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Effects of Hormonal Combinations on Nuclear Maturation and Parthenogenesis in Korean Black Goat Oocytes

Sang Hwan Kim[1,2*]

[1]*School of Animal Life Convergence Science, Hankyong National University, 327, Jungang-ro, Ansung, Gyeonggi-do, 17579, Republic of Korea;* [2]*Institute of Applied Humanimal Science, Hankyong National University, 327, Jungang-ro, Ansung, Gyeonggi-do, 17579, Republic of Korea.*

Abstract | The Korean black goat (*Capra hircus coreanae*) is a traditional livestock breed valued for its functional and medicinal meat. However, low reproductive efficiency and limited genetic improvement systems limit its industrial potential. This study investigated the effects of four in vitro maturation (IVM) protocols on nuclear maturation and parthenogenetic embryo development of immature oocytes collected from black goats in Gyeonggi-do, Korea. Oocytes were cultured under four hormonal conditions: a combination of EGF, FSH, GTH, and LH (G1); EGF, GTH, and LH without FSH (G2); low-dose FSH and LH (T1); and T1 supplemented with estradiol (T2). After maturation, nuclear stages (GV, GVBD, MI, MII) were assessed by Hoechst 33258 staining. Parthenogenetic activation was induced using 7% ethanol, chosen for its efficiency and simplicity in small ruminants, followed by incubation in 6-DMAP and embryo culture in mSOF medium. The G1 group showed the highest MII rate (54.83%) and blastocyst formation rate (45%), whereas T1 and T2 groups showed significantly lower maturation (28.13%, 32.26%) and cleavage rates. Cumulus expansion and polar body extrusion were positively associated with oocyte competence. Statistical analysis using a general linear model and Duncan's multiple range test ($p < 0.05$) confirmed that comprehensive hormonal supplementation, including FSH and GTH, significantly improves nuclear maturation and developmental potential. These results provide valuable data for optimizing IVEP systems tailored to native Korean black goats.

Keywords | Korean black goat, In vitro maturation, Parthenogenesis, Embryo development, FSH, Hormonal stimulation

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***Correspondence** | Sang Hwan Kim, School of Animal Life Convergence Science, Hankyong National University, 327, Jungang-ro, Ansung, Gyeonggi-do, 17579, Republic of Korea; **Email:** immunoking@hknu.ac.kr
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INTRODUCTION

The Korean black goat (*Capra hircus coreanae*) has been traditionally regarded as a medicinal livestock breed. Its meat valued for its functional and medicinal properties is widely consumed as a functional food due to traditional use in Korean medicine and folk remedies, including the enhancement of vitality and the prevention of disease, based on long-standing practices (Park *et al.*, 2018). While historically popular among middle-aged and elderly consumers, recent years have seen increasing demand among younger demographics who recognize its value as a high-protein,

low-fat food. According to the Korean Statistical Information Service (KOSIS, 2023), the domestic black goat population increased from approximately 290,000 in 2010 to over 450,000 in 2022, reflecting rising demand driven by national interest in health and wellness. Despite this growth, reproductive efficiency in black goats remains relatively low compared to other livestock species, and the lack of a systematic genetic improvement framework hinders the sustainable utilization of genetic resources (Kim *et al*., 2022).

In vitro embryo production (IVEP) technologies have garnered attention as a potential solution to improve the reproductive efficiency and industrial viability of black goats. IVEP involves the in vitro maturation (IVM), fertilization, and culture of immature oocytes to generate preimplantation embryos and serves as a fundamental platform for applications such as genetic resource conservation, elite selection, and cloning (Khatir *et al*., 2018). As a genetically distinct indigenous breed, the Korean black goat exhibits unique mitochondrial haplotypes and microsatellite marker profiles, emphasizing the need for tailored biotechnological interventions. Developing an efficient IVEP system for this breed is therefore critical for both genetic diversity preservation and the advancement of reproductive biotechnology.

Recent studies on IVM in black goats have explored various strategies to enhance oocyte developmental competence, including optimization of culture environments, regulation of oxidative stress, and supplementation with hormones and growth factors. Assareh *et al*. (2022) reported that sequential treatment with C-type natriuretic peptide (CNP), prostaglandin E2 (PGE2), and amphiregulin (AREG) improved both nuclear and cytoplasmic maturation. Wang *et al*. (2025) demonstrated that supplementation with N-acetyl-L-cysteine (NAC) reduced reactive oxygen species (ROS) accumulation, improved mitochondrial function, and enhanced embryonic development. Similarly, Podda *et al*. (2025) showed that the use of a three-dimensional liquid marble culture system facilitated mitochondrial distribution and ATP generation, promoting cytoplasmic maturation. These findings underscore that IVM is a complex process involving not only meiotic progression but also the biochemical and structural readiness of the ooplasm.

However, IVEP efficiency in black goats remains suboptimal, with blastocyst formation rates generally reported to be lower (20–40%) compared to 50% or higher in well-established caprine IVEP systems (Menéndez-Blanco *et al*., 2020; Khatir *et al*., 2018). First, asynchronous nuclear and cytoplasmic maturation is a major impediment to embryo development, particularly with frequent developmental arrest at the 8- to 16-cell stage (Menéndez-Blanco *et al*., 2020). Second, oocyte competence is influenced by follicular origin, and oocytes derived from small follicles exhibit markedly reduced maturation and developmental capacity (Lonergan *et al*., 1994). Third, variability in hormonal responsiveness due to season, age, and follicular environment makes standardizing IVM protocols challenging for this breed (Paramio and Izquierdo, 2016).

Hormonal regulation during IVM is therefore essential. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) are known to influence various aspects of oocyte maturation, including cumulus expansion, meiotic progression, and cytoplasmic organelle distribution (Chohan and Hunter, 2003; Singh *et al*., 2015). FSH enhances cumulus–oocyte communication and increases metaphase II (MII) rates (Alvarez *et al*., 2019), while estradiol contributes to cytoskeletal rearrangement and spindle integrity (Choi *et al*., 2021). In addition, recent studies have investigated the role of oocyte-secreted factors such as growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) in modulating cumulus cell responsiveness (Silva *et al*., 2018). In native Korean black goats, where follicular response and oocyte competence are known to vary due to breed-specific traits, the role of hormonal supplementation during IVM becomes particularly relevant.

Given these insights, there is a pressing need to develop an IVM system specifically tailored to the reproductive physiology and genetic background of Korean black goats, rather than adopting protocols designed for other breeds. For example, studies have shown distinct differences in follicular dynamics, estrous cyclicity, and seasonal reproductive patterns between Korean native goats and exotic breeds. Most previous research has focused on exotic or crossbred goats, and investigations into optimal IVM conditions for native Korean black goats remain extremely limited (Kim *et al*., 2022).

Accordingly, the present study evaluated the effects of hormonal supplementation, specifically FSH and estradiol, during IVM on nuclear maturation rates and subsