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Effects of insulin-like growth factor-1 and fibroblast growth factor-2 on the *in vitro* maturation of bovine oocytes

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As of now, the leading method of reproducing highly productive animals in animal husbandry is production of cattle embryos in in vitro conditions, in particular transvaginal ultrasound-guided oocyte aspiration with further in vitro maturation, which is currently more popular than the in vivo embryo production. Despite the broad introduction of the technique, this process is artificial, meaning limited percentage of matured oocytes, and therefore lower final yield of embryos suitable for transplantation. Augmenting the success rate of oocyte cultivation in in vitro conditions would mean a greater effectiveness of reproductive technologies. Therefore, the objective of our study was to improve the culture medium for maturation of bovine oocytes by comparing the effects of different concentrations of insulin-like growth factor (IGF-1) and the fibroblast growth factor 2 (FGF2). For this purpose, the cumulus cell-oocyte complexes were divided into six experimental groups: control and groups with different concentrations (50 and 100 ng/cm^3) of insulin-like and fibroblast growth factors and their combination, comprising 50 ng/cm^3 of each. The effects of the growth factors were assessed by analyzing the morphological maturity of the oocytes (the cumulus expansion index and percentage of oocytes at the MII stage) and also the parameters of embryonic development (the percentage of cleavage and yield of blastocysts). The study revealed that insulin-like growth factor, regardless of concentration, provided no statistically significant increase in the percentage of blastocysts formed, compared with the control. Meanwhile, addition of 50 ng/cm^3 of fibroblast growth factor produced a significant, although not always statistically significant, increase in the studied parameters. The greatest support of maturation was achieved in the group with the combination of growth factors, which provided the highest level of blastulation, accounting for 31.7 ± 2.9%, and a significant difference compared with the control. An optimal strategy for increasing the effeciency of maturation of bovine oocytes in in vitro conditions was a combined enrichment of the culture medium with of 50 ng/cm^3 of IGF and 50 ng/cm^3 of FGF 2, leading to the highest yield of mature oocytes that were competent for fertilization and further embryonic development of gametes.

Keywords: reproductive biotechnologies; fertility; IVM; cumulus cell-oocyte complex; COCs; IGF-1; FGF2.

Introduction

Bovine cattle forms the main segment of the global industry of embryo transfer with a 93.2% share in the total number of yielded embryos among productive animals (Viana, 2024a). The analysis of the advancement tendencies in this sphere revealed and validated the global introduction of *in vitro* embryo production. Thus, 2016 became a turning point, because according to the reports of the International Embryo Transplantation Society (IETS), the number of embryos grown in vitro for the first time exceeded the number of those collected *in vivo* and has continued to increase with an average rate of 12% (Viana, 2024b). As of now, transvaginal aspiration of oocytes under USD (OPU) control and further in vitro production of embryos comprises 72.7% of the total nuber of obtained bovine embryos around the world (Reddy et al., 2023). It has to be noted that oocyte aspiration using OPU can be carried out weekly without negative impact on the animals' reproductive function (Pieterse et al., 1988). The mentioned tendencies clearly demonstrate that selective farms are transitioning from MOET protocols to the IVP technology, and this transformation notably correlates with the increase in the latter's efficiency (Ferré et al., 2020; Kovpak et al., 2023; Valchuk et al., 2023).

Oocyte aspiration is the initial, key stage of production of bovine cattle embryos in *in vitro* conditions. On industrial scale, they are produced through transviganal aspiration of oocytes under USD (OPU) control (Ferré et al., 2023). However, oocytes obtained using this method are morphologically and functionally immature, being in the prophase of first meiotic division (Bevers et al., 1997). This condition, in turn, requires oocytes to be further matured in *in vitro* conditions to reach competence for fertilization (Kovpak et al., 2022; Sakaguchi, 2025). Oocyte maturation is a complex process, which involves both nuclear and cytoplasmic changes in gametes (Ferré et al., 2020). Nuclear maturation includes renewal and progression of meiosis, which begins with the prophase of first meiotic division through germinal vesicle breakdown, with the end point during metaphase of the second meiotic division (Hyttel et al., 1997). This process is a result of a balance between factors supporting meiotic arrest and maturation stimulators (Jamnongjit & Hammes, 2005). At the same time, cytoplasmic maturation occurs, which comprises a series of ultrastructural and biochemical changes in cytoplasm, including accumulation of mRNA, proteins, substrates, and nutrients, which provide the oocyte with energy and regulatory resources that it requires for fertilization and support of early embryonic development (Watson, 2007). Other indirect morphologic parameters considered during evaluation of oocyte maturity include the degree of expansion of cumulus cells, presence of polar body, and increase in perivitelline space (Kruip et al., 1983).

