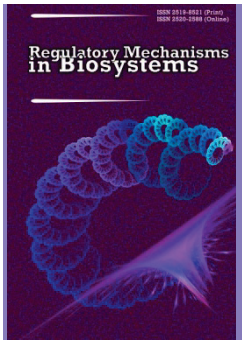

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Concentration of progesterone in the blood serum and size of the corpus luteum as criteria for selection of recipient cows for embryo transfer

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Pregnancy rate is the main factor influencing the productivity and economic efficiency of animal farming. Transfer of bovine cattle embryos is currently one of the most promising methods of overcoming the problem in the reproduction sphere. That is why the objective of our research was to identify progesterone concentration in blood serum and size of the corpus luteum in the cows on the day of embryo transfer in order to improve the strategy of selecting recipient animals, and, as a result, increase the conception rate. All animals admitted to the experiment were divided into three groups based on concentration of progesterone in the blood serum, being <2.5 ng/cm^3 in the first, 2.5 to 5.0 ng/cm^3 in the second, and >5 ng/cm^3 in the third group. Progesterone concentrations in the blood serum and sizes of corpora lutea were determined on the day of embryo transfer (7th day after estrus). The animal was considered pregnant according to a positive result of ultrasound examination. Based on the study results, we concluded that measuring the progesterone content in the blood serum and the size of the corpus luteum are not interchangeable. We found that sizes of corpora lutea did not correlate with concentrations of progesterone in the blood serum of the experimental recipient cows. However, the size of the corpus luteum was of significant predictive value for pregnancy rate. We saw that decrease in its diameter below 15 mm, even against the background of high progesterone concentration, was a negative criterion for selection of the recipient animals. Optimal progesterone concentration in blood serum of the experimental animals was within 2.5–5.0 ng/cm^3, leading to the highest pregnancy rate in the groups – 46.7. The hormone parameter outside the indicated range led to a significant decline in the pregnancy rate in the recipient animals. Therefore, when selecting recipient cows prior to transfer, both progesterone concentration in the blood serum and the diameter of the corpus luteum should be taken into account, which should not be beyond the proposed ranges. The data presented and analyzed in the article can help improve the efficiency of transfer of bovine cattle embryos for scientific and industrial purposes.

Keywords: reproductive biotechnology; bovine cattle; progesterone; corpus luteum; ovaries; pregnancy.

Introduction

Cattle farming is a well-established and heavily financed sphere of animal husbandry worldwide. It solves the most important global problem – provision of the population with food products, in particular dairy and meat products (Gorelik et al., 2020).

Currently, cattle farming should concentrate its efforts on increasing the population of highly productive cattle, since the total number of cows in Ukraine has been tending to decline. In particular, the population of cattle decreased 3.7-fold between 2020 and 2023 (from 4,958.3 thou to 1,355.2 thou individuals, respectively), while on farms it plummeted 4.7-fold (from 1,851.0 to 390.4 thou individuals, respectively) (Kruglyak et al., 2020; Elfeel et al., 2022). The production system in animal farming has to be profitable and minimize ecological and social impacts, and also ensure the optimal health and wellbeing of the animals (Wathes et al., 2008). This can only be achieved using technological production methods.

Breeding in cattle farming is a laborious process that is crucial to production intensity, duration of using genetically valuable animals, obtaining quality of products from them, and therefore profitability. Transfer of embryos of cattle is one of the most effective methods of producing high-potential calves (Kovpak et al., 2022b; Santos et al., 2023). Its effectiveness is high enough to enhance genetic selection, increase conception rates, and optimize schemes of cross breeding in cattle farming (Dochi, 2019; Kovpak et al., 2022a), as was evidenced in rapid increase in production (Viana, 2020) and improvement of methods of producing and storing cattle embryos (Ferré et al., 2020; Kovpak et al., 2022c; Kovpak et al., 2023).

Survival of embryos is the main factor influencing the productivity and economic efficiency in all systems of production milk and meat of ruminants (Diskin & Morris, 2008). Rate of embryo loss in cattle is highest in the early embryonic period (up to the 16th day), before determining pregnancy (Diskin & Morris, 2008; Lonergan, 2011). In turn, the rate of successful pregnancy was observed to be improved through programs of synchronization that induce ovulation, development of the corpus luteum, and the consequent hormonal changes in the recipient (Choi et al., 2023).

During embryo transfer, the cattle must have a functional corpus luteum and a sufficient concentraton of progesterone (Kanazawa et al., 2016). Those factors play an important role in identification and support of pregnancy (Kastelic et al., 1990; Hasler, 2001; Berger et al., 2017). Embryo transfer programs usually consider the presence of the corpus luteum in a recipient cow as the main criterion to conclude on the animal's suitability to carry an embyo (Morotti et al., 2014; Pugliesi et al., 2019), because the size of the corpus luteum can be evaluated directly by transrectal palpation or ultrasound research of the animal. There are data pointing to the importance of the size of the corpus luteum for a successful embryo implantation (Vasconcelos et al., 2001; Stevenson & Britt, 2017; Bonato

et al., 2022), because its diameter correlated with progesterone concentration in the blood plasma (Kanazawa et al., 2016). This regularity is explained by the fact that luteocytes that are derived from thecal and granulose cells are the main primary producers of progesterone (Ohara et al., 1987). However, there are studies that disprove the statement that the size of the corpus luteum is a predictive criterion for pregnancy detection (Siqueira et al., 2013; Smith et al., 2018). And even though there are inconsistencies regarding effects of the size of the corpus luteum on success of pregnancy, there still is a tendency to prefer recipients with a larger corpus luteum (Santos et al., 2023).

