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Optimization of the *in Vitro* Fertilization System in Cỏ Goat Oocytes

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Abstract

This study aimed to optimization of the *in vitro* fertilization system in Cỏ goat oocytes to achieve the maximum possible blastocyst development rate. In *Experiment 1*, we assessed the effects of IVF media on the *in vitro* fertilization of Cỏ goat oocytes. There was no significant difference in the cleavage, blastocyst, or hatching rates between TALP-Fert and BO-IVF media. *Experiment 2* was performed to assess the concentration of sperm in the *in vitro* fertilization of Cỏ goat oocytes. The matured Cỏ goat oocytes were fertilized in BO-IVF for four sperm concentrations: 5×10^5, 1×10^6, 2×10^6 and 3×10^6 sperm/ml. The blastocyst rate of 2×10^6 sperm/ml and 3×10^6 sperm/ml groups was higher than that of 5×10^5 sperm/ml and 1×10^6 sperm/ml groups ($P < 0.05$). *Experiment 3* was performed to assess the IVF duration on the *in vitro* fertilization of Cỏ goat oocytes. The matured Cỏ goat oocytes were fertilized in BO-IVF with sperm concentration of 3×10^6 sperm/ml for 18, 20, 22 and 24 h. The cleavage, blastocyst, and hatching blastocyst rates of 18 h group were lower than those of 20, 22 and 24 h groups ($P < 0.05$). The difference in cleavage, blastocyst and hatching blastocyst rates between 20, 22 and 24 h groups was not statistically significant ($P > 0.05$). In conclusion, the matured Cỏ goat oocytes were fertilized in BO-IVF with sperm concentration of 3×10^6 sperm/ml for 20 hours, which is suitable for the *in vitro* Cỏ goat embryo production.

Keywords

Cỏ Goat Oocytes, *In Vitro* Fertilizaition Media, *In Vitro* Fertilizaition Duration, Sperm Concentration, *In Vitro* Embryos

1. Introduction

The goat is one of the most important domestic farm animals and provides a large number of products, such as milk, meat and hides. *In vitro* embryo production

technology is an important tool for genetic improvement in goats and provides a source of low-cost embryos for basic research and for commercial application of biotechnologies, such as nuclear transfer, transgenesis, embryo sexing, and stem cells. *In vitro* goat embryo production includes three steps: *in vitro* maturation of goat oocytes, *In Vitro* Fertilization (IVF) of mature goat oocytes, and *in vitro* goat embryo culture [1]. IVF is a complex procedure whose success depends on several factors, such as the sperm concentration, fertilization media and IVF duration used.

The fertilization media used for IVF are the Synthetic Oviductal Fluid (SOF) medium used in ovine [2], Brackett and Oliphant (BO) medium used in caprine [3], and the Tyrode's Albumin Lactate Pyruvate (TALP) medium supplemented with hypotaurine widely used in goat [4]. For IVF, the final sperm concentration used in the IVF drop can vary from 0.5×10^6 cells/ml to 12 - 15×10^6 cells/ml, depending on the IVF used [5] [6]. According to Souza-Fabjan *et al.* (2023) [1], co-culture time is too short or long can reduce the IVF rate. These authors showed that a shorter oocyte-sperm incubation reduced IVF efficiency, but using longer co-culture time could result in higher ROS concentrations and lower blastocyst rate. In IVF goats, the duration of sperm and oocyte co-culture is inconsistent between the studies. Sperm and oocytes of goats are co-cultured for 16 to 24 hours, depending on the laboratory.

Cỏ goat is an indigenous goat breed, and they are an important genetic resource for conservation of native Vietnamese goat biodiversity. Cỏ goats are a goat breed commonly raised in Vietnam for meat production. Some of the advantages of Cỏ goats include small in size and popular for disease resistance, high reproduction, limited food requirement and high adaptability in Vietnam's climate [7]. The development of *in vitro* production of goat embryos is able to support the propagation of this animal, however, IVF experiments on Cỏ goat in Vietnam have not been done in the past. Therefore, in this study, we optimized the *in vitro* fertilization system in Cỏ goat oocytes.

2. Materials and Methods

All the experimental procedures used in this study were performed in accordance with Vietnam legislation and according to Decision No. 5814/QĐ-BNN-KHCN of the Ministry of Agriculture and Rural Development in Vietnam on December 27.2023.

2.1. Reagents and Chemicals

All reagents and chemicals used in this study were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Petri dishes used for cell culture are originated from Corning Inc. (Corning, NY, USA).

2.2. Collection of Cỏ Goat Ovaries and Cumulus Oocytes Complexes (COCs)

Cỏ goat ovaries were transported to the laboratory in Dullbeco Phosphate Buffer

Saline (DPBS) supplemented with antibiotics within 2 hours after collection from the slaughterhouse. Oocytes were collected from 2 - 8 mm diameter follicles on the ovarian surface using an aspiration method with a 5 ml syringe containing Tyrode's Albumin Lactate Pyruvate-HEPES (TALP-HEPES) oocyte collection solution supplemented with serum and an 18G needle. After aspiration, the TALP-HEPES medium with harvested oocytes was transferred to a Petri dish, and oocytes were searched by using a stereo microscope and evaluated according to the standards of Wani *et al.* (2000) [8]. After evaluation, cumulus oocytes complexes were selected based on: 1) uniform cytoplasm and 2) the presence of at least three compact surrounding layers of cumulus cells.

2.3. *In Vitro* Maturation of Goat Oocytes

The COCs of Cỏ goat oocytes were washed three times in IVM medium either TCM 199 supplemented with 10% Fetal Cafl Serum (FCS), 50 ng/ml Follicle Stimulating Hormone (FSH), 10 ng/ml Epidermal Growth Factor (EGF), 100 µM cysteamine, 100 units/ml penicillin G potassium + 0.1 mg/ml streptomycin sulphate, and then transferred to 4-well plates containing 500 µl of the *in vitro* maturation medium per well for 22 hours, under conditions of 38.5˚C, 5% CO_2, and saturated air humidity (50 oocytes per well).

2.4. *In Vitro* Fertilization (IVF) and Embryo Culture (IVC)

The IVF and IVC procedures were performed using the method of Widayati and Pangestu (2020) [9] with some modifications. Matured Cỏ goat oocytes were washed in *in vitro* fertilization (BO-IVF) medium two times. Thawed semen was centrifuged at 320 × g for 5 min in BO sperm washing medium. The supernatant was removed, and sperm pellet was diluted in BO sperm washing medium. Oocytes and sperm were incubated in IVF medium from 18 to 24 h depending on the experiment, at 38.5˚C under 5% CO_2, 5% O_2 and humidified air. After duration co-culture, the cumulus cells surrounding the oocyte were removed by repeated pipetting in TALP-HEPES medium + 0.2% hyaluronidase. The oocytes without cumulus cells were washed in SOF medium two times and cultured in SOF medium + 2.5% Fetal Bovine Serum (FBS) in an incubator at 38.5˚C under 5% CO_2, 5% O_2 and humidified air for 7 days.

2.5. Evaluation of Embryo Cell Number

The cell numbers in blastocyst at Day 7 after IVF were evaluated by staining with Hoechst 33342. The embryos of Cỏ goat were washed in Phosphate Buffered Saline (PBS) medium supplemented with 0.3% Polyvinylpyrrolidone (PVP) solution. Next, the oocytes were transferred into a staining solution (Hoechst 33342 + Absolute ethanol in a 1:9 ratio) and left overnight at 4˚C. After incubation overnight at 4˚C in the staining solution, the embryos were washed in absolute ethanol and then transferred to a Glycerol solution. Subsequently, the embryos were transferred to a glass slide, each embryo in a drop, and aligned along the length of the

slide. A cover slip was placed over the slide, and the embryos were examined under a fluorescence microscope. The cell numbers count under a fluorescence microscope.

2.6. Experimental Design (Figure 1, Figure 2)

Experiment 1. The effect of IVF media on the in vitro fertilization of Cỏ goat oocytes

In this experiment, matured Cỏ goat oocytes were divided into two IVF media: 1) TALP-Fert and 2) BO-IVF medium. Matured Cỏ goat oocytes and sperm were co-cultured for 20 hours and placed in an incubator at 38.5˚C, in 5% CO_2, 5% O_2 and in saturated air humidity. The embryo developmental competence of Cỏ goat oocytes was evaluated by examination of cleavage, blastocyst and hatching rates at Day 2, Day 6, and Day 7 after IVF, respectively, and total cells per blastocyst counted on Day 7. Eight replications were performed.

