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The Influence of Platelet Concentrate on the Development of Cattle Embryos in an *In Vitro* System

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Abstract. There are many studies on the improvement of the conditions for cultivating bovine embryos *in vitro*. However, the issue of co-cultivation of platelets and cow embryos is understudied, although platelet activation leads to the release of a considerable amount of biologically active substances into the culture medium and their co-cultivation with embryos can positively affect the growth and development of the latter. Therefore, the purpose of this study was to investigate the effect of different platelet concentrations in the *in vitro* culture system on the development of cow embryos. Fertilized zygotes (total number 180) were divided into 5 groups: Group 1 – culture medium without platelet addition (control); Group 2 – medium for cultivation with the addition of 10×10^6 platelets/cm^3; Group 3 – final concentration in the system is 20×10^6 platelets/cm^3; Group 4 – 50×10^6 platelets/cm^3; Group 5 – 100×10^6 platelets/cm^3. According to the results, it was found that co-cultivation of embryos with platelets in the *in vitro* system is effective. Thus, a correlation was found between improved embryo development indicators and an increase in platelet concentration. The optimal platelet concentration was 50×10^6/cm^3, which allowed obtaining a 13.9% higher level of blastulation, 15.7% higher average embryo size, and 2.5% higher average number of cells in the blastocyst compared to the control. At the same time, the platelet concentration of 100×10^6/cm^3 led to a significant decrease in the indicators under study, compared with the group without platelets. Therefore, co-cultivation of cow embryos with platelets is advisable since it allows improving the development indicators of cow embryos. The data analysed and presented in this paper will increase the efficiency of cultivation of bovine embryos for both scientific and industrial purposes

Keywords: biotechnology of ruminant reproduction, growth factor, division, blastocyst

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INTRODUCTION

Despite the widespread study of methods used for the cultivation of bovine embryos, there are still ways to improve them. Optimisation of cultivation parameters *in vitro* allows increasing the percentage of embryos suitable for transplantation. Therefore, scientists continue to work on improving the protocols of assisted reproductive technologies in cattle breeding.

In veterinary embryology, the use of blood platelets or biologically active substances released during the cultivation of bovine embryos is still understudied. This study is the first to analyse the effect of diverse platelet concentrations on the percentage of blastulation, cell number, and size of cow blastocysts *in vitro*.

Bovine embryo transplantation is a biotechnological method used for programs of accelerated reproduction of highly productive animals and herd improvement by actively using the genetic potential of females (Dochi, 2019). In cattle breeding, there are 2 methods of obtaining embryos: *in vivo* and *in vitro*. Until 2016, *in vivo* cattle embryo production was dominant (Viana, 2020). However, with the development of the branch, obtaining oocytes by ovum pick up (OPU) allowed obtaining a more considerable number of embryos produced *in vitro* per individual animal, compared with indicators after multiple ovulation embryo transfer (MOET) (Hasler *et al*., 2003; Ferré *et al*., 2020). Notably, the technologies of genomic selection used by selective breeding companies additionally increase the demand for embryos obtained from genetically desirable animals obtained *in vitro* since the use of this technique allows reducing the intervals between generations and increasing genomic growth (Aardema *et al*., 2022).

In addition, *in vitro* embryo production technology has made great strides since the first extracorporeal fertilisation calf was born at the University of Pennsylvania in 1981 (Brackett *et al*., 1986). Considering the above, the ratio of bovine embryos obtained *in vitro* (IVP) to embryos obtained *in vivo* has gradually changed (1 million to 378,769 embryos in 2019, respectively) (Viana, 2020). Today, biotechnology of cow reproduction is a profitable business of international level, which tends to increase the percentage of obtaining embryos by *in vitro* fertilisation (O'Neill, 2005).