It has to be noted that the size of the corpus luteum is considered prognostic data for such an important indicator as progesterone. Therefore, a large portion of early embrionic mortality is related to insufficient concentration of circulating progesterone (Lonergan, 2011). Progesterone is a steroid hormone that is produced by the corpus luteum during an estral cicle and later by the placenta during pregnancy. It affects many aspects of reproduction of cattle (Colazoet al., 2017). Increase in progesterone after ovulation leads to expression of endometrial transcriptomes, necessary for successful implantation of bovine embyos, whereas its low concentration delays the uterus receptivity (Forde et al., 2011; Forde et al., 2012). Moreover, concentration of progesterone in blood serum is the factor the embryo growth depends on, as well as its generation of interferon tau, which affects identification of pregnancy by a mother organism (Mann & Lamming, 2001; Carter et al., 2008; Lonergan & Sánchez, 2020), and also secretion of histotrophs in the uterus lumen (Lonergan, 2011). Suboptimal concentrations of the hormone can cause high embryonic mortality during first three weeks of pregnancy, during which mother's endometrium recognizes embryos (Dunne et al., 2000; Berg et al., 2010). Unfortunately, the procedure that is based on the analysis of progesterone in blood plasma or milk is difficult to employ in diagnostics at farms due to relatively high cost of analysis and the temporal interval between blood withdrawal and release of the results.

As of now, there are no standardized recommendations regarding selection of recipient cows on the day of embryo transfer. The data regarding correlation between sizes of corpora lutea and progesterone concentrations in the blood serum of cattle are scattered and there are no convincing data about their effects on identification of pregnancy. Therefore, the objective of our study was to identify correlation between progesterone concentration in blood serum and the size of the corpus luteum of the recipient cows on the day of transfer (7th day of the sexual cycle) and pregnancy rates. Additionally, we checked for presence of correlation between the diameter of the corpus luteum and progesterone concentration in the blood of the recipient cows.

Materials and methods

Synchronization protocol. The studies were performed in 2020–2023. In total, the study involved 38 Ukrainian Black-Red Dairy cows, grown at the farm of Zoloti Luky Ltd., Ukraine, Vinnytsia Oblast, Illintsi District, Pariivka village, Sadova Str., 18. The experiments on the animals were carried out in accordance with the Law of Ukraine on Protection of Animals from Abuse (Art. 230 as of 2006), the General Ethical Prinicipals of Experiments on Animals, approved by the National Congress of Bioethics, and European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986). The condition of maintenance of the animals adhered to the Order of the Ministry of Developent of Economics, Trade, and Agricultural Farming of Ukraine on Approval of Requirements for Wellbeing of Agricultural Animals during their Maintanance (# 224 as of 2021).

The animals were held in stables, monitored by one or two qualified workers. The conditions the cows were held in were maximally close to the natural, meeting their basic, psychological, and social needs. Temperature, dust concentration, relative moisture, air temperature, and air quality in the stables corresponded to the parameters that are not harmful to the health and minimized the discomfort and stress to the animals. To provide a healthy microclimate in the cowsheds, an air exchange rate was organized of 40–60 times in summer, 10 times in winter, and 15–20 times in spring and autumn. The cows had free 24 h access to high-quality balanced diets and clean water in correspondence to the standards and requirements. The cows were examined every day. In cases when the animals showed signs of disease, they were immediately treated in order to mitigate suffering and pain. During the experiment, we showed humanity to the animals, minimized stress and pain during the procedures, causing no long-lasting harm to their health.

The estrus was synchronized using Estrofan (Bioveta a.s., Czech Republic), a synthetic analogue Prostaglandin F2α (PGF2α) and Surfaron (Reagent Ltd, Ukraine), a synthetic analogue of gonadotropin releasing hormone (GnRH), according to the scheme below (Fig. 1).

Identificaion of sizes of corpora lutea in the recipient cows prior to the experiment. Clinical transrectal ultrasound examination of the ovaries was performed for all the animals on the 7th day of estral cycle (Fig. 2). Luteum size was determined by measuring the maximal area of transverse section of the corpus luteum.

According to results of the study, we culled animals with bones and corpus luteum smaller than 0.5 cm, which indicated absence of the reaction to hormonal stimulation. Therefore, only animals that could be potential recipients were used in the experiment. The examination was carried out using ultrasound diagnostics in B regime (Kaixin KX5200, KHP).

Identification of progesterone concentration in blood serum of cows. Blood was withdrawn from the jugular vein of 32 experimental animals on the day of embryo transfer. Prior to blood withdrawal, we performed a sanitary treatment of the injection region. We collected 4 cm^3 of blood from each cow using the standard method and placed it into vacuum sterile test tubes (Vacutainer PREMIUM) containing silicon dioxide (SiO_2) (Greiner Bio-One, Austria). No later than 2 h after blood sampling, the test tubes were delivered to the laboratory, at the temperature of +4 °C during transportation. Blood was subjected to centrigugation at 300 g for 10 min at +25 °C temperature, leading to division of blood into layers: the lowest being erythrocytes, the middle comprising leukocytes, and the upper consisting of serum. In a laminar box, in aseptic conditions, we sampled no less than 1 cm^3 of serum, transferred it into sterile test tubes (Biosigma, Italy) and froze it at the temperature of –15 °C in a domestic refrigerator (Nord, Ukraine). The samples were sent to the Biolaits Ltd Experimental Center of Diagnostics and Laboratory Assistance (Ukraine, Ternopil, Podilska st., 46), adhering to the cold regime, where progesterone concentration was measured using the enzyme-linked fluorescence assay (ELFA).

The available literature contains conflicting data about correlation between the corpus luteum and progesterone concentration in the blood serum of cattle. Particularly, these data were determined for the recipient cows on the 7th day of estral cyce and the results were analyzed for correlation between the size of the corpus luteum and progesterone concentration in the blood serum of the experimental animals. To systematize the data, all the recipients (n = 32) were divided into three groups (Table 2), depending on progesterone concentration in blood serum.

1 group (n = 7) – progesterone concentration was below 2.5 ng/cm^3.

2 group (n = 15) – progesterone concentration ranged 2.5–5.0 ng/cm^3 (control).

3 group (n = 10) – progesterone concentration was above 5.0 ng/cm^3.

Obtaining oocytes of cattle. The ovaries were sampled at the slaughter house from clinically healthy cows. The collected organs were transported in a thermos at 30–33 °C temperature. In less than 3 h after sampling, the ovaries were delivered to the Scientific Research Laboratory the Center of Reproductology of Animals with Bank of Sperm and Embryos of the National University of Life and Environmental Sciences of Ukraine. The procedures with the ovaries and cells were carried out in an Esco SC2-4A1 laminar box of Class II biological safety (type A2) (ESCO, Germany) on a heating table at 37 °C temperature.