Although IVM of animal oocytes is a common practice, it is important to keep in mind that this process is absolutely unnatural. This method is different from *in vivo* oocyte maturation in two principally important moments (Gilchrist & Thompson, 2007). First of all, cumulus cell-oocyte complexes (COCs) are usually collected from antral follicles of average size, with oocytes having not yet completed capacitation and with no complete molecular and cellular apparatus, necessary for the support of early embryogenesis (Hyttel et al., 1997). By being mechanically removed from the follicle, COCs lose their natural environment, and thus meiosis in hindered and meiotic formation of oocyte spontaneously begins in *in vitro* conditions (Ali et al., 2006).

In *in vitro* conditions, oocytes complete nuclear maturation to the MII stage in 20–24 hours, after which they become ready for fertilization (Leibfried-Rutledge et al., 1987). It has to be noted that maturation of nucleus occurs automatically in most conditions of cultivation at a rate over 85% (Sirard, 2018), although it does not guarantee high level of blastocyst yield. At the same time, cytoplasmic maturation takes place, which is more dependent on exogenic impact (Watson, 2007). Paracrine interactions between oocyte and somatic cells through gap junctions and factors that are transmitted via follicle fluid are decisive for a successful maturation of oocytes (Rodgers & Irving-Rodgers, 2010). However, we have only a limited understanding of the nature and diversity of compounds transmitted between cumulus cells and oocyte through gap junctions during this final maturation phase (Gilchrist et al., 2004), and the data on how dynamic this process is and whether dynamic changes of levels of transmitted molecules affect the competence of development are quite limited (Gilchrist & Thompson, 2007).

Despite the limitations and disadvantages, the IVM of oocytes is a highly effective method for the technology of artificial reproduction of bovines. Therefore, the objective of this study was to optimize the culture system of *in vitro* oocyte maturation (IVM) by comparing the effects of different concentrations of exogenic growth factors – insulin-like growth factor (IGF-1) and fibrobast growth factor (FGF2).

Materials and methods

The studies were carried out at the National University of Life and Environmental Sciences of Ukraine at the Research and Education Laboratory Center of Reproduction of Animals with Sperm and Embryo Bank during 2024–2025. The experiment required no ethical approval because the initial material (bovine ovaries) were obtained postmortem at a commercial slaughter house.

Obtaining cumulus cell-oocyte complexes. The bovine ovaries were removed from clinically healthy animals. The biological material was transported to the laboratory in standardized conditions, with the delivery period of no longer than three hours and the temperature maintained at 30–33 °C to minimize thermal shock. All further procedures with the material after it had been delivered to the laboratory were performed in a laminar box to ensure sterility. The ovaries were rinsed four times with subsequent change of sterile Dulbecco's Phosphate Buffered Saline (Sigma, USA), heated to 37–38 °C, to which we added kanamycin sulfate to prevent bacterial contamination (Sigma, USA) in a final concentration of 0.075 mg/cm^3. All the procedures were performed on a heated table at a controlled temperature of 37 °C. Removal of COCs was performed by dissection of antral follicles (d = 2–8 mm) using a safe blade. Then, we examined them under a SZ51 stereomicroscope (Olympus, Japan). For the further experimental studies, we selected only COCs with dense whole cumulus, homogeneous rounded ooplasm, integral translucent membrane, and no morphological features of atresia (Fig. 1).

After removal, the cumulus cell-oocyte complexes were six times additionally rinsed in a fresh bovine oocyte and embryo manipulation medium Wash Medium (EmbryoCloud, Spain) to provide maximal sterility prior to cultivation. To achieve the goal, identifying the effects of different growth factors on oocyte maturation, we studied the following media:

1) maturation medium – medium for in vitro maturation of bovine oocytes (EmbryoCloud, Spain);

2) maturation medium + 50 ng/cm^3 of insulin-like growth factor (IGF-1) (Sigma, USA);

3) maturation medium + 100 ng/cm^3 of IGF-1;

4) maturation medium + 50 ng/cm^3 of fibroblast growth factor (FGF2) (Sigma, USA);

5) maturation medium + 100 ng/cm^3 of FGF2;

6) maturation medium + 50 ng/cm^3 of IGF-1 + 50 ng/cm^3 of FGF2.

The COCs were incubated under in vitro conditions for 20–24 hours. For the cultivation, we used a four-well dish (Oosafe, USA). We added 300 mm^3 of maturation medium and covered it with mineral oil (Origio, Denmark) to prevent evaporation. To each well we added 20–22 COCs and placed the dish in a CO_2 incubator for cultivation under controlled conditions (temperature of 38.5 °C, CO_2 – 5%).

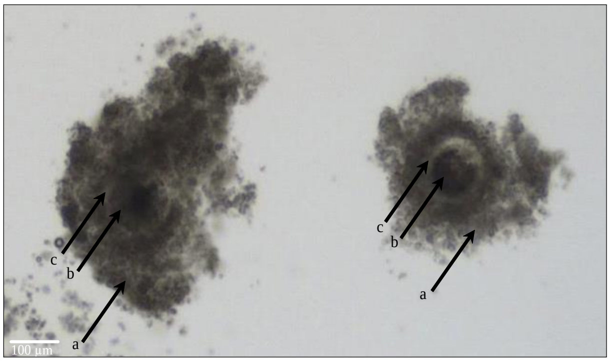

Fig. 1. Cumulus cell-oocyte complexes of bovine cattle, selected for maturation: cumulus cells (*a*), ooplasm (*b*), translucent membrane (*c*)

Evaluation of maturation of the cumulus cell-oocyte complexes. To assess the expansion of cumulus, the images of the COCs were taken twice: before (0 hours) and after maturation (20–24 hours). The photos were taken using an inverted research microscope Nicon Eclipse Ti2-U equipped with a Lykos DTS laser-assisted hatching system (Hamilton Thorne, USA). All the images were obtained in the same resolution (100×). The cumulus expansion was assessed by direct comparison of paired images and assigning points according to the five-point Likert scale (zero to four), as described by Raes et al. (2024). Zero point characterized the absence of expansion; one indicated a separation of only peripheral layers of cumulus; two indicated that the expansion included the outer half of the cumulus oophorus; three indicated further expansion to, but not beyond, corona radiate; and four indicated expansion, including corona radiata (Fig. 2).

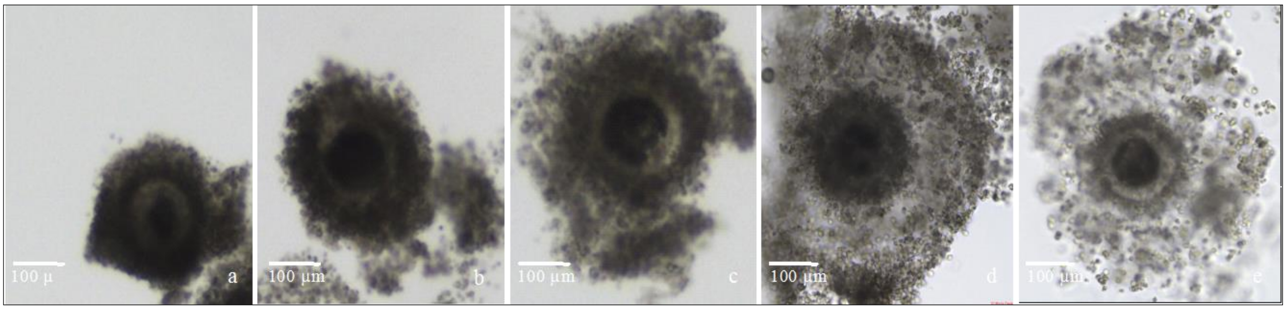

Fig. 2. Visual method of evaluating cumulus cell-oocyte complexes after maturation: 0 points (*a*), 1 point (*b*), 2 points (*c*), 3 points (*d*), 4 points (*e*)

To quantify the degree of expansion, we calculated the Cumulus Expension Index (CEI). This parameter was determined as a mean of points for all evaluated oocytes in each repetition of the experimental group. It was calculated according to the formula:

$$CEI = \frac{(N_0 \times 0)+(N_1 \times 1)+(N_2 \times 2)+(N_3 \times 3)+(N_4 \times 4)}{N_{tot}},$$

where N_0, N_1, N_2, N_3, and N_4 – number of oocytes assigned a corresponding point, N_{tot} – total number of evaluated oocytes.

Fertilization and cultivation. Bull sperm was prepared for fertilization according to the standardized multistage method, which included washing, selection of motile fraction, and capacitation induction.

For primary washing of the spermatozoids from cryopreservative and plasm, to 1 cm^3 of Sperm Wash Medium (EmbryoCloud, Spain) of bovine spermatozoa, which was preheated to a room temperature of 20–25 °C, we added a sperm dose, defrozen on a water bath, after which centrifigutation was performed at a centrifugal force of 200 g for 5 min. The sediment obtained after centrifugation was rinsed. Once washed, the sediment was transferred to the bottom of test tube with 1 cm^3 of Swim-up Medium (EmbryoCloud, Spain) of bovine spermatozoa for the selection of the most motile fraction of spermatozoids using the swim-up method. One-hour incubation is suffice for vital motile spermatozoids to migrate in the upper layers of the medium, separating from dead and pathological gametes that remain on the bottom. The obtained supernatant (motile fraction) was selected and transferred to a new portion of the medium for capacitation induction, which lasted for four hours. After the capacitation was completed, the spermatozoids were centrifuged again (200 g, 5 min), the supernatant was removed and resuspended in 1 cm^3 of Fertilization Medium of bovine oocytes (EmbryoCloud, Spain). To determine the working concentration of spermatozoids, we counted them in a Goriayev's chamber.

After the cumulus matured and was evaluated, the bovine oocytes were co-cultured with the prepared fraction of spermatozoids in a Fertilization Medium of bovine oocytes (EmbryoCloud, Spain) in four-well dishes (Oosafe, USA). To each well, we added 300 mm^3 of the medium covered with mineral oil (Origio, Denmark). In the wells, we placed 5–10 COCs and motile spermatozoids in a calculation of 5×10^3 per oocyte. The co-cultivation lasted for 18–20 hours. Then, the oocytes were freed from cumulus cells by mechanical multiple pipetting denudation with a 140 µm-diameter denudation pipette (Cook, USA). For the further embyonic development, the cleaned oocytes were transferred to the system for cultivation. Using a drop culture system, three oocytes were placed in Petri dishes (Oosafe, USA) with 65 mm^3 microdrops of Culture Medium for bovine embryo development (EmbryoCloud, Spain) under mineral oil (Origio, Denmark). Cultivation was carried out in a CO2 incubator at a temperature of 38.5 °C with 5% CO_2 and 5% O_2.

Nuclear maturation of the oocytes was identified by morphologically evaluating the gametes 18–20 hours after the beginning of co-cultivation of the cumulus cell-oocyte complexes with spermatozoids. The main criterion of maturity was visualization of polar bodies (Fig. 3). The evaluation was performed using SZ51 stereomicroscope (Olympus, Japan).

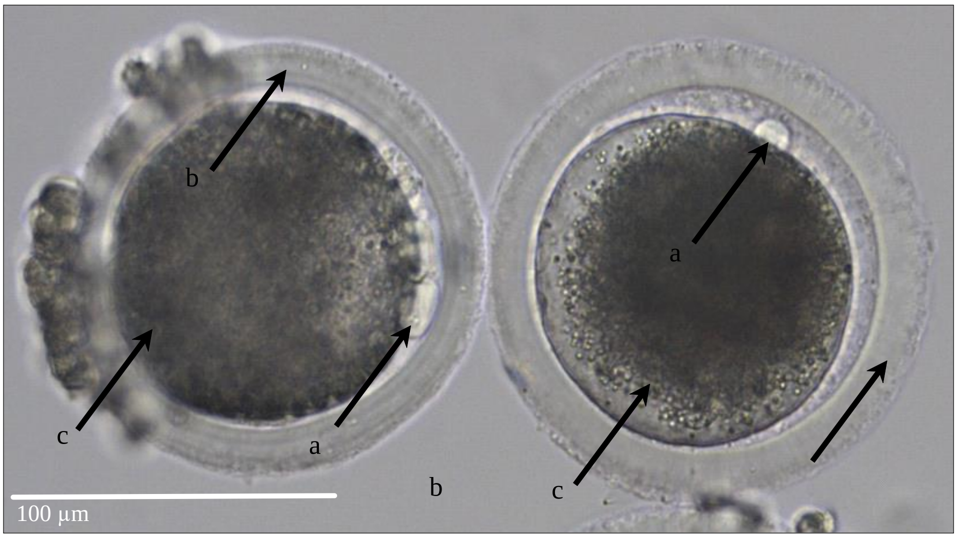

Fig. 3. Morphologic identification of mature bovine oocytes: polar body (*a*), translucent membrane (*b*), ooplasm (*c*)

The fertilization of oocytes was evaluated according to cleavage from 2- to 8-cell stage 48 hours after the contact with spermatozoids. On day 8 of cultivation, under the stereomicroscope, we examined the obtained blastocysts, estimating their percentage from the overall number of fertilized oocytes.

Statistical data analysis. The results are presented as mean ± standard deviation (x ± SD). The difference was considered statistically significant at $P < 0.05$. To compare the values among the groups, we employed the one-factor disperse analysis (ANOVA) with subsequent aposterior Tukey's Test (Tukey's HSD Test).

Results

The results of the conducted studies indicate that enrichment of the culture medium for in vitro oocyte maturation with exogenous growth factors, IGF1 and FGF2, significantly affected the development of oocytes and early embryogenesis. It has to be noted that cumulus expansion, effectiveness of nuclear maturation of oocytes (visualization of polar bodies), and vitality at initial stages of embryogenesis (beginning of cleavege and ability to form blastocyst) were affected by both concentration of the mentioned factors and their complex application (Table 1).

Table 1
Effects of enriching IVM medium with the growth factors on the success rate of oocyte maturation and further development of bovine emryos (x ± SD, n = 3)

Growth factor	Concentration, ng/cm^3	Cumulus expansion index	MII oocytes, %	Cleavage, %	Blastocysts, %
Without addition (control)	0	2.81 ± 0.21 ^{a}	61.5 ± 2.9 ^{a}	49.0 ± 3.1 ^{a}	17.9 ± 3.7 ^{a}
IGF-1	50	3.04 ± 0.18 ^{b}	66.0 ± 4.8 ^{ab}	54.7 ± 5.8 ^{a}	22.3 ± 3.2 ^{a}
	100	2.92 ± 0.06 ^{ab}	61.8 ± 1.8 ^{a}	50.0 ± 5.0 ^{a}	20.0 ± 4.7 ^{a}
FGF2	50	3.13 ± 0.13 ^{b}	73.5 ± 5.1 ^{c}	62.6 ± 3.3 ^{b}	27.7 ± 5.0 ^{ab}
	100	2.97 ± 0.12 ^{ab}	69.8 ±4.4 ^{cb}	58.7 ± 5.92 ^{ab}	23.7 + 4.5 ^{a}
IGF-1 + FGF2	50+50	3.28 ± 0.26 ^{c}	76.2 ± 5.2 ^{c}	69.3 ± 3.7 ^{c}	31.7 ± 2.9 ^{b}

Note: the percentages are calculated from the initial number of oocytes in the groups; letters indicate significant differences among the groups within a column ($P < 0.05$), according to the Tukey's Test.

Therefore, the cumulus expansion index, as a criterion for evaluation of the morphological maturity of oocytes, demonstrated a statistically significant effect of the culture medium. The average level of CEI varied the lowest in the control group to the highest in the group with addition of both IGF-1 and + FGF2. The complex effect of the growth factors provided a 0.47 increase in the cumulus expansion index, compared with the control group ($P < 0.05$). The groups affected by either of the tested growth factors had higher average parameters than the control (3.04–3.13), but no statistically significant increase was observed.

After addition of the growth factors to culture medium, we observed a positive effect on the nuclear maturation of oocytes, although their effectiveness varied broadly. Thus, introduction of IGF-1 led to a statistically insignificant result compared with the control in cases of both studied concentrations: 100 ng/cm^3 of IGF-1 provided only a 0.3% increase in MII oocytes and a 1% increase in the cleavage, whereas 50 ng/cm^3 produced 4.5% and 5.7% increases, respectively. On the other hand, the use of FGF2 demonstrated better, statistically significant, results, compared with the control: Addition of 100 ng/cm^3 of FGF2 increased the percentage of MII oocytes by 8.3% and the cleavage by 9.7%, while the addition of 50 ng/cm^3 caused greater gains in the studied parameters, measuring 12.0% and 13.6% respectively. Coupling the mentioned growth factors led to the highest gains in the studied parameters ($P < 0.05$): IGF-1 and FGF2 combined caused a 14.7% increase in the percentage of MII oocytes and a 20.3% increase in the cleavage.

Yield of blastocysts (as a final parameter of embryonic development in *in vitro* conditions) demonstrated the efficacy of the culture schemes. The greatest increase in this parameter was delivered by the combined use of the growth factors, reaching 31.7%, or a 13.8% statistically significant increase compared with the control. A notable result was also observed after adding 50 ng/cm^3 of FGF2 – increase in the formed blastocysts accounted for 9.8% compared with the control. At the same time, IGF1 in either concentrations and FGF2 in a dose of 100 ng/cm^3 provided no statistically significant gain in the percentage of blastocysts in comparison with the control group.