However, despite noteworthy progress in the development of techniques for obtaining embryos *in vitro*, the ratio of embryos suitable for transplantation to used ova is still quite low. Thus, Ferré *et al*. (2020) indicate that only 20-40% of fertilized eggs reach the blastocyst development stage on Day 7. Lonergan & Fair (2008) states that only 30-35% of zygotes can develop into characteristics when their transfer to recipient cows is successful. Inaba *et al*. (2016) noted the effect of sperm on the further development of embryos, and the percentage of blastocysts obtained in their study was within 22-44%. By investigating the kinetic patterns of embryos reaching the blastocyst stage, Oliveira *et al*. (2019) obtained 29.9% blastocysts, and in the studies of Sirard *et al*. (1988), the percentage of obtaining embryos suitable for transplantation was 28%.

Given the low level of cattle embryos reaching a stage sufficient for transplantation, scientists continue to work on perfecting cultivation conditions. The production of bovine embryos *in vitro* involves achieving the closest parameters of the oviduct environment (Besenfelder *et al*., 2020). Clearly, the conditions of cultivation are still unable to provide all the advantages of development in the reproductive organs of the female. However, several culture systems and environments have been proposed that allow approaching physiological conditions. Thus, Lopera-Vásquez *et al*. (2016) used a conditioned medium obtained from bovine oviduct epithelial cell lines and noted its positive effect on the quality of embryos obtained *in vitro*. Goovaerts *et al*. (2009) indicate an increase in the percentage of blastocysts during the co-cultivation of embryos and cumulus cells. Chen *et al*. (2017) proposes to culture zygotes on mammalian oviductal epithelial cells, which generate oviductal fluid surrogates, to the blastocyst stage without the addition of embryo culture medium. Leivas *et al*. (2011) and Soto-Moreno *et al*. (2021) recommend adding foetal bovine serum to the culture medium, while Rizos *et al*. (2003) indicate its lack of effectiveness and negative impact on cryotolerance of embryos. Cañón-Beltrán *et al*. (2021) demonstrate a positive effect of nobiletin on the pre-implantation development of bovine embryos *in vitro* and improvement of the quality of produced blastocysts.

As the data given above suggest, there is currently a large amount of information on the improvement of the conditions for the cultivation of bovine embryos *in vitro*, but it is extremely diverse and often the conclusions of scientists are contradictory. That is why, *the purpose of this study* is to investigate the effect on the development of cattle embryos of co-cultivation with different concentrations of platelets in the commercial environment Minitube (Germany) and practical implementation of the obtained results.

MATERIALS AND METHODS

The study was conducted during 2020-2022 based on the Educational and Scientific Laboratory "Centre for Animal Reproductology with Sperm and Embryo Bank" of the National University of Life and Natural Sciences of Ukraine. Animal experiments were conducted in compliance with the requirements of the Law of Ukraine "On the 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 agreed with the provisions of the "European Convention for the Protection of Vertebrates Used for Experiments and Other Scientific Purposes" (Strasbourg, 1986).

Production of zygotes *in vitro*

1.1. Maturation

Ovaries from clinically healthy cows were selected in slaughterhouses and delivered to the laboratory in a thermos at 30-33°C no more than 3 hours after sampling. In the laboratory, the ovaries were washed 4 times in a sterile phosphate-salt solution of Dulbecco (Sigma, USA) with the addition of 0.075 mg/cm^3 kanamycin sulphate (Sigma, USA) (solution temperature 37-38°C). The cumulus cell-oocyte complexes (COCs) from the antral follicles (2-8 mm in size) of the ovaries of cows were removed in a laminar box by dissecting the follicles with a safety razor blade in an oocyte collection medium consisting of 5 cm^3 TL HEPES (Minitube, Germany) with the addition 30 mg of bovine serum albumin (BSA) (Sigma, USA). Removal of COCs, their selection and setting for maturation, fertilisation, and subsequent cultivation were also performed in a sterile box. After evaluation under a stereomicroscope SZ51 (Olympus, Japan), COCs of 120-130 μm with solid dense cumulus, intact transparent shell and homogeneous unvacuolized ooplasm of regular rounded shape were selected, without visible morphological signs of atresia. The extracted COCs were washed 6 times in an oocyte collection medium. The COCs were selected and washed on a heating table at 37°C. Oocytes were matured *in vitro* for 22-24 hours in 4-well plates (Oosafe, USA). 300 mm^3 of medium was added to each well, which included 4.5 cm^3 of the initial solution of maturation medium TCM 199 (Minitube, Germany), 0.5 cm^3 of bovine estrous serum, 0.125 IU. FSH (follicle-stimulating hormone) and 0.125 IU of luteinising hormone (LH) (50 mm^3 "Pluset" (Laboratories Calier S.A., Spain)) and 0.125 IU of FSH ("FSH-super" (Agrobiomed, RF)) and 50 mm^3 of an antibiotic-antimycotic (Sigma, USA) were covered with mineral oil (Origio, Denmark), 25 COCs were added and cultivated in a CO_2 incubator at 38.5°C and 6% CO_2 and 5% O_2.

