

Comparative assessment of the biological effectiveness of a domestic medium for bovine oocyte aspiration

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Abstract

Background and Aim. *In vitro* embryo production is an important reproductive biotechnology for accelerating genetic improvement in cattle. The efficiency of this technology largely depends on the quality and developmental competence of cumulus–oocyte complexes (COCs) recovered during ovum pick-up (OPU). Aspiration media play an important role in maintaining oocyte viability during collection and subsequent handling. This study aimed to evaluate the biological efficacy of a newly developed domestic aspiration medium for bovine oocyte recovery and to compare its performance with that of a commercial aspiration medium.

Materials and Methods. The study was conducted using six physiologically healthy Galloway donor cows aged 1–4 years. Oocyte aspiration was performed twice in each donor at a 14-day interval. The recovered COCs were assigned to experimental and control groups according to the aspiration medium used. A commercial aspiration medium was used in the control group, whereas a newly developed domestic medium based on modified Dulbecco's medium was used in the experimental group. COC quality was evaluated immediately after aspiration by morphological assessment and after 24 h of *in vitro* maturation using fluorescence and polarization microscopy. Nuclear maturation, cumulus morphology, reactive oxygen species (ROS) levels, mitochondrial activity, and apoptotic changes were assessed. Differences were considered statistically significant at $p < 0.05$.

Results. A total of 125 COCs were recovered using the commercial medium and 121 using the experimental medium, with mean yields of 20.8 ± 3.6 and 20.5 ± 3.2 COCs per OPU procedure, respectively. Morphologically normal COCs accounted for 81.6% in the control group and 80.1% in the experimental group, with no statistically significant differences between the groups. After *in vitro* maturation, the proportion of oocytes reaching metaphase II and the presence of the meiotic spindle were comparable between groups. No significant differences were detected in cumulus morphology, ROS levels, mitochondrial activity, or apoptotic changes. The proportion of oocytes with high mitochondrial activity was 76.4% in the control group and 74.2% in the experimental group.

Conclusion. The newly developed domestic aspiration medium demonstrated biological efficacy comparable to that of the commercial medium and maintained the morphological quality, nuclear maturation, mitochondrial activity, and overall viability of bovine oocytes. These findings indicate that the medium is promising for further evaluation in OPU and *in vitro* embryo production. However, validation in a larger number of donor animals, including assessment of fertilization, cleavage, blastocyst development, embryo transfer, and pregnancy outcomes, is required before conclusions can be drawn regarding its broader practical application.

Keywords: cattle; cumulus-oocyte complexes; embryo transfer; *in vitro* embryo production; oocyte aspiration; oocyte aspiration medium.

Introduction

In the Russian Federation, cattle breeding is a key sector of the animal husbandry industry as a whole. The effectiveness of its further development depends largely on the implementation of modern biotechnological procedures, one of which is the production of *in vitro* embryos. This technology makes it possible to significantly accelerate genetic progress in herds, make fuller use of the genetic potential of outstanding animals, and produce more offspring from them.

The first step in *in vitro* embryo production technology is ovum pick-up (OPU), during which cumulus-oocyte complexes (COCs) are obtained from the cow's ovaries using minimally invasive methods, which are then used to produce embryos [1]. This step is critical, as it largely determines the potential of the oocyte for subsequent development, fertilization, and culture of the resulting zygote [2]. During OPU, the oocyte is exposed to various stress factors, including fluctuations in environmental pH, mechanical stress, oxidative stress, and temperature deviations from the physiological norm [3]. To maintain its ability to further develop, the use of a special fluid, which is an aspiration medium, is necessary [4].

Currently, imported commercial oocyte aspiration media are widely used in the Russian Federation; however, they are expensive, and their availability depends on foreign supplies. An alternative is the use of laboratory-prepared experimental media of varying compositions, the preparation of which requires specialized equipment and resources. Domestic commercial aspiration media, in turn, are virtually nonexistent.

To date, studies have been conducted that provide sufficient information on the impact of aspiration medium components such as HEPES buffer [5, 6], various antioxidants, and various salts for maintaining osmotic pressure [7] on COC quality. However, given the lack of domestic commercial media, a comprehensive evaluation of the effectiveness of a domestic aspiration medium relative to its commercial counterpart is a promising direction.

Consequently, it was decided to conduct an experimental study of a newly developed domestic medium for oocyte aspiration. Its overall effectiveness was compared with that of a commercial medium used for this stage of *in vitro* embryo production. The obtained results fill the existing gap in data on domestic alternatives to imported aspiration media.

The objective of the study was to evaluate the biological effectiveness of a new domestic aspiration medium for use in *in vitro* bovine embryo production technology based on the data obtained during the experimental study.

Materials and Methods

The study was conducted from March to April 2026. The ovum pick-up (OPU) procedures were performed at the Smolensk Galloway Breeding Center, LLC, and the laboratory stage was conducted in the embryology laboratory of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy. The study subjects were cumulus-oocyte complexes (COCs) obtained from physiologically healthy Galloway cows aged 1 to 4 years with no estrous cycle disorders.

Oocyte aspiration was performed twice in each donor at a 14-day interval [8], once using the commercial medium and once using the experimental medium. The order of media application was balanced: in three animals the experimental medium was used for the first aspiration, and in the other three the commercial medium. In the control group, OPU was performed using the commercial aspiration medium OPU Medium (cat. No. 51001, IVF Bioscience, UK). According to the manufacturer, it is a serum-free medium enriched with vitamins and amino acids and supplemented with heparin and gentamicin. The quantitative composition of the medium is not disclosed by the manufacturer. OPU media maintain a pH of 7.2 - 7.4 and an osmolality close to that of the internal environment of the body, which is 290 - 310 mOsm/kg. In the experimental group, a new domestic medium developed for bovine oocyte aspiration was used.

The domestic medium was prepared on the basis of modified Dulbecco's phosphate-buffered saline (mDPBS), in which the NaCl concentration was reduced to 114 mM to compensate for the osmotic contribution of the buffer components. It contained a buffer system consisting of HEPES and TES; sodium heparin to prevent blood clotting; polyvinyl alcohol, used instead of bovine serum albumin [7] to prevent adhesion of oocytes to the walls of the needle and tubes; gentamicin to prevent bacterial

contamination; and L-cysteine and L-carnitine as antioxidants. The quantitative composition of the medium is given in Table 1. The pH of the medium was adjusted to 7.4 with 1 M NaOH; the osmolality of the medium was 290 mOsm/kg.

Table 1. Composition of the experimental oocyte aspiration medium

Component	Concentration	Amount per 1 L of medium
NaCl	114 mM	6.66 g
KCl	2.68 mM	0.20 g
Na ₂ HPO ₄ (anhydrous)	8.1 mM	1.15 g
KH ₂ PO ₄	1.47 mM	0.20 g
HEPES	25 mM	5.96 g
TES	10 mM	2.29 g
Polyvinyl alcohol	1.5 mg/mL	1.5 g
Sodium heparin	7.5 IU/mL	7.500 IU
L-cysteine	0.2 mM	24.2 mg
L-carnitine	0.5 mM	80.6 mg
Gentamicin	75 µg/mL	75 mg
NaOH (1 M solution)	to pH 7.4	0.65 g

After OPU, the resulting aspirate, according to standard protocols [9, 10], was subjected to primary filtration, placed in a transport medium, and transported in a thermal container at a temperature of 37, 38 °C for no more than 3 hours [11, 12]. The cumulus-oocyte complexes were then washed in a special medium, after which they underwent an initial morphological assessment under a stereomicroscope at ×40 and ×80 magnification [13]. Morphologically normal COCs were identified as oocytes with uniform, dark-granulated cytoplasm without visible vacuoles or with minor cytoplasmic heterogeneity and with 2-4 layers of cumulus cells. These were immediately transferred to *in vitro* oocyte maturation medium in 100-µl droplets covered with a layer of sterile mineral oil [14].

After 24 hours, the COCs were re-evaluated for quality. Nuclear maturity of oocytes was assessed by fluorescence microscopy by performing complete denudation using gentle pipetting, fixation, and DAPI staining, after which the metaphase stage II was determined [14]. The presence of the meiotic spindle was also assessed using polarized light microscopy [15]. The degree of expansion, compactness, and the presence of degenerative signs of the cumulus were separately recorded. In addition, the level of reactive oxygen species was determined using the fluorescent probe DCFH-DA, and mitochondrial activity was assessed using the fluorescent dye MitoTracker [16]. The level of apoptosis was also determined using the TUNEL assay [17].

Statistical data processing was performed using Statistica 10.0 and Microsoft Excel 2019. Quantitative data with a normal distribution were presented as mean ± standard deviation (SD). Normality was assessed using the Shapiro-Wilk test. COC yield per procedure was compared using a linear mixed model with medium and aspiration order as fixed factors and animal as a random effect. Indicators assessed at the level of individual oocytes were compared using pooled group data with Pearson's χ^2 test and Fisher's exact test. Fluorescence intensity was compared using Student's t-test for independent samples. Differences between groups were considered statistically significant at a probability level of $p < 0.05$.

Results

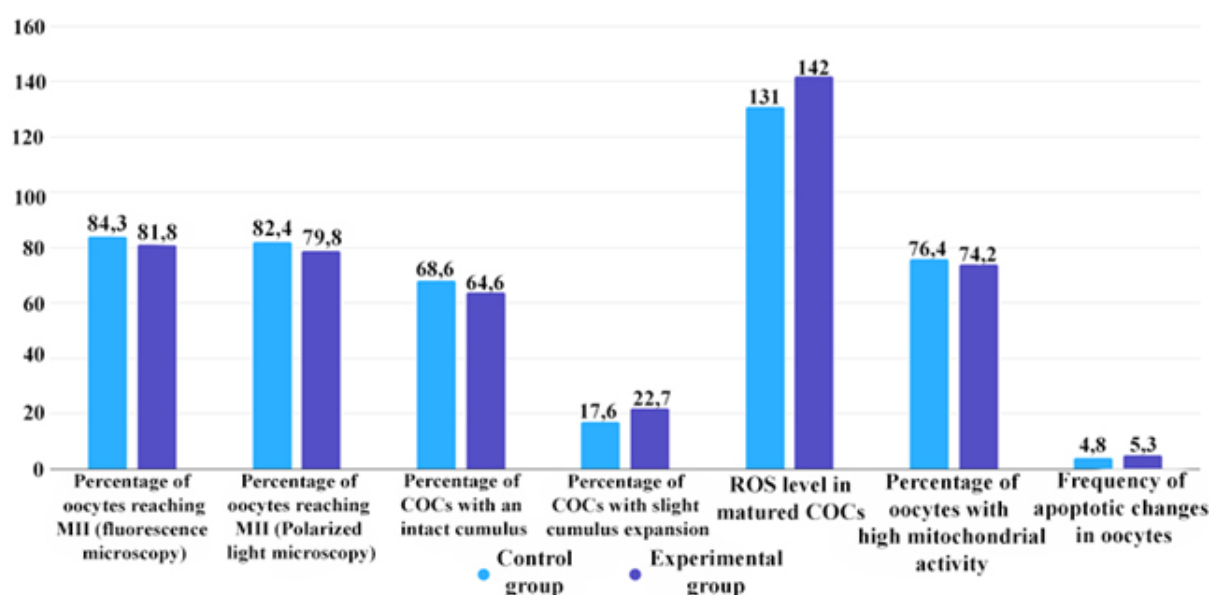
During oocyte aspiration using a commercial medium, 125 COCs were obtained, while 121 COCs were obtained in the experimental group using a new domestic aspiration medium. The average yield of complexes per OPU procedure was 20.8 ± 3.6 in the control group and 20.2 ± 3.2 in the experimental group. Differences in the number of oocytes retrieved were not statistically significant ($p = 0.28$), nor was the effect of aspiration order ($p = 0.82$). A preliminary assessment of the COCs was conducted, and the results are presented in Table 2.

Table 2. Distribution of COCs by morphological grade in the control and experimental groups

Control group	Number of COCs	Proportion of COCs, %	Experimental group	Number of COCs	Proportion of COCs, %
Total	125	100	Total	121	100
1 grade	64	51.2	1 grade	59	48.8
2 grade	38	30.4	2 grade	38	31.4
3 grade	14	11.2	3 grade	15	12.4
4 grade	9	7.2	4 grade	9	7.4
Morphologically normal COCs	102	81.6	Morphologically normal COCs	97	80.2

The total proportion of grade 1 and 2 complexes in the experimental group was 80.2%, which is 1.4% lower than in the control group. Statistical analysis revealed no significant differences between the groups in the distribution of COCs by grade ($\chi^2 = 0.17$; $p = 0.98$). The obtained data are consistent with published data, according to which, when using OPU, the proportion of morphologically intact cumulus-oocyte complexes ranges from 70 to 85% of the total number of retrieved oocytes [18, 19].

During a secondary assessment of cumulus-oocyte complexes, the data presented in Figure 1 were obtained. The results of the secondary assessment of cumulus-oocyte complexes are presented in Figure 1.