Discussion

The success of the methods of *in vitro* oocyte maturation directly depends on the competence of the cell to develop further (Gilchrist & Thompson, 2007). To enhance the effectiveness of IVM, researchers are actively studying the practicality of enriching culture media with various growth factors.

One of the growth factors currently used by researchers the most in culture systems for *in vitro* oocyte maturation is insulin-like growth factor type 1 (Sirotkin, et al., 1998; Kaya, et al., 2018; de Santi Phelippe Nunes et al., 2025), which is a paracrine and endocrine modulator of the ovaries' functional activity. It affects a large number of processes occurring in the ovaries: growth of follicles, hormonal secretion, atresia, cell differentiation, steroidogenesis, and oocyte maturation (Mazerbourg et al., 2003). It was found that IGF-1 has effects on intermediate metabolism, proliferation, growth and differentiation of cells, and is a powerful stimulator of oocyte maturation and embryonic development (Xia et al., 1994; Luciano et al., 2000). Sirotkin et al. (2000) noted that this growth factor promoted nuclear maturation of swine oocytes. At the same time, Sakaguchi et al. (2002) reported an enhancement of meiosis in bovine oocytes.

Depending on the follicle's development stage, IGF-1 stimulates the proliferation or differentiation of granulosa cells and plays a key role in the ovaries' sensitivity to the follicle-stimulating hormone (Mazerbourg & Monget, 2018). In addition, using IGF-1 during maturation reduces apoptotic degeneration of oocytes (Wasielak & Bogacki, 2007). While studying COCs in *in vitro* conditions, Mazerbourg et al. (2003) observed that IGF-1 effectively inhibited the apoptosis and stimulated the proliferation of granulosa cells (GCs). In bovine cattle and mice, IGF-1 is a major driving force behind the increase in the sensitivity of small antral follicles to the action of hormones and is instrumental in their transition to gonadotropin-dependent follicle stage (Mazerbourg & Monget, 2018). It was determined that insulin-like growth factor that expresses in bovine ovaries (Shiomi-Sugaya et al., 2015) can accelerate the maturation and enhance the quality of oocytes in *in vitro* conditions (Yu et al., 2012). The presented data underline the potential use of IGF-1 to improve the results of IVM.

Despite the significant scientific interest, the role of exogenic IGF-1 in the system of maturation of bovine oocytes in *in vitro* conditions remains a subject of active discussion (Ascari et al., 2017; Yang et al., 2022; de Santi Phelippe Nunes et al., 2025). The available studies demonstrate variable and often contradicting results regarding the effectiveness of this growth factor. In particular, reports about the influence of IGF-1 on the percentage of nuclear maturation of oocytes vary from increase (Lorenzo et al., 1994) to no observed changes at all (Ascari et al., 2017). A similar nonhomogeneity was also observed on early stages of embryonic development. Palma et al. (1997) and Green & Day (2013) reported that addition of IGF-1 increased the percentage of the blastocyst formation, whereas Block et al. (2008) and de Santi Phelippe Nunes et al. (2025) pointed to the absence of a significant effect on this parameter. Such conflicting data require additional experimental verification.