1.2. Preparing sperm for fertilisation

Bull sperm was prepared for fertilisation using density gradients "Origio Gradient Series" (Origio, Denmark) and sperm capacitation medium (Minitube, Germany). The components of the gradient were heated to room temperature (20-25°C), all other reagents were equilibrated in a CO_2 incubator at 38.5-39.0°C and 5% CO_2 for at least 2 hours. The gradient was prepared by carefully layering 1 cm^3 of "Origio Gradient 40" on 1 cm^3 of "Origio Gradient 80", after which sperm previously thawed in a water bath was carefully introduced. The resulting system was centrifuged at a centrifugal force of 300 g for 20 minutes. The supernatant was removed, and the sediment was transferred with a new sterile nozzle into a test tube with 2 cm^3 of medium for sperm preparation and capacitation, which included 5 cm^3 of basic solution for capacitation (Minitube, Germany), 30 mg of BSA (Sigma, USA), 0.55 mg of sodium pyruvate (Sigma, USA) and 50 mm^3 of antibiotic-antimycotic (Sigma, USA) and centrifuged at a centrifugal force of 300 g for 5 minutes, most of the supernatant was removed. The procedure was repeated twice. After washing, the sediment was transferred to the bottom of a test tube with 1 cm^3 of a new portion of medium for the preparation and capacitation of spermatozoa. The mobile sperm fraction was obtained using the swim-up method described by Parrish *et al.* (1986). Incubation for 1 hour was sufficient for mobile spermatozoa to rise to the upper layers of the medium, while dead and pathological ones stayed at the bottom of the test tube. Motile spermatozoa were capacitated in the medium for preparation and capacitation for 4 hours of exposure to heparin (Sigma, USA) at a concentration of 20 μg/cm^3 in a CO_2 incubator at 38.5°C with 6% CO_2 and 5% O_2. After capacitation, the spermatozoa were centrifuged at a centrifugal force of 200 g for 5 minutes. The supernatant was removed and 1 cm^3 of fertilisation medium was added, and the concentration of spermatozoa was counted in the Goryaev chamber.

1.3. Co-cultivation of oocytes with spermatozoa

After maturation, the oocytes were co-cultured with spermatozoa. Oocytes were fertilised in 4-well plates (Oosafe, USA) in 300 mm^3 of medium, which included 5 cm^3 of fertilisation medium (Minitube, Germany), 30 mg of BSA, 0.11 μg of sodium pyruvate, 0.2 mg of heparin, and 50 mm^3 of an antibiotic-antimycotic (Sigma, USA). The oocytes were covered with mineral oil (Origio, Denmark) for 18 hours after the addition of capacitated spermatozoa (at the rate of 1×10^6 motile spermatozoa/cm^3). The number of oocytes in the well ranged from 5 to 10.

2. Obtaining bovine platelets

Blood was obtained from the jugular vein from 8 clinically healthy cows that had not received medication in the preceding two months. Preliminarily, surgical treatment of several centimetres of skin near the jugular vein was performed. From each cow, 20 cm^3 of blood was collected by a standard method into sterile Vacutainer Plus vacuum tubes with EDTA (BD, USA). The test tubes were transported at 4°C to the laboratory no more than 2 hours after selection. All stages of obtaining platelets from whole blood were carried out in a laminar box under aseptic conditions. The blood was transferred to sterile centrifuge tubes and subjected to centrifugation at 100 g within 30 minutes, which caused the blood to divide into three layers: red blood cells at the lowest level, white blood cells at the middle level, and platelet-rich plasma (PRP) at the top. The top layer was selected, transferred to new sterile test tubes, and the platelet count in the suspension was calculated using a Goryaev chamber. Platelet-rich plasma was re-centered at 300 g for 15 minutes, after which the sediment was selected. 1 cm^3 of embryo culture medium was carefully added to the platelet sediment and centrifuged at 300 g for 10 minutes. The sediment was selected, and a medium for embryo culture was added to the sediment at the rate of 1×10^9 platelets/ml.

3. Embryo cultivation

After co-culture, bovine oocytes with loose enlarged cumulus were released from cumulus cells by gentle pipetting in 0.1% hyaluronidase solution (Sigma, USA). Then the oocytes were washed from the enzyme in 5-6 drops of TL HEPES (Minitube, Germany) and transferred to a culture medium comprising 5 cm^3 of culture medium with pyruvate (Minitube, Germany), 0.5 cm^3 of bovine estrous serum, 200 mm^3 of essential amino acids (Sigma, USA), 50 mm^3 of substitute amino acids and 50 mm^3 of antibiotic-antimycotic (Sigma, USA). Fertilized oocytes were discovered by division from the 2- to 8-cell stage 48 hours after contact with spermatozoa. 48 hours after fertilisation, the zygotes were randomly divided into 5 groups and platelet concentrate was added in different amounts. Embryos were cultivated in microdrops of 100 mm^3 (6 embryos per drop) under a layer of mineral oil (Origio, Denmark) in culture dishes (Oosafe, USA) in a CO_2 incubator at 38.5°C with 6% CO_2 and 5% O_2.

4. Embryo development evaluation

Embryo quality was evaluated on Day 7 under a Zeiss Axio Observer A inverted microscope (Carl Zeiss, Germany), photos and size measurements were made using the Octax NaviLase laser system (OCTAX Microscience GmbH, Germany).

The number of nuclei in the embryo was counted under a DMR microscope (Leica, Germany) at a magnification of ×400, ×1000, and photographed with a Canon DS126291 camera (Canon, Taiwan). To visualize the nuclei, the embryo was stripped of its transparent shell using a laser system, after which it was transferred to superfrost slides (Thermo Scientific, USA) in a drop with a solvent (0.2% Tween20 (Sigma, USA) in 0.01 m HCl (Sigma, USA), ph 2.0) for lysis of the blastomere envelope. The process was monitored under an inverted microscope, adding a new portion of solvent if necessary. After lysis of the blastomere shell, the solvent was selected as much as possible and the nuclei were sequentially washed with a phosphate buffer solution (Sigma, USA), 70% ethyl alcohol (Sigma, USA) and 100% ethyl alcohol (Sigma, USA). Glass samples were air-dried and stained with an industrial smear kit "Leucodif 200" (Erba Lachema, Czech Republic), according to the manufacturer's instructions.

5. Statistical processing

The reliability of the results was evaluated according to the criteria proposed by Brzhevska *et al.* (2019). Statistical processing of the obtained results of experimental studies was performed according to N.A. Plokhinsky (1970) and E.V. Montseviciute-Eringen (1964) using the Microsoft Excel data analysis package (Lesnikova & Kharchenko, 2002). Arithmetic mean values and their errors were found, and the probability of difference between parallel data sets was figured out. In all cases, the difference was considered reliable at $P<0.05$.

RESULTS AND DISCUSSION

The clinical benefit of platelet concentrates has been reported to be fourfold higher than that of whole blood (Marx, 2001) (platelet concentrations in bovine blood range within 160-800×10^6/ml (Roland *et al.*, 2014)), but strong scientific evidence that preparations with a platelet content below these are less effective (Cole *et al.*, 2010). Considering the specific cultivation conditions, where the presence of platelets is atypical, it was decided to create systems for culturing bovine embryos using a lower platelet concentration than in whole blood. Thus, 48 hours after fertilisation, the zygote (total number 180) was randomly divided into 5 groups (the study in each group was repeated three times, (n=3):

Group 1 – culture medium without platelet addition (control).

Group 2 – the cultivation system included 90 mm^3 medium for cultivating embryos with the addition of 11×10^6 platelets/cm^3 and 10 mm^3 medium where cattle embryos were cultivated for 48 hours to activate platelets with clot formation.

Group 3 – final concentration in the system is 20×10^6 platelets/cm^3;

Group 4 – final concentration in the system is 50×10^6 platelets/cm^3;

Group 5 – final concentration in the system is 100×10^6 platelets/cm^3 (Table 1).

***Table 1**. The influence of the concentration of platelets in the culture system on the development of bovine embryos in vitro (M±m, n=3)*

Group	Platelet count, platelets/cm^3	Number of zygotes, pcs	Blastocysts, %	Number of cells in the blastocyst, pcs	Blastocyst size, μm
1	No platelets (control)	36	38.8±2.4	173.3±10.3	195.0±3.4
2	10×10^6	36	41.6±3.6	201.1±3.4***	203.2±2.2**
3	20×10^6	36	50.0±3.6 *	215.6±2.7***	213.2±2.3***
4	50×10^6	36	52.7±2.4**	224.4±4.8***	225.6±4.8***
5	100×10^6	36	33.3±0.0*	153.1±4.2***	188.4±2.2*

***Note:** * – P<0.05, ** – P<0.01, *** – P<0.001 compared to the control group (medium without platelet addition)*
***Source:** compiled by the authors*

McCarrel & Fortier (2009) indicated that platelet degranulation begins within 10 minutes after exposure to clotting factors. Therefore, in this study, embryos were introduced into the culture medium 30 minutes after the start of aggregation to prevent the embryo from being involved in the clot formation. Aggregated platelets were left in the culture system (Fig. 1) because the data of McCarrel & Fortier (2009) indicate that the main secretion of growth factors occurs within the first hour, but their constant release is noted throughout the entire period of viability of platelets (7 days).

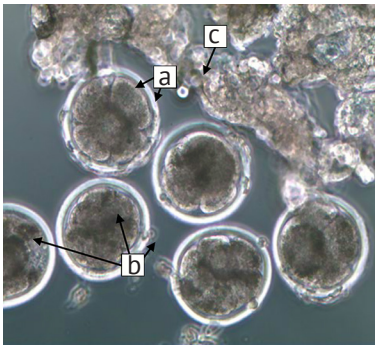

Figure 1. *Culture system with the addition of allogeneic platelets (second group, third day after fertilisation)*
Note: *a) 4-cell embryo; b) 8-cell embryo; c) aggregated platelets. Native preparation, Mag.: ×200*

The effect of platelets on the development of bovine embryos was assessed comprehensively, the percentage of blastulation, and the number of cells (Fig. 2) and blastocyst sizes (Fig. 3) were found.

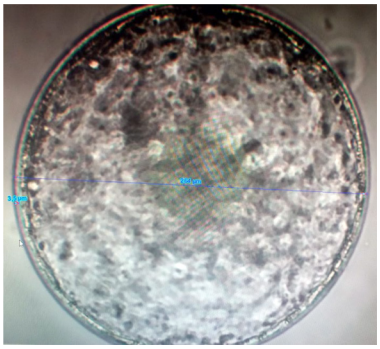

Figure 2. *Bovine blastocyst*
Note: *Cycle Day – 7, Stage Code – 7, Quality Code – 1 (according to the classification proposed by Bó & Mapletoft (2013))*

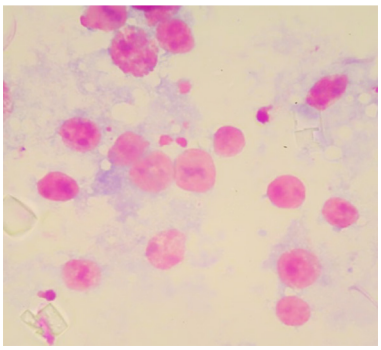

Figure 3. *Embryo nuclei are visualized after blastomere lysis*
Note: *Stained with "Leukodif 200". Mag.: ×1000*

When studying the influence of the concentration of platelets in the culture system on the development of bovine embryos *in vitro*, differences were found. Thus, the addition of 20×10^6 and 50×10^6 platelets/cm^3 contributed to a significant increase in the number of blastocysts by 11.2% ($P<0.05$) and 13.9% ($P<0.01$), respectively, compared to the control (38.8±2.4%). While the addition of 100×10^6 platelets/cm^3 resulted in a significant decrease in blastulation percentage on Day 7 by 5.5% ($P<0.05$) compared to the control.

In addition, in the groups, the authors noted a correlation between the percentage of blastulation on Day 7, the size and number of cells in the embryo. Thus, adding 10×10^6 platelets/cm^3 to the culture system increased the average number of cells in the blastocyst by 16.0% ($P<0.001$), 20×10^6 platelets/cm^3 – by 24.4% ($P<0.001$), 50×10^6 platelets/cm^3 – by 29.5% ($P<0.001$), compared to the control (173.3±10.3 cells). A similar pattern was noted when measuring blastocyst sizes, e.g., the addition of 10×10^6 platelets/cm^3 increased the size of the embryo by 4.2% ($P<0.01$), 20×10^6 platelets/cm^3 – by 9.3% ($P<0.001$), 50×10^6 platelets/cm^3 – by 15.7% ($P<0.001$), compared to the control (195.0±3.4 μm). An increase in the concentration of platelets in the culture system to 100×10^6/cm^3 led to the opposite effect. Thus, the average number of cells in the blastocyst decreased by 11.7% ($P<0.001$), and its average size decreased by 3.4% ($P<0.05$), comparing with control (173.3±10.3 cells and 195.0±3.4 μm, respectively).

Therefore, adding platelets to the culture medium for cow embryos is effective. The concentration of 50×10^6 platelets/cm^3 is best and allows maximising the number and improving the quality of bovine embryos produced *in vitro*.

Platelets are small disc-shaped non-nuclear cells formed by bone marrow megakaryocytes and circulating in the blood, playing a crucial role in supporting vascular integrity and regulating haemostasis (Ghoshal & Bhattacharyya, 2014; Bos-Mikich *et al*., 2019). They are the second most common component of blood after red blood cells, their size ranges from 2 to 5 μm (Michelson *et al*., 2019). Platelets hold three distinct types of granules: α-granules, dense or δ-granules, and lysosomes (Koupenova *et al*., 2018; Morrell *et al*., 2014; Semple *et al*., 2011). Platelet α-granules hold proteins, chemokines, cytokines, and growth factors collected in platelets by megakaryocytes and are necessary for normal platelet function. Δ-granules hold small molecules such as ADP, serotonin, polyphosphates, glutamate, histamine, and calcium necessary for haemostasis (Koupenova *et al*., 2017; Morrell *et al*., 2014). Platelet lysosomes hold glycohydrolases and enzymes that break down glycoproteins, glycolipids, and glycosaminoglycans (Ciferri *et al*., 2014; Morrell *et al*., 2014).

The activation of platelets leads to morphological changes caused by the reorganisation of the cortical actin cytoskeleton from a disk-like shape to a significantly enlarged spherical shape (Bender & Palankar, 2021). Exocytosis of granules is initiated by the activation of surface receptors, which leads to an increase in the level of intracellular Ca^{2+} and activation of phosphokinase C (PKC) (Manne *et al*., 2017). After stimulation, platelets free the contents of their granules releasing growth factors such as platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF 1), fibroblast growth factor (FGF), haepatocyte growth factor (HGF) (Drago *et al*., 2013), which can increase the proliferative activity of cells (Mazurkevych *et al*., 2021). B_1 and β_3 integrins: e.g., $\alpha_2\beta_1$ (GPIa/IIa) and $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) (Ludwig *et al*., 2022). Seven antimicrobial peptides: Platelet Factor 4 (PF-4), RANTES, Connective Tissue Activating Peptide 3 (CTAP-3), Platelet Basic Protein, Thymosin β-4 (Tβ-4), fibrinopeptide B (FP-B), fibrinopeptide A (FP-A) (Tang *et al*., 2002) and other molecules. Therefore, apart from their crucial role in coagulation and maintenance of haemostasis after mechanical damage to the vascular system, platelets, due to the many bioactive molecules in their granules and the expression of various receptors on their surfaces, can perform a wide range of other essential functions (Schlesinger, 2018).

There are few clinical reports of platelet use in veterinary medicine. Usually, platelet-rich plasma is used to treat lesions of the musculoskeletal system of horses (Brossi *et al*., 2015; Malyuk *et al*., 2021), skin wounds in dogs Farghali *et al*., 2019), intestinal wounds in pigs (Fresno *et al*., 2010), ovarian hypofunction in cows (Cremonesi *et al*., 2020), etc. This study offers a novel approach to the use of platelet concentrate based on current knowledge about its regenerative effect due to its high content of growth factors and cytokines (Bendinelli *et al*., 2010).

Embryos of all mammalian species studied to date produce and secrete platelet activation factor (PAF) (O'Neill, 2005) at elevated levels of 1 to 100 ng of PAF/embryo in 24 hours (Ammit & O'neill, 1991; Roudebush *et al*., 2002). Co-culture of the embryo with platelets will activate the latter and release biologically active substances into the culture medium, which can positively affect the growth and development of the embryo. However, a sharp decrease in platelet activation factor can adversely affect embryo development since the physiologically important target for PAF released by the embryo is itself (O'Neill, 2005). Therefore, an important task when introducing platelets into the culture system is to find their number to ensure a positive effect on the development of bovine embryos.

In the available literature sources, some publications have been found on the effect of platelet-rich plasma (PRP) on embryo development *in vitro* (Thibodeaux *et al*., 1993; Lange-Consiglio *et al*., 2015; Ramos-Deus *et al*., 2020). A specific feature of these studies is that PRP was used as a substitute for the components of the standard culture medium. Whereas, in the study presented above, platelets were introduced as an added component to the culture system.

Analysing the data obtained during the study, several features were noted. In Group 5 (100×10^6 platelets/cm^3), a decrease in the percentage of blastulation and embryo quality was observed, which is probably caused by a high platelet count in the medium. DeLong *et al.* (2012) claim that excessive platelet counts can lead to cellular apoptosis, suppression, and desensitisation

of growth factor receptors, and have an inhibitory effect. Tang *et al*. (2002) notes that a significant increase in the content of growth factors in the environment also inhibits the development of the embryo. At that time, the quality of embryos in Groups 2, 3 and 4 was higher than in the first (control), which may be related to growth factors released by platelets (platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), epithelial growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF), haepatocyte growth factor (HGF)) (Drago *et al*., 2013). Lange-Consiglio *et al.* (2015) believe that biologically active substances released by platelets during activation can stimulate the development of bovine embryos and the proliferation of trophoblast cells during cultivation *in vitro*. Larson *et al.* (1992) point out that after platelet aggregation, which occurs when they are added to the culture medium, some glycoproteins and fibronectin are released, providing the extracellular matrix necessary for embryo development to the blastocyst stage.