Prior to extraction of cumulus-oocyte complexes (COCs), the extracted ovaries were rinsed in a sterile Dulbecco's buffer solution (Sigma, USA), heated up to 37–38 °C, with 4-times addition of antibiotic kanamycin sulfate in the amount of 0.075 mg/cm^3 (Sigma, USA). Sampling of COCs was performed only from follicules that varied in size within 2–8 mm (antral follicles). They were dissociated using a safety razor in a TL HEPES (5 cm^3 of stock solution) holding medium for oocytes (Minitube, Germany) with addition of 30 mg of bull serum albumin (Sigma, USA). The COCs were evaluated under a SZ51 steromicroscope (Olympus, Japan). For maturation, we selected 120–130 μm-sized COCs with complete dense cumulus, with a translucent membrane without signs of dama-

ge, with homogenous regular-form ooplasm, with no vacuoli and visible morphological signs of atresia. The selected COCs were rinsed 6 times in the TL HEPES holding medium for oocytes and placed for maturation in the 4-well dishes (Oosafe, USA) for 22–24 h in a CO_2-incubator (at 38.5 °C, containing 6% CO_2 and 5% O_2 in the gas mixture) in calculation of 25 COCs and 300 mm^3 of maturation medium for oocytes TCM 199 per well. Maturation medium for cattle oocytes TCM 199 included 4.5 cm^3 of stock solution (Minitube, Germany), 0.5 cm^3 of estral serum of cows, 0.125 IU of LH (luteinizing hormone), and 0.125 IU of FSH (follicle-stimulating hormone) in the content of 50 mm^3 of Pluset (Laboratories Calier S.A., Spain) and 0.125 IU of FSH in the content of FSH Super (Agrobiomed, RF) and 50 mm^3 of antimycotic antibiotic (Sigma, USA). The system of evaporation prevention was covered by mineral oil (Origio, Denmark).

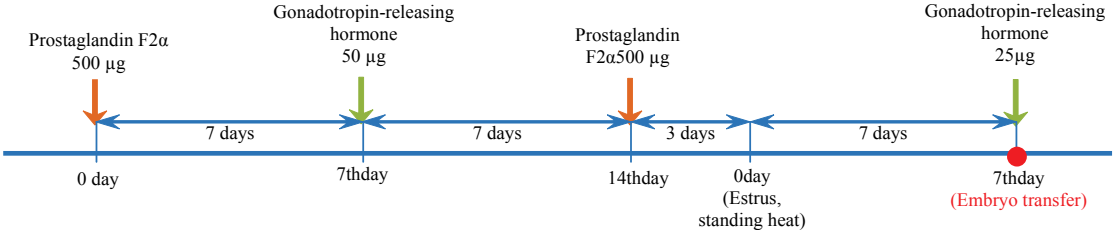

Fig. 1. Scheme of hormonal synchronization of the recipient cows

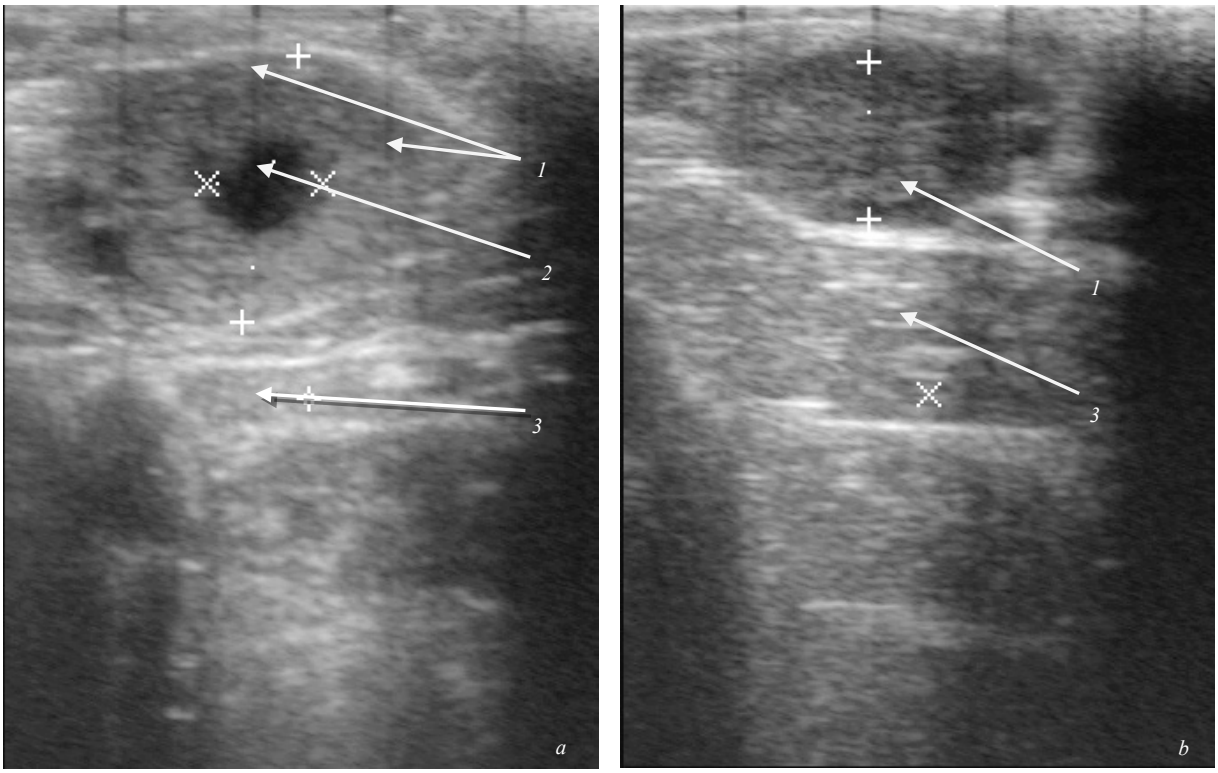

Fig. 2. Ultrasound image of corpus luteum of the bovine cattle on the 7th day after ovulation (*a*, *b*): tissue of the corpus luteum (*1*); cavity of the corpus luteum (*2*); tissue of the ovary (*3*)

