Scientific Research Publishing

Open Journal of Animal Sciences, 2026, 16(1), 13-24
https://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Influence of Fetal Calf Serum and Culture Media on the *in Vitro* Development of Cỏ Goat Embryos

Van Khanh Nguyen[1*], Huong Thi Thu Vu[1], Au Thi Hoang[1], Yen Thi Kim Pham[1], Dat Van Le[1], Nguyen Thi Le Huong[1], Nguyen Thi Nhien[2], Nguyen Thi Van Anh[3], Nguyen Thi Vinh Ha[3], Lan Doan Pham[1]

[1]Key Laboratory of Animal Cell Biotechnology, National Institute of Animal Sciences, Hanoi, Vietnam
[2]Faculty of Biotechnology, Vietnam National University of Agriculture, Hanoi, Vietnam
[3]Faculty of Biomedical Science, Phenikaa University, Hanoi, Vietnam
Email: *cotihin@gmail.com

How to cite this paper: Nguyen, V.K., Vu, H.T.T., Hoang, A.T., Pham, Y.T.K., Le, D.V., Huong, N.T.L., Nhien, Ng.T., Anh, N.T.V., Ha, N.T.V. and Pham, L.D. (2026) Influence of Fetal Calf Serum and Culture Media on the *in Vitro* Development of Cỏ Goat Embryos. *Open Journal of Animal Sciences*, **16**, 13-24.
https://doi.org/10.4236/ojas.2026.161002

Received: November 20, 2025
Accepted: December 9, 2025
Published: December 12, 2025

Abstract

This study examined the effects of fetal calf serum (FCS) and different culture media on the *in vitro* development of Cỏ goat embryos. In *Experiment* 1, no statistically significant differences were detected in the cleavage rates of Cỏ goat oocytes cultured in SOFaa medium supplemented with FCS across the 0-hour, 48-hour, and 120-hour post-fertilization time points, as well as in the control group ($P > 0.05$). In contrast, the 0-hour group exhibited a significantly higher blastocyst formation rate and greater average cell number per blastocyst compared to the other groups ($P < 0.05$). Remarkably, hatching blastocysts were detected exclusively in the 0-hour group, indicating enhanced developmental competence under this condition. In *Experiment* 2, based on the findings from *Experiments* 1, Cỏ goat embryos were cultured in *in vitro* culture (IVC) medium supplemented with fetal calf serum (FCS) at 0 hours post-fertilization. No statistically significant difference was observed in the cleavage rates of Cỏ goat oocytes following *in vitro* fertilization between the SOFaa and CR1aa groups ($P > 0.05$). However, the SOFaa group demonstrated a significantly higher blastocyst formation rate and greater average cell number per blastocyst compared to the CR1aa group (37.04% vs. 24.98% and 136.08 vs. 125.37, respectively; $P < 0.05$). In conclusion, supplementing SOFaa medium with fetal calf serum at 0 hours post-fertilization is effective for enhancing the *in vitro* production of Cỏ goat embryos.

Keywords

In Vitro Cỏ Goat Embryos, Fetal Calf Serum, Culture Media

1. Introduction

In vitro embryo production (IVEP) refers to the generation of embryos outside the maternal organism through the retrieval of oocytes, their fertilization with sperm under laboratory conditions, and the subsequent culture of the developing embryos within a regulated environment. The initial successful birth of offspring from *in vitro*-derived embryos represented a pivotal advancement, highlighting the essential contribution of *in vitro* embryo technologies to the progress of the goat industry [1]. Ensuring embryo viability during the *in vitro* culture process remains a cornerstone for the effective application of *in vitro* embryo production (IVEP) methodologies [2]. *In vitro* embryo culture media are critical to the successful development of goat embryos, encompassing a wide range of formulations—from chemically defined media containing basic salts to complex, undefined media enriched with serum and other uncharacterized components. In the majority of studies on *in vitro* embryo production (IVEP) in goats, presumptive zygotes cultured *in vitro* using complex media and serum supplementation [3]. The efficiency of blastocyst formation and overall embryonic development is significantly affected by the composition of the *in vitro* culture media. In the context of goat embryo production, commonly employed media include Brackett and Oliphant (BO), Tyrode's Albumin Lactate Pyruvate (TALP), synthetic oviduct fluid (SOF), and Charles Rosenkrans (CR) medium. These formulations are routinely enriched with fetal calf serum (FCS), amino acids, and antioxidants to promote optimal embryonic growth and viability.