**Figure 1.** Comparison of the experimental and control groups by indicators of nuclear and cytoplasmic oocyte maturation

Following IVM, matured oocytes were examined using polarized light microscopy. The meiotic spindle was visualized in approximately 80% of oocytes in both the control and experimental groups. Fluorescence microscopy then showed that the proportion of oocytes reaching metaphase II was 2.5 percentage points higher in the control group ($p > 0.05$); the results of fluorescence and polarized light microscopy were comparable. Both media provided an acceptable level of nuclear maturation typical of standard IVM protocols, which is consistent with studies on *in vitro* oocyte maturation [20, 21].

The secondary evaluation of COCs also included assessment of morphological changes in the cumulus. Overall cumulus morphology after IVM remained within the physiological norm in both groups; no statistically significant differences were found between the two groups [22]. Additionally, ROS levels were measured, revealing that the average fluorescence intensity was slightly higher in the experimental group. The difference compared to the control group averaged 10.5 arbitrary units, which is statistically insignificant. These results are consistent with published data on the permissibility

of moderate fluctuations in oxidative stress levels without significantly affecting oocyte maturation [23].

Mitochondrial activity was also assessed, but no statistically significant differences were found: the proportion of oocytes with intense mitochondrial fluorescence was 76.4% in the control group and 74.2% in the experimental group. The absence of differences in these data indicates the maintenance of the effectiveness of the oocyte mitochondrial system, confirming their cytoplasmic maturation.

Finally, apoptotic changes were assessed and occurred at a low frequency. This is also consistent with published data showing that apoptosis levels remain minimal under optimal oocyte retrieval conditions [24, 25].

Limitations of this study include the small number of donor animals, which limited the total number of cumulus-oocyte complexes studied, limiting the statistical power of the study. Furthermore, only an early assessment of oocyte quality indicators was conducted; however, monitoring the progress of *in vitro* embryo production up to their transfer to recipients could have yielded more reliable results.

The obtained results demonstrated that the domestic medium exhibits comparable performance to a commercial one. The obtained results are within the range of values for standard commercial oocyte aspiration media [26, 27]. These data may indicate the need for further research on the domestic medium. The absence of statistically significant differences between groups suggests that the experimental medium is successfully performing its functions. The observed tendency towards a slight decrease in individual indicators requires further verification of the data, for which it is worth considering the possibility of repeating a similar experiment on a larger sample with different donors, as well as longer-term monitoring of the obtained oocytes [28].

Discussion

The comparable biological responses observed with the experimental and commercial aspiration media indicate that the composition of the newly developed domestic medium provides an appropriate microenvironment for bovine oocytes during OPU and subsequent handling. The buffer system based on HEPES and TES is intended to maintain stable physicochemical conditions, while polyvinyl alcohol protects oocytes from undesirable adhesion to aspiration equipment. The inclusion of L-cysteine and L-carnitine as antioxidant components may contribute to maintaining cellular homeostasis during oocyte recovery and maturation. Importantly, the experimental medium did not produce detectable adverse effects on nuclear or cytoplasmic maturation, oxidative status, mitochondrial function, or apoptotic processes. These observations suggest that replacement of the commercial aspiration medium with the developed formulation did not compromise the principal biological characteristics associated with oocyte quality and developmental competence. From a practical perspective, the development of a domestically produced aspiration medium may reduce dependence on imported commercial products and improve the accessibility of OPU-based reproductive technologies. Nevertheless, the relatively small number of donor animals and the assessment of oocytes only during the early stages of *in vitro* embryo production should be considered when interpreting the findings. Further studies involving a larger and more diverse donor population, fertilization, embryo culture, blastocyst development, and subsequent embryo transfer are required to comprehensively evaluate the reproductive efficiency of the developed medium.

Conclusions

The experiment assessed the biological efficacy of a new domestic aspiration medium for use in *in vitro* bovine embryo production technology, confirming its suitability for further testing.

The number of oocytes recovered, their quality, and maturation parameters obtained using the new domestic medium were comparable to those obtained using a commercial medium.

No statistically significant differences were found between the two groups, indicating the comparability of the domestic medium with its commercial counterpart.

These results indicate that the tested medium is promising for further evaluation in OPU procedures. Validation in a larger and more diverse donor population is required, together with assessment of fertilization, cleavage, blastocyst development, embryo transfer, and pregnancy outcomes. Further studies should also evaluate whether optimization of the medium composition is warranted.

Authors' Contributions

Conceptualization: AA, YK, DS; methodology: AA, YK, SF and DS; investigation: AA, YK and DS; data curation: AA and YK; statistical analysis: YK and DS; writing original draft preparation: AA and YK; writing review and editing: SF and DS; supervision: SF. All authors have read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

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