Preparation of sperm for fertilization. To select progressively mobile spermatozoids from sequenced bull sperm, we used a system of density gradients, accurately coating 1 cm^3 of Origio Gradient 40 per 1 cm^3 of Origio Gradient 80. The reactives were preheated up to the room temperature (20–25 °C). Onto the gradients on the walls of test tubes, defrosted sperm was placed. Then, centrifugation was performed at 300 g centrifugal force for 20 minutes. We gathered supernatant, and added 2 cm^3 of sperm preparation medium TL for swim-up (Minitube, Germany) to the sediment. The medium was equilibrated for no less than 2 h in the CO_2-incubator at 38.5–39.0 °C and 5% CO_2. Sperm preparation medium TL for swim-up comprised 5 cm^3 of stock solution (Minitube, Germany), 30 mg of BSA (Sigma, USA), 0.55 mg of sodium pyruvate (Sigma, USA), and 50 mm^3 of antimycotic antibiotic (Sigma, USA).

The obtained suspension was centrifuged at the centrifuging force of 300 g for 5 min, after which we gathered the supernatant and repeated the procedure. After two-phase rinsing, carefully layer 1 cm^3 of the liquefied semen in a tube underneath 1 cm^3 of sperm preparation medium TL for the swim-up. The mobile fraction of spermatozoids was separated using the swim-up method (Parrish et al., 1986) in one hour. This time was enough for the normal spermatozoids to ascend to the upper layer of the medium, while dead and pathological ones stayed at the bottom of the test tube. After the swim-up was completed, 0.5 cm^3 of the supernatant was taken to a new test tube and centrifued at 300 g centifuging force for 5 min. Then, the supernatant was removed. To the obtained sediment, we added 1 cm^3 of the TL fertilization medium (Minitube, Germany) and counted spermatozoids in a Goryaev's chamber.

Co-culturing of oocytes and spermatozoids was carried out in the 4-well dishes (Oosafe, USA) for 18 h. The number of COCs in a well ranged 5 to 10, depending on sizes of the complexes, and such of spermatozoids was in calculation of 1×10^6 of mobile spermatozoids/cm^3. Co-culturing occurred in a 300 mm^3 medium per well, comprising 5 cm^3 of stock solution TL fertilization medium (Minitube, Germany), 30 mg of BSA, 0.11 μg of sodium pyruvate, 0.2 mg of heparine, and 50 mm^3 of antimycotic antibiotic (Sigma, USA). The system was covered by mineral oil (Origio, Denmark).

Cultivation of bovine cattle embryos. After co-cultivation, the cumulus became swollen, allowing it to be freed from likely zygotes by soft pipetting. The cleared likely zygotes were transferred into the cultivation dishes (Oosafe, USA) with 65 mm3 microdroplets (3 embryos in one droplet) under a layer of mineral oil (Origio, Denmark). Cultivation was

performed in the CO_2-incubator at the temperature of 38.5 °C with 6% CO_2 and 5% O_2 in the gas mixture. The embryos were cultivated in a culture medium SOF+, comprising 5 cm^3 of stock solution (Minitube, Germany), 0.5 cm^3 of estral serum of cows, 200 mm^3 of essential aminoacids (Sigma, USA), 50 mm^3 of non-essential aminoacids (Sigma, USA), and 50 mm^3 of antimycotic antibiotic (Sigma, USA). Fertilization was identified 48 h after the contact with spermatozoids, detecting cleavage, from 2 to 8-cell stage. The embryos reached the morule stage on the 4th day and the blastocyst stage on the 6–8th days.

Vitrification. Only blastocysts were subjected to vitrification – cycle day 7, stage code 6 (expressed in differention of trophoblast and embryoblast, blastocoel was distinctive, the embryo occupied most of the perivitelline space), qualitycode 1 (the embryo was symmetric, with spherical cellular mass, corresponded to the expected development stage, slight irregularities, over 85% of cellular material had undamaged vital embryonic mass; the zona pellucida was smooth, with no concaves and flat surfaces), recommended according to the classification of the International Embryo Transfer Society (IETS) (Rocha et al., 2016). Quality of the embryos was determined under a Zeiss Axio Observer A inverted microscope (Carl Zeiss, Germany).

For cryoconservation of the bovine embryo, we used Vitrification Kit 101 (Cryotech, Japan), which included a 3-well Vitri dish, Cryote and equilibration solution (ES), and vitrification solution (VS). Before using, the reactives were heated up to the room temperature (20–25 °C). Prior to cryoconservation, a cooling rack (Cryotech, Japan) was filled with liquid nitrogen and marked Cryotec. For the manipulations with blastocytes, we used a Stripper micro pipette (CooperSurgical, USA) with 300 μm Flexipet flexible polycarbonate pipettes (Cook, USA). We added 300 μm of ES to the first well and 300 μm of VS to the second and third. Using a micropipette, the embryos were placed on the surface of equilibration medium for 15 min. After the equilibration period, the embryos were aspirated with a small amount of ES and were transferred to the second well (VS) for 30 sec. The pipette was rinsed from ES and then the blastocyst was 5–6 times aspirated with pipette and retuned to the bottom of the well. Once the embryo stopped surfacing, it was introduced to the third well (VS) for 20 sec. The pipette was once again rinsed using vitrification solution, and the embryo was given time to completely settle to the bottom of the well. The blastocyst was aspirated with a small amount of VS (less than 0.1 μm) and transferred onto the Cryotec near a black mark. The surplus amount of the solution was removed with a micropipette. Under a SZ51 microscope (Olympus, Japan), we checked for presence of blastocysts on the Cryotec and quickly submerged it into liquid nitrogen. While in nitrogen, Cryotec was covered by a protective lid. The embryos were transferred into a dewar for further storage.

