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Effects of Culture Media and Duration on In Vitro Maturation and Production of Cỏ Goat Embryos

Van Khanh NGUYEN*, Huong Thi Thu VU, Au Thi HOANG, Yen Thi Kim PHAM, Dat Van LE, Lan Anh Thi NGUYEN, Huu Xuan QUAN, and Lan Doan PHAM

Key Laboratory of Animal Cell Biotechnology, National Institute of Animal Sciences, Hanoi, Vietnam

*Author for correspondence; Email: cotihin@gmail.com

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This study aimed to evaluate the effects of maturation media and duration on the in vitro maturation (IVM) of Cỏ goat oocytes for the in vitro production of Cỏ goat embryos. In Experiment 1, the highest percentage of matured oocytes and blastocyst rates were observed in the IVM medium consisting of tissue culture medium (TCM) 199 + 10% fetal calf serum (FCS) + 50 ng/mL follicle stimulating hormone (FSH) + 100 µM cysteamine + 10 ng/mL epidermal growth factor (EGF) + 100 units/mL penicillin G potassium + 0.1 mg/mL streptomycin sulphate ($P < 0.05$). In Experiment 2, Cỏ goat oocytes were cultured in the same IVM medium for 20, 22, and 24 h, respectively. The matured oocyte and blastocyst rates of the 20-h group were lower than those of the 22- and 24-h groups (61.56% vs. 76.81% and 73.48%; 13.76% vs. 24.75% and 24.72% respectively; $P < 0.05$), while there was no difference between the 22- and 24-h groups ($P > 0.05$). In conclusion, the TCM 199 medium supplemented with FCS, FSH, cysteamine, EGF, and a maturation duration of 22 or 24 h are suitable for the in vitro maturation and subsequent embryo development of Cỏ goat oocytes.

Keywords: Cỏ goat oocytes, in vitro maturation media, in vitro maturation duration, in vitro production of embryos

INTRODUCTION

Biotechnology techniques improve and increase the number of livestock breeds such as bovine, pig, and goat (Suprayogi et al. 2018). In vitro embryo production is a key tool for goat genetic improvement. In vitro fertilization (IVF) goat embryo production includes steps such as in vitro maturation (IVM) of oocytes, IVF, and embryo culture until the blastocyst stage (Avelar et al. 2012). In vitro matured goat oocyte is a critical resource to generate in vitro goat embryos. The rate of in vitro blastocyst formation ranges from 30% to 50% in animals (Fernandes-Franca et al. 2020). The IVM process is one of the critical steps in IVF goat embryo production procedure (Fernandes-Franca et al. 2020). A stable in vitro oocyte maturation culture system is needed to produce quality mature oocytes for in vitro production of embryos.

IVM goat oocyte competence depends on several factors such as the maturation culture medium, supplements added to IVM culture, and maturation duration (Crocomo et al. 2020). The process of oocyte maturation includes both the maturation of their nucleus and cytoplasm (Sun and Nagai 2003). If IVM does not support cytoplasmic maturation, the development of in vitro embryos will be reduced. Moreover, asynchrony in the maturation of the nucleus and cytoplasm of oocytes reduces the number of in vitro embryos produced. The IVM medium plays a crucial role in synchronizing the maturation of the nucleus and cytoplasm of oocytes. Currently, IVM media for oocytes are categorized into three types: (1) undefined medium often containing serum or follicular fluid, (2) semi-defined medium containing albumin as a substitute for serum and follicular fluid, and (3) defined medium composed of known components, without albumin, serum, and follicular fluid (Farin et al. 2001). Each type of medium has its advantages and disadvantages. Thus, selecting an appropriate IVM medium suited to the experimental conditions of each laboratory can enhance in vitro goat embryo production efficiency.

In addition, the duration of IVM is also a factor that affects on IVM efficiency of goat oocytes. If the IVM duration is too short, the nucleus and cytoplasm of the oocyte do not have sufficient time to complete the maturation process. Conversely, if the IVM duration is too long, the nucleus of the oocyte may degenerate after completing the maturation. Each

livestock species has different requirements for the time it takes for the nucleus and cytoplasm of the oocyte to complete their maturation. In cattle, IVM duration for oocytes typically ranges from 20 – 24 h, while in pigs, it takes 40 – 44 h for the nucleus and cytoplasm of the oocyte to complete maturation. Choosing the appropriate IVM medium can enhance efficient in vitro goat embryo production.

The Cỏ goat is an indigenous breed in Vietnam and is an important genetic resource for the conservation of native Vietnamese goat biodiversity. Cỏ goats are small in size and popular for their disease resistance, high reproduction, and adaptability to Vietnam's climate (Pham and Nguyen 2015). However, data regarding IVM and in vitro embryo production in Cỏ goats are lacking. Therefore, this study aimed to determine the optimum culture media and IVM duration of Cỏ goat oocytes and their correlation with in vitro embryo production of this goat breed.

MATERIALS AND METHODS

All the experimental procedures in this study were performed in accordance with Vietnam legislation and according to Decision No. 1137/QĐ-BNN-KHCN of the Ministry of Agriculture and Rural Development of Vietnam on March 28, 2023.

Reagents and Chemicals

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

Collection of Cỏ Goat Ovaries and Cumulus-oocyte Complexes (COCs)

Goat ovaries (Fig. 1) were collected from a 5–6-mo-old prepubertal female Cỏ goat at a local slaughterhouse, kept in a flask containing Dulbecco's phosphate buffered saline (DPBS) supplemented with antibiotics and transported to the laboratory within 2 h at 35°C – 37°C. The ovaries were washed in DPBS supplemented with antibiotics five times. Thereafter, these ovaries were trimmed of surrounding tissues and washed again with DPBS solution. Oocytes were aspirated from follicles 2 – 6 mm in diameter on the ovarian surface with a 5-mL syringe containing Tyrode's Albumin Lactate Pyruvate-HEPES (TALP-h) oocyte collection solution supplemented with serum and an 18G needle. After aspiration, the entire solution in the syringe was dispensed into a Petri dish, and oocytes were searched by using a stereo microscope and evaluated according to the standards of Wani et al. (2000). After evaluation, cumulus oocytes complexes were selected based on uniform cytoplasm and the presence of at least three compact surrounding layers of cumulus cells (Fig. 2).

