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OBTAINING BOVINE CATTLE EMBRYOS *IN VITRO* AT DIFFERENT TIME PARAMETERS OF THE ACTIVATION ONSET OF *IN VITRO* OOCYTE MATURATION

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Aim. To investigate the effect of using different time parameters of starting *in vitro* activation of oocyte maturation (activation onset) on the viability and further development of oocyte-cumulus complexes (OCC) of cows, the efficiency of oocyte fertilization, and the production and further *in vitro* development of embryos. **Methods.** Biotechnological, embryological, morphological, and cytogenetic methods, as well as methods of variation statistics were used in the research. The ovaries of the Ukrainian black-and-white dairy cows ($n=28$) were kept at the temperature of +38°C in the physiological solution. The OCC were obtained by incising visible antral follicles with a blade. The period from the moment of incising the antral follicles to placing the OCC for cultivation was 30–40 min in all the groups. The embryos with more than 16 cells were classified as morulae and the embryos with blastocoel — as blastocysts. All the experimental groups were analyzed in three repeats ($n=3$). The statistical processing of the data was conducted using the reliability criterion χ^2, and the differences were deemed statistically significant at $p<0.05$. **Results.** The study was conducted from 2021 to 2024. The effect of the activation procedure and different terms of its start on the biological completeness and further development of cow oocytes of Ukrainian black-and-white dairy breed (n-28) after meiotic maturation *in vitro* was studied. The coefficient and index of the fragmentation of the obtained *in vitro* embryos were analyzed for different time parameters of the activation onset. It was shown that the efficiency of obtaining bovine cattle embryos *in vitro* and their further development depended on the time of activation onset. The least number of gametes at the metaphase-2 stage (48.8%) and the most cells with degenerated chromatin (44.0%) were obtained when using the most delayed time of activation onset of *in vitro* maturation of oocytes (≥ 8 hours). In case of activation 2, 4, 6, and 8 hours after the start of cultivation, the rate of morula-blastocyst formation was 11.1, 9.0, 2.9, and 0.0%, respectively. **Conclusions.** With an increase in the time interval between the start of culture and the activation of oocytes up to 8 hours, the number of the formed *in vitro* bovine embryos decreased.

Keywords: bovine cattle, oocyte-cumulus complex, *in vitro* activation, oocyte maturation, *in vitro* fertilization, embryo.

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INTRODUCTION

Assisted reproductive technologies in animal breeding are widely used in genetic improvement programs and in the replication of animals with a desired genotype, as they are a powerful tool for accelerating genetic progress. Over the past decades, some progress has been made in the efficient obtaining of embryos of farm animals *in vitro*. Obtaining a large number of embryos *in vitro* envisages the use of animal ovaries after ovariectomy, as this approach ensures the collection of the maximum number of oocytes (Martín-Maestro A et al. 2020). Unlike those

obtained *in vivo*, oocytes from the ovaries of slaughtered animals demonstrate a reduced potential for *in vitro* development. In addition, slaughterhouses are usually located at different distances from research laboratories. Storing ovaries for long periods of transportation over long distances has a negative effect on the viability of oocytes. Given that oocyte quality determines the developmental competence of embryos after fertilization, maintaining the integrity of oocytes from the moment of animal ovariectomy to the moment of embryological manipulations with the cells is of key importance.

However, the time interval between the slaughter of the animal and the extraction of oocytes from the follicles should be taken into account. After slaughter, the ovary loses its oxygen and energy supply due to the interruption of blood flow, which in turn leads to an ischemic state of the ovaries (Lopes C. et al., 2009). During the transportation of ovaries from the slaughterhouse to the research laboratory, the ovaries and oocytes may be exposed to significant temperature changes, which may affect the quality of the gametes in terms of nuclear maturation and developmental competence after *in vitro* maturation (IVM) and fertilization (IVF) (Febretrisiana A et al. 2015). Oocyte quality is an essential factor affecting the developmental competence of embryos obtained *in vitro*. As for bovine cattle, ovaries collected at slaughterhouses are the main source of oocytes for the production of *in vitro* embryos by *ex vivo* fertilization.