Thawing. Prior to thawing of blastocysts, we used a Warming Kit 102 (Cryotech, Japan), which included a 4 well Warm Plate dish, cultivation (TS), diluent (DS), and washing (WS) solutions. Prior to using DS, WS was heated up to the room temperature (20–25 °C) and TS was heated in a thermostat at 37 °C for 3 h. To the second well, we introduced 300 μm of DS, and also 300 μm of WS was added to each the third and fourth, and TS was introduced to the first well right before thawing. Using a pincette, in the conditions of liquid nitrogen, we took a protective lid off the Cryotec and quickly (for 1 s) submerged the embryo to the first well for 1 min. Then, using micropipette, we transferred the embryo to the bottom of the second well and left it there for 3 min. Afterwards, the embryo was transferred for 5 min into the third well and for 1 min to the fourth. After thawing, blastocysts were transferred to a SOF+ culture medium.

Embyo transfer. Prior to transfer, an embryologist evaluated the embryos, and under control of a binocular magnifying glass selected 2 in each catheter. The embryo transfer was performed by a qualified veterinarian after assessing the recipient. The embryos were introduced into the upper third of the uterine horn ipsilaterally to the corpus luteum using a tool for embryo transfer (Deep model, for non-shortened catheters, Minitube, Germany). Transplantation of the embryos was performed for 32 recipient cows.

Identification of pregnancy. All the animals that had undergone embryo transfer were checked for pregnancy 28 days later. We used an Alertys Ruminant ruminant pregnancy test (Idexx, USA), which by the method of immunoenzymatic analysis revealed the pregnancy markers – glycoproteins, which form during pregnancy (PAG). The procedure was performed according to the manufacturer's instructions. However, the final confirmation of pregnancy was ultrasound visualization of embryo, carried out on the 40th day after embryo transplantation. Conception rate was estimated as a ratio of pregnant females to all the recipient animals.

Statistical analysis of the data was performed using ANOVA software; the results are presented in the tables as x ± SE (mean ± standard error). To compare the difference of mean parameters between the control and experimental groups, we used the Tukey test, where the differences were considered statistically significant at $P < 0.05$ for all the data.

Results

According to the study results, there was no correlation between the size of the corpus luteum and concentration of progesterone in the blood serum of the experimental animals (Table 1). Therefore, at significant differences in progesterone concentration for the groups, the average diameter of the corpus luteum ranged 19.0–19.5 mm. However, it has to be noted that in the third group of experimental animals, we saw cows with corpus luteum diameters exceeding 15 mm, which were found not to be pregnant on the 40th day after embryo transfer, despite a high concentration of progesterone in the blood serum at the moment of embryo transfer.

The next objective of the study was to identify what effect progsterone concentration in the blood serum of the recipient cows had on pregnancy rate of the experimental animals (Table 1).

Table 1
Effects of progesterone concentration in blood serum of the recipient cows and diameter of corpus luteum on the 7th day after estrus on pregnancy rate (x ± SE, N = 32)

Concentration of progesterone in blood serum	Progesterone, ng/cm^3	Diameter of corpus luteum, mm	Pregnancy, %
<2.5 ng/cm^3	2.10 ± 0.23^b	19.0 ± 1.5^a	28.6
2.5–5 ng/cm^3	3.63 ± 0.34^a	19.0 ± 1.5^a	46.7
>5 ng/cm^3	5.89 ± 0.46^c	19.5 ± 3.4^a	20.0

Note: letters indicate significant differences between the groups in a column ($P < 0.001$) according to the results of Tukey's test.

The highest pregnancy rate (46.7%) was found in the group of animals with progesterone concentration in the blood serum in the range of 2.5–5.0 ng/cm^3. Visualization of the fetus on the 40th day after transplantation was recorded in 7 of 15 recipient cows. At the same time, decrease in the concentration of the examined steroid below 2.5 ng/cm^3 led to 18.1% decrease in pregnancy rate (2 of 7 recipient cows at the moment of ultrasound control were pregnant), compared with the control (46.7%). The group least suitable for embryo transplantation consisted of recipient cows with progesterone concentration in blood serum above 5 ng/cm^3. The pregnancy rate in this group was 26.7% lower (2 pregnant cows of 10) than in the control group.

Discussion

Despite the existing data that the size of the corpus luteum correlated with concentration of progesterone in the blood serum (Ohara et al., 1987; Kanazawa et al., 2016), this study has not found this pattern. However, there are reports that can explain the data we found in our study. In particular, Niswender et al. (2000), when studying hemodynamics of the corpus luteum at different stages of its development, noted that progesterone concentration in blood serum depended not only on amount of the steroidogenic tissue (size of corpus luteum), but also blood flow to the corpus luteum and the ability of the steroidogenic tissue to synthesize progesterone. Sartori et al. (2002) researched decline in pregnancy rate in highly productive animals, revealing that the lactating cows had larger corpora lutea than heifers and dry cows against the background of similar progesterone level, which they explained by high metabolism of steroids in highly productive cows. Sangsritavong et al. (2002), when studying effects of high levels of fodder consumption by highly productive cows on blood circulation in the liver, revealed that increased blood circulation in the liver as a result of high level of fodder consumption in highly productive dairy cows during lactation enhanced metabolism of progesterone

with no effect on the size of the corpus luteum. Donadeu et al. (2020), in turn, indicated that an essential role in regulation of lutein angiogenesis, survival and proliferation of lutein cells, and also steroidogenesis is played by microRNA (miRNA). Analysis of the obtained data and data from the literature sources may suggest that using the size of the corpus luteum as a prognostic parameter of progesterone concentration in blood serum of cows is outdated.

However, we should note that Choi et al. (2023) reported that pregnancy rate after embryo transfer can be increased by selecting recipients with optimal size of corpus luteum (15 < corpus luteum ≤ 25 mm), correlating with other parameters. The obtained data should be taken into account when selecting recipient cows.

