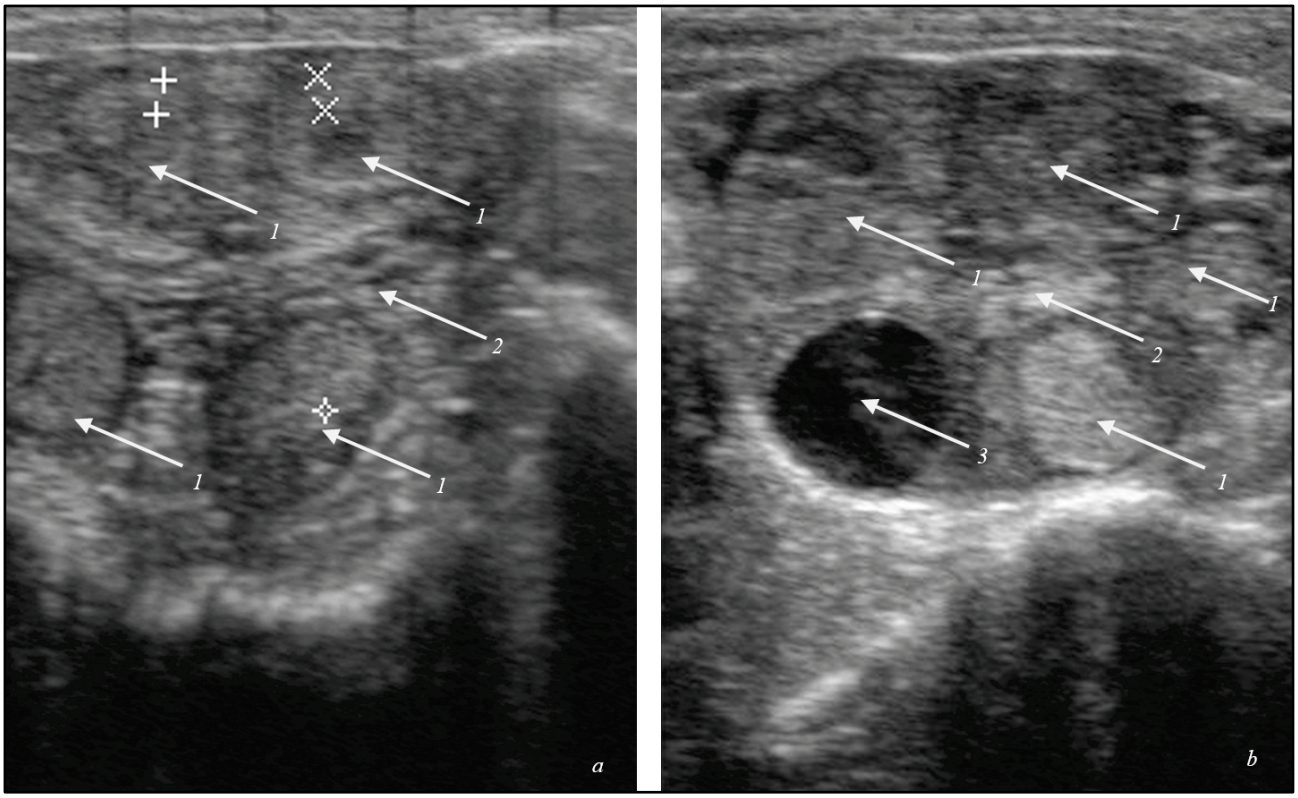

Fig. 3. Ultrasound image of a cow's ovary on the 5th day after the first insemination under stimulation of superovulation (*a*, *b*): corpus luteum (*1*); ovarian tissue (*2*); a follicle that did not ovulate (*3*)

Fig. 4. Bovine embryos of different stages of development: compact morula (Stage code 4) (*a*); early blastocyst (Stage code 5) (*b*); expanded blastocyst (Stage Code 7) (*c*), expanded blastocyst collapse (Stage Code 7) (*d*)