CONCLUSIONS

Studies of the effect of platelets on the preimplantation development of bovine embryos indicate that the latter can also affect the percentage of blastulation and cell proliferation. The authors suggest that blood plates introduced into the culture system before embryos enrich the environment with factors necessary for its development. However, different platelet concentrations in the culture system cause divergent effects. Thus, with the addition of $10\times10^6/cm^3$ platelets to the medium, an increase in the percentage of blastulation by 2.8%, cell proliferation by 16.0% ($P<0.001$), and the size of blastocysts by 4.2% ($P<0.01$) was noted, compared with control. With an increase in the concentration of platelets to $20\times10^6/cm^3$, improvement in the development indicators of bovine embryos was observed, compared with the $10\times10^6/cm^3$ group. The best concentration is 50×10^6 platelets/cm^3, which allows maximising the number and improving the quality of bovine embryos produced *in vitro*. Thus, at the specified concentration of blood plates, the percentage of blastulation increased by 13.9% ($P<0.01$), the number of cells in the blastocyst – 29.5% ($P<0.001$), and the size of blastocysts increased by 15.7% ($P<0.001$) compared to the group without platelet addition. At that time, the addition of 100×10^6 platelets/cm^3 leads to a sharp decrease in all the indicators under study, e.g., the average number of cells in a blastocyst decreased by 11.7% ($P<0.001$), and its average size – by 3.4% ($P<0.05$), compared to control.

This study offers a new strategy for *in vitro* embryo culture and opens the possibility of using platelets in future assisted reproductive technology programs in veterinary medicine as a way to increase the blastulation rate and the quality of the embryos obtained.

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Вплив концентрату тромбоцитів на розвиток ембріонів великої рогатої худоби у системі *in vitro*

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Анотація. Існує велика кількість досліджень щодо удосконалення умов культивування ембріонів великої рогатої худоби *in vitro*. Проте, питання співкультивування тромбоцитів та ембріонів корів мало вивчене, хоча активація тромбоцитів призводить до вивільнення у культуральне середовище значної кількості біологічно активних речовин і їх співкультивування з ембріонами може позитивно вплинути на ріст і розвиток останніх. Тому, метою роботи було дослідження впливу різних концентрацій тромбоцитів у культуральній системі *in vitro* на розвиток ембріонів корів. Запліднені зиготи (загальна кількість 180) поділили на 5 груп: 1 група – середовище для культивування без додавання тромбоцитів (контроль); 2 – середовище для культивування з додаванням 10×10^6 тромбоцитів/см^3; 3 – кінцева концентрація у системі 20×10^6 тромбоцитів/см^3; 4 – 50×10^6 тромбоцитів/см^3; 5 група – 100×10^6 тромбоцитів/см^3. За результатами встановлено, що співкультивування ембріонів з тромбоцитами у системі in vitro є ефективним. Так, відмічали кореляцію покращення показників розвитку ембріонів зі збільшенням концентрації тромбоцитів. Оптимальною концентрацією тромбоцитів виявилася 50×10^6/см^3, що дозволило отримати на 13.9% вищий рівень бластуляції, на 15.7% – середній розмір ембріонів і на 2.5% – середню кількість клітин в бластоцисті, порівнюючи з контролем. У той час концентрація тромбоцитів 100×10^6/см^3 призвела до достовірного зниження досліджуваних показників, у порівнянні з групою без тромбоцитів. Отже, співкультивування ембріонів корів з тромбоцитами є доцільним, адже дозволяє покращити показники розвитку ембріонів корів. Проаналізовані та представлені в статті дані дозволять підвищити ефективність культивування ембріонів великої рогатої худоби як в наукових, так і виробничих цілях

Ключові слова: біотехнологія відтворення жуйних, фактор росту, ділення, бластоциста