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Influence of Different Environments on Oocyte Maturation and Development of Bovine Embryos *in Vitro*

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Abstract. According to the International Embryo Technology Society, the number of bovine embryos produced by *in vitro* fertilization technology is increasing every year. However, despite the large volumes of their production, the effectiveness of this method is still low and needs to be improved. Therefore, the purpose of this study was to compare the effectiveness of two commercial media – Origio Sequential Series (Origio, Denmark) and a panel of products manufactured by Minitube (Germany) in terms of oocyte maturation and development of bovine embryos *in vitro.* At the first stage of the study, a comparative evaluation of oocyte maturation media was performed: based on TCM 199 (Minitube) and Universal (Origio) culture media. At the second stage, the protocols for culturing bovine embryos were compared: Minitube and the two-stage Origio culture protocol with changing media. Therewith, it was found that the use of TCM 199 medium for oocyte maturation is more effective compared to Universal. Thus, at 48 hours of cultivation (the initial stage of embryo development), 64.3 ± 1.0 and 60.3 ± 1.4% of 2-8 cell embryos were obtained, and on Day 8 – 25.3 ± 1.0 and 20.0 ± 0.6% of blastocysts, respectively. The results of a comparison of bovine embryo culture protocols showed that when using both Minitube and Origio media, the percentage of division and the percentage of resulting embryos corresponded to their known values. It was found that the Minitube cultivation protocol is more effective than Origio. At 48 hours, the number of embryos obtained using the Minitube culture protocol was 1.3% higher compared to Origio, on Day 6 – by 7.8%, and on Day 8 – by 3.8%. The results obtained are a necessary component of the development of successful processes to produce bovine embryos *in vitro* with further implementation in the ruminant reproduction biotechnology

Keywords: embryonic development, Minitube and Origio culture media, cultivation protocols, ruminant reproduction biotechnology

Introduction

Animal husbandry plays an important role in agriculture and the economy of Ukraine. It provides profit and work for producers and other individuals working in value chains [1]. Currently, there is a well-known forecast that the world's population will exceed 9.2 billion by 2050, and this in turn will require an increase in food production by 50% [2]. However, it is impossible to achieve the required production volumes using conventional methods of animal reproduction biotechnology. To create volumes sufficient to meet the needs of the constantly growing population, the livestock industry needs to use modern methods of reproduction biotechnology, which contribute to obtaining greater efficiency from livestock productivity. Thus, genetic advance in dairy cattle breeding leads to an increase in the

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availability of milk, which provides nutrients to a growing population, without requiring an increase in the total number of animals that produce milk.

Programs to improve the genetic merits of the herd based on breeding are used to reproduce cows for many generations. However, the rate of change in individual traits as a result of breeding is relatively low (0.5-3.0% per year) [3]. At the same time, reproductive biotechnology for inducing changes can occur at an accelerated rate by increasing the intensity of selection without limiting the rate of reproduction.

A heifer is normally born with 10,000-35,000 healthy follicles and ova in the ovaries [4], but a typical beef cow will have no more than 10 calves in its lifetime, and dairy cows have even lower productivity. Scientific and biotechnological advances in animal reproduction in recent decades have led to the development of various methods, commonly referred to as assisted reproductive technologies. Their main task is to increase the offspring of genetically valuable animals and spread them all over the world [5]. When using these technologies, the average number of calves from a donor cow increases from 1 to more than 10 per year, which ensures an increase in reproduction rates and an increase in the genetic progress of the herd [6]. Additionally, it is possible to combine the selection of not only females, but also males according to the productivity of daughters. Thus, Rutledge [7] offers support for a herd of F1 cattle without the need to transport many pure-bred animals and apply further crossbreeding schemes using artificial insemination protocols *in vitro*. Such protocols allow fertilizing a cow's ova with the sperm of several bulls, effectively allowing the offspring of different producers to be born from the same cow within a year. It is also possible to implement the opposite scheme, when one sperm straw is used to fertilize oocytes obtained from several distinct donor cows, which allows the maximum use of valuable sperm samples [3].

Notably, the reproduction biotechnology in cattle breeding has been developing for a long time [8]. Thus, the first superovulations in cattle were implemented by Casida *et al*. as early as 1943 [9]. In the 1950s, Rowson performed the first successful transfer of embryos into cows in Cambridge, and the first commercial embryo transfer was performed as early as 1972 [10]. Successful *in vitro* fertilization in cattle was first reported in 1977 by Iritani and Niwa using sperm that had been capacitated in the oviduct or uterus of cows during oestrus or in the uterus of rabbits [11]. The birth of a calf after *in vitro* fertilization first took place in 1981 at the University of Pennsylvania [12].

Presently, the commercial transfer of bovine embryos is a significant international business, and most bovine embryos in the world are produced by *in vitro* fertilization technology [13]. Even though the methods used in the biotechnology of reproduction of cows are sufficiently studied, there are opportunities for their perfection. Therefore, scientists around the world continue to work on improving the protocols of *in vitro* fertilization of cattle.

The purpose of this study was to analyse the effect of two commercial panels of products – Origio (Origio, Denmark) and Minitube (Germany) on the effectiveness of oocyte maturation and development of bovine embryos *in vitro*.

Literature Review

Embryo production *in vitro* is rapidly developing because it has a great potential for strengthening genetic selection, increasing fertilization and optimizing crossbreeding schemes in beef and dairy cattle production systems [14]. According to Parrish *et al*. [15] in 1986, embryos that were obtained outside the body accounted for only 30% of the total number of cow embryos transferred worldwide. Statistics compiled by the International Society for Embryo Transplantation in 2011 showed that 732,862 embryos that were suitable for transfer were obtained by superovulation, and 572,342 embryos were obtained *in vivo* and transferred worldwide [16]. Moreover, of the latter, Europe, and North America account for 19% and 43% of their total number, respectively [16]. And in 2017, statistics showed that the number of IETS (International Embryo Technology Society) embryos, which were created *in vitro* in the entire world, was greater, compared to MOET programs (Multiple Ovulation Embryo Transfer – programs where embryos are washed from bovine uterine horns) [17].

However, despite the large volume of production of bovine embryos *in vitro*, the percentage ratio of the number of embryos suitable for transplantation to the number of used ova stays quite low. Thus, Lonergan and Fair indicate that only 30-35% of fertilized ova can reach a certain stage of development or meet high quality when their transfer to recipient cows is successful [18]. Similar data are highlighted by Ferré *et al*. [19], who claim that on Day 7 of cultivation, only 20-40% of probable zygotes reach the blastocyst stage.

Optimization of cultivation parameters *in vitro* allows increasing the percentage of embryos suitable for transplantation. That is why scientists around the world are working to solve this issue and develop various methodological approaches. The *in vitro* cultivation conditions to produce bovine embryos are aimed at maximally imitating the specific features of the oviduct to successfully manage this process outside the body [20]. This led to the creation of distinct types of culture systems using standard media, conditioned media [21], or by adding liquid from oviducts obtained during oestrus [22]. Goovaerts *et al*. [23] co-cultured zygotes with culture of cumulus cells, Chen *et al*. [24] – with the culture of epithelial cells of the oviducts of mammals, Ferraz *et al*. [25] – with oviduct cell culture on a chip. Rizos *et al*. [26] investigated the effect of serum on embryo development and cryotolerance, claiming that its absence has a positive effect. In turn, Leivas *et al*. [27] and Soto-Moreno *et al*. [28] indicate a positive effect of fetal bovine serum on the development of bovine embryos *in vitro*. Zullo *et al*. [29] claim that crocetin increases the quality and quantity of blastocysts from cows under *in vitro culture conditions*. As the above information suggests, there is presently significant information on the cultivation of bovine embryos, but most of these data differ from each other.

However, along with the development of innovative approaches to the cultivation of bovine embryos and changes in the composition of the culture medium, commercial environments are also being improved [30]. Comparing the effectiveness of the latter and determining the best one is a necessary component of developing successful protocols for obtaining embryos *in vitro*, with subsequent implementation in production.

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. 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/cm³ kanamycin sulphate (Sigma, USA) (solution temperature 37-38°C). The cumulus cell-oocyte complexes 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 including 5 cm³ TL HEPES (Minitube, Germany) with the addition 30 mg of bovine serum albumin (Sigma, USA). Removal of cumulus cell-oocyte complexes, their selection and setting for maturation, fertilization, and subsequent cultivation were also performed in a sterile box (Fig. 1).

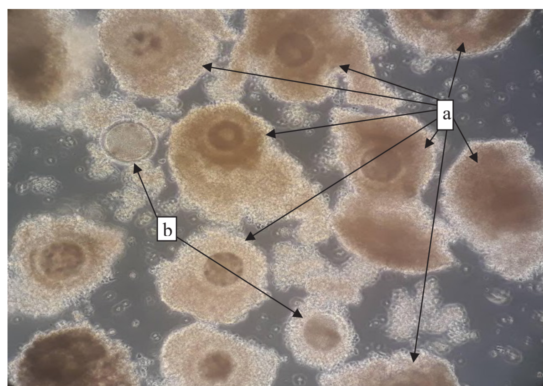

Figure 1. Cumulus cell-oocyte complexes of cows

Note: a – suitable for further cultivation; b – subject to culling. Native preparation. Mag.: ×80

After evaluation under a stereomicroscope SZ51 (Olympus, Japan), cumulus cell-oocyte complexes (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 (see Fig. 1).

The extracted COCs were washed 6 times in an oocyte collection medium. These complexes were selected and washed on a heating table at 37°C.

Bull sperm was prepared for fertilization 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 on 1 cm³ of "Origio Gradient 40" and on 1 cm³ 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 precipitate was transferred with a new sterile tip to a 2 cm³ tube media for preparation and capacitation of spermatozoa. The specified medium consisted of 5 cm³ base capacitation solution (Minitube, Germany), 30 mg bovine serum albumin (Sigma, USA), 0.55 mg sodium pyruvate (Sigma, USA) and 50 mm³ antibiotic-antimycotic (Sigma, USA). Then the contents of the test tube were centrifuged at a centrifugal force of 300 g for 5 minutes. Most of the supernatant was removed, and the procedure was repeated twice. After washing, the sediment was transferred to the bottom of a test tube with 1 cm³ 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*. [15]. Incubation for 1 hour was sufficient for mobile spermatozoa to rise to the upper layers of the medium, while dead and pathologically altered 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/cm³ in a CO_2 incubator at 38.5-39.0°C and 5% CO_2. After capacitation, the spermatozoa were centrifuged at a centrifugal force of 200 g for 5 minutes. The supernatant was removed and 1 cm³ of fertilization medium was added, and the concentration of spermatozoa was counted in the Goryaev chamber.

At the first stage of the study, two media for oocyte maturation were compared:

1. The first medium included 4.5 cm³ of initial solution of the maturation medium TCM 199 (Minitube, Germany), 0.5 cm³ of cow oestrus serum, 0.125 IU of follicle-stimulating hormone (FSH) and 0.125 IU of luteotrophic hormone (LH) (50 mm³ of "Pluset", Laboratories Calier S.A., Spain), 0.125 IU of FSH ("FSH-Super", Agrobiomed, RF) and 50 mm³ of antibiotic-antimycotic (Sigma, USA).
2. The second medium included 4.5 cm³ of Universal (Origio, Denmark), 0.5 cm³ of cow oestral serum, 0.125 IU of FSH and 0.125 IU of LH (50 mm³ "Pluset", Laboratories Calier S.A., Spain) and 0.125 IU of FSH ("FSH-Super", Agrobiomed, Russia) and 50 mm³ of antibiotic-antimycotic (Sigma, USA).

Oocytes were matured *in vitro* for 22-24 hours in 4-well plates (Oosafe, USA). 300 mm³ of medium was introduced into each well, covered with mineral oil (Origio, Denmark), 25 COCs were added and cultivated in a CO_2 incubator at 38.5°C and 5% CO_2. After cultivation, the oocytes were co-cultured with spermatozoa. The specified medium included 5 cm³ base capacitation solution (Minitube,

Germany), 30 mg of bovine serum albumin, 0.11 µg of sodium pyruvate, 0.2 mg of heparin, and 50 mm^3 of antibiotic-antimycotic (Sigma, USA). Oocytes were fertilized in 4-well plates (Oosafe, USA). 300 mm^3 of the medium was covered with mineral oil (Origio, Denmark) for 18 h 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. 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. On Day 7 of cultivation, the resulting blastocysts were evaluated under a stereomicroscope and their percentage was determined.

Since TCM 199 maturation medium turned out to be more effective, further studies were conducted using it.

At the second stage of the study, two protocols for culturing bovine embryos were compared:

1. Minitube cultivation protocol (n = 50):

– oocytes were co-cultured with spermatozoa for 18 h in a fertilization medium including 5 cm^3 of base capacitation solution (Minitube, Germany), 30 mg of bovine serum albumin, 0.11 µg of sodium pyruvate, 0.2 mg of heparin, and 50 mm^3 of antibiotic-antimycotic (Sigma, USA). Subsequently, the embryos were transferred to the embryo culture medium;

– the medium for embryo cultivation included 5 cm^3 of culture medium with pyruvate (Minitube, Germany), 0.5 cm^3 of bovine oestrous 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), where they were cultured for 8 days.

2. Two-stage Origio cultivation protocol (n = 50) with changing media:

– oocytes were co-cultivated with spermatozoa for 18 hours in Sequential Fert medium (Origio, Denmark) with the addition of 10 mm^3/cm^3 of an antibiotic-antimycotic (Sigma, USA), after which the selected embryos were transferred to the medium for their cultivation;

– in Sequential Cleav medium (Origio, Denmark) with the addition of 10 mm^3/cm^3 antibiotic-antimycotic (Sigma, USA), embryos were cultured for 48 hours until their 4-8-cell stage;

– in Sequential Blast medium (Origio, Denmark) with the addition of 10 mm^3/cm^3 of an antibiotic-antimycotic (Sigma, USA), the embryos were further cultured for up to 8 days.

Embryos were cultivated in microdrops of 65 mm^3 (3 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 5% CO_2 and 5% O_2.

The effectiveness of diverse bovine embryo culture protocols was evaluated on days 6 and 8. Therewith, the percentage of blastocysts obtained was found under a stereomicroscope.

Statistical processing of the obtained experimental results was performed according to N.A. Plokhinsky [31] and E.V. Montsevichyute-Eringeme [32] using the Microsoft Excel data analysis package [33]. Arithmetic mean values and their errors were found, and the probability of difference between parallel data sets was determined. In all cases, the difference was considered reliable at $P < 0.05$.

Results and Discussion

Maturation of ova outside the body or *in vitro* Maturation (IVM) is an important stage of the technology of obtaining bovine embryos *in vitro*. Its improvement will considerably increase the efficiency of *in vitro* fertilization and, as a result, increase the percentage ratio of the number of embryos suitable for transplantation to the number of ova used. Along with the bovine-adapted maturing medium TCM 199 (with the addition of bovine oestrous serum, FSH and LH), this study presents the effectiveness of the Universal medium (with the addition of bovine oestrous serum, FSH and LH in the same concentration), which is normally used in humanitarian medicine (Table 1).