In Vitro Maturation of Goat Oocytes

The COCs of Cỏ goat oocytes were washed three times in IVM medium and then transferred to 4-well plates containing 500 µL of IVM medium per well for a duration depending on the experiment, under conditions of 38.5°C, 5% CO_2, and saturated air humidity (10–50 oocytes per well).

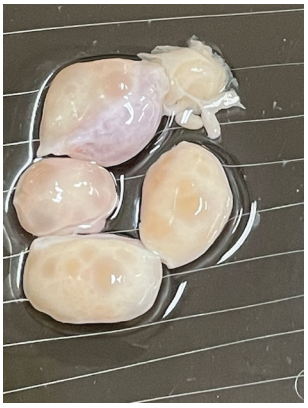

Fig. 1. Cỏ goat ovaries.

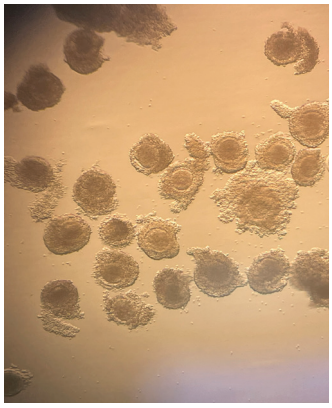

Fig. 2. Cumulus-oocyte complexes (COCs) of Cỏ goat oocytes before maturation (eyepiece * objective is 5x/0.12).

In Vitro Fertilization (IVF) and In Vitro Embryo Culture (IVC)

The IVF and IVC procedures were performed according to the method of Widayati and Pangestu (2020) with some modifications. In vitro matured Cỏ goat oocytes were washed in Brackett and Oliphant (BO) medium two times. Two frozen straws of goat sperm were pre-thawed at room temperature (25ºC, 30 s) followed by thawing in a water bath (37ºC, 1 min). These straws were cut at both ends by a pair of sterile scissors and the sperm was slowly released into a 15-mL tube containing BO sperm washing medium. Thereafter, frozen-thawed goat sperm was centrifuged at 320 x g for 5 min in BO sperm washing medium. The supernatant was removed and sperm pellet was diluted in BO sperm washing medium. A final concentration used for IVF of Cỏ goat oocytes was 1 x 106 sperm/mL. Oocytes and sperm were co-incubated in BO-IVF medium for 20 h and placed in an incubator at 38.5ºC under 5% CO2, 5% O2, and humidified air. After 20 h of co-incubation, the cumulus cells surrounding the oocyte were removed by repeated pipetting in TALP-h medium + 0.2% hyaluronidase. The oocytes without cumulus cells were washed in synthetic oviduct fluid (SOF) medium two times and cultured in SOF medium + 2.5% fetal bovine serum (FBS) in an incubator at 38.5ºC under 5% CO2, 5% O_2, and humidified air for 7 d.

Evaluation of Viable Oocytes and Nuclear Status After In Vitro Maturation

After in vitro maturation, the oocytes with no apparent signs of lysis and with evenly granulated cytoplasm and expanded cumulus cells were considered viable oocytes (Fig. 3). Then, cumulus cells of the viable oocytes were removed by vortex-

ing in TALP-h medium + 1 mg/mL hyaluronidase to observe the first polar body (PB1) under a stereo microscope (Fig. 4). Thereafter, the oocytes with PB1 were washed in phosphate buffered saline (PBS) + 0.3% polyvinylpyrrolidone (PVP) solution. Next, these oocytes were transferred into a staining solution (Hoechst 33342 + absolute ethanol in a 1:9 ratio) and left overnight at 4°C. After overnight incubation at 4°C in the staining solution, the oocytes were washed in absolute ethanol and then transferred to a glycerol solution. Subsequently, the oocytes were moved to a glass slide with each oocyte in a drop and aligned along the length of the slide. A cover slip was placed over the slide, and the oocytes were examined under a fluorescence microscope. Mature oocytes are defined as metaphase II (MII) oocytes (Fig. 5), which completed the first meiotic division and extruded the first polar body (PB1) (Shani et al. 2023).

Evaluation of Embryo Cell Number

The cell numbers of blastocysts at Day 7 after IVF were evaluated by staining with Hoechst 33342 as described above. All blastocysts observed in the experiments were stained with Hoechst 33342. The cell numbers were counted under a fluorescence microscope.

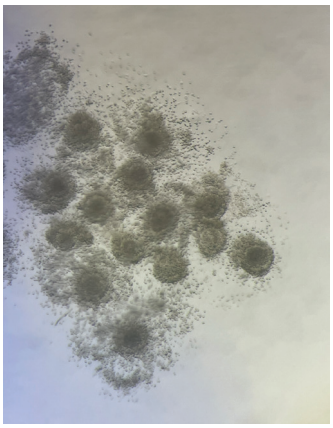

Fig. 3. Cỏ goat oocytes after maturation in medium (3) (eyepiece * objective is 5x/0.12).

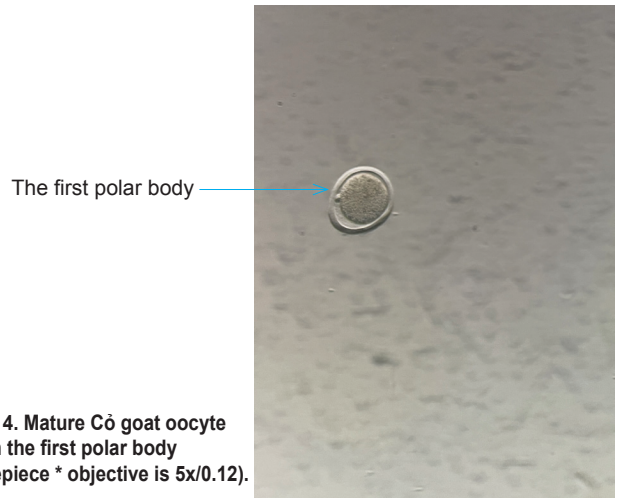

Fig. 4. Mature Cỏ goat oocyte with the first polar body (eyepiece * objective is 5x/0.12).

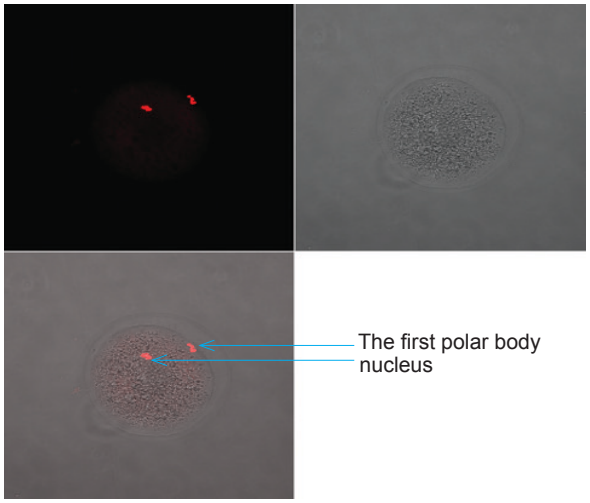

Fig. 5. Nucleus of matured Cỏ goat oocyte at Metaphase II (eyepiece * objective is 10x/0.25).

Experimental Design

Experiment 1: The effect of maturation media on the in vitro maturation of Cỏ goat oocytes and in vitro production of Cỏ goat embryos

In this experiment, the maturation medium was used according to Wang et al. (2007) with some modifications. In brief, Cỏ goat oocytes were divided into three different maturation culture media: (1) tissue culture medium 199 (TCM 199) + 10% fetal calf serum (FCS) + 100 units/mL penicillin G potassium + 0.1 mg/mL streptomycin sulphate; (2) TCM 199 + 10% FCS + 100 µM cysteamine + 10 ng/mL epidermal growth factor (EGF) + 100 units/mL penicillin G potassium + 0.1 mg/mL streptomycin sulphate; and (3) TCM 199 + 10% FCS + 50 ng/mL follicle stimulating hormone (FSH) + 100 µM cysteamine + 10 ng/mL EGF + 100 units/mL penicillin G potassium + 0.1 mg/mL streptomycin sulphate. After 24 h of maturation, matured Cỏ goat oocytes were fertilized in BO-IVF medium, then embryo culture 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 (Fig. 6), Day 6, and Day 7 (Fig. 7) after IVF, respectively, and the number of cells per blastocyst were counted blastocyst on Day 7. The cleavage, blastocyst, and hatching blastocyst rates were calculated based on the total. The average number of cells per blastocyst was calculated based on the total cells of blastocyst observed (Fig. 8). Eight replications were performed.

Experiment 2: The effect of maturation duration on the in vitro maturation of Cỏ goat oocytes and in vitro production of Cỏ goat embryos

In this experiment, Cỏ goat oocytes were cultured in the selected maturation medium from Experiment 1 for 20, 22, and 24 h. After maturation, Cỏ goat oocytes were fertilized in BO-IVF medium, then embryo culture was performed as described above. The embryo developmental competence of Cỏ goat oocytes was evaluated as described in Experiment 1. Eight replications were performed.

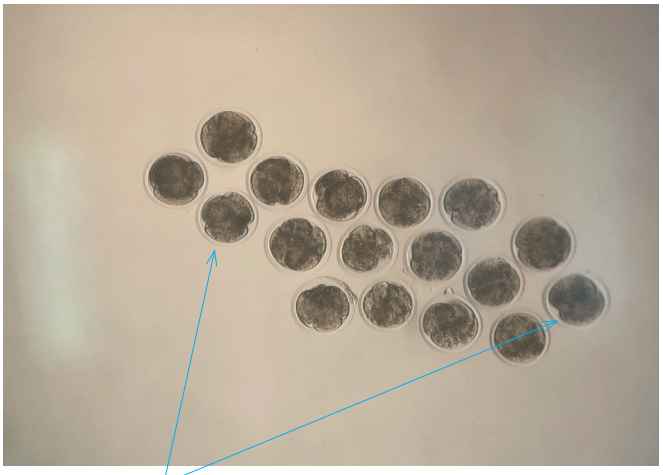

Fig. 6. 2–4 cells embryos of Cỏ goat at Day 2 after in vitro fertilization (IVF) (eyepiece * objective is 5x/0.12).

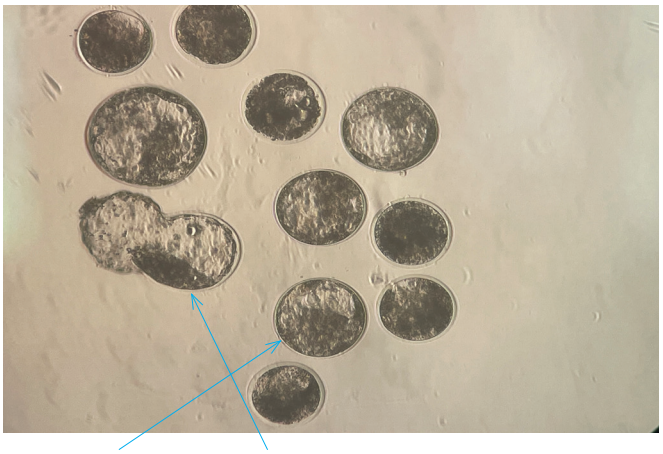

Fig. 7. Blastocyst and hatching blastocyst of Cỏ goat at Day 7 after IVF (eyepiece * objective is 10x/0.25).

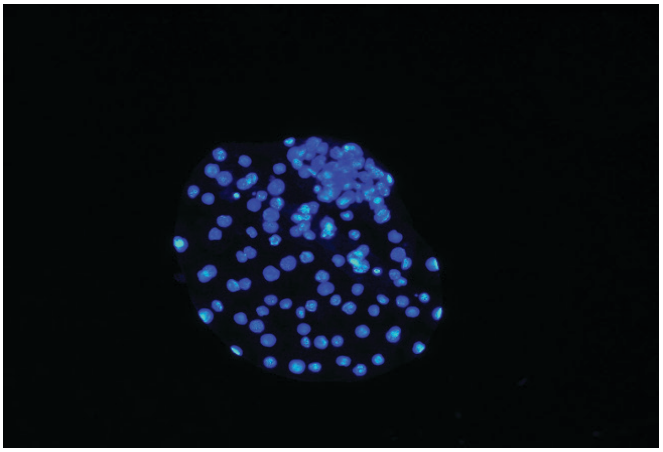

Fig. 8. Cỏ goat blastocyst stained with Hoechst 33342 (eyepiece * objective is 40x/0.65).

Statistical Analysis

Data were expressed as mean ± SEM values and analyzed 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.

RESULTS

The Effect of Maturation Culture Media on the In Vitro Maturation of Cỏ Goat Oocytes and In Vitro Production of Cỏ Goat Embryos

The results indicate no significant difference in the survival rate of Cỏ goat oocytes after maturation among the three IVM media for goat oocytes at 90.21%, 89.92%, and 92.46%, respectively. Surviving oocytes were identified as having no apparent signs of lysis and with evenly granulated cytoplasm and expanded cumulus cells. Although there was no difference in the survival rate of oocytes among the three in vitro maturation media, there was a significant difference in the rate of mature oocytes after maturation. The highest percentage of mature oocytes was observed in medium 3 (74.33%) and the lowest was in medium 1 (47.24%) (Table 1).

Table 1. The effect of maturation media on the in vitro maturation of Cỏ goat oocytes.

Medium	Total	Surviving (% total)	Matured Oocytes (% total)
1	102	92	48
		90.21 ± 1.34	47.24^{a} ± 1.62
2	108	97	66
		89.92 ± 1.12	61.28^{b} ± 1.56
3	105	97	78
		92.46 ± 1.25	74.33^{c} ± 1.08

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

(1): TCM 199 + 10% FCS (v/v); (2): TCM 199 + 10% FCS (v/v) + 100μM Cysteamine + 10ng/mL EGF; (3): TCM 199 + 10% FCS (v/v) + 50ng/mL FSH + 100μM Cysteamine + 10ng/mL EGF

The results assessing the influence of the maturation culture media on in vitro Cỏ goat embryo production are shown in Table 2. There was no significant difference in the cleavage and blastocyst rates among the three IVM culture media for goat oocytes. The blastocyst rate in medium 3 was significantly higher than that of medium 1 and 2 (24.42% vs 11.44% and 13.08%, respectively).

Table 2. The effect of maturation media on the in vitro production of Cỏ goat embryos.

Medium	Total	Cleaved (% total)	Blastocyst (% total)	Hatching Blastocyst	Average Number of Cells per Blastocyst
1	98	54	11	2	13
		55.31 ± 2.18	11.44[a] ± 2.63	2.32 ± 1.78	133.42 ± 2.46
2	101	60	18	3	21
		59.62 ± 2.35	13.08[a] ± 2.16	3.04 ± 1.97	134.08 ± 2.76
3	99	66	24	4	28
		66.96 ± 2.12	24.42[b] ± 2.34	4.26 ± 2.03	134.26 ± 2.88

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

(1): TCM 199 + 10% FCS (v/v); (2): TCM 199 + 10% FCS (v/v) + 100µM Cysteamine + 10ng/mL EGF; (3): TCM 199 + 10% FCS (v/v) + 50ng/mL FSH + 100µM Cysteamine + 10ng/mL EGF

The Effect of Maturation Duration on the In Vitro Maturation of Cỏ Goat Oocytes and In Vitro Production of Cỏ Goat Embryos

Based on the results of Experiments 1 and 2, the maturation medium consisting of TCM 199 + 10% FCS + 50 ng/mL FSH + 100 µM cysteamine + 10 ng/mL EGF was used. The results are shown in Tables 3 and 4.

IVM duration did not affect the survival rate of oocytes but impacted the maturation of goat oocytes after IVM. The survival rates of oocytes after in vitro maturation for the three durations (20, 22, and 24 h) were 91.02%, 91.21%, and 92.35%, respectively ($P > 0.05$). The maturation rate of oocytes from the 20-h group was lower compared to the 22- and 24-h groups (61.56% vs. 76.81% and 73.48%, respectively), whereas the maturation rate at IVM duration of the 22-h group was higher than that of the 24-h group. However, this difference was not statistically significant ($P > 0.05$) (Table 3).

Table 3. The effect of in vitro maturation duration on the in vitro maturation of Cỏ goat oocytes.

Maturation Duration	Total	Surviving	Matured Oocytes
		(% total)	(% total)
20 h	109	99	67
		91.02 ± 1.51	61.56[a] ± 1.86
22 h	112	102	86
		91.21 ± 1.67	76.81[b] ± 2.01
24 h	116	107	85
		92.35 ± 1.82	73.48[b] ± 1.94

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

The results in Table 4 show no significant difference in the cleaved and blastocyst rates among the 20-, 22-, and 24-h groups. The blastocyst rate of the 20-h group was lower than that of the 22- and 24-h groups (13.76% vs. 24.75% and 24.72%, respectively; $P < 0.05$). The difference of the blastocyst rate between the 22- and 24-h groups was not statistically significant ($P > 0.05$).

Table 4. The effect of in vitro maturation duration on the in vitro production of Cỏ goat embryos.