Experiment 2. The effect of sperm concentration on the in vitro fertilization of Cỏ goat oocytes

In this experiment, matured Cỏ goat oocytes were fertilized in the selected fertilization medium from *Experiment 1* for four sperm concentrations: 5×10^5, 1×10^6, 2×10^6 and 3×10^6 sperm/ml. Cỏ goat oocytes and sperm were co-cultured for 20 hours and placed in an incubator at 38.5˚C, in 5% CO_2, 5% O_2 and in saturated air humidity. The IVF of matured Cỏ goat oocytes was performed as described above. The embryo developmental competence of Cỏ goat oocytes was evaluated by examination of cleavage, blastocyst and hatching rates at Day 2, Day 6, and Day 7 after IVF, respectively, and total cells per blastocyst counted on Day 7. Eight replications were performed.

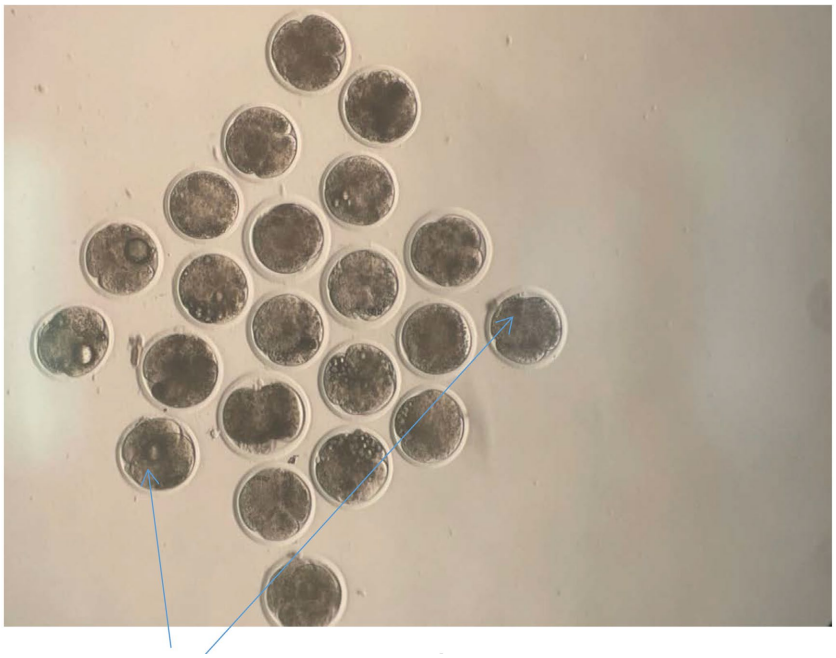

Figure 1. 2 - 4 cells embryo of Cỏ goat at Day 2 after IVF (eyepiece * objective is 5×/0.12).

Figure 2. Blastocyst and hatching blastocyst of Cỏ goat at Day 7 after IVF (eyepiece * objective is 10×/0.25).

Experiment 3. The effect of IVF duration on the in vitro fertilization of Cỏ goat oocytes

In this experiment, Cỏ goat oocytes were fertilized in the selected fertilization medium, sperm concentration from *Experiment 1* and *Experiment 2* for 18, 20, 22 and 24 hours at 38.5˚C, in 5% CO_2, and in saturated air humidity. The IVF of Cỏ goat oocytes was performed as described above. The embryo developmental competence of Cỏ goat oocytes was evaluated by examination of cleavage, blastocyst and hatching rates at Day 2, Day 6, and Day 7 after IVF, respectively, and total cells per blastocyst counted on Day 7. Eight replications were performed.

2.7. Statistical Analysis

All data were expressed as mean ± SEM values and analysed by ANOVA, followed by Tukey's multiple comparisons test, using GraphPad Prism software (Version 7.02 for Windows, GraphPad Software, La Jolla, California, USA. $P < 0.05$ was defined as the significance level.

3. Results

3.1. Experiment 1. The Effect of IVF Media on the *in Vitro* Fertilization of Cỏ Goat Oocytes

The results in Table 1 indicated no significant difference in the cleavage and blastocyst and hatching rates and the average number of cells/blastocyst of Cỏ goat oocytes after fertilization between two IVF media: TALP-Fert and BO-IVF (67.84%

vs. 68.92%, 23.82% vs. 24.76%, 4.36% vs. 4.02% and 132.96 vs. 135.08, respectively; $P > 0.05$).

Table 1. Influence of IVF media on the *in vitro* fertilization of Cỏ goat oocytes.

Medium	No. of fertilized oocytes	No. of cleavage (% total)	No. of blastocyst (% total)	No. of hatching blastocyst (% total)	The average number of cells/blastocyst
TALP-Fert	98	66 67.84 ± 2.34	23 23.82 ± 2.15	4 4.36 ± 1.98	132.96 ± 2.58
BO-IVF	102	70 68.92 ± 2.22	25 24.76 ± 2.05	4 4.02 ± 2.18	135.08 ± 2.21

Eight replications were performed. Percentage data are shown as mean ± SEM.

3.2. Experiment 2. The Effect of Sperm Concentration on the *in Vitro* Fertilization of Cỏ Goat Oocytes

Based on the results of *Experiment 1*, in *Experiment 2* we used the BO-IVF medium to evaluate the effect of sperm concentration on the *in vitro* fertilization of Cỏ goat oocytes. The results are shown in Table 2. The cleavage rate of 5×10^5 group was lower than that of 1×10^6, 2×10^6 and 3×10^6 groups (55.96% vs. 69.98%, 76.48%, 76.95%; respectively, $P < 0.05$). The blastocyst rate of 2×10^6 and 3×10^6 groups was higher than that of 5×10^5 and 1×10^6 groups (35.92% and 35.96% vs. 14.72% and 24.01%; respectively, $P < 0.05$). The difference in blastocyst and hatching blastocyst rates between 2×10^6 and 3×10^6 groups were not statistically significant ($P > 0.05$).

Table 2. Influence of sperm concentration on the *in vitro* fertilization of Cỏ goat oocytes.

Concentration (sperm/ml)	No. of fertilized oocytes	No. of cleavage (% total)	No. of blastocyst (% total)	No. of hatching blastocyst (% total)	The average number of cells/blastocyst
5×10^5	114	63 55.96^a ± 2.07	16 14.72^a ± 2.31	3 2.96 ± 1.75	133.24 ± 2.24
1×10^6	119	83 69.98^b ± 2.03	28 24.01^b ± 2.42	4 3.89 ± 2.76	134.98 ± 2.38
2×10^6	118	90 76.48^b ± 1.99	42 35.92^b ± 2.14	6 5.48 ± 2.35	135.01 ± 2.46
3×10^6	112	86 76.95^b ± 2.58	40 35.96^b ± 2.67	6 5.62 ± 2.32	135.78 ± 2.51

Eight replications were performed. Percentage data are shown as mean ± SEM. a and b in the same column differ significantly ($P < 0.05$).

3.3. Experiment 3. The Effect of IVF Duration on the *in Vitro* Fertilization of Cỏ Goat Oocytes

Based on the results of *Experiment 1 and Experiment 2*, in *Experiment 3*, matured Cỏ goat oocytes were fertilized in the BO-IVF medium with a concentration of 3 ×

10^6 sperm/ml in each IVF droplet.

The results in Table 3 indicate that the cleavage, blastocyst, and hatching blastocyst rates of 18 hours group were lower than that of 20-, 22- and 24-hour groups (54.16% vs. 76.22%, 77.21% and 77.09%; 17.64% vs. 35.67%, 35.82% and 35.96%; 3.58% vs. 5.92%, 6.36, 6.31%; respectively, $P < 0.05$). The difference in cleavage, blastocyst and hatching blastocyst rates between 20-, 22- and 24-hour groups were not statistically significant ($P > 0.05$).

Table 3. Influence of IVF duration on the *in vitro* fertilization of Cỏ goat oocytes.

IVF duration (hours)	No. of fertilized oocytes	No. of cleavage (% total)	No. of blastocyst (% total)	No. of hatching blastocyst (% total)	The average number of cells/blastocyst
18	115	62 54.16^a ± 2.31	20 17.64^a ± 2.06	4 3.58 ± 2.33	132.98 ± 2.46
20	121	92 76.22^b ± 2.26	43 35.67^b ± 2.41	7 5.92 ± 2.09	134.89 ± 2.14
22	117	90 77.21^b ± 2.54	41 35.82^b ± 2.28	7 6.36 ± 2.47	135.12 ± 2.71
24	116	89 77.09^b ± 2.72	41 35.96^b ± 2.45	7 6.31 ± 2.39	134.86 ± 2.67

Eight replications were performed. Percentage data are shown as mean ± SEM. a and b in the same column differ significantly ($P < 0.05$).

4. Discussion

The results of this study indicate that IVF media does not affect on the *in vitro* fertilization of Cỏ goat. There was no significant difference in the cleavage and blastocyst and hatching rates and the average number of cells/blastocyst of Cỏ goat oocytes after fertilization between TALP-Fert and BO-IVF media (Table 1, $P > 0.05$). The quality of sperm has an important role in the success of the IVF process and *in vitro* embryo production. During the IVF process, there are some changes in membrane properties, intracellular ion concentrations, enzyme activity, protein modifications that can affect the IVF process [10]. To improve fertilization ability and capacitation of sperm as well as increase the number of fertilized oocytes, adding of supplements to the IVF media is necessary. According to Kharche *et al.* (2009) [11], when using fetal calf serum instead of bovine serum albumin in IVF media will increase the cleavage and blastocyst rates of goat oocytes. Heparin and hypotaurine are agents to improve the ability of sperm fertilization *in vitro* [12] [13].

In this study, Heparin was added to the BO-IVF media to improve the effect of IVF of goat oocytes. Cox and Alfaro (2007) [14] also showed that heparin is a potent glycosaminoglycan that has been shown to improve the fertilizing ability of bovine, goat and sheep when used in IVF medium. The cause is that the presence of heparin in the IVF media will induce capacitation of sperm in the *in vitro* embryo production,

increased calcium uptake, increased intracellular free calcium and decreased calmodulin concentration in spermatozoa [15].

Hypotaurine is also an antioxidant, which can induce capacitation of sperm during IVF process, and it is often oxidized to taurine to remove hydroxyl radicals in the *in vitro* fertilization system [16]. According to Pons-Rejraji *et al.* (2021) [17], the presence of heparin and hypotaurine in the IVF media will increase viability and progressive motility, as well as reduce the rate of sperm that undergo chromatin decondensation, DNA fragmentation and nuclear vacuolization, which is why in our study, we added Hypotaurine to TALP-Fert medium and heparine to BO-IVF medium.

In *Experiment 2*, our results are similar to Palomo *et al.* (2010) [18]. According to Palomo *et al.* (2010) [18], sperm concentration affects the *in vitro* fertilization of prepubertal goat oocytes. However, there is no consensus among studies on the optimal of sperm concentration for IVF in goat. Palomo *et al.* (2010) [18] reported the optimum sperm concentration is 4×10^6 sperm/ml, but in our study and the report of Pawshe *et al.* (1994) [19], the optimum sperm concentration is 2×10^6 or 3×10^6 sperm/ml. The reason for these different results might be the different evaluation criteria, the quality of sperm between the studies. For optimum concentration sperm, Palomo *et al.* (2010) [18] used the penetration rate as evaluation criteria, whereas we and Pawshe *et al.* (1994) [19] used cleavage and blastocyst and hatching blastocyst rates.

The correlation between the polyspermy rate and sperm concentration present during IVF process show that reducing sperm concentration may be minimize polyspermy in IVF (Gil *et al.* 2008) [20]. The quality of sperm frozen-thawed is a necessary requirement to increase the fertilized oocytes rate. During *in vitro* fertilization, when using sperm frozen, high sperm concentration is essential to maintain the motility of sperm and increase the number of sperm capable of fertilization. Therefore, this could be the reason why the optimal of sperm concentration in this study was lower than that of the report of Palomo *et al.* (2010) [18]. When increasing sperm concentration from 5×10^6 to 10×10^6 sperm/ml, the fertilized oocytes rate decreases and the polyspermy rate increase. Therefore, in this study, we also limited sperm concentration to a maximum of 3×10^6 sperm/ml.

In this study, we aimed to optimize *in vitro* fertilization for Cỏ goat oocytes in order to optimize the production of blastocyst goat embryo by IVF. Optimization of the IVF duration means the maximization of efficacy as measured by blastocyst stage embryos after subsequent IVF. The results of *Experiment 3* showed that IVF duration affect the *in vitro* fertilization of Cỏ goat. Our results are similar to Cognié *et al.* (2003) [21] and Tan *et al.* (2011) [22]. According to Cognié *et al.* (2003) [21] and Tan *et al.* (2011) [22], IVF duration affect the *in vitro* fertilization of goat oocytes. The period of sperm-oocytes exposure in goat is still controversial, and there is no consensus on the optimal IVF duration in goat between studies: 20 h [21], 24 h [23] [24], 16 - 20 h [14], 18 h [3] and 12 h [25]. Tan *et al.* (2011) [22] showed that the cleavage rate of 8 - 14 h group was higher than the 18 - 24 h group, whereas

in the report of Cognié *et al.* (2003) [21], the cleavage and blastocyst rates of 17 h group were higher than the 3 h group. Differences between studies may be due to the quality of sperm and matured oocytes, IVF medium, IVF protocol…

According to Van Soom *et al.* (2002) [26], the presence of cumulus cells around the oocytes at the time of fertilization appears to increase the blastocyst yield. dos Santos-Neto *et al.* (2020) [27] found beneficial effects of cumulus cells during *in vitro* fertilization in goat. The cumulus cells act as a physical barrier in polyspermy control, and they may exert an additional benefit in the gamete interaction. Even, Yanagimachi (2022) [28] suggested that progesterone secreted by cumulus cells can activate the acrosome reaction of sperm during IVF. That is why, in this study to increase the rate of Cỏ goat blastocyst, we used the IVF process with cumulus cells in Cỏ goat oocytes.

According to Mauchart *et al.* (2023) [29], increasing the co-incubator time of sperm oocytes can also cause cytotoxic damage due to increased amount of reactive oxygen species (ROS). Similarly, Gualtieri *et al.* (2021) [30] also found that using frozen sperm during IVF process produced increased amounts of ROS. ROS are by-products of metabolism and cell death, which may be related to longer fertilization duration. ROS has played an important role as it increases DNA fragmentation, modifies the cytoskeleton and produces a loss of fluidity, integrity and competence of the sperm membrane to participate in the membrane events associated with fertilization [30]. Fan *et al.* (2023) [31] showed that reducing the co-incubator time of sperm oocytes improves the viability of human embryo, possibly due to a decrease in potential damage from sperm metabolic waste products. Therefore, determining the optimal co-incubator time of sperm oocytes will improve the efficiency of *in vitro* goat embryo production.

5. Conclusion

In conclusion, the present study reveals that the matured Cỏ goat oocytes were fertilized in BO-IVF medium with sperm concentration of 3×10^6 sperm/ml for 20 h, which is suitable for the *in vitro* Cỏ goat embryo production.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Souza-Fabjan, J.M.G., Leal, G.R., Monteiro, C.A.S., Batista, R.I.T.P., Barbosa, N.O. and Freitas, V.J.F. (2023) *In Vitro* Embryo Production in Small Ruminants: What Is Still Missing? *Animal Reproduction*, **20**, e20230055.

https://doi.org/10.1590/1984-3143-ar2023-0055

[2] Wang, Z., Lin, P. and Yu, S. (2013) Effects of Ghrelin on Developmental Competence and Gene Expression of *in Vitro* Fertilized Ovine Embryos. *Theriogenology*, **79**, 695-701. https://doi.org/10.1016/j.theriogenology.2012.11.026

[3] Kharche, S.D., Goel, A.K., Jindal, S.K., Goel, P. and Jha, B.K. (2011) Birth of Twin Kids Following Transfer of *in Vitro* Produced Caprine Embryos. *Indian Journal Animal Science*, **81**, 1132-1134.

[4] Hammami, S., Morató, R., Romaguera, R., Roura, M., Catalá, M., Paramio, M., *et al.* (2012) Developmental Competence and Embryo Quality of Small Oocytes from Prepubertal Goats Cultured in IVM Medium Supplemented with Low Level of Hormones, Insulin-Transferrin-Selenium and Ascorbic Acid. *Reproduction in Domestic Animals*, **48**, 339-344. https://doi.org/10.1111/j.1439-0531.2012.02160.x

[5] Bai, J., Hou, J., Guan, H., Yan, F., Cui, X., Liu, L., *et al.* (2008) Effect of 2-Mercaptoethanol and Cysteine Supplementation during *in Vitro* Maturation on the Developmental Competence of Oocytes from Hormone-Stimulated Lambs. *Theriogenology*, **70**, 758-764. https://doi.org/10.1016/j.theriogenology.2008.04.053

[6] Herrick, J.R., Lane, M., Gardner, D.K., Behboodi, E., Memili, E., Blash, S., *et al.* (2005) Metabolism, Protein Content, and *in Vitro* Embryonic Development of Goat Cumulus-Oocyte Complexes Matured with Physiological Concentrations of Glucose and L-Lactate. *Molecular Reproduction and Development*, **73**, 256-266. https://doi.org/10.1002/mrd.20407

[7] Dang, P.K. and Mui, N.B. (2015) Physical Appearance and Growth Performance of Indigenous Goat Co, F1 (Bach Thao × Co) and Three Way Crossbred Goat [Boer × (Bach Thao × Co)] Raised in Nho Quan, Ninh Binh Province. *Journal Science & Development*, **13**, 551-559.

[8] Wani, N.A., Wani, G.M., Khan, M.Z. and Salahudin, S. (2000) Effect of Oocyte Harvesting Techniques on *in Vitro* Maturation and *in Vitro* Fertilization in Sheep. *Small Ruminant Research*, **36**, 63-67. https://doi.org/10.1016/s0921-4488(99)00097-8

[9] Widayati, D.T. and Pangestu, M. (2020) Effect of Follicle-Stimulating Hormone on Bligon Goat Oocyte Maturation and Embryonic Development Post *in Vitro* Fertilization. *Veterinary World*, **13**, 2443-2446. https://doi.org/10.14202/vetworld.2020.2443-2446

[10] Jin, S. and Yang, W. (2016) Factors and Pathways Involved in Capacitation: How Are They Regulated? *Oncotarget*, **8**, 3600-3627. https://doi.org/10.18632/oncotarget.12274

[11] Kharche, S.D., Goel, A.K., Jindal, S.K., Yadav, E.N., Yadav, P., Sinha, R., *et al.* (2009) Effect of Serum Albumin Supplementation on *in Vitro* Capacitation and Fertilization of Caprine Oocytes. *Small Ruminant Research*, **81**, 85-89. https://doi.org/10.1016/j.smallrumres.2008.11.013

[12] Ledda, S., Kelly, J.M., Nieddu, S., Bebbere, D., Ariu, F., Bogliolo, L., *et al.* (2019) High *in Vitro* Survival Rate of Sheep *in Vitro* Produced Blastocysts Vitrified with a New Method and Device. *Journal of Animal Science and Biotechnology*, **10**, Article No. 90. https://doi.org/10.1186/s40104-019-0390-1

[13] Rahman, M.M., Rahman, M.M., Juyena, N.S. and Bhuiyan, M.M.U. (2020) Optimization of *in Vitro* Fertilization Technique for Oocytes of Indigenous Zebu Cows. *Journal of Animal Reproduction and Biotechnology*, **35**, 142-148. https://doi.org/10.12750/jarb.35.2.142

[14] Cox, J. and Alfaro, V. (2007) *In Vitro* Fertilization and Development of OPU Derived Goat and Sheep Oocytes. *Reproduction in Domestic Animals*, **42**, 83-87. https://doi.org/10.1111/j.1439-0531.2006.00735.x

[15] Rahmatullah, R., Setiadi, M.A. and Supriatna, I. (2023) Heparin and Hypotaurine Supplementation Improve the Fertilization Rate of Sheep Oocytes Matured in Media Containing L-Carnitine *in Vitro*. *Jurnal Kedokteran Hewan—Indonesian Journal of Veterinary Sciences*, **16**, 121-126. https://doi.org/10.21157/j.ked.hewan.v16i4.27339

[16] Chen, P.R., Spate, L.D., Leffeler, E.C., Benne, J.A., Cecil, R.F., Hord, T.K., *et al.* (2020) Removal of Hypotaurine from Porcine Embryo Culture Medium Does Not Impair Development of *in Vitro*-Fertilized or Somatic Cell Nuclear Transfer-Derived Embryos at Low Oxygen Tension. *Molecular Reproduction and Development*, **87**, 773-782. https://doi.org/10.1002/mrd.23393

[17] Pons-Rejraji, H., Vorilhon, S., Difrane, A., Dollet, S., Bourgne, C., Berger, M., *et al.* (2021) Beneficial Effects of Hypotaurine Supplementation in Preparation and Freezing Media on Human Sperm Cryo-Capacitation and DNA Quality. *Basic and Clinical Andrology*, **31**, Article No. 26. https://doi.org/10.1186/s12610-021-00144-6

[18] Palomo, M.J., Mogas, T., Izquierdo, D. and Paramio, M.T. (2010) The Influence of Sperm Concentration, Length of the Gamete Co-Culture and the Evolution of Different Sperm Parameters on the *in Vitro* Fertilization of Prepubertal Goat Oocytes. *Zygote*, **18**, 345-355. https://doi.org/10.1017/s0967199410000055

[19] Pawshe, C.H., Totey, S.M. and Jain, S.K. (1994) A Comparison of Three Methods of Recovery of Goat Oocytes for *in Vitro* Maturation and Feritilization. *Theriogenology*, **42**, 117-125. https://doi.org/10.1016/0093-691x(94)90668-9

[20] Gil, M.A., Almiñana, C., Roca, J., Vázquez, J.M. and Martínez, E.A. (2008) Boar Semen Variability and Its Effects on IVF Efficiency. *Theriogenology*, **70**, 1260-1268. https://doi.org/10.1016/j.theriogenology.2008.06.004

[21] Cognié, Y., Baril, G., Poulin, N. and Mermillod, P. (2003) Current Status of Embryo Technologies in Sheep and Goat. *Theriogenology*, **59**, 171-188. https://doi.org/10.1016/s0093-691x(02)01270-0

[22] Tan, W.L., Wan Khadijah, W.E. and Abdullah, R.B. (2011) Effect of Sperm Insemination Duration on *in Vitro* Fertilisation (IVF) Performance in Goats. *Malaysian Journal of Science*, **30**, 105-111. https://doi.org/10.22452/mjs.vol30no2.4

[23] Nagar, D. and Purohit, G.N. (2005) Effect of Epidermal Growth Factor on Maturation and Fertilization *in Vitro* of Goat Follicular Oocytes in a Serum Free or Serum Supplemented Medium. *Veterinarski Arhiv*, **75**, 459-467.

[24] Palomo, M.J., Quintanilla, R., Izquierdo, M.D., Mogas, T. and Paramio, M.T. (2016) Variability in *in Vitro* Fertilization Outcomes of Prepubertal Goat Oocytes Explained by Basic Semen Analyses. *Zygote*, **24**, 831-838. https://doi.org/10.1017/s0967199416000162

[25] Malakar, D., Das, S.K., Mukesh, M., Sodhi, M. and Goswami, S.L. (2007) Production of Kids from *in Vitro* Fertilized Goat Embryos and Their Parentage Assessment Using Microsatellite Markers. *Asian-Australasian Journal of Animal Sciences*, **20**, 842-849. https://doi.org/10.5713/ajas.2007.842

[26] Van Soom, A., Tanghe, S., De Pauw, I., Maes, D. and De Kruif, A. (2002) Function of the Cumulus Oophorus before and during Mammalian Fertilization. *Reproduction in Domestic Animals*, **37**, 144-151. https://doi.org/10.1046/j.1439-0531.2002.00345.x

[27] dos Santos-Neto, P.C., Vilariño, M., Cuadro, F., Barrera, N., Crispo, M. and Menchaca, A. (2020) Cumulus Cells during *in Vitro* Fertilization and Oocyte Vitrification in Sheep: Remove, Maintain or Add? *Cryobiology*, **92**, 161-167. https://doi.org/10.1016/j.cryobiol.2020.01.002

[28] Yanagimachi, R. (2022) Mysteries and Unsolved Problems of Mammalian Fertilization and Related Topics. *Biology of Reproduction*, **106**, 644-675.

https://doi.org/10.1093/biolre/ioac037

[29] Mauchart, P., Vass, R.A., Nagy, B., Sulyok, E., Bódis, J. and Kovács, K. (2023) Oxidative Stress in Assisted Reproductive Techniques, with a Focus on an Underestimated Risk Factor. *Current Issues in Molecular Biology*, **45**, 1272-1286. https://doi.org/10.3390/cimb45020083

[30] Gualtieri, R., Kalthur, G., Barbato, V., Longobardi, S., Di Rella, F., Adiga, S.K., *et al.* (2021) Sperm Oxidative Stress during *in Vitro* Manipulation and Its Effects on Sperm Function and Embryo Development. *Antioxidants*, **10**, Article No. 1025. https://doi.org/10.3390/antiox10071025

[31] Fan, Y., Wu, Z., Peng, F., Peng, H., Liang, X. and Zhu, S. (2023) Brief and Long Co-Incubation of Sperm and Oocytes for *in Vitro* Fertilization: A Meta-Analysis of Randomized Controlled Trials. *BMC Pregnancy and Childbirth*, **23**, Article No. 200. https://doi.org/10.1186/s12884-023-05490-z