Results

When stimulating controlled superovulation with the average recommended dose of follicle-stimulating hormone (1000 IU) in donor cows with different concentrations of anti-Müllerian hormone in blood serum, significant differences in the number of flushed embryos were noted (Table 1). A reliable correlation was noted between an increase in the level of anti-Müllerian hormone and an increase in the number of embryos obtained in experimental animals. The next step of the study was the study of the effect on the results of superovulation stimulation of the dose of follicle-stimulating hormone in cows with different levels of anti-Müllerian hormone in blood serum (Table 1). The control was a group with a medium level of AMH (from 0.1 ≥ to ≥ 0.25) and a medium dose of FSH – 1000 IU (the dose recommended by the manufacturer of the drug for stimulation of superovulation for dairy breeds of cows). In the control group, during the flushing process, 9.7 ± 0.3 embryos were obtained, of which 96.5% were categorized as high-quality. When the dose of FSH was reduced by 20% in groups of animals with a medium level of AMH, we noted a decrease in the total number of embryos obtained by 17.5% and transplantable by 17.2%, compared to the control group. At that time, increasing the dose of follicle-stimulating hormone to 1500 IU led to a significant decrease in the number of flushed embryos by 27.8%.

In groups of animals with a low level of anti-Müllerian hormone, a significant decrease in all studied indicators was noted, compared to the control. It should be noted that the optimal dose of follicle-stimulating hormone for animals with AMH concentration < 0.1 ng/cm^3, which allowed 5.0 ± 0.6 embryos to be obtained suitable for transplantation (this indicator is 48.4% lower than that in the control group), turned out to be 1000 IU. The least effective dose for animals with a low level of AMH was 800 IU of FSH, which led to production of 72.2% fewer embryos than in the control group, and only 62.5% of them were suitable for further transplantation. Somewhat better results were noted in the group of animals with an increased dose of FSH – 55.7% fewer embryos compared to the control group, 76.9% of which were characterized as high-quality.

In the group of animals with a high level of AMH and a low dose of FSH, a decrease in the number of flushed embryos was noted by 24.7% compared to the control, however, it is worth noting that 95.4% of them were suitable for further manipulations. While when 1000 IU of follicle-stimulating hormone was administered to animals with AMH > 0.25 ng/cm3, a significant increase in both flushed embryos by 57.7% and high-quality embryos by 53.8% was noted, compared to the control. When the dose of FSH was increased to 1500 IU in the group of animals with a high concentration of anti-Müllerian hormone in the blood serum, the largest number of embryos was obtained 21.7 ± 1.9, which was 123.7% more than in the control group, but the percentage of high-quality embryos from the total number was only 27.6 (the number of high-quality embryos was 35.5% lower than in the control group).

Table 1
The effect of the dose of follicle-stimulating hormone on the effectiveness of stimulation of superovulation depending on the concentration of anti-Müllerian hormone in the blood serum of donor cows (x ± SE, n = 3)

AMH concentration, ng/cm^3	FSH dose, IU	Number of corpora lutea, pcs	Total number of embryos, pcs	Number of high-quality embryos, pcs
< 0.1 (low level)	800	2.7 ± 0.3***	2.7 ± 0.3***	1.7 ± 0.3***
	1000	5.0 ± 0.6*	5.0 ± 0.6*	5.0 ± 0.6***
	1500	4.3 ± 0.9**	4.3 ± 0.9**	3.3 ± 0.3***
From 0.1 ≥ to ≥ 0.25 (medium level)	800	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 0.3
	1000 (control group)	9.7 ± 0.3	9.7 ± 0.3	9.3 ± 0.3
	1500	8.0 ± 1.0	7.0 ± 0.4*	7.0 ± 0.4*
> 0.25 (high level)	800	7.3 ± 0.9	7.3 ± 0.9	7.0 ± 0.6*
	1000	15.3 ± 0.9**	15.3 ± 0.9**	14.3 ± 0.3***
	1500	22.3 ± 1.8***	21.7 ± 1.9***	6.0 ± 0.6***

Notes: * – P < 0.05; ** – P < 0.01 *** – P < 0.001 vs. control group (ANOVA followed with Bonferroni post hoc test).