Fetal calf serum (FCS) remains the most widely employed growth supplement in cell culture systems due to its rich composition of bioactive components, including growth factors, hormones, amino acids, proteins, vitamins, inorganic salts, and antibodies [4]. The precise composition of fetal calf serum (FCS) remains incompletely characterized, and its biochemical variability arising from differences between individual donor foetuses can significantly influence cell culture outcomes [5]. Fetal calf serum (FCS) is commonly integrated into *in vitro* embryo culture media owing to its abundant repertoire of bioactive compounds such as antioxidants, growth factors, and essential nutrients—that facilitate cellular proliferation and enhance embryonic development [6].

Pre-implantation stage embryos can be cultured in a variety of media, ranging from simple formulations containing balanced salt solutions and carbohydrates to more complex compositions, such as those used in tissue culture systems [2]. Numerous studies have substantiated the use of sequential embryo culture media as an effective strategy to accommodate the evolving physiological requirements of embryos during both early and later stages of development. The selection of culture media, along with targeted supplementation at specific developmental phases, plays a critical role in optimizing the efficiency of *in vitro* blastocyst production. While fetal calf serum (FCS) has been shown to improve blastocyst development and hatching rates [7], its use remains subject to variability due to undefined components. Exposure of early-stage embryos to fetal calf serum (FCS)

has been demonstrated to negatively impact blastocyst quality [8], as serum exhibits biphasic effects significantly inhibiting the initial cleavage division while markedly enhancing development at later stages [9]. Moreover, the inclusion of fetal calf serum (FCS) in *in vitro* embryo culture has been associated with elevated lipid accumulation in embryos, which negatively impacts cryopreservation outcomes. Consequently, the use of FCS in *in vitro* embryo culture remains a subject of ongoing debate.

The Cỏ goat is an indigenous breed native to Vietnam and represents a valuable genetic resource for the conservation of the country's goat biodiversity. Characterized by their small stature, Cỏ goats are widely recognized for their disease resistance, high reproductive efficiency, and strong adaptability to Vietnam's climatic conditions [10]. This study aimed to produce high-quality *in vitro* Cỏ goat embryos suitable for cryopreservation and subsequent embryo transfer.

2. Material and Methods

All experimental procedures conducted in this study complied with Vietnamese legislation and adhered to Decision No. 2056/QĐ-BNN-KHCN, issued by the Ministry of Agriculture and Rural Development of Vietnam on June 28, 2024.

2.1. Reagents and Chemicals

All chemicals were supplied by Sigma-Aldrich (St. Louis, MO, USA).

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

Ovaries from 5 - 6 month-old pre-pubertal female Cỏ goats were collected at a local slaughterhouse and promptly transferred into sterile flasks containing Dulbecco's phosphate-buffered saline (DPBS) supplemented with antibiotics. The samples were transported to the laboratory within 2 hours, maintained at a temperature of 35˚C - 37˚C. Upon arrival, the ovaries were washed five times with antibiotic-supplemented DPBS, trimmed of surrounding connective tissues, and rinsed again with fresh DPBS. Oocytes were retrieved from surface follicles measuring 2 - 6 mm in diameter using a 5 mL syringe equipped with an 18-gauge needle, preloaded with Tyrode's Albumin Lactate Pyruvate-HEPES (TALP-HEPES) collection medium supplemented with serum. The aspirated follicular contents were transferred into Petri dishes, where oocytes were identified under a stereo microscope and assessed based on the morphological criteria described by Wieczorek *et al.* (2020) [11]. Cumulus-oocyte complexes (COCs) were selected according to two key features: 1) homogeneous cytoplasmic appearance and 2) the presence of at least three tightly packed layers of cumulus cells.

2.3. *In Vitro* Maturation of Goat Oocytes

Oocyte maturation in Cỏ goats was carried out following the protocol described by Van *et al.* (2024) [12]. Cumulus-oocyte complexes (COCs) obtained from adult

goats were rinsed three times in an *in vitro* maturation (IVM) medium composed of Tissue Culture Medium 199 (TCM-199), supplemented with 10% fetal calf serum (FCS), 50 ng/mL follicle-stimulating hormone (FSH), 10 ng/mL epidermal growth factor (EGF), 100 μM cysteamine, 100 IU/mL penicillin G potassium, and 0.1 mg/mL streptomycin sulfate. The COCs were then cultured in 4-well plates, with each well containing 500 μL of IVM medium and from 10 to 50 oocytes per well. Maturation was carried out for 24 hours, under controlled conditions of 38.5˚C, 5% CO_2, and saturated air humidity.