Maturation duration	Total	Cleaved (% total)	Blastocyst (% total)	Hatching Blastocyst	Average Number of Cells per Blastocyst
20 h	112	68	15	4	19
		60.98 ± 1.84	13.76[a] ± 2.23	3.65 ± 2.24	134.12 ± 2.87
22 h	106	71	26	4	30
		67.04 ± 2.01	24.75[b] ± 2.32	3.94 ± 2.19	135.01 ± 2.45
24 h	118	78	29	4	33
		66.46 ± 2.21	24.72[b] ± 1.98	3.56 ± 2.33	135.12 ± 2.48

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

DISCUSSION

The results shown in Tables 1 and 2 indicate that supplementing hormones to the IVM media for Cỏ goat oocytes significantly increases the maturation rate of oocytes and blastocyst of Cỏ goat. During the maturation process, the oocyte undergoes several changes in molecule and structure, so the IVM medium must have the necessary components for the maturation of nucleus and cytoplasm (Leal et al. 2018).

The addition of the combination of FSH and EGF hormones resulted in a significantly higher maturation rate in comparison to using FSH alone (74.33% vs. 61.28%, respectively). These findings are consistent with the reports of Hatif and Abdulla (2016) and Wang et al. (2007). According to Hatif and Abdulla (2016), culturing goat oocytes in TCM 199 medium containing a combination of hormones enhances the efficiency of IVM of goat oocytes. Wang et al. (2007) also found that culturing goat oocytes in TCM 199 + EGF and FSH resulted in a higher maturation rate compared to culturing in TCM 199 with only FCS.

Cumulus cells surrounding the oocyte act as a mediator for nutrient exchange between the oocyte and IVM medium; hence, the maturation process of the oocyte is influenced by the layer of cumulus cells surrounding it. The presence

of hormones such as FSH or EGF in the maturation culture supports the expansion of the cumulus cell layer and stimulates the nuclear maturation of the oocyte, thereby increasing the rate of oocyte maturation in vitro (Lounas et al. 2024).

According to Romaguera et al. (2010) and Herrick et al. (2004), the current IVM media for goat oocytes commonly use TCM 199 or SOF supplemented with various gonadotrophin hormones. Gonadotropic hormones such as FSH, luteinizing hormone (LH), equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG), and 17β-estradiol are frequently used for the in vitro maturation of goat oocytes (Rahman et al. 2011). The purpose of supplementing gonadotrophin hormones is to improve the maturation of the nucleus and cytoplasm as well as the expansion of the cumulus cell surrounding the oocyte.

FSH plays a key role in stimulating the development of oocytes. A characteristic expression of matured oocyte is the expansion of the surrounding cumulus cells and the appearance of PB1 (Sauerbrun-Cutler et al. 2015). The presence of FSH stimulates the nuclear and cytoplasm maturation. Furthermore, FSH supports the expansion of cumulus cells, thereby enhancing fertilization efficiency and subsequent development of the oocytes (Widayati and Pangestu 2020). The presence of FSH and LH in IVM medium affects the development of oocytes—without FSH, the oocyte may cease development (Tetkova et al. 2019). Additionally, culturing oocytes in an IVM media supplemented with hormones improves division and development up to the blastocyst stage of the fertilized oocyte (Mastromonaco and Gonzales-Grajales 2020). The presence of FSH in IVM of porcine oocytes enhanced nuclear maturation and increased blastocyst rate (Lima et al. 2018). Abdoon et al. (2001) also showed that FSH in IVM of buffalo oocytes intensified cleavage and blastocyst rates. The influence of FSH on oocytes involves stimulating cumulus cells to produce cyclic adenosine monophosphate (cAMP), which plays a significant role in the maturation of meiotic oocytes. It maintains meiotic arrest through inactivation of maturation promoting factor and stimulates cAMP-dependent protein kinase A (Pan and Li 2019). FSH is very important for competence of the MII stage and the fertilization and subsequent development of oocytes. Hence, FSH was added to IVM medium of Cỏ goat oocytes.

Glutathione (GSH) is an antioxidant that plays an important role in protecting against reactive oxygen species (ROS) and pronuclear formation (Dominko and Đikić 2018; Shirazi et al. 2018). The use of antioxidants in IVM media such as cysteamine is an important tool that can limit the formation of ROS. Gulo et al. (2020) showed that cysteamine supplementation during maturation increased the nuclear maturation rate of sheep oocyte. Cysteamine supplementation during IVM could also increase the GSH synthesis ability of oocytes, facilitating the replacement of sperm protamins by histones during the male pronuclear formation (Canel et al. 2018). Also, according to Cognié et al. (2004), when added to the IVM media, cysteamine increased the blastocyst rate of oocytes from adult goats.

The results of Experiment 2 are in agreement with those reported by Cognié et al. (2004), Herrick et al. (2004), Phua et al. (2015), and Nursadida et al. (2024), who also found that the maturation duration impacts the IVM rate of goat oocytes. Phua et al. (2015) even suggested that maturation time also affects in vitro goat embryo production efficiency.

However, there is no consensus among studies on the exact time required for goat oocytes to complete maturation of their nucleus and cytoplasm. Herrick et al. (2004) reported the highest rates of goat oocytes reaching the MII stage after 18 – 20 h of maturation (70% – 80%). Cognié et al. (2004) observed that PB1 appears within 16 – 24 h of maturation. Rho et al. (2001) and Rahman et al. (2007) also found that the rate of goat oocytes in the MII stage after 27 h was higher than after 24 h of culture. Sharma et al. (1996) even suggested that maturation of goat oocytes in TCM 199 medium + 20% oestrus goat serum (OGS) for 32 h resulted in a 71.6%-matured goat oocytes. Nursadida et al. (2024) suggested that the highest rate of matured goat oocytes was found in the 22-h group but did not significantly differ from the 24-h group, similar to this study's findings. Nonetheless, most of the reports on in vitro goat embryo production involve mature goat oocytes in vitro for 22 – 24 h (Cognié et al. 2004; Rodríguez-Dorta et al. 2007; de Souza et al. 2013). Therefore, in this study, IVM duration of Cỏ goat oocytes was limited to a maximum of 24 h.