It should be noted that immediately after the ovary is separated from the animal (either via surgery or after slaughter), blood flow ceases, and the main mechanism of negative effect during ischemia is hypoxia; cells with a high metabolic rate, including those that form ovarian tissue, are damaged very quickly (Martín-Maestro A et al. 2020). In this situation, the production of reactive oxygen species in the follicle may increase (Guibert EE et al. 2011), which overloads endogenous antioxidant systems and leads to oxidative stress and oocyte damage (Liang LF et al. 2017). To reduce the harmful effect of reactive oxygen species on oocytes, Torres-Osorio V et al. (Torres-Osorio V et al. 2019) used antioxidants during IVM, and Sánchez-Ajofrín I et al. (Sánchez-Ajofrín I et al. 2020) added them to the medium for transporting ovaries. Recent studies have shown that the subsequent development of oocytes after *in vitro* fertilization is significantly affected by the interval between obtaining ovaries immediately after slaughter and removing oocytes from the follicles (Goodarzi A et al. 2018, Sofi KA et al. 2022). It has been proven that the environment and temperature at which ovaries are transported significantly affect the maturation of cow oocytes. In addition, the transportation medium affected the rate of fertilization and obtaining embryos (Bohlooli Sh et al., 2015, Febretrisiana A et al., 2015). Comparing different schemes of storage and transportation of ovaries before the activation onset of *in vitro* oocyte maturation, it was found that the quality of oocytes determined the competence of embryo development after fertilization. The preservation of the integrity of oocytes from the moment of female ovariectomy to the moment of embryological manipulations with cells is of key importance for the vital potential of the oocyte (Evecen M et al., 2010, 2018; Yoshida T et al., 2022).

Understanding the reproductive processes involved in ovarian folliculogenesis and applying this knowledge to different species is important for the conservation of biodiversity. The investigation into cryopreservation of tissues has enabled scientists to create tissue banks for scientific research and biomedical applications (Whaley et al. 2021). The preservation of ovarian and follicular tissue depends mainly on the methods of collection, transportation, and storage (Duncan et al., 2016; Barberino et al., 2019). The limited knowledge about the transportation of ovaries of different species has meant that there are currently no established standards for the transportation of ovaries and that this assessment has been largely empirical (Vilela JMV et al., 2021). Therefore, the research into the optimal medium, time, and temperature of transportation is necessary for successful preservation of tissue viability.

It is crucial for effective production of bovine embryos *in vitro* and their further development not only to select the composition of the culture medium, the quality and stage of oocyte development, but also to consider the timing of the activation onset of *in vitro* oocyte maturation, which to some extent expands the access and terms of using the basic biological material for further biotechnological manipulations with cells. In the sources of information available to us, we did not come across publications in which the authors would study the transportation time and the time they spent on manipulations with OCC before setting for maturation. Therefore, the purpose of the research was to obtain bovine embryos *in vitro* and their further development, considering the effect of

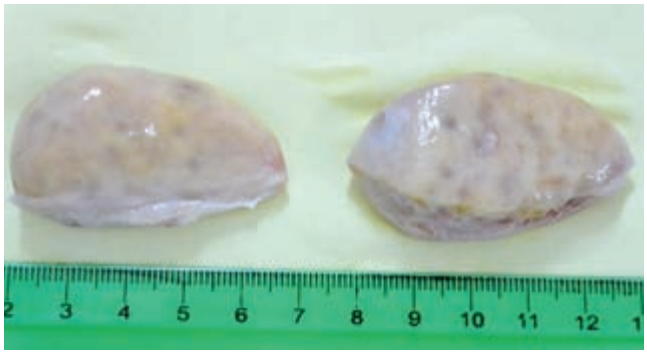

Fig. 1. Cow ovaries at the stage of follicular growth

various time parameters of the activation onset of *in vitro* oocyte maturation.

MATERIALS AND METHODS

The obtained cow ovaries (**Fig. 1**) were transported to the laboratory in the physiological solution (0.9% NaCl) supplemented with penicillin (100 IU/ml) and streptomycin sulfate (100 μg/ml), at a temperature of about +38°C for 1–1.5 h. The ovaries of Ukrainian black-and-white dairy cows ($n = 28$) were randomly divided into four experimental groups: A ($n = 14$), B ($n = 14$), C ($n = 14$), and D ($n = 14$), and stored in a thermostat with physiological solution at a temperature of +38°C. The studies were conducted from 2021 to 2024; each experiment had three ($n = 3$) repeats.

Cow oocyte-cumulus complexes (OCC) (**Fig. 2**) were obtained by incising visible antral follicles with a blade, washed with Dulbecco's medium (D 5773 «Sigma-Aldrich»), caught with an embryological pipette, and evaluated in terms of morphological features under a microscope (MBS-9, USSR). The time from the moment of incision of the antral follicles to the placement of the OCC for cultivation in all groups was about 30–40 minutes. The studies used cow oocytes with homogeneous fine-grained ooplasm, intact transparent membrane, dense or partially loosened cumulus (Kovtun S.I. et al., 2010). The time from obtaining the ovaries to the antral follicle incision in the groups was as follows: A — 2 h, B — 4 h, C — 6 h, D — 8 h before OCC activation onset prior to *in vitro* maturation.