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

The *in vitro* fertilization (IVF) procedure was carried out in accordance with the protocol established by Van *et al.* (2025) [13]. *In vitro* matured oocytes derived from Cỏ goats were rinsed twice in Brackett and Oliphant (BO) medium. Two frozen straws of goat semen were initially pre-thawed at room temperature (25˚C for 30 seconds), followed by complete thawing in a water bath at 37˚C for 1 minute. Both ends of the straws were aseptically severed, and the sperm was gently expelled into a 15 mL centrifuge tube containing BO sperm washing medium. The sperm suspension was centrifuged at 320 × g for 5 minutes, after which the supernatant was discarded and the pellet resuspended in fresh BO sperm washing medium. The final sperm concentration was adjusted to 3×10^6 sperm/mL for fertilization. Oocytes and sperm were co-incubated in BO-IVF medium for 20 hours at 38.5˚C in an incubator maintained under a gaseous environment of 5% CO_2, 5% O_2, and humidified air. Post-incubation, cumulus cells were removed by repeated pipetting in TALP-HEPES medium supplemented with 0.2% hyaluronidase. The denuded oocytes were then washed twice in *in vitro* culture (IVC) medium and subsequently cultured under identical incubation conditions for a period of 7 days.

2.5. Evaluation of Embryo Cell Number

Blastocyst cell numbers on Day 7 post-IVF were assessed using Hoechst 33342 nuclear staining. Following initial staining, blastocysts were rinsed in phosphate-buffered saline (PBS) supplemented with 0.3% polyvinylpyrrolidone (PVP). Subsequently, they were immersed in a staining solution consisting of Hoechst 33342 and absolute ethanol at a 1:9 ratio and incubated overnight at 4˚C. Post-incubation, the blastocysts were washed with absolute ethanol and transferred into a glycerol solution. Each blastocyst was individually mounted onto a glass slide in a separate droplet and oriented longitudinally. A cover slip was then applied, and total cell numbers were assessed using fluorescence microscopy.

2.6. Experimental Design

Experiment 1: *Influence of fetal calf serum supplementation timing on in vitro Cỏ goat embryo production*

In this study, *in vitro* maturation (IVM) of Cỏ goat oocytes was performed us-

ing the culture medium formulation developed by Van *et al.* (2024) [12]. After 24 hours of maturation, the oocytes were fertilized in Brackett and Oliphant (BO-IVF) medium, as previously described. Following co-incubation, the resulting presumptive zygotes were thoroughly washed and subsequently cultured in Synthetic Oviductal Fluid supplemented with amino acids (SOFaa) medium. Fetal calf serum (FCS) was added to the SOF medium at a concentration of 2.5% at three distinct time points: 0 hours, 48 hours, and 120 hours post-fertilization. Embryo developmental competence was evaluated by measuring the cleavage rate on Day 2, blastocyst formation rate on Day 6, and hatching rate on Day 7 following *in vitro* fertilization (IVF). On Day 7, total cell numbers per blastocyst were quantified using fluorescence microscopy. Cleavage, blastocyst, and hatching rates were calculated relative to the total number of oocytes cultured, while the average cell count per blastocyst was determined based on the cumulative number of cells observed across all assessed blastocysts. Six replications were performed.

Experiment 2: *Influence of in vitro culture medium on in vitro Cỏ goat embryo production*

In this experiment, Cỏ goat oocytes were matured *in vitro* using the IVM medium formulation established by Van *et al.* (2024) [12]. Following 24 hours of maturation, the oocytes were fertilized in Brackett and Oliphant (BO-IVF) medium as previously described. After co-incubation, the presumptive Cỏ goat zygotes were washed and cultured in either Synthetic Oviductal Fluid amino acids (SOFaa) or C. Rosenkrans 1 amino acids (CR1aa) medium. Fetal calf serum (FCS) was incorporated into each medium at a concentration of 2.5%, based on the optimal supplementation time point identified in *Experiment* 1. The embryo developmental competence of Cỏ goat oocytes was evaluated as described in *Experiment* 1. Six replications were performed.

2.7. Statistical Analysis

Data were presented as mean ± SEM and statistically analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparisons test. All analyses were performed using GraphPad Prism software (Version 7.02 for Windows; GraphPad Software, La Jolla, CA, USA), with a significance threshold set at $P < 0.05$.

3. Results

Experiment 1: *Influence of fetal calf serum supplementation timing on in vitro Cỏ goat embryo production*

As presented in **Table 1**, no statistically significant differences were observed in the cleavage rates of Cỏ goat oocytes among the 0-hour, 48-hour, 120-hour, and control groups following *in vitro* fertilization ($P > 0.05$). In contrast, the 0-hour group exhibited a significantly higher blastocyst formation rate and greater average cell number per blastocyst compared to the other groups (**Table 1**; $P < 0.05$). Remarkably, hatching blastocysts were detected exclusively in the 0-hour group (**Table 1**).

Table 1. The effect of FCS supplementation timing on *in vitro* Cỏ goat embryo production.

FCS supplementation time	Total	Cleaved (% total)	Blastocysts (% total)	Hatching blastocysts (% total)	The average number of cells per blastocysts
0 hour	52	40 77.16 ± 2.35	19 36.86[a] ± 2.08	4 8.06 ± 2.18	23 135.76[a] ± 2.28
48 hours	58	44 76.21 ± 2.32	11 19.34[b] ± 2.36	0	11 124.35[b] ± 2.39
120 hours	56	43 76.94 ± 2.24	8 14.82[b] ± 2.16	0	8 123.42[b] ± 2.76
Control (without FCS)	57	43 76.08 ± 2.42	8 14.56[b] ± 2.41	0	8 123.36[b] ± 2.54

Six replications were performed. Percentage data are shown as mean ± SEM. Values with different superscripts in the same column differ significantly ($P < 0.05$).

Experiment 2: *Influence of in vitro culture medium on in vitro Cỏ goat embryo production*

Based on the findings from *Experiments* 1, in *Experiments* 2 fetal calf serum (FCS) will be supplemented into the *in vitro* culture medium for Cỏ goat embryos at 0 hours post-IVF. As shown in Table 2, there was no statistically significant difference in the cleavage rate of Cỏ goat oocytes following *in vitro* fertilization among the SOFaa and CR1aa groups ($P > 0.05$). Although both media were supplemented with fetal calf serum (FCS), however, the SOFaa group exhibited a significantly higher blastocyst formation rate and average cell number per blastocyst compared to the CR1aa group (37.04% vs. 24.98% and 136.08 vs. 125.37, respectively; $P < 0.05$).

Table 2. The effect of *in vitro* culture medium on *in vitro* Cỏ goat embryo production.

Medium	Total	Cleaved (% total)	Blastocyst (% total)	Hatching blastocyst	The average number of cells per blastocyst
(1)	114	88 77.98 ± 2.34	42 37.04[a] ± 2.45	9 8.02 ± 2.18	51 136.08[a] ± 2.26
(2)	116	88 76.24 ± 2.41	28 24.98[b] ± 2.15	4 3.72 ± 2.17	32 125.37[b] ± 2.96

Six replications were performed. Percentage data are shown as mean ± SEM. Values with different superscripts in the same column differ significantly ($P < 0.05$). 1): SOFaa + 2.5% FCS (v/v, at 0 hour post IVF); 2): CR1aa + 2.5% FCS (v/v, at 0 hour post IVF).

4. Discussion

The utilization of oocytes derived from 5 - 6-month-old prepubertal goats may be advantageous compared to those obtained from older animals, as prepubertal does yield a greater number of oocytes, and their *in vitro* developmental competence being comparable to that of adult goats. Therefore, in this study, oocytes obtained from 5 - 6-month-old prepubertal goats were utilized. As shown in Table 1, sup-

plementation of fetal calf serum (FCS) in the *in vitro* culture medium for Cỏ goat embryos significantly improved both the blastocyst formation rate and the occurrence of hatching blastocysts. However, no statistically significant differences in cleavage rates were observed among groups supplemented with FCS at 0, 48, or 120 hours post-IVF, or in the absence of FCS. These results align with previous findings by Abdel-Wahan *et al.* (2018) [14] and Goel *et al.* (2016) [2]. Abdel-Wahan *et al.* (2018) [14] reported no significant variation in cleavage rates of bovine oocytes when FCS was added at either 20 or 42 hours post-IVF. Similarly, Goel *et al.* (2016) [2] found that FCS supplementation during the cleavage phase of goat oocytes post-IVF did not significantly affect cleavage rates (**Figure 1**). Notably, Goel *et al.* (2016) [2] demonstrated that FCS supplementation during the post-IVF culture phase led to a significantly higher blastocyst yield ($P < 0.05$) compared to media lacking FCS or supplemented at later stages. Therefore, the findings of this study suggest that the supplementation of fetal calf serum (FCS) to the *in vitro* culture medium at 0 hours post-IVF account for the higher rate of hatching blastocysts observed, compared to supplementation at later time points (**Table 1**).