Goat oocytes are commonly matured in vitro for 24–27 h at 38°C–39°C in 5% CO2 (Samaké et al. 2000). According to Sharma et al. (1996), IVM time for goat oocytes is longer compared to sheep or cattle. The difference in the time required for goat oocytes to complete maturation depends on the maturation culture medium and conditions (Cognié et al. 2004), the size of the follicle, and the developmental stage of the follicle before oocyte retrieval (Rho et al. 2001). The IVM duration influenced oocyte maturation rate and results in the ageing of oocytes and increased genetic risks if culture duration is excessive (La et al. 2019).

This study aimed to optimize IVM medium and culture duration for immature Cỏ goat oocytes to increase the blastocyst rate. The rate of cleavage of the 22-h group in this study was higher than that of Wang et al. (2007) (67.04% vs. 63.3%). Moreover, the blastocyst rate of the 22-h group was also higher than that of Wang et al. (2007) and Mondal et al. (2008) (24.75% vs. 22.6% and 16.27%, respectively). Similarly, the blastocyst rate of the 24-h group in this study was higher than that of Widayati and Pangestu (2020) (24.72% vs. 22.28%,

respectively). This might be due to the different IVM medium and maturation time among the studies. According to Phua et al. (2015), an IVM duration of 18–20 h improved embryo yield, which may explain why the blastocyst rate was higher than that of Wang et al. (2007) and Mondal et al. (2008).

In this study, all blastocysts were assessed for cell numbers. The results in Tables 2 and 4 show no significant difference in the average number of cells per blastocyst among treatments. The average number of cells per blastocyst ranged from 133.42 to 135.12 (Tables 2 and 4) and was higher than that of Veshkini et al. (2018) (114.7). The difference between these results may be due to the difference of IVM medium.

This study used TCM 199 with FCS, EGF, and FSH, while Wang et al. (2007) and Veshkini et al. (2018) used TCM 199 with FCS and FSH and Mondal et al. (2008) used TCM 199 with FCS. Besides the supplementation of gonadotrophin hormones like FSH, various growth factors are also added to IVM medium to enhance maturation efficiency, among which EGF is the most commonly used and supplemented (Zhang et al. 2013). De Souza et al. (2013) even suggested that EGF addition to the goat oocyte IVM media may eliminate the need for serum supplementation. Gall et al. (2004) also observed that EGF presence in the goat oocyte IVM media regulates the maturation of the nucleus and cytoplasm. According to Grupen et al. (1997), using EGF at a concentration of 10 ng/mL in IVM media for pig oocytes resulted in a higher maturation rate compared to no EGF use (88% vs. 70%, respectively). Hsieh et al. (2007) showed that EGF may be the factor stimulating LH activity, thereby supporting oocyte maturation.

EGF is a factor that stimulates the synthesis of intracellular glutathione. Using IVM with EGF, Abeydeera et al. (2000) achieved better cytoplasmic maturation. Thongkittidilok et al. (2015) showed that EGF is important for mammalian embryo production. EGF also encouraged maturation of the cytoplasm, which improved embryo development rates (Abeydeera et al. 2020). This might explain this study's higher maturation and blastocyst rates of Cỏ goat oocytes matured in medium supplement with EGF, FSH, and cysteamine in comparison to the said supplement with EGF and cysteamine or FCS alone.

CONCLUSION

Experiments were conducted to evaluate the effects of maturation media and duration on the in vitro maturation (IVM) of Cỏ goat oocytes for in vitro production of Cỏ goat embryos. Results revealed that the rate of blastocyst was 24.75% at 22 h IVM and 24.72% at 24 h IVM. This demonstrates that the tissue culture medium (TCM) 199 supplemented with fetal calf serum (FCS), follicle stimulating hormone (FSH), cysteamine, and epidermal growth factor (EGF) is suitable for the in vitro maturation and subsequent embryo development of Cỏ goat oocytes.

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REFERENCES CITED

ABDOON AS, KANDIL OM, OTOI T, SUZUKI T. 2001. Influence of oocyte quality, culture media and gonadotropins on cleavage rate and development of in vitro fertilized buffalo embryos. Anim Reprod Sci. 65(3–4):215–223. doi:10.1016/s0378-4320(01)00079-3.

ABEYDEERA LR, WANG WH, CANTLEY TC, RIEKE A, MURPHY CN, PRATHER RS, DAY BN. 2000. Development and viability of pig oocytes matured in a protein-free medium containing epidermal growth factor. Theriogenology. 54(5):787–797. doi:10.1016/S0093-691X(00)00390-3.

AVELAR SRG, MOURA RR, SOUSA FC, PEREIRA AF, ALMEIDA KC, MELO CHS, TELES-FILHO ACA, BARIL G, MELO LM, TEIXEIRA DIA, FREITAS VJF. 2012. Oocyte production and in vitro maturation in Canindé goats following hormonal ovarian stimulation. Anim Reprod. 9(1):27–32. https://www.animal-reproduction.org/article/5b5a605ff7783717068b470f.

CANEL NG, SUVÁ M, BEVACQUA RJ, ARIAS ME, FELMER R, SALAMONE DF. 2018. Improved embryo development using high cysteamine concentration during IVM and sperm co-culture with COCs previous to ICSI in bovine. Theriogenology. 117:26–33. doi:10.1016/j.theriogenology.2018.05.017.

COGNIÉ Y, POULIN N, LOCATELLI Y, MERMILLOD P. 2004. State-of-the-art production, conservation and transfer of in-vitro-produced embryos in small ruminants. Reprod Fertil Dev. 16(4):437–445. doi:10.10371/RD04029.

CROCOMO LF, ARIU F, BOGLIOLO L, BEBBERE D, LEDDA S, BICUDO SD. 2020. Roscovitine use for the delay of meiotic progression in prepubertal sheep oocytes. Vet Sci. 55:1–8. doi:10.1590/S1678-3921.pab2020.v55.01585.