Then, selected OCC ($n = 670$) were washed three times with maturation medium and cultured in four-well plates for 24 h at a temperature of +38.5°C, 5% CO2 in the air, in drops of medium 199 (M 2520 «Sigma-Aldrich») with 10% (v/v) bovine serum (F 7524 «Sigma»), which was previously inactivated at +56°C for 30 minutes, 2.5 μg/ml FSH, 1.0 μg/ml estradiol (E 2758 «Sigma»), 2.5 IU/ml luteinizing hormone, 2.0 mM sodium pyruvate (P 5280 «Sigma»), 2.92 mM calcium lactate (L 2000 «Sigma»), 40 μg/ml gentamicin. After cultivation, some oocytes ($n = 350$) were freed from cumulus cells by careful pipetting, then they were transferred to 0.9% hypotonic solution of 3-substituted sodium citrate for 10 min at room temperature and fixed on a previously degreased dry glass slide with Carnoy's fixative (methanol : glacial acetic acid = 3 : 1). The preparations were stained with 2.0% (v/v) Giemsa stain solution (32884 «Fluka») and analyzed using a light microscope (Jenaval «Carl Zeiss»). The maturation rate was determined as the number of oocytes with an obvious polar body and metaphase II plate (M II) compared to the total number of analyzed oocytes. The remaining oocytes at the M II stage with the first polar body were characterized as mature oocytes and subjected to *in vitro* fertilization. After IVM, oocytes

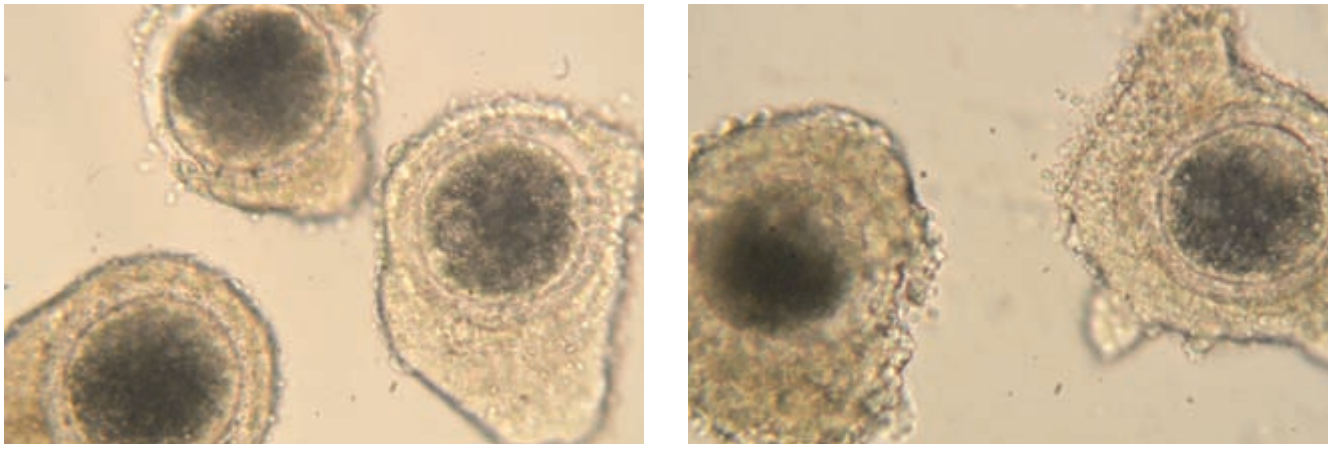

Fig. 2. Cow OCC, suitable for *in vitro* cultivation. Magnification 56× (vol. 4×, oc. 14×)

were freed from cumulus cells by careful pipetting, and the resulting bare oocytes ($n = 320$) were used for *in vitro* fertilization.

Frozen bull sperm was used for *in vitro* fertilization of cow eggs. Sperm pellets were thawed in a water bath (+37°C) in 2 ml of Sperm-TALP medium (Parrish J et al. 1989) for 10–12 s. Sperm was separated from seminal plasma and diluent by the «swim-up» method. Sperm capacitation was performed with heparin (100 U/ml) in Sperm-TALP medium. Before insemination, the oocytes that matured outside the body were partially freed from their surrounding cumulus cells mechanically (pipetting through a smaller diameter pipette). The coincubation of eggs and sperm, with a concentration of approximately $1–3\times10^6$ sperm/ml, was carried out in a thermostat at a temperature of 38.5°C, 5% CO_2, in drops of Fert.-TALP medium (Parrish J et al., 1988). After 12–18 hours of coincubation, the probable zygotes ($n = 110–130$) were washed free of adherent sperm and transferred to drops of CDM (culture development medium) for further cultivation after *in vitro* fertilization at a temperature of 38.5°C, 5% CO_2 in the air. The CDM medium consisted of TCM 199 supplemented with 20% (v/v) fetal bovine serum (F 7524 «Sigma»), 2.0 mM sodium pyruvate (P 5280 «Sigma»), 2.92 mM calcium lactate (L 2000 «Sigma»), and 40 μg/ml gentamicin.

The embryos with more than 16 cells were classified as morulae, and the embryos with a blastocoel as blastocysts. The cytogenetic preparations of gametes after *in vitro* fertilization were prepared as follows: the embryos were placed in 0.8% hypotonic solution of 3-substituted sodium citrate for 2 min and fixed with a mixture of methanol : glacial acetic acid: deionized water = 3 : 2 : 1 on dry, degreased glass and stained with a 2.0% solution of Giemsa stain (32884 «Fluka») followed by the analysis under a light microscope (Jenaval «Carl Zeiss»).

All experimental groups were analyzed in three repeats ($n = 3$). The statistical data processing was performed according to the χ^2 criterion using the ×7 software package version 2.0.0.9, and differences were considered statistically significant at $p < 0.05$.

RESULTS

It was determined (**Table 1**) that there was a dependence between the time of the activation onset of *in vitro* oocyte maturation and the further development of female gametes *in vitro*. Increasing the time of activation onset of *in vitro* oocyte maturation from 2 to 8 hours leads to a significant decrease (from 82.7% to 48.8%) in the rate of maturation of cow oocytes outside the body to metaphase-2 of meiosis. The rate of the number of gametes with degenerated chromatin in these experimental groups increased from 12.4% to 44.0%, respectively.

The morphological and cytogenetic analysis of cow oocytes *in vitro* was performed at different time parameters of the activation onset, and their biological value after meiotic maturation outside the body was determined. The studies were aimed at obtaining bovine cattle embryos *in vitro* and their further development (**Table 2**).

According to the results of morphological and cytogenetic analyses of OCC, previously cultured outside the body for 24 hours, at different time parameters of their activation onset, different levels of *in vitro* embryo formation were found (**Fig. 3, 4**). The biological adequacy of *ex vivo* maturation of cow oocytes, obtained from the ovaries at different time parameters of their activation onset, was checked by

Table 1. The analysis of the *in vitro* development of cow oocytes at different time parameters of their activation onset

Experiment groups	Activation onset, h.	Number of cultivated cells	Number of cells:					
			in metaphase-2 of meiosis		in other stages of meiosis		with degenerated chromatin	
			n	%	*n*	%	*n*	%
A	2	81	67	$82.7^{a} \pm 4.2$	4	4.9±2.4	10	$12.4^{e} \pm 3.7$
B	4	89	67	$75.3^{ad} \pm 4.6$	9	10.1±3.2	13	$14.6^{eh} \pm 3.7$
C	6	96	59	$61.5^{b} \pm 5.0$	10	10.4 ± 3.1	27	$28.1^{f} \pm 4.6$
D	8	84	41	$48.8^{c} \pm 5.5$	6	7.2±2.8	37	$44.0^{g} \pm 5.4$

Note 1. In this table and the following table one, different superscripts indicate the probable difference between the indices.
Note 2. b : d; f : h; f : g — $P < 0.05$; a : b; e : f — $P < 0.01$; a : c; e : g — $P < 0.001$.
Note 3. Cultivation period — 24 h.

Table 2. The efficiency of obtaining bovine cattle embryos *in vitro* at different time parameters of the activation onset

Experiment groups	Number of cells to be fertilized *in vitro*	Number of embryos at the stages:									
		2 cells		3–4 cells		5–8 cells		9–16 cells		morula + blastocyst	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
A	81	34	$42.0^{a} \pm 5.5$	29	$35.8^{c} \pm 5.3$	24	$29.6^{f} \pm 5.1$	19	$23.5^{i} \pm 4.7$	9	$11.1l \pm 3.5$
B	89	38	$42.7^{a} \pm 5.2$	32	$36.0^{c} \pm 5.1$	25	$28.1^{f} \pm 4.8$	17	$19.1^{ij} \pm 4.2$	8	$9.0l^{mo} \pm 3.0$
C	70	22	$31.4^{ab} \pm 5.5$	18	$25.7^{ce} \pm 5.2$	12	$17.1^{fh} \pm 4.5$	7	$10.0^{j} \pm 3.6$	2	$2.9^{mn} \pm 1.9$
D	80	15	$18.6^{b} \pm 4.4$	7	$8.6^{d} \pm 3.2$	4	$5.0^{g} \pm 2.4$	1	$1.3^{k} \pm 1.2$	0	$0.0^{n} \pm 0.0$

Note 1. * — activation onset, h: A — 2, B — 4, C — 6, D — 8.
Note 2. d : e; g : h; i : j; j : k; l : m — $P<0.05$; n : o — $P<0.01$; a : b; c : d; f : g; i : k; l : n — $P<0.001$.