Inferring from the results of the conducted study, addition of IGF-1 (both 50 and 100 ng/cm^3) to culture medium for maturation of bovine oocytes provided no effective enhancement of the studied parameters, compared with the control. However, IGF-1 in a concentration of 50 ng/cm^3 promoted a significant increase in the cumulus expansion index. The obtained values correlate with the studies of Lorenzo et al. (1994). The available values can be explained by the fact that IGF-1 activates cumulus cells through IGF-1R receptors, stimulating them to synthesize hyaluronic acid, which accumulates in extracellular matrix between cumulus cells and thus induces its expansion (Nemcová et al., 2007). Insulin-like growth factor-1 is able to promote growth, proliferation, and differentiation of many types of cells, both in *in vivo* and *in vitro* conditions, and also inhibit cell apoptosis (Mazurkevych et al., 2021). It has to be noted that IGF-1R is a tyrosine kinase receptor, which activates the PI3K/Akt pathway (Sato et al., 2018), the main antiapoptic and proliferative pathway in most of the cells (Zhuang et al., 2011), including cumulus cells (Artini et al., 2017). Cumulus cells that surround oocytes are crucial for maturation and fertilization of mammal oocytes (Coticchio et al., 2015). Moreover, studies point to a correlation between adequate expansion of cumulus and high potential of the oocyte development (Rose-Hellekant et al., 1998; Nevoral et al., 2015), whereas the degree of cumulus expansion is considered an important indicator of oocyte quality (Raes et al., 2024). However, based on our data, the improvement of cumulus phenotype is only one of a number of maturation parameters that do not guarantee improvement of the quality of oocyte itself. Thus, increase in the cumulus expansion index in the group with addition of IGF-1 did not increase the percentage of MII oocytes. This suggests that IGF-1 can activate the processes in cumulus cells that promote their expansion, but do not provide a quality maturation of oocyte, which affects its competence for further development. The yielded data confirm that oocyte competence for fertilization and complete embryonic development depend not only on completion of meiosis (MII), but also on accumulation of cytoplasmic resources (mytochondria, organelles, mRNA) (Babayev & Seli, 2015; Cheng et al., 2022). The study by Dai et al. (2022) corrobrated that optimal doses of IGF-1 improved the development of preantral follicles in *in vitro* conditions, but on the other hand provided no competence of oocytes for further development. Nonetheless, there are studies that indicate that addition of IGF-1 to culture medium was associated with increase in the percentages of MII oocytes and obtained blastocysts. Noteworthy is that its effectiveness has been often recorded against the background of other additional factors (granulosa cells, other growth factors) (Moreira et al., 2002; Yang et al., 2022).

An interesting fact is that increasing the IGF-1 concentration to 100 ng/cm^3—dose typically chosen by researchers (Moreira et al., 2002; Ascari et al., 2017; Yang et al., 2022)—did not provide better results, but quite the opposite, was less effective compared with 50 ng/cm^3. The data obtained allign with the results of Shah et al. (2025), who reported that a higher efficiency for IVM of bovine oocytes was produced by a 50 ng/cm^3 dose rather than a 100 ng/cm^3 dose. The coherent results point to a dose-dependent, biophase effect of this growth factor. Dai et al. (2022) reported that excessive concentrations of IGF-1 are able to enhance the expression of genes that regulate apoptosis, thereby increasing the vulnerability of granulosa cells and inducing their death. Yang & Rajamahendran (2000) confirmed that adding a low concentration of insulin-like growth factor (10 ng/cm^3) inhibited the apoptosis in the granular bovine cells, whereas a higher concentration (100 ng/cm^3) promoted apoptosis. Based on the obtained data and analysis of the literature, we may state that IGF-1 is a dose-dependent biophase regulator of IVM, and its use as a single component has limited effectiveness for increasing the competence of bovine oocytes and improvement of their further development.

While the influence of IGF-1 on the competence of bovine oocytes has been a focus of plethora of studies, studies of the role of FGF2 in the systems of *in vitro* oocyte maturation are less common, underlining the necessity of additional research on its potential. Our interest to studying FGF2 as a modulator of oocyte competence in the process of *in vitro* maturation is due to its role in the follicle's physiology. For instance, FGF2 is a paracrine regulator of follicle genesis because it is expressed by granulosa and theca-cells throughout the development period (Berisha et al., 2006). Assidi et al., (2010) reported that FGF2 expression increased after luteinizing hormone peaked in bovine cumulus cells, indicating the involvement of FGF2 in mechanisms that are at the core of periovular maturation of COCs. Bieser et al. (1998) observed a modulating effect of FGF2 on extracellular proteolysis during IVM of bovine COCs. Furthermore, there was observed a sharp increase in the expression of FGF2 (FGF2R) receptors in bovine cumulus cells, driven by the action of FSH during IVM, suggesting their increased sensitivity to FGF2, which preceeds ovulation (Caixeta et al., 2013). Fibroblast growth factor 2 regulates steroidogenesis of granulosa cells and strengthens the competence of oocyte development (Kanke et al., 2025). However, the main function of the fibroblast growth factor in follicle is stimulation of proliferation and prevention of apoptosis of granulosa cells (Tilly et al., 1992; Barros et al., 2019).