Table 1. The influence of different environments on the maturation and further development of oocytes *in vitro*, M ± m, n = 3

No. seq.	Composition of the medium for oocyte maturation	Number of COCs, pcs	Number of experiments	Division, %	Development to the blastocyst stage (Day 8 of cultivation)	
					% of the number of oocytes	% of the number of embryos
1	4.5 cm^3 of the initial solution of maturation medium TCM 199, 0.5 cm^3 of bovine oestrous serum, 0.25 IU FSH and 0.125 IU LH	100	3	64.3 ± 1.0	25.3 ± 1.0	38.8 ± 2.1
2	4.5 cm^3 of Universal, 0.5 cm^3 of bovine oestrous serum, 0.25 IU FSH and 0.125 IU LH	100	3	60.3 ± 1.4	20.0 ± 0.6**	33.2 ± 0.7*

Note: * $P < 0.05$, ** $p < 0.01$, compared to the indicators of medium No. 1

A comparison of the two maturation media, TCM 199 and Universal, did not reveal a statistically significant difference in the percentage of embryos at the stage of 2-8 cells at 48 hours of cultivation, although it was 4% higher when using medium No. 1 compared to medium No. 2. At the same time, on Day 8 of oocyte culture, a significant difference ($P < 0.01$) between the groups was established. Thus, in the group of dishes, the basis of which medium for maturation was TCM 199, the number of blastocysts was 25.3 ± 1.0% per 100 taken COCs. When using a medium for

oocyte maturation, which included Universal, the indicator was lower by 5.3% ($P < 0.01$) compared to the indicators of medium No. 1 (per 100 COCs).

The above pattern was also observed when calculating the percentage of blastocysts from the number of embryos. Thus, on Day 8 of cultivation, it was noted that the values of this indicator in Group No. 2 decreased by 5.6% ($P < 0.05$) compared to Group No. 1. Analysing the data obtained, the use of TCM 199 medium for maturation of oocytes is more effective compared to Universal both at the initial stages of embryo development – division, and at the following stages – up to the blastocyst stage.

Syngina *et al*. consider 30.6% the best result in obtaining blastocysts [34]. Inaba *et al*. indicate that the percentage of blastocysts obtained is also substantially affected by the sperm used for fertilization. Therefore, these indicators in their study ranged within 22-44% [35]. Sirard *et al*. [36] reported that culturing oocytes in medium containing TCM 199 supplemented with 10% heat-treated fetal calf serum, FSH (0.5 μg/cm^3), LH (5 μg/cm^3), and estradiol-17beta (1 μg/cm^3) is the most effective of those studied because the frequency of obtaining late morulae or blastocysts was 28%. According to the research results, when using both TCM 199 and Universal as a medium for oocyte maturation (which were not previously used for bovine oocyte maturation), the percentage of divisieon and the percentage of embryos obtained were within the data presented by other scientists [34-36].

Presently, the production of embryos *in vitro* has become the dominant method of producing bovine embryos for transplantation to female recipients. However, the protocols to produce embryos *in vitro* are not agreed upon. Thus, the proportion of embryos developing to the blastocyst stage is usually less than 40%. While embryos developing *in vivo* become blastocysts in 50-80% [37].

One of the strategies for improving *in vitro* embryo production programs is to change the cultivation conditions, which will stimulate development and improve the ability of embryos to implant. There is evidence [38] that changes in the culture medium can lead to the loss of important regulatory factors for embryos. It is known that changes in the cultivation environment do not lead to the accumulation of toxic substances [38].

A comparative evaluation of the cultivation protocols of two different producers was performed: Minitube, which does not involve changing the environment during cultivation, and Origio with a two-stage cultivation protocol with changing “Sequential Cleav” to “Sequential Blast” for 48 hours of incubation.

TCM 199 medium was used for oocyte maturation because according to the results of the study given above, it turned out to be the most effective. After purification from cumulus (Fig. 2), oocytes were transferred to the Minitube culture medium using the first protocol and “Sequential Cleav” – using the second protocol. At 48 hours, the first differences in embryo development were observed, which lasted up to Days 6 and 8 (Table 2).

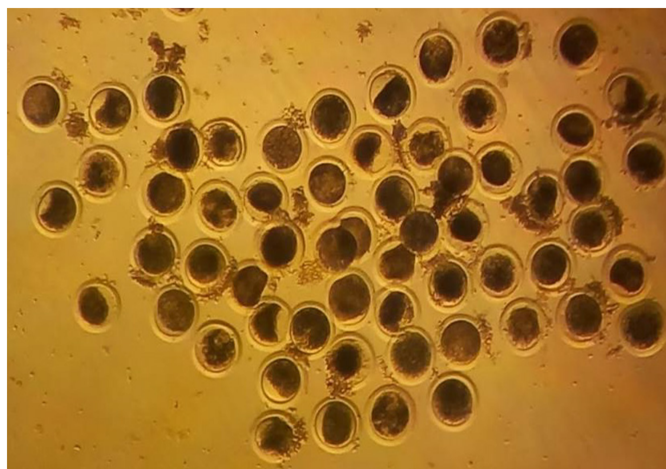

Figure 2. Bovine oocytes after co-cultivation with spermatozoa and purification from cumulus cells. Native preparation. Mag.: ×80

Table 2. Comparative evaluation of embryo culture protocols and their further development *in vitro*, M ± m, n = 3

No. seq.	Bovine embryo cultivation protocol	Number of COCs, pcs	Number of experiments	Division, %	Development to the blastocyst stage, % of the number of embryos	
					(Day 6)	(Day 8)
1	**Minitube** (medium for fertilization (18 hours) and medium for cultivation (up to 8 days)	100	3	64.0 ± 1.7	35.9 ± 2.1*	38.8 ± 0.8*
2	**Origio** (“Sequential Fert” (18 hours), “Sequential Cleav” (48 hours) and “Sequential Blast” (up to 8 days)	100	3	62.7 ± 1.5	28.1 ± 1.8	35.0 ± 1.1

Note: * $P < 0.05$, compared to the indicators of Protocol No. 2

The results presented in Table 2 suggest that using the first protocol of cultivation of 2-8 cell embryos was 1.3% more compared to Protocol No. 2 (Origio scheme). Therewith, there is no statistically significant difference between the indicators.

On Day 6 of cultivation, 7.8% more blastocysts were obtained from the number of embryos using Protocol No. 1 compared to Protocol No. 2. On Day 8 of cultivation, 3.8% more blastocysts were observed from the total number of embryos using Protocol No. 1 compared to Protocol No. 2.

Thus, on Day 8 of cultivation, an average of 25 blastocysts per 100 COCs were obtained using the Minitube protocol. The use of Origio media resulted in 23 blastocysts. The results show that the Minitube protocol is significantly more effective than Origio for the cultivation of bovine embryos.

In the embryo, the number of cells and their placement in the blastocyst are considered indicators of the embryo's developmental potential [39-41]. That is why the described studies found the percentage of blastocysts from the number of embryos on Days 6 and 8 of cultivation.

Thus, on Day 6 of cultivation, it was found that the embryos are at various stages of development (Fig. 3).

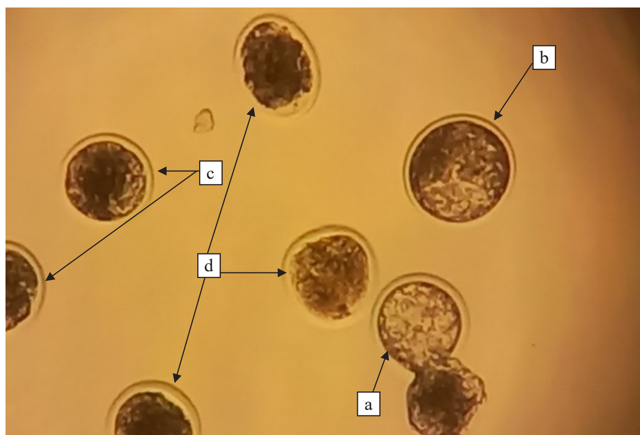

Figure 3. Bovine embryos at various stages of development (Day 6 of cultivation)

Note: a) blastocyst emerging from the zona pellucida; b) expanded blastocyst; c) early blastocyst; d) morula. Native preparation. Mag.: ×200

Thus, Oliveira *et al*. [42], investigating the kinetic laws of success and the reasons for the inability of embryos to reach the blastocyst stage, obtained the maximum percentage of blastocyst yield – 29.9%. While in Stoliarova and Leontieva [43], using KSOM medium with the addition of 1 mM glutamine and bovine serum albumin at a concentration of 1 mg/cm^3, this indicator was 22.3%. According to the study results, the use of the bovine embryo cultivation protocol, both Minitube and Origio, which was not previously used for the cultivation of bovine embryos, the percentage of division and the percentage of obtained embryos were within the data presented by other scientists [42; 43].