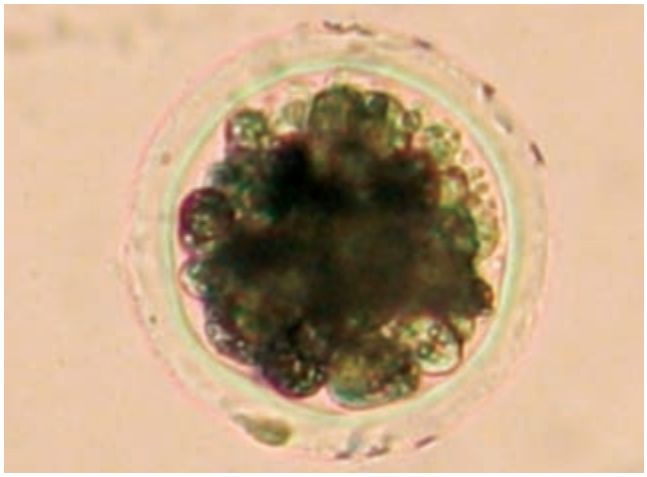

Fig. 3. The embryo, formed in vitro, at the morula stage. Magnification 56× (vol. 4×, oc. 14×)

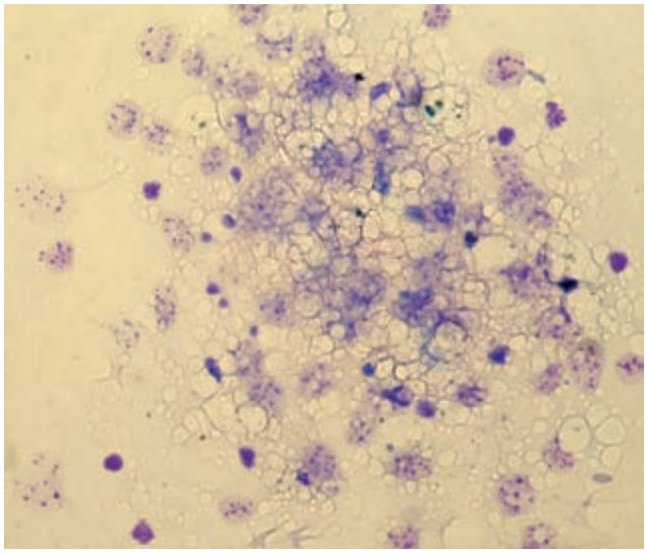

Fig. 4. The cytogenetic preparation of a blastocyst, obtained *in vitro* ($n=65$). Magnification 1000× (vol. 100×, oc. 10×)

their *in vitro* fertilization. In case of the activation onset in 2 h (group A) and the subsequent fertilization of cow eggs that matured *ex vivo*, the number of embryos obtained *in vitro* is 42.0%.

According to the results of fertilization of cow oocytes, matured *in vitro*, if the activation onset was in 4 h (group B), the index of the number of embryos obtained *in vitro* did not change, 42.7%, and in 6 h, it decreased insignificantly (31.4%, group C). The cultivation of cow oocytes, in case of their activation onset in 8 h (group D), led to a significant decrease in the level of embryo fragmentation — 18.6%.

During the study of the fragmentation coefficient (the number of embryos at the corresponding stage of development from the total number of embryos), it was found that the fragmentation coefficient of 2-cell embryos increased from 29.6 to 36.1% in the experimental groups and was the highest in group D — 55.6% (**Fig. 5**). After 48 h of cultivation, the embryo fragmentation rate in the experimental groups was almost the same (A — 25.2%, B — 26.7%, C — 29.5%, D — 25.9%). Further cultivation of embryos up to 72 h led to a decrease in the fragmentation rate in the experimental group D (14.8%) and was almost the same in groups A, B, and C (20.9, 20.8, 19.7%, respectively). On the fourth day of cultivating the embryos, obtained at different time parameters of the activation onset of *in vitro* oocyte maturation, and cow eggs, fertilized *ex vivo*, a decrease in the fragmentation coefficient was observed from 16.5 to 3.7% in experimental groups A, B, C, and D, respectively. After 120 h of embryo cultivation, different fragmentation coefficients were observed in all experimental groups, from 7.8 to 0.0%, respectively.