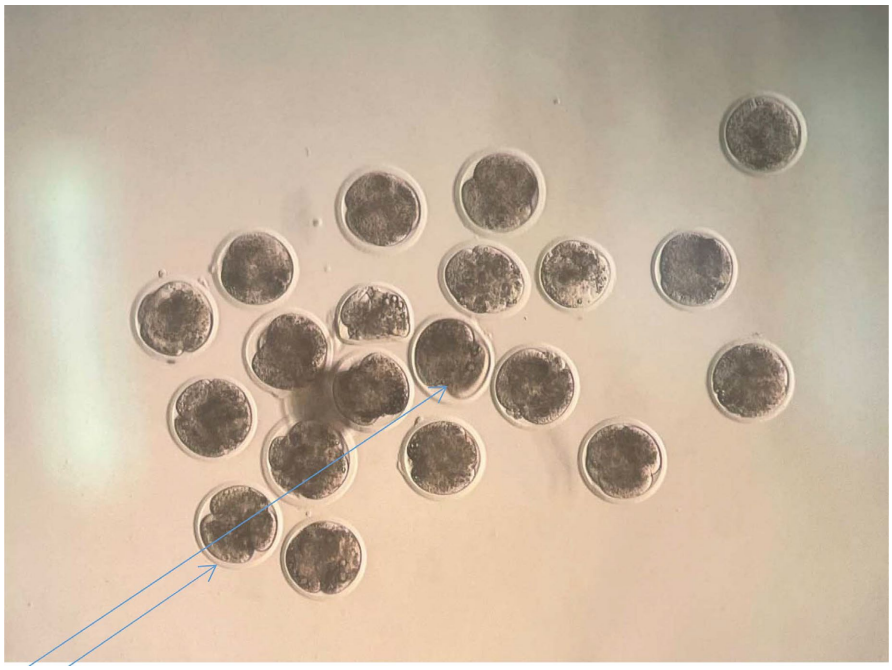

Figure 1. Cỏ goat embryos at the 2 - 4 cell stage, cultured in SOFaa medium supplemented with 2.5% fetal calf serum (FCS) at 0 hours post-*in vitro* fertilization (IVF), were observed on Day 2 post-IVF.

Extensive researches has explored strategies to improve culture media for *in vitro* embryo production in domestic animals, with particular emphasis on the supplementation of sera, hormones, and somatic cells [15]. Among these, serum supplementation during oocyte maturation has demonstrated considerable benefits, largely attributed to its high concentration of growth factors that play a crucial role in modulating oocyte maturation. In addition to its antioxidant properties

and its capacity to prevent zona pellucida hardening, serum also provides a rich source of essential components—including hormones, minerals, trace elements, lipids, and detoxifying agents—that collectively contribute to enhanced embryonic development [16]. According to Sena-Netto *et al.* (2020) [17], supplementation of fetal calf serum (FCS) to the embryo culture medium at 0 hours post-IVF significantly accelerates the progression to the morula stage. While the exact mechanisms by which FCS influences embryonic development are not yet fully elucidated, its positive effects are presumed to be linked to its rich content of growth factors, amino acids, and antioxidant properties. These components contribute to an increased blastocyst formation rate and enhance the overall quality of embryos cultured in FCS supplemented media compared to those cultured without FCS.

Although fetal calf serum (FCS) is known to support the development of *in vitro* embryos, Sudano *et al.* (2011) [18] reported that its inclusion in embryo culture media led to an increase in lipid accumulation within embryos, which negatively affected their suitability for cryopreservation. Furthermore, Ghaedrahmati *et al.* (2024) [19] found no significant differences in blastocyst formation rates when using varying concentrations of FCS (2.5%, 5%, and 10%) in the culture medium. Based on these findings, fetal calf serum (FCS) was incorporated at a concentration of 2.5% (v/v) into the *in vitro* culture medium for Cỏ goat embryo development, with the objective of enhancing embryo cryosurvival without compromising blastocyst yield.

Blastocyst quality is a critical determinant of pregnancy success following embryo transfer [8]. In this study, embryo quality was assessed using two key indicators: the percentage of hatching blastocysts (**Figure 2**) and the average cell number per blastocyst (**Figure 3**). The highest hatching rate (8.06%) was recorded in embryos cultured in Synthetic Oviductal Fluid supplemented with amino acids (SOFaa) and fetal bovine serum (FCS) administered at 0 hours post-fertilization. Furthermore, embryos cultured under this condition exhibited a significantly greater average cell number per blastocyst compared to those cultured with FCS supplementation at later time points.

The results of **Table 2** in this study are in agreement with those of Wan *et al.* (2008) [20], who demonstrated that Synthetic Oviductal Fluid supplemented with amino acids (SOFaa) is more conducive to the *in vitro* development of ovine IVF embryos compared to CR1aa medium. SOFaa is widely utilized for cattle, sheep and goat embryo culture due to its composition, which closely mimics oviduct fluid and includes essential components such as hormones, growth factors, and amino acids. These constituents enhance the developmental competence of embryos prior to transfer. SOFaa medium represents a flexible culture system that can be tailored to support various species and embryonic developmental stages through the incorporation of specific additives such as amino acids, serum, and growth factors.