DE SOUZA JMG, DUFFARD N, BERTOLDO MJ, LOCATELLI Y, CORBIN E, FATET A, FREITAS VJF, MERMILLOD P. 2013. Influence of heparin or the presence of cumulus cells during fertilization on the in vitro production of goat embryos. Anim Reprod Sci. 138(1–2):82–89. doi:10.1016/j.anireprosci.2013.02.012.

DOMINKO K, ĐIKIĆ D. 2018. Glutathionylation: a regulatory role of glutathione in physiological processes. Arh Hig Rada Toksikol. 69:1–24. doi:10.2478/aiht-2018-69-2966.

FARIN PW, CROSIER AE, FARIN CE. 2001. Influence of in vitro systems on embryos survival and fetal development in cattle. Theriogenology. 55(1):151–170. doi:10.1016/s0093-691x(00)00452-0.

FERNANDES-FRANCA PH, OLIVEIRA-SANTOS MV, OLIVEIRA-LIRA GP, AZEVEDO-BORGES A, FERNANDES-PEREIRA A. 2020. Influence of different protein supplements on the recovery and in vitro maturation of bovine oocytes. Rev. Colomb Cienc Pecu. 33:172–181. doi:10.17533/udea.rccp.v33n3a01.

GALL L, CHENE N, DAHIREL M, RUFFINI S, BOULESTEIX C. 2004. Expression of epidermal growth factor receptor in the goat cumulus-oocyte complex. Mol Reprod Dev. 67(4):439–445. doi:10.1002/mrd.20040.

GRUPEN CG, NAGASHIMA H, NOTTLE MB. 1997. Role of epidermal growth factor and insulin-like growth factor–I on porcine oocyte maturation and embryonic development in vitro. Reprod Fertil Dev. 9(6):571–575. doi:10.1071/r96115.

GULO FDK, KARJA NWK, SETIADI MA. 2020. Cysteamine in maturation medium enhances nuclear maturation and fertilization rate of sheep oocytes in vitro. Hayati J Biosci. 27(4):290–295. doi:10.4308/hjb.27.4.290.

HATIF SA, ABDULLA OM. 2016. In vitro maturation of goat oocyte with deferent hormonal additives and in vitro fertilization by buck and ram sperm. Indian J Res. 5(8):178–180. https://journals.indexcopernicus.com/api/file/viewByFileId/614060.

HERRICK JR, BEHBOODI E, MEMILI E, BLASH S, ECHELARD Y, KRISHER RL. 2004. Effect of macromolecule supplementation during in vitro maturation of goat oocytes on developmental potential. Mol Reprod Dev. 69(3):338–346. doi:10.1002/mrd.20141.

HSIEH M, LEE D, PANIGONE S, HORNER K, CHEN R, THEOLOGIS A, LEE DC, THREADGILL DW, CONTI M. 2007. Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. Mol Cell Biol. 27(5):1914–1924. doi:10.1128/MCB.01919-06.

LA X, ZHAO J, WANG Z. 2019. Clinical application of in vitro maturation of oocytes. In: Wu B, Feng HL, editors. Embryology-theory and practice. London (UK): IntechOpen Limited. doi:10.5772/intechopen.87773.

LEAL GR, MONTEIRO CAS, SOUZA-FABJAN JMG, VASCONCELOS COP, NOGUEIRA LAG, FERREIRA AMR, SERAPIÃO RV. 2018. Role of cAMP modulator supplementations during oocyte in vitro maturation in domestic animals. Anim Reprod Sci. 199:1–14. doi:10.1016/j.anireprosci.2018.11.002.

LIMA GL, LUZ VB, LIMA LF, ROCHA RMP, CASTRO SV, CASTELO TS, RODRIGUES A, FIGUEIREDO JR, SILVA AR. 2018. Interactions between different media and follicle-stimulating hormone supplementation on in vitro culture of preantral follicles enclosed in ovarian tissue derived from collared peccaries (Pecari tajacu Linneaus, 1758). Reprod Domest Anim. 53(4):880–888. doi:10.1111/rda.13179.

LOUNAS A, BRETON Y, LEBRUN A, LAFLAMME I, VERNOUX N, SAVAGE J, TREMBLAY ME, PELLETIER M, GERMAIN M, RICHARD FJ. 2024. The follicle-stimulating hormone triggers rapid changes in mitochondrial structure and function in porcine cumulus cells. Sci Rep. 14:436. doi:10.1038/s41598-023-50586-3.

MASTROMONACO GF, GONZALES-GRAJALES AL. 2020. Reproduction in female wild cattle: influence of seasonality on ARTs. Theriogenology. 150:396–404. doi:10.1016/j.theriogenology.2020.02.016.

MONDAL A, KHANDOKER MAMY, MONDAL MA, RAHMAN AHMS, APU AS, PERVAGE S. 2008. In vitro production of goat embryo in Bangladesh. Bang J Anim Sci. 37(1):1–9. doi:10.3329/bjas.v37i1.9859.

NURSADIDA M, WIDJIATI W, MJUSTOFA I, SAFITRI E, SUSILOWATI S, SUPRAYOGI TW. 2024. The effects of duration of the in vitro maturation process on the maturation level and apoptosis of Kacang goat. Vet Stanica. 55(1):79–85. doi:10.46419/vs.55.1.8.

PAN B, LI J. 2019. The art of oocyte meiotic arrest regulation. Reprod Biol Endrocin. 17(1):8. doi:10.1186/s12958-018-0445-8.

PHAM KD, NGUYEN BM. 2015. Physical appearance and growth performance of indigenous goat Co, F1 (Bach Thao x Co) and three way crossbred goat [Boer x (Bach Thao x Co)] raised in Nho Quan, Ninh Binh Province. J Sci Devel. 13(4):551–559. https://vjol.info.vn/index.php/hvnn/article/view/31251/26521.

PHUA ACY, RAHMAN MM, WAN KHADIJAH WE, ABDULLAH RB. 2015. Influence of in vitro maturation duration on embryo yield in goats. Mal J Anim Sci. 18(2):23–28. https://www.msap.my/pdf/mjas_18_2/2.Phua-influence_r4.pdf.