In addition to the coefficient of embryo fragmentation after *in vitro* fertilization, the index of embryo

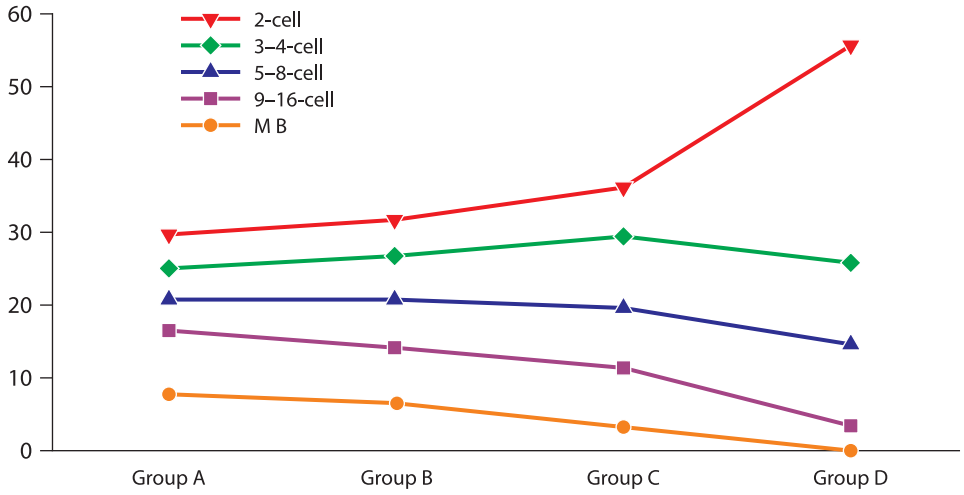

Fig. 5. The fragmentation coefficient for *in vitro* embryos at different time parameters of the activation onset of *in vitro* maturation of cow oocytes

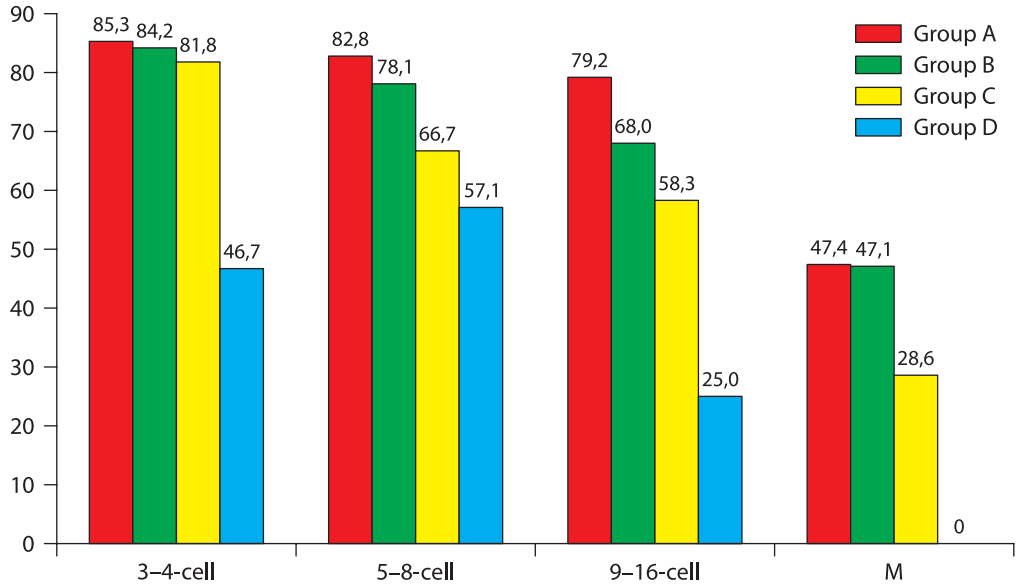

Fig. 6. *In vitro* embryo fragmentation index at different time parameters of the activation onset of *in vitro* maturation of cow oocytes

fragmentation (the ratio of the number of embryos at a certain stage to the next stage of their development) is no less informative. The analysis of the subsequent fragmentation of bovine embryos obtained from matured and fertilized eggs at different time parameters of the activation onset of *in vitro* maturation of oocytes (**Fig. 6**) detected significant fluctuations in the fragmentation index.

The fragmentation index of 3-4-cell embryos was the lowest in group D — 46.7%, compared with groups A, B, and C (85.3, 84.2, 81.8%, respectively), which is related to the effect of different time parameters of the activation onset of *in vitro* maturation of oocytes. Further cultivation to 5–8-cell embryos led to a significant decrease in the fragmentation index in groups A, B, and C (82.8, 78.1, and 66.7%, respectively) and an increase in group D to 57.1%. The largest decrease in the fragmentation index of 9–16-cell embryos was observed in group D (25.0%). After 120 h of embryo cultivation, a decrease in the

fragmentation index was observed in group B to 28.6% and in experimental group D to 0.0%.

DISCUSSION

Ovaries obtained from slaughterhouses are the main source of oocytes for obtaining bovine embryos *in vitro*. Storing ovaries without any blood supply can affect the quality of oocytes, influencing the extracellular environment surrounding the oocytes. Oocytes in the ovaries are at the diplotene stage in a dormant state for a long time, depending on the mammalian species. To transition from a dormant state to *in vitro* development, oocytes must be activated, usually using hormonal drugs (FSH, LH). In our studies on obtaining bovine embryos *in vitro* from reactivated OCC, we delivered ovaries within 2 h (Trotsky PA et al. 2021) and 6 h (Ladyka V et al. 2023) without consideration of the time spent on manipulations with OCC. When analyzing the development of cow oocytes at different time parameters of their activation onset *in vitro* (Trotsky PA et al. 2024), the time spent on manipulations with OCC was taken into account. Our study results demonstrate that in the case of the activation onset of *in vitro* maturation of cow oocytes within 2–8 h, the number of cells that matured to metaphase-2 decreased significantly, and the percentage of oocytes with degenerated chromatin increased considerably. These results indicate that prolonged transportation may cause follicular fluid acidosis due to ischemia in the ovary, leading to degeneration of oocytes in the follicles. This result is in agreement with the results of (Sofi KA et al., 2022, Febretrisiana A et al., 2015) who reported that the time and temperature of ovarian transportation may have a negative effect on the subsequent oocyte maturation.

However, Özdas OB et al. (Özdas OB et al. 2006) investigated the effect of ovarian transportation temperatures of +32°C and +4°C on oocyte maturation. They found that the temperature of ovarian transportation did not affect the developmental adequacy of the obtained OCC. Some studies demonstrate that storing ovaries at a low temperature of +4°C before oocyte retrieval did not affect their subsequent *in vitro* maturation (Bohlooli Sh et al., 2015; Goodarzi A. et al., 2018; Yoshida T et al., 2022). However, the authors of these publications studied the effect of transporting ovaries in a semi-complex medium containing HEPES (Bohlooli Sh et al., 2015), and with the addition of different doses of melatonin as an antioxidant to the transportation medium (Goodarzi A. et al., 2018).

Yoshida T et al. (Yoshida T et al., 2022) tested the effectiveness of the physiological solution, Euro-Collins solution (EC), and ET-Kyoto solution (ET-K) as media for transporting ovaries. They found that the ovarian transportation medium affects oocyte maturation and subsequent embryo development.

Based on our data on the use of different time parameters for the activation onset of *in vitro* oocyte maturation, we believe that transporting ovaries in the physiological solution provides the most optimal conditions, as well as the ability of oocytes to undergo subsequent development. This assumption is confirmed in the publications (Evecen M et al., 2018; Martín-Maestro A et al., 2020), which showed an increase in the level of maturation and fertilization of *in vitro* matured oocytes in case of transporting ovaries in the physiological solution. The results of our studies on the presence of ovaries at temperatures above +30°C are consistent with the data obtained by Wongsrikeao P et al. (Wongsrikeao P et al., 2005), Evecen M et al. (Evecen M et al., 2010, 2018). However, in the studies of these authors, matured OCC demonstrated better development results, but the quality of embryos obtained *in vitro* did not depend on the temperature of transportation and medium.

Thus, the analysis of the study results showed different efficiency rates of obtaining bovine cattle embryos *in vitro* using different time parameters of the activation onset of *in vitro* maturation of oocytes. The comparative analysis of different time parameters demonstrated a significant difference between groups in terms of such indices as maturation to metaphase-2 of meiosis and the number of cells with degenerated chromatin. Therefore, in case of increasing the time of the activation onset of the maturation of bovine oocytes from 2 to 8 h, it is necessary to take into account that only 48.8% of gametes will restore meiotic maturation to the metaphase-2 stage of meiosis. It should be noted that the studied activation onset times are used in the system of preservation and rational use of the bovine cattle gene pool *in vitro*.

The conducted studies will be useful in the reproductive technology system to maintain the appropriate quality of oocytes, meiotic maturation, fertilization rate, and *in vitro* embryo obtaining for their further implementation (Tellado M et al., 2014, Febretrisiana A et al., 2015, Barberino RS et al., 2019, Franko et al., 2024).

Understanding the reproductive processes involved in ovarian folliculogenesis and applying this know-

ledge to different species is important for the preservation of biodiversity. The development of such methods is particularly important in cases when biological material is obtained in areas where animals are few or far from research laboratories, as well as in cases of selecting biological material from wild animals (Barberino RS et al., 2019).

Our assumption that the adequacy of OCC for development is affected by manipulations with them before further cultivation is consistent with the publications of Kouamo J et al. (Kouamo J et al., 2019) who found that cow ovaries can be stored at a temperature of +5°C for 9 h without any significant biochemical changes in the follicular fluid, the number and quality of oocytes suitable for cultivation. And Fonseca Dias SD et al. (Fonseca Dias SD et al., 2022) did not find a statistically significant difference between the maturation indices of cow oocytes obtained by the puncture from ovaries at the slaughterhouse and those obtained by the puncture from the same ovaries in the laboratory after transportation for 6 h. The comparison of general biological properties with other animal species is consistent with Vilela JMV et al. (Vilela JMV et al., 2021), who analyzed the transportation of domestic and wild animal ovaries and made a generalized conclusion. Duncan FE et al. (Duncan FE et al., 2016) considered the data obtained during the transportation of animal ovaries as a model that can be applied to the transportation of human ovaries.