The CR1aa medium is composed of inorganic salts (NaCl, KCl), a buffering agent ($NaHCO_3$), and metabolic energy substrates including lactic acid, sodium

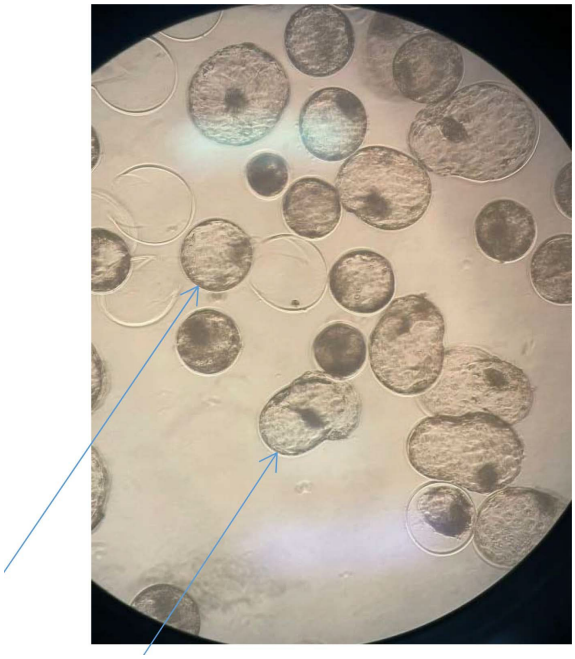

Figure 2. Cỏ goat blastocysts and hatching blastocysts, cultured in SO-Faa medium supplemented with 2.5% fetal calf serum (FCS) at 0 hours post-*in vitro* fertilization (IVF), were observed on Day 7 post-IVF.

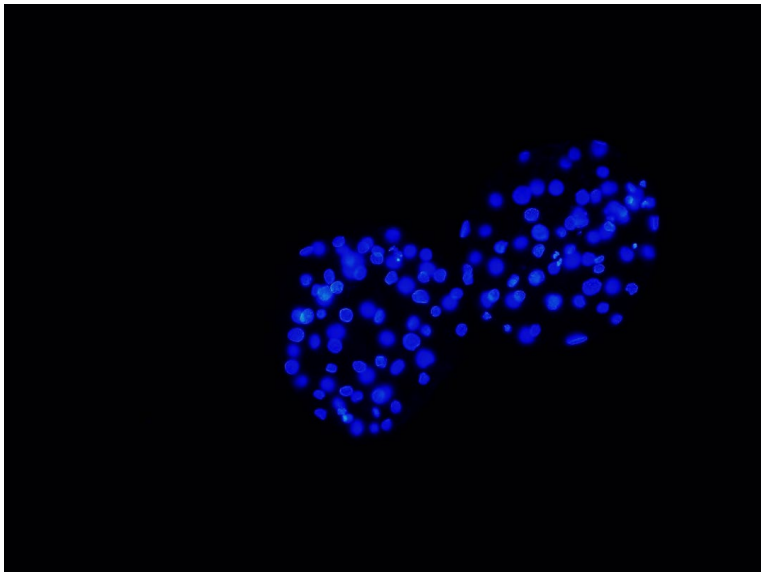

Figure 3. Cỏ goat hatching blastocyst stained with Hoechst 33342.

pyruvate, and glutamine. Moreover, the medium provides both essential and non-essential amino acids, which are critical for supporting embryonic development [21]. Notably, CR1aa medium lacks glucose but is supplemented with a complete profile of essential and nonessential amino acids to sustain embryo viability and growth. During the transition from the 8-cell stage to the blastocyst, nonessential amino acids and glutamine promote blastocyst formation and facilitate hatching. Conversely, essential amino acids have been shown to enhance blastocyst cell

numbers and facilitate cellular differentiation processes that promote the formation of the inner cell mass (ICM) [22].

The developmental competence of mammalian embryos post-implantation is significantly influenced by cellular death mechanisms, particularly apoptosis, occurring during the preimplantation stages. Apoptotic activity has been documented in both *in vivo* and *in vitro* embryos across a range of domestic species, including equine, porcine, ovine, caprine, and bovine [23]. Suboptimal culture conditions that compromise cell proliferation and elevate apoptosis rates in early-stage embryos are correlated with reduced implantation success, increased fetal resorption, and lower birth weights during post-implantation development [24]. Wan *et al.* (2008) [20] further reported that embryos cultured in CR1aa medium exhibited a significantly higher incidence of apoptotic signals and greater numbers of apoptotic cells per embryo compared to those cultured in SOFaa medium. Although the underlying mechanism remains unclear, it is hypothesized that certain components in CR1aa medium may induce embryonic cell apoptosis. The findings reported by Wan *et al.* (2008) are consistent with our results. Although apoptosis was not assessed in this study, the observation that blastocysts cultured in CR1aa medium contained fewer cells than those cultured in SOFaa medium supports this hypothesis. These findings may explain the enhanced performance of the SOFaa medium relative to the CR1aa medium, as evidenced by improved blastocyst formation, higher hatching rates, and increased average cell numbers per blastocyst observed in this study (**Table 2**).

5. Conclusion

In conclusion, supplementing SOFaa medium with fetal calf serum at 0 hours post-fertilization is effective for enhancing the *in vitro* production of Cỏ goat embryos.

Acknowledgements

The research was carried out at the Key Laboratory of Animal Cell Technology. The authors would like to thank the Vietnamese Ministry of Science and Technology for supporting the project.

Conflicts of Interest

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

References

[1] Crozet, N., De Smedt, V., Ahmed-Ali, M. and Sevellec, C. (1993) Normal Development Following *in Vitro* Oocyte Maturation and Fertilization in the Goat. *Theriogenology*, **39**, 206. https://doi.org/10.1016/0093-691x(93)90061-9

[2] Goel, P., Goel, A.K., Bhatia, A.K. and Kharche, S.D. (2016) Comparative Study on Effect of Different Embryo Culture Media on *in Vitro* Blastocyst Production in Goats. *The Indian Journal of Animal Sciences*, **86**, 1115-1120. https://doi.org/10.56093/ijans.v86i10.62325

[3] Sangale, R.R., Pawshe, C.H., Ambalkar, M.B., Deshmukh, S.G. and Ingawale, M.V. (2024) Comparative Efficiency of Different Culture Media on *in Vitro* Embryonic Development of Goat Oocytes. *Indian Journal of Animal Research*. https://doi.org/10.18805/ijar.b-5220

[4] Subbiahanadar Chelladurai, K., Selvan Christyraj, J.D., Rajagopalan, K., Yesudhason, B.V., Venkatachalam, S., Mohan, M., *et al.* (2021) Alternative to FBS in Animal Cell Culture—An Overview and Future Perspective. *Heliyon*, **7**, e07686. https://doi.org/10.1016/j.heliyon.2021.e07686

[5] Lee, D.Y., Lee, S.Y., Yun, S.H., Jeong, J.W., Kim, J.H., Kim, H.W., *et al.* (2022) Review of the Current Research on Fetal Bovine Serum and the Development of Cultured Meat. *Food Science of Animal Resources*, **42**, 775-799. https://doi.org/10.5851/kosfa.2022.e46

[6] Sovernigo, T., Adona, P., Monzani, P., Guemra, S., Barros, F., Lopes, F., *et al.* (2017) Effects of Supplementation of Medium with Different Antioxidants during *in Vitro* Maturation of Bovine Oocytes on Subsequent Embryo Production. *Reproduction in Domestic Animals*, **52**, 561-569. https://doi.org/10.1111/rda.12946

[7] McElroy, S.L., Kim, J.H., Kim, S., Jeong, Y.W., Lee, E.G., Park, S.M., *et al.* (2008) Effects of Culture Conditions and Nuclear Transfer Protocols on Blastocyst Formation and mRNA Expression in Pre-Implantation Porcine Embryos. *Theriogenology*, **69**, 416-425. https://doi.org/10.1016/j.theriogenology.2007.10.010

[8] Rizos, D., Gutiérrez-Adán, A., Pérez-Garnelo, S., de la Fuente, J., Boland, M.P. and Lonergan, P. (2003) Bovine Embryo Culture in the Presence or Absence of Serum: Implications for Blastocyst Development, Cryotolerance, and Messenger RNA Expression. *Biology of Reproduction*, **68**, 236-243. https://doi.org/10.1095/biolreprod.102.007799

[9] Pinyopummintr, T. and Bavister, B.D. (1994) Development of Bovine Embryos in a Cell-Free Culture Medium: Effects of Type of Serum, Timing of Its Inclusion and Heat Inactivation. *Theriogenology*, **41**, 1241-1249. https://doi.org/10.1016/0093-691x(94)90481-w

[10] 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.

[11] Wieczorek, J., Koseniuk, J., Skrzyszowska, M. and Cegła, M. (2020) L-OPU in Goat and Sheep—Different Variants of the Oocyte Recovery Method. *Animals*, **10**, Article 658. https://doi.org/10.3390/ani10040658

[12] Nguyen, V.K. (2024) Effects of Culture Media and Duration on *in Vitro* Maturation and Production of Cỏ Goat Embryos. *The Philippine Agricultural Scientist*, **103**, 309-317. https://doi.org/10.62550/gcd03037024

[13] Nguyen, V.K., Vu, H.T.T., Hoang, A.T., Pham, Y.T.K., Le, D.V., Huu, Q.X., *et al.* (2025) Optimization of the *in Vitro* Fertilization System in Cỏ Goat Oocytes. *Open Journal of Animal Sciences*, **15**, 39-50. https://doi.org/10.4236/ojas.2025.151003

[14] Abdel-Wahab, A., Abdel Aziz, R.L., Helmy, N.A. and Ibrahim, S.S. (2018) The Impact of Using Newborn Bovine Serum as Fetal Calf Serum Substitute in the *in Vitro* Bovine Embryos Production System. *Porto Biomedical Journal*, **3**, e3. https://doi.org/10.1016/j.pbj.0000000000000003

[15] Pawshe, C.H., Palanisamy, A., Taneja, M., Jain, S.K. and Totey, S.M. (1996) Comparison of Various Maturation Treatments on *in Vitro* Maturation of Goat Oocytes and Their Early Embryonic Development and Cell Numbers. *Theriogenology*, **46**, 971-

982. https://doi.org/10.1016/s0093-691x(96)00261-0

[16] Gstraunthaler, G. (2003) Alternatives to the Use of Fetal Bovine Serum: Serum-Free Cell Culture. *ALTEX*, **20**, 257-281. https://doi.org/10.14573/altex.2003.4.257

[17] Sena-Netto, S.B., Sprícigo, J.F.W., Leme, L.O., Guimarães, A.L.S., Caixeta, F.M.C., Dode, M.A.N., *et al.* (2020) The Replacement of Fetal Bovine Serum with Bovine Serum Albumin during Oocyte Maturation and Embryo Culture Does Not Improve Blastocyst Quality after Slow Freezing Cryopreservation. *Biopreservation and Biobanking*, **18**, 171-179. https://doi.org/10.1089/bio.2019.0059

[18] Sudano, M.J., Paschoal, D.M., da Silva Rascado, T., Magalhães, L.C.O., Crocomo, L.F., de Lima-Neto, J.F., *et al.* (2011) Lipid Content and Apoptosis of *in Vitro*-Produced Bovine Embryos as Determinants of Susceptibility to Vitrification. *Theriogenology*, **75**, 1211-1220. https://doi.org/10.1016/j.theriogenology.2010.11.033

[19] Ghaedrahmati, A., Mamouei, M. and Zandi, M. (2024) Low Serum Concentration in Ovine Embryo Culture Media. *Gene, Cell and Tissue*, **11**, e146911. https://doi.org/10.5812/gct-146911

[20] Wan, P., Hao, Z., Zhou, P., Wu, Y., Yang, L., Cui, M., *et al.* (2009) Effects of SOF and CR1 Media on Developmental Competence and Cell Apoptosis of Ovine *in Vitro* Fertilization Embryos. *Animal Reproduction Science*, **114**, 279-288. https://doi.org/10.1016/j.anireprosci.2008.09.020

[21] Rosenkrans, C.F. and First, N.L. (1994) Effect of Free Amino Acids and Vitamins on Cleavage and Developmental Rate of Bovine Zygotes *in Vitro*. *Journal of Animal Science*, **72**, 434-437. https://doi.org/10.2527/1994.722434x

[22] Steeves, T.E. and Gardner, D.K. (1999) Temporal and Differential Effects of Amino Acids on Bovine Embryo Development in Culture1. *Biology of Reproduction*, **61**, 731-740. https://doi.org/10.1095/biolreprod61.3.731

[23] Pomar, F.J.R., Teerds, K.J., Kidson, A., Colenbrander, B., Tharasanit, T., Aguilar, B., *et al.* (2005) Differences in the Incidence of Apoptosis between *in Vivo* and *in Vitro* Produced Blastocysts of Farm Animal Species: A Comparative Study. *Theriogenology*, **63**, 2254-2268. https://doi.org/10.1016/j.theriogenology.2004.10.015

[24] Gjorret, J.O. (2003) Chronology of Apoptosis in Bovine Embryos Produced *in Vivo* and *in Vitro*. *Biology of Reproduction*, **69**, 1193-1200. https://doi.org/10.1095/biolreprod.102.013243