RAHMAN A, ABDULLAH RB, WAN-KHADIJAH WE. 2007. Intracytoplasmic sperm injection of in vitro matured goat oocyte with abnormal ooplasmic morphology. Proceedings of the 28th Malaysian Society of Animal Production Annual Conference: Kuching, Malaysia.

RAHMAN ANMA, ABDULLAH RB, WAN-KHADIJAH WE. 2011. In vitro maturation of oocytes with special reference to goat: a review. Biotechnology. 7(4):599–611. doi:10.3923/biotech.2008.599.611.

RHO GJ, HAHNEL AC, BETTERIDGE KJ. 2001. Comparisons of oocyte maturation times and of three methods of sperm preparation for their effects on the production of goat embryos in vitro. Theriogenology. 56(3):503–516. doi:10.1016/s0093-691x(01)00581-7.

RODRÍGUEZ-DORTA N, COGNIÉ Y, GONZÁLEZ F, POULIN N, GUIGNOT F, TOUZÉ JL, BARIL G, CABRERA F, ALAMO D, BATISTA M, GRACIA A, MERMILLOD P. 2007. Effect of coculture with oviduct epithelial cells on viability after transfer of vitrified in vitro produced goat embryos. Theriogenology. 68(6):908–913. doi:10.1016/j.theriogenology.2007.07.004.

ROMAGUERA R, MORATÓ R, JIMÉNEZ-MACEDO AR, CATALÁ M, ROURA M, PARAMIO MT, PALOMO MJ, MOGAS T, IZQUIERDO D. 2010. Oocyte secreted factors improve embryo developmental competence of COCs from small follicles in prepubertal goats. Theriogenology. 74(6):1050–1059. doi:10.1016/j.theriogenology.2010.04.035.

SAMAKÉ S, AMOAH EA, MOBINI S, GAZAL O, GELAYE S. 2000. In vitro fertilization of goat oocytes during the non-breeding season. Small Rum Res. 35(1):49–54. doi:10.1016/S0921-4488(99)00065.

SAUERBRUN-CUTLER MT, VEGA M, KELTZT M, MCGOVERN PG. 2015. In vitro maturation and its role in clinical assisted reproductive technology. Obstet Gynecol Surv. 70(1):45–57. doi:10.1097/OGX.0000000000000150.

SHANI AK, HAHAM LM, BALAKIER H, KUZNYETSOVA I, BASHAR S, DAY EN, LIBRACH CL. 2023. The developmental potential of mature oocytes derived from rescue in vitro maturation. Fertil Steril. 120(4):860–869. doi:10.1016/j.fertnster.2023.05.163.

SHARMA GT, MAJUMDAR AC, BONDE SW. 1996. Chronology of maturational events in goat oocytes cultured in vitro. Small Ruminant Res. 22(1):25–30. doi:10.1016/0921-4488(96)00859-0.

SHIRAZI A, GOLESTANFAR A, BASHIRI M, AHMADI E, SHAMS-ESFANDABADI N. 2018. Male pronuclear formation and embryo development following intracytoplasmic injection of ovine pretreated sperm. Avicenna J Med Biotechnol. 10(1):41–48. https://pubmed.ncbi.nlm.nih.gov/29296266/.

SUN QY, NAGAI T. 2003. Molecular mechanisms underlying pig oocyte maturation and fertilization. J Reprod Dev. 49(5):347–359. doi:10.1262/jrd.49.347.

SUPRAYOGI TW, RIMAYANTI, HERNAWATI T, SUHARSONO, SUSILOWATI S. 2018. The addition of L-arginine in capacitation media to motility, viability, and spermatozoa capacity of goats. Int J ChemTech Res. 11(3):13–18. doi:10.20902/IJCTR.2018.110303.

TETKOVA A, SUSOR A, KUBELKA M, NEMCOVA L, JANSOVA D, DVORAN M, DEL LLANO E, HOLUBCOVA Z, KALOUS J. 2019. Follicle-stimulating hormone administration affects amino acid metabolism in mammalian oocytes. Biol Reprod. 101(4):719–732. doi:10.1093/biolre/ioz117.

THONGKITTIDILOK C, THARASANIT T, SONGSASEN N, SANANMUANG T, BUARPUNG S, TECHAKUMPHU M. 2015. Epidermal growth factor improves developmental competence and embryonic quality of singly cultured domestic cat embryos. J Reprod Dev. 61(4):269–276. doi:10.1262/jrd.2014-167.

VESHKINI A, MOHAMMADI-SANGCHESHMEH A, GHANEM N, ABAZARI-KIA AH, MOTTAGHI E, KAMALEDINI R, DELDAR H, OZTURK I, GASTAL EL. 2018. Oocyte maturation with royal jelly increases embryo development and reduces apoptosis in goats. Anim Reprod. 15(2):124–134. doi:10.21451/1984-3143-2017-AR986.

WANG ZG, XU ZR, YU SD. 2007. Effects of oocyte collection techniques and maturation media on in vitro maturation and subsequent embryo development in Boer goat. Czech J Anim Sci. 52(1):21–25. doi:10.17221/2327-CJAS.

WANI NA, WANI GM, KHAN MZ, SALAHUDIN S. 2000. Effect of oocyte harvesting techniques on in vitro maturation and in vitro and fertilization in sheep. Small Ruminant Res. 36(1):63–67. doi:10.1016/s0921-4488(99)00097-8.

WIDAYATI DT, PANGESTU M. 2020. Effect of follicle-stimulating hormone on Bligon goat oocyte maturation and embryonic development post in vitro fertilization. Vet World. 13(11):2443–2446. doi:10.14202/vetworld.2020.2443-2446.

ZHANG H, WU B, LIU H, QIU M, LIU J, ZHANG Y, QUAN F. 2013. Improving development of cloned goat embryos by supplementing α-lipoic acid to oocyte in vitro maturation medium. Theriogenology. 80(3):228–233. doi:10.1016/j.theriogenology.2013.03.027.