Normal concentration of progesterone in blood plasma on the 7th day after heat ranged 1 to 6 ng/mL (Niemann et al., 1985; Bhoraniya et al., 2012; Barnwell et al., 2015), which correlates with our data (Table 3). Taking into account the absence of unaminous opinion among the researchers regarding the effects of progesterone concentration on pregnancy rate (Lonergan & Sánchez, 2020), an additional study was performed in the conditions of a farm in Ukraine. There are data that the pregnancy rate decreased after progesterone concentration in blood serum during embryo transfer (on the 7th day) decreased below 2.5 ng/cm^3 (López et al., 1995), 2.0 ng/cm^3 according to the data of Remsen et al. (1982) and Niemann et al. (1985), 1 ng/cm^3 (after transfer of fresh embryos) or 3 (after transfer of cryoconserved embryos) according to Stubbings & Walton (1986). Increase in its concentration above 5 ng/cm^3 also negatively affected the pregnancy rate (Niemann et al., 1985; López et al.,1995; Nogueira et al., 2004). However, it has to be noted that Choi et al. (2023) reported that progesterone level a day prior to embryo transfer had no statistically significant effect on the pregnancy rate, but its concentration was usually higher than in the pregnant recipients. Silva et al. (2002) found no significant differences in progesterone values in plasma on days 0, 4, and 7 between the pregnant and non-pregnant recipients. Carter et al. (2010) reported that a high concentration of progesterone had no effect on the early development of embryo in the reproductive organs of a female.

Based on the data of other researchers, we selected an optimal, in our opinion, division of animals into groups, which would allow increasing the pregnancy rate without excessive culling of recipient animals. Embryo transfer to the cows with progesterone level within 2.5–5.0 ng/cm^3 on the 7th day led to 46.7% pregnancy rate, which is high enough to economically substantiate use of this biotechnological method at farms. Analysis of the data suggest a conclusion that progesterone concentraton in blood serum above 2.5 ng/cm^3 allows provision of a necessary expression of endometrial transcriptomes and creation of conditions for embryo implantation and its further development. However, increase in progesterone above 5 ng/cm^3 led to decrease in the pregnancy rate. This pattern is explained by the fact that progesterone concentration in the blood regulates the expression of progesterone receptors (PGR) in endometry (Lonergan & Sánchez, 2020). Therefore, animals with high concentrations of the hormone undergo early loss of PGR (Okumu et al., 2010), i.e. maturation of endometry abnormally accelerates, leading to decline in endometry receptivity (Labarta et al., 2011; Fatemi &Van Vaerenbergh, 2015; Xu et al., 2022) and then the lowest implantation percentage (Al-Azemi et al., 2012; Xu et al., 2012), because time of decrease in PGR regulation is an essential event for a mother to recognize pregnancy (Bazer et al., 2011). Vice versa, low or suboptimal progesterone concentrations delay loss of progesterone receptors and, therefore, slow down identification of receptivity of the uterus to implantation (Forde et al., 2011).

Analysis of the literature and data obtained in the research may suggest that optimization of the criteria of selecting recipient cows prior to embryo transplantation requires a complex approach, because such parameters as size of corpus luteum and progesterone concentration in the blood serum cannot be completely interchangeable.

Conclusion

The obtained data suggest that a complex approach is optimal for evaluation of the recipient cows. On the day of embryo transplantation, both sizes of the corpus luteum and progesterone concentration in the blood serum have to be accounted for. Accounting for those parameters would allow the most receptive animals to be selected, and, as a result, increase the pregnancy rate. In conclusion, sizes of the corpus luteum are not a prognostic criterion of progesterone concentration in blood serum of the recipient cows on the 7th day of the estral cycle. A corpus luteum smaller than 15 mm, even in the presence of high progesterone concentration, did not provide the conditions necessary for transfer and further development of embryo. Optimal concentration of progesterone in the blood serum of the recipient cows was 2.5–5.0 ng/cm^3 (mean of 3.7 ± 0.13 ng/cm^3), which produced a 46.7% pregnancy rate in the group. Decrease in concentration of this hormone in blood serum below 2.5 ng/cm^3 decreased the pregnany rate in the group to 28.6%. At the same time, increase in progesterone concentration in blood serum above 5.0 ng/cm^3 had the most pronounced effect on decrease in the pregnancy rate of the recipient cows, because its percentage in this group was 20. The proposed strategy of selection of recipient cows on the 7th day of estral cycle can increase the pregnancy rate. The presented results can be important for commercial programs of embryo transfer, because it allows the costs for transfer to be reduced.

References

Al-Azemi, M., Kyrou, D., Kolibianakis, E. M., Humaidan, P., Van Vaerenbergh, I., Devroey, P., & Fatemi, H. M. (2012). Elevated progesterone during ovarian stimulation for IVF. Reproductive BioMedicine Online, 24(4), 381–388.

Barnwell, C. V., Farin, P. W., Whisnant, C. S., Alexander, J. E., & Farin, C. E. (2015). Maternal serum progesterone concentration and early conceptus development of bovine embryos produced *in vivo* or *in vitro*. Domestic Animal Endocrinology, 52, 75–81.

Bazer, F. W., Spencer, T. E., Johnson, G. A., & Burghardt, R. C. (2011). Uterine receptivity to implantation of blastocysts in mammals. Frontiers in Bioscience, 2, 745–767.

Berg, D. K., van Leeuwen, J., Beaumont, S., Berg, M., & Pfeffer, P. L. (2010). Embryo loss in cattle between Days 7 and 16 of pregnancy. Theriogenology, 3(2), 250–260.

Berger, H., Lietzau, M., Tichy, A., & Herzog, K. (2017). Pregnancy outcome is influenced by luteal area during diestrus before successful insemination but not by milk production level. Theriogenology, 104, 115–119.

Bhoraniya, H. L., Dhami, A. J., Naikoo, M., Parmar, B. C., & Sarvaiya, N. P. (2012). Effect of estrus synchronization protocols on plasma progesterone profile and fertility in postpartum anestrous Kankrej cows. Tropical Animal Health and Production, 44(6), 1191–1197.

Bonato, D. V., Ferreira, E. B., Gomes, D. N., Bonato, F. G. C., Droher, R. G., Morotti, F., & Seneda, M. M. (2022). Follicular dynamics, luteal characteristics, and progesterone concentrations in synchronized lactating Holstein cows with high and low antral follicle counts. Theriogenology, 179, 223–229.

Carter, F., Forde, N., Duffy, P., Wade, M., Fair, T., Crowe, M. A., Evans, A. C., Kenny, D. A., Roche, J. F., & Lonergan, P. (2008). Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reproduction, Fertility and Development, 20(3), 368–375.