Discussion

According to reports by the International Embryo Technology Society (IETS, 2023), the bovine embryo industry has grown significantly over the past 20 years (Kovpak et al., 2022b; Viana, 2023). The high variability of donors still hinders the prediction of the number of embryos obtained, which is why the main efforts of scientists are directed to the development of recommendations for the selection of donor cows and optimization of superovulation stimulation schemes (Mapletoft & Bó, 2011). AMH has been shown to be an important hormone, especially for the female reproductive organs, and is currently considered the best biomarker for controlled ovarian stimulation (Pilsgaard et al., 2018; Bedenk et al., 2020). Assisted reproductive technology in livestock still favors antral follicle count (AFC) as a marker of ovarian reserve (Taneja et al., 2000). However, a growing number of scientists point to the significant effectiveness of determining the anti-Mullerian hormone in donor cows precisely for predicting the response of the ovaries to hormonal stimulation. Thus, Rico et al. (2009) in their studies demonstrate that AMH is a reliable endocrine marker of the population of small antral gonadotropin-sensitive follicles in cows and its concentration in plasma affects the efficiency of the donors' response to superovulation induction. Monniaux et al. (2010) state that the concentration of AMH in the blood plasma of cattle is positively correlated with the number of corpora lutea and the quality of embryos obtained after ovarian stimulation. Hirayama et al. (2017) indicate that the number of flushed eggs/embryos after multiple ovulation differs significantly among groups of donors with different concentrations of circulating anti-Müllerian hormone (high – AMH ≥ 0.488 ng/cm^3, medium – AMH – 0.487–0.119 ng/cm^3 and low – AMH ≤ 0.118 ng/cm^3), while a proportional increase in the indicated indicators was noted. Koca et al. (2024) noted that in cows with a high level of AMH (0.339 ± 0.015 ng/cm^3), a significantly higher number of embryos in general and high-quality embryos in particular were flushed, compared to groups with a medium (0.215 ± 0.012 ng/cm^3) and low level of the hormone (0.116 ± 0.004 ng/cm^3, $p < 0.05$). Mossa & Ireland (2019), in their research, indicate that cows with low AMH levels have a reduced response of granulosa, thecal and luteal cells to FSH and consequently a poor response to superovulation compared to animals with high AMH. The results obtained during the research correlate with the data presented above, which once again confirms the feasibility of measuring the concentration of anti-Müllerian hormone in blood serum for the selection of egg/embryo donor cows.

However, the level of anti-Müllerian hormone is only one of the factors on which the effectiveness of superovulation stimulation depends. Since ovarian hormone stimulation is an assisted reproductive technology technique designed to provide long-term maintenance of FSH concentrations by exogenously administering this hormone to promote the development of gonadotropin-dependent follicles to pre-ovulatory size (Clark et al., 2022), the first candidate for investigation is the response of the ovaries to different doses of the specified hormone. FSH performs a number of functions in the follicle by interacting with various signaling pathways (synthesis of steroid hormones, cell proliferation and survival, induction of LH receptor expression and downregulation of FSH receptors), which contribute to its normal functioning and the development of quality oocytes (Gloaguen et al., 2011). During the physiological estrus cycle, the increase in the concentration of endogenous FSH is responsible for the recruitment and growth of the follicle, while its subsequent decrease – for the selection of a single dominant (Adams et al., 1992; Ginther et al., 2000). Administration of exogenous FSH stimulates the growth of recruited follicles, which in turn allows a large number of follicles to acquire a dominant phenotype (Bó & Mapletoft, 2014). Given the broad and complex effects of FSH on follicular function, the effects of the exogenous hormone are likely to be dose-dependent (Kanitz et al., 2002; Barati et al., 2006; Clark et al., 2021). In cattle breeding, the question of the effect of the follicle-stimulating hormone dose on the response of the ovaries during superovulation is being actively studied (Karl et al., 2021), however, the available literature still lacks reports on the comprehensive study of the response of egg donor cows to different doses of FSH based on the concentration of circulating anti-Müllerian hormone in blood serum (Hayden et al., 2023).