CONCLUSIONS

The efficiency of obtaining bovine cattle embryos *in vitro* using different time parameters of the activation onset of *in vitro* oocyte maturation was studied. It was shown that by increasing the time of the activation onset of *in vitro* maturation of cow oocytes from 2 to 8 h and their subsequent cultivation, the number of gametes obtained at metaphase-2 of meiosis decreases from 82.7% to 48.8%, and the number of cells with degenerated chromatin increases (from 12.4% to 44.0%). There is also a decrease in the number of embryos formed *in vitro* at later stages of development from 11.1% to 0%, respectively. At the activation onset of maturation in 6 and 8 h, the fragmentation rate of *in vitro* embryos decreased by 10.6% and 23.4%, compared to 2 h, and at the activation onset in 4 h it was 42.7%.

The results obtained are relevant for solving the problem related to obtaining bovine cattle embryos *in vitro*. In case of obtaining bovine cattle embryos *in vitro* at different time parameters of the activation onset of *in vitro* oocyte maturation, many factors can affect the quality of matured oocytes, and therefore the rate of embryo development. However, further attention should be paid to the manipulations with OCC before placing them for maturation, the media in which OCC and embryos are cultivated, and the procedures to which gametes and embryos are subjected. The addition of antioxidants during ovarian transportation and cultivation may contribute to improving the quality of oocytes and their adequacy for embryo development. In the future, the main attention will be focused on molecular genetic analysis of embryos at different stages of development and optimization of cultivation media for each stage of the development of embryos obtained *in vitro* at different time parameters of the activation onset of *in vitro* oocyte maturation.

Adherence to ethical principles. The Commission on the treatment of animals in scientific research at the M.V. Zubets Institute of Animal Breeding and Genetics, the National Academy of Agrarian Sciences of Ukraine, Minutes No. 5, dated July 18, 2025.

Conflict of interests. The authors declare the absence of any conflicts of interests.

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ОДЕРЖАННЯ ЕМБРІОНІВ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ *IN VITRO* ЗА РІЗНИХ ЧАСОВИХ ПАРАМЕТРІВ ПОЧАТКУ АКТИВАЦІЇ *IN VITRO* ДОЗРІВАННЯ ООЦИТІВ

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Мета. Вивчити вплив використання різних часових параметрів початку активації *in vitro* дозрівання ооцитів (початок активації) на життєздатність і подальший розвиток ооцит-кумулюсних комплексів (ОКК) корів, результативність запліднення ооцитів, одержання та подальший розвиток *in vitro* ембріонів. **Методи.** При проведенні досліджень застосовано біотехнологічні, ембріологічні, морфологічні та цитогенетичні методи, а також методи варіаційної статистики. Яєчники корів української чорно-рябої молочної породи ($n = 28$) зберігали за температури +38°С з фізіологічним розчином. ОКК отримували шляхом надрізу лезом видимих антральних фолікулів. Час від моменту надрізу антральних фолікулів до постановки ОКК на культивування в усіх групах становив 30–40 хвилин. Класифікували ембріони з більш ніж 16 клітинами як морули та ембріони з бластоцелем як бластоцисти. Усі експериментальні групи були проаналізовані у трьох повторах ($n = 3$). Статистичну обробку даних проводили за критерієм відповідності χ^2, а відмінності вважали статистично значущими за $p < 0,05$. **Результати.** Дослідження проведені з 2021 по 2024 роки. Вивчено вплив активації та різних термінів її початку на біологічну повноцінність і подальший розвиток ооцитів корів української чорно-рябої молочної породи ($n = 28$) після мейотичного дозрівання *in vitro*. Проаналізовано коефіцієнт та індекс дроблення отриманих *in vitro* ембріонів за різного часу початку активації. Показано, що ефективність одержання ембріонів великої рогатої худоби *in vitro* та їх подальший розвиток залежить від часу початку активації. Найменше гамет на стадії метафази-2 (48,8%) та найбільше клітин з дегенерованим хроматином (44,0%) отримано за використання найбільш відтермінованого часу початку активації дозрівання *in vitro* ооцитів (≥ 8 год.). За активації через 2, 4, 6 та 8 годин від початку культивування показник формування морул-бластоцист становив 11,1, 9,0, 2,9 та 0,0% відповідно. **Висновок.** Зі збільшенням проміжку часу початку проведенням активації ооцитів до 8 годин кількість сформованих *in vitro* ембріонів великої рогатої худоби зменшується.

Ключові слова. Велика рогата худоба, ооцит-кумулюсний комплекс, активація *in vitro*, дозрівання ооцитів, запліднення *in vitro*, ембріон.