Carter, F., Rings, F., Mamo, S., Holker, M., Kuzmany, A., Besenfelder, U., Havlicek, V., Mehta, J. P., Tesfaye, D., Schellander, K., & Lonergan, P. (2010). Effect of elevated circulating progesterone concentration on bovine blastocyst development and global transcriptome following endoscopic transfer of *in vitro* produced embryos to the bovine oviduct. Biology of Reproduction, 83(5), 707–719.

Choi, W., Ro, Y., Choe, E., Hong, L., Lee, W., & Kim, D. (2023). Evaluation of *corpus luteum* and plasma progesterone the day before embryo transfer as an index for recipient selection in dairy cows. Veterinary Sciences, 10(4), 262.

Colazo, M. G., López Helguera, I., Behrouzi, A., Ambrose, D. J., & Mapletoft, R. J. (2017). Relationship between circulating progesterone at timed-AI and fertility in dairy cows subjected to GnRH-based protocols. Theriogenology, 94, 15–20.

Diskin, M. G., & Morris, D. G. (2008). Embryonic and early foetal losses in cattle and other ruminants. Reproduction in Domestic Animals, 43(2), 260–267.

Dochi, O. (2019). Direct transfer of frozen-thawed bovine embryos and its application in cattle reproduction management. The Journal of Reproduction and Development, 65(5), 389–396.

Donadeu, F. X., Sanchez, J. M., Mohammed, B. T., Ioannidis, J., Stenhouse, C., Maioli, M. A., Esteves, C. L., & Lonergan, P. (2020). Relationships between size, steroidogenesis and miRNA expression of the bovine *corpus luteum*. Theriogenology, 145, 226–230.

Dunne, L. D., Diskin, M. G., & Sreenan, J. M. (2000). Embryo and foetal loss in beef heifers between day 14 of gestation and full term. Animal Reproduction Science, 58(1–2), 39–44.

e Silva, J. C., Da Costa, L. L., & Silva, J. R. (2002). Plasma progesterone profiles and factors affecting embryo-fetal mortality following embryo transfer in dairy cattle. Theriogenology, 58(1), 51–59.