It is reasonable to assume that an increase in the dose of follicle-stimulating hormone will lead to an increase in the number of oocytes/embryos obtained, but the presented research and a number of scientific publications refute this theory. Studies indicate a negative effect of high doses of FSH on the number of corpora lutea (Karl et al., 2021), the concentration of circulating progesterone (Saumande & Chupin, 1986) and estradiol (Kanitz et al., 2002; Karl et al., 2021), the ovulation rate (Saumande & Chupin, 1986; Karl et al., 2021). High doses cause premature luteinization of follicles of ovulation size (Clark et al., 2022), induce premature cumulus expansion and dysregulation of oocyte maturation (Karl et al., 2023) and reduce the number of transplantable embryos obtained (Sugano & Watanabe. 1997). At that time, Kanitz et al. (2002) states that increasing the dose of FSH to a certain level can increase the number of antral follicles, and each drug containing follicle-stimulating hormone has its own optimal dose range, which must be tested in field studies.

It is worth noting that during the research we noted differences in the reaction of donor cows with different concentrations of AMH in the blood serum to an increase in the dose of follicle-stimulating hormone. Thus, in the groups with normal and low AMH, we noted a decrease in all studied indicators, while in the group of animals with AMH > 0.25 ng/cm^3, we noted a significant increase in the number of corpora lutea and the total number of embryos against the background of a significant decrease in their quality, compared with control. Currently, there are no studies in veterinary medicine that would explain this phenomenon, but in human medicine, a high level of AMH in the blood serum of patients is one of the factors that increase the risk of developing ovarian hyperstimulation syndrome (OHSS) (Jayaprakasan et al., 2012; Sun et al., 2021). Stewart et al. (2014) indicate that extraction of >15 oocytes significantly increases the risk of OHSS and suggests the use of less aggressive stimulation protocols, especially in patients with a high level of response and ovarian reserve. In turn, in patients who developed the specified syndrome, an increase in the number of corpora lutea and oocytes was noted, while the percentage of high-quality oocytes and the level of their fertilization decreased, compared to the group without complications (Aboulghar et al., 1997). Analyzing the received and literature data, we can conclude that the use of high doses of FSH for stimulation of donor cows is economically unprofitable, and in the case of animals with a high level of AMH, it can negatively affect their health and further reproductive qualities. Reducing the dose of FSH predictably led to a decrease in all studied indicators, both

compared with the control group and within the group with different levels of AMH. The obtained data are confirmed by the study of Kanitz et al. (2002), who state that a decrease in exogenous FSH leads to a decrease in the number of ovulations. At the same time, Barati et al. (2006) indicate that season, dose of Folltropin-V and time of onset of heat in *Bos indicus* do not affect the number of transplantable embryos obtained. Son et al. (2007) when reducing the dose and number of *Bos taurus* coreanae treatments also did not note changes in the number of embryos obtained. The difference between the research results and the data presented by Barati et al. (2006) and Son et al. (2007) may be related to the specific response of different cattle breeds to hormonal stimulation, but this assumption needs further study and clarification.

The obtained results indicate that the dose of follicle-stimulating hormone and the level of anti-Müllerian hormone in the blood serum significantly affect the results of controlled superovulation and the quality of the obtained embryos in cattle.

Conclusion

Determining the concentration of anti-Müllerian hormone in the blood serum of donor cows before the start of superovulation stimulation makes it possible to predict the number of embryos obtained. Thus, an increase in the concentration of the hormone leads to a proportional increase in the number of embryos obtained. However, it is equally important to determine the optimal dose of exogenous follicle-stimulating hormone, which will allow the maximum number of high-quality embryos to be obtained in a particular cow, based on the level of its anti-Müllerian hormone. Reducing the dose of follicle-stimulating hormone to 800 IU led to a decrease in the number of flushed embryos in all experimental groups, an increase to 1500 IU caused a decrease in the quality of embryos, which was especially critical in the group of animals with a high level of anti-Müllerian hormone. By contrast, the 1000 IU dose of follicle-stimulating hormone in the study was optimal for all the studied groups, which helped to obtain the maximum number of embryos suitable for transplantation.

The authors declare that they have no conflict of interest.

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