Considering the aforementioned, exogenic addition of FGF2 to IVM environment is justified, as additionally confirmed by the data yielded in our study. Introduction of FGF2 (especially at a concentration of 50 ng/cm^3) was found to lead to statistically significant improvements in the key parameters of maturation, as compared with the control group. It should be noted that over the process of the study, addition of FGF2 to culture medium caused no significant cumulus expansion, which is consistent with the data of Barros et al. (2019) and Zhang & Ealy (2012). However, cumulus likely considerably improves the quality of extracellular matrix (ECM) and cell vitality. This assumption is based on the data of Barros et al. (2019), who pointed to increased resistance of cumulus to disassociation, noting qualitative changes in ECM. At the same time, Zhang & Ealy (2012) reported that FGF2 reduced the percentage of apoptic nuclei in cumulus cells, opposing the spontaneous cellular death during IVM (Ikeda et al., 2003). Therefore, the ability of FGF2 to provide the functionality of cumulus cell-oocyte complex through survival of the cells and quality of ECM was found to be more significant for the competence of oocytes rather than for the achievement of maximal cumulus expansion (Brown et al., 2017; Raes et al., 2024).

A substantial increase in MII oocytes (12%) following the addition of 50 ng/cm^3 of FGF2 evidences its participation in the mechanisms of oocyte maturation, which is consistent with the data of Barros et al. (2019), Zhang & Ealy (2012), and Du et al. (2021). The effectiveness of this growth factor for increasing the competence of oocytes during IVM can be explained not only by the support of cumulus vitality, but also by a direct activation of meiotic signal pathways. Therefore, according to Du et al. (2021), addition of FGF2 increased the expression of recombinant bone morphogenetic protein 15 (BMP15) or growth differentiation factor (GDF9), which promote effective transfer of metabolites from cumulus cells to bovine oocyte, thereby enhancing its development competence (Sudiman et al., 2014; Sugimura et al., 2014). Moreover, this growth factor induces the FGF2/FGFR autocrine-paracrine loop, which prevents the inhibition of c-Mos/MAPK and maturation-promoting factor (MPF) (Du et al., 2021), which is important for renewal of meiosis. Fibroblast growth factor 2 intensifies the phosphorylation of ERK1/2 in the bovine granulosa cells (Barros et al., 2019), and this signal pathway is considered the main trigger of meiosis renewal (Fan et al., 2007). Thus, FGF2 acts as a powerful signal modulator, which promotes a successful completion of meiosis and increase in the competence of bovine oocytes.

The results of further fertilization and cultivation of bovine oocytes, matured in *in vitro* conditions with addition of FGF2, demonstrated a significant increase in the parameters of cleavage and formation of blastocysts. The obtained data are consistent with the reports of Zhang & Ealy (2012) and Fields et al. (2011), confirming the role of FGF2 as a modulator of the competence of bovine oocytes. Because FGF2 was introduced exclusively into the environment for IVM, we can state that its impact is long-lasting, optimizing the functional state of oocyte already before fertilization. This particular increased development competence, acquired during IVM, is directly correlated with higher parameters of blastocyst yield. This confirms the statement that oocyte quality, formed during the maturation stage, is decisive for the success of embryonic development.

We should note that similarly to IGF-1, increasing the FGF2 concentration to 100 ng/cm^3 led to a reduction of the effectiveness for the key parameters of development, compared with 50 ng/cm^3. The data obtained confirm its biphase dose-dependent action pattern, where excessive concentration, which is beyond the limits of an optimal physiological range, can cause low or even inhibiting response of the cells.

However, we obtained optimal results only after the complex application of the growth factors. The peak result confirms that oocytes matured under the influence of IGF-1+FGF2 acquire the most complete competence of development. Insulin-like growth factor 1 (through the pathway PI3K/Akt) enhances the proliferation and antiapoptic defense (Artini et al., 2017; Sato et al., 2018), while FGF2 acts as a powerful modulator of meiosis and quality of ECM. Their synergic impact ensures not only a successful meiosis completion of the oocyte, but also the best metabolic and molecular readiness for overcoming the activation of embryonic genome and formation of a vital blastocyst.

Conclusions

The study demonstrated that optimization of culture medium for maturation of bovine oocytes by adding exogenous growth factors is an effective strategy of enhancing the efficiency of artificial reproduction technologies in animal farming. Combined application of IGF1 and FGF2 provided the highest results according to all measured parameters: cumulus expansion index, percentage of MII occytes, cleavage, and blastocyst yield. Individual use of FGF2 positively affected the early stages of oocytes' development and embryogenesis, while IGF1 alone provided no statistically significant increase in the end yield of blastocysts, but promoted an expansion of cumulus and supported the cell vitality.

Therefore, to achieve maximum IVM effectiveness, which is directly correlated with further embryonic development, it is practical to enrich the culture medium with a balanced combination of IGF1 and FGF2, which complexely support the nuclear and cytoplasmic maturation of oocytes.

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