Elfeel, A., Husyatinska, O., & Susol, R. (2022). Current state and prospects of the development of the dairy industry in Ukraine. Agrarian Bulletin of the Black Sea Littoral, 104, 20–31.
Fatemi, H. M., & Van Vaerenbergh, I. (2015). Significance of premature progesterone rise in IVF. Current Opinion in Obstetrics and Gynecology, 27(3), 242–248.
Ferré, L. B., Kjelland, M. E., Strøbech, L. B., Hyttel, P., Mermillod, P., & Ross, P. J. (2020). Recent advances in bovine *in vitro* embryo production: Reproductive biotechnology history and methods. Animal, 14(5), 991–1004.
Forde, N., Beltman, M. E., Duffy, G. B., Duffy, P., Mehta, J. P., O'Gaora, P., Roche, J. F., Lonergan, P., & Crowe, M. A. (2011). Changes in the endometrial transcriptome during the bovine estrous cycle: Effect of low circulating progesterone and consequences for conceptus elongation. Biology of Reproduction, 84(2), 266–278.
Forde, N., Mehta, J. P., Minten, M., Crowe, M. A., Roche, J. F., Spencer, T. E., & Lonergan, P. (2012). Effects of low progesterone on the endometrial transcriptome in cattle. Biology of Reproduction, 87(5), 124.
Gorelik, O. V., Likhodeevskaya, E., Zezin, N. N., Sevostyanov, M. Y., & Leshonok, O. I. (2020). The use of inbreeding in dairy cattle breeding. IOP Conference Series: Earth and Environmental Science, 548(8), 082013.
Hasler, J. F. (2001). Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. Theriogenology, 56(9), 1401–1415.
Kanazawa, T., Seki, M., Ishiyama, K., Kubo, T., Kaneda, Y., Sakaguchi, M., Izaike, Y., & Takahashi, T. (2016). Pregnancy prediction on the day of embryo transfer (Day 7) and Day 14 by measuring luteal blood flow in dairy cows. Theriogenology, 86(6), 1436–1444.
Kastelic, J. P., Bergfelt, D. R., & Ginther, O. J. (1990). Relationship between ultrasonic assessment of the *corpus luteum* and plasma progesterone concentration in heifers. Theriogenology, 33(6), 1269–1278.
Kovpak, V. V., Kovpak, O. S., Derkach, S. S., Valchuk, O. A., Zhuk, Y. V., & Masalovych, Y. S. (2023). Influence of calcium ionophore on the fertilization of bovine oocytes and their further embryonic development. Regulatory Mechanisms in Biosystems, 14(1), 137–144.
Kovpak, V. V., Kovpak, O. S., Valchuk, O. A., Zhuk, Y. V., & Derkach, S. S. (2022). Specifics of vitrification of *in vitro*-produced cattle embyos at various development stages. Regulatory Mechanisms in Biosystems, 13(3), 265–271.
Kovpak, V., Kovpak, O., Babii, Y., Derkach, S., & Masalovych, Y. (2022). Influence of different environments on oocyte maturation and development of bovine embryos *in vitro*. Ukrainian Journal of Veterinary Sciences, 13(3), 17–24.
Kovpak, V., Kovpak, O., Derkach, S., Masalovych, Y., & Babii, Y. (2022). The influence of platelet concentrate on the development of cattle embryos in an *in vitro* system. Scientific Horizons, 25(9), 9–18.
Kruglyak, O. V., Chornoostrovets, N. M., Kulakova, M. B., & Martynyuk, I. S. (2020). Development of genetic resources of dairy cattle breeding in Ukraine. Animal Breeding and Genetics, 60, 47–53.
Labarta, E., Martínez-Conejero, J. A., Alama, P., Horcajadas, J. A., Pellicer, A., Simón, C., & Bosch, E. (2011). Endometrial receptivity is affected in women with high circulating progesterone levels at the end of the follicular phase: A functional genomics analysis. Human reproduction, 26(7), 1813–1825.
Lonergan, P. (2011). Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology, 76(9), 1594–1601.
Lonergan, P., & Sánchez, J. M. (2020). Progesterone effects on early embryo development in cattle. Journal of Dairy Science, 103(9), 8698–8707.
López, J. R. A., Holy, L., Arceo, A. M. A., & Zaldívar, J. M. M. (1995). Influence of plasma progesterone level on pregnancy rates in bovine embryo transfer recipients. Veterinaria México, 26(2), 103–106.
Mann, G. E., & Lamming, G. E. (2001). Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction, 121(1), 175–180.
Morotti, F., Sanches, B. V., Pontes, J. H. F., Basso, A. C., Siqueira, E. R., Lisboa, L. A., & Seneda, M. M. (2014). Pregnancy rate and birth rate of calves from a large-scale IVF program using reverse-sorted semen in *Bos indicus*, *Bos indicus-taurus*, and *Bos taurus* cattle. Theriogenology, 81(5), 696–701.
Niemann, H., Sacher, B., & Elsaesser, F. (1985). Pregnancy rates relative to recipient plasma progesterone levels on the day of nonsurgical transfer of frozen/thawed bovine embryos. Theriogenology, 23(4), 631–639.
Niswender, G. D., Juengel, J. L., Silva, P. J., Rollyson, M. K., & McIntush, E. W. (2000). Mechanisms controlling the function and life span of the *corpus luteum*. Physiological Reviews, 80(1), 1–29.
Nogueira, M. F., Melo, D. S., Carvalho, L. M., Fuck, E. J., Trinca, L. A., & Barros, C. M. (2004). Do high progesterone concentrations decrease pregnancy rates in embryo recipients synchronized with PGF2alpha and eCG? Theriogenology, 61(7–8), 1283–1290.
Ohara, A., Mori, T., Taii, S., Ban, C., & Narimoto, K. (1987). Functional differentiation in steroidogenesis of two types of luteal cells isolated from mature human *corpora lutea* of menstrual cycle. The Journal of Clinical Endocrinology and Metabolism, 65(6), 1192–1200.
Okumu, L. A., Forde, N., Fahey, A. G., Fitzpatrick, E., Roche, J. F., Crowe, M. A., & Lonergan, P. (2010). The effect of elevated progesterone and pregnancy status on mRNA expression and localisation of progesterone and oestrogen receptors in the bovine uterus. Reproduction, 140(1), 143–153.
Parrish, J. J., Susko-Parrish, J. L, Leibfried-Rutledge, M. L., Critser, E. S., Eyestone, W. H., & First, N. L. (1986). Bovine *in vitro* fertilization with frozen-thawed semen. Theriogenology, 25(4), 591–600.
Pugliesi, G., Dalmaso de Melo, G., Silva, J. B., Carvalhêdo, A. S., Lopes, E., de Siqueira Filho, E., Silva, L. A., & Binelli, M. (2019). Use of color-Doppler ultrasonography for selection of recipients in timed-embryo transfer programs in beef cattle. Theriogenology, 135, 73–79.
Remsen, L. G., Roussel, J. D., & Karihaloo, A. K. (1982). Pregnancy rates relating to plasma progesterone levels in recipient heifers at day of transfer. Theriogenology, 18(3), 365–372.
Rocha, J. C., Passalia, F., Matos, F. D., Maserati Jr., M. P., Alves, M. F., Almeida, T. G., Cardoso, B. L., Basso, A. C., & Nogueira, M. F. (2016). Methods for assessing the quality of mammalian embryos: How far we are from the gold standard? JBRA Assisted Reproduction, 20(3), 150–158.
Sangsritavong, S., Combs, D. K., Sartori, R., Armentano, L. E., & Wiltbank, M. C. (2002). High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17beta in dairy cattle. Journal of Dairy Science, 85(11), 2831–2842.
Santos, G. M. G. D., Junior, L. B., Silva-Santos, K. C., Ayres Dias, J. H., Dias, I. D. S., Seneda, M. M., & Morotti, F. (2023). Conception rate and pregnancy loss in fixed-time cattle embryo transfer programs are related to the luteal blood perfusion but not to the corpus luteum size. Theriogenology, 210, 251–255.
Sartori, R. G. J. M., Rosa, G. J. M., & Wiltbank, M. C. (2002). Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. Journal of Dairy Science, 85(11), 2813–2822.
Siqueira, L. G. B., Areas, V. S., Ghetti, A. M., Fonseca, J. F., Palhao, M. P., Fernandes, C. A. C., & Viana, J. H. M. (2013). Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. Journal of Dairy Science, 96(10), 6461–6472.
Smith, M. F., Geisert, R. D., & Parrish, J. J. (2018). Reproduction in domestic ruminants during the past 50 year: Discovery to application. Journal of Animal Science, 96(7), 2952–2970.
Stevenson, J. S., & Britt, J. H. (2017). A 100-year review: Practical female reproductive management. Journal of Dairy Science, 100(12), 10292–10313.
Stubbings, R. B., & Walton, J. S. (1986). Relationship between plasma progesterone concentrations and pregnancy rates in cattle receiving either fresh or previously frozen embryos. Theriogenology, 26(2), 145–155.
Vasconcelos, J. L. M., Sartori, R., Oliveira, H. N., Guenther, J. G., & Wiltbank, M. C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56(2), 307–314.
Viana, J. (2020). 2019 statistics of embryo production and transfer in domestic farm animals: Divergent trends for IVD and IVP embryos. Embryo Technology Newsletter, 4, 7–26.
Wathes, C. M., Kristensen, H. H., Aerts, J. M., & Berckmans, D. (2008). Is precision livestock farming an engineer's daydream or nightmare, an animal's friend or foe, and a farmer's panacea or pitfall? Computers and Electronics in Agriculture, 64(1), 2–10.
Xu, B., Li, Z., Zhang, H., Jin, L., Li, Y., Ai, J., & Zhu, G. (2012). Serum progesterone level effects on the outcome of *in vitro* fertilization in patients with different ovarian response: An analysis of more than 10,000 cycles. Fertility and Sterility, 97(6), 1321–1327.
Xu, Y., Zhang, J., Li, A., Yang, N., Cui, N., Hao, G., & Gao, B. L. (2022). Impact of elevated progesterone in late follicular phase on early pregnancy outcomes and live birth rate after fresh embryo transfers. Frontiers in Cell and Developmental Biology, 10, 855455.