Conclusions

Recent advances in assisted reproductive technologies are a powerful tool that can be used to improve and address the challenges of animal husbandry in the future. Thus, as a result of using the Minitube maturation medium for 48 hours of cultivation, 4% more embryos were obtained at the 2-8 cell stage, compared to Universal, which is 64.3 ± 1.0 and 60.3 ± 1.4%, respectively. On Day 8 of cultivation, when using the TCM 199 oocyte maturation medium, a larger number of blastocysts per 100 cumulus cell-oocyte complexes was observed compared to Universal, namely: 25.3 ± 1.0 and 20.0 ± 0.6, respectively. At 48 hours of cultivation using the Minitube protocol, 1.3% more 2-8 cell embryos were obtained compared to the Origio scheme. Therewith, the specified indicator was 64.0 ± 1.7 and 62.7 ± 1.5%, respectively. On Day 6 of cultivation, there are 7.8% more blastocysts from the number of embryos using the Minitube culture protocol compared to the Origio protocol. Using the Minitube bovine embryo culture protocol, 38.8 ± 0.8 blastocysts were obtained on Day 8 of the study, which is 3.8% more than Origio.

Applying these results to ruminant reproduction biotechnology will help manage the limited availability of resources and the increased need for food production because producers will be able to cause rapid genetic changes to create the next generation of animals.

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Вплив різних середовищ на дозрівання ооцитів та розвиток ембріонів великої рогатої худоби в умовах *in vitro*

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Анотація. Згідно з даними International Embryo Technology Society, кількість ембріонів великої рогатої худоби, які виробляються за технологією запліднення в умовах in vitro, зростає з кожним роком. Однак, незважаючи на великі обсяги їх виробництва, ефективність цього методу залишається низькою і потребує удосконалення. Тому, метою дослідження було порівняти ефективність двох комерційних середовищ – Origio Sequential Series (Origio, Данія) і панелі продуктів виробництва Minitube (Німеччина) щодо дозрівання ооцитів та розвитку ембріонів великої рогатої худоби в умовах *in vitro*. На першому етапі дослідження проведено порівняльну оцінку середовищ для дозрівання ооцитів: на основі культуральних середовищ TCM 199 (Minitube) та «Universal» (Origio). На другому – здійснено порівняння протоколів культивування ембріонів великої рогатої худоби: Minitube та двоступеневого протоколу культивування Origio зі зміною середовищ. При цьому встановлено, що використання середовища TCM 199 для дозрівання ооцитів є ефективнішим у порівнянні з «Universal». Так, на 48 год культивування (початковий етап розвитку ембріона) отримано 64,3 ± 1,0 і 60,3 ± 1,4 % 2-8 клітинних ембріонів, а на 8 добу – 25,3 ± 1,0 і 20,0 ± 0,6 % бластоцист, відповідно. Результати порівняння протоколів культивування ембріонів великої рогатої худоби показали, що за використання середовищ як Minitube, так і Origio відсоток ділення та відсоток отриманих ембріонів відповідали їх відомим значенням. При цьому, встановлено, що протокол культивування Minitube є ефективнішим за Origio. Зокрема, на 48 год кількість отриманих ембріонів за використання протоколу культивування Minitube була на 1,3 % більшою порівняно з Origio, на 6 добу – на 7,8 %, а на 8 добу – на 3,8 %. Отримані результати є необхідною складовою розробки успішних процесів виробництва ембріонів великої рогатої худоби в умовах in vitro з подальшим впровадженням у біотехнологію відтворення жуйних

Ключові слова: ембріональний розвиток, культуральні середовища Minitube і Origio, протоколи культивування, біотехнологія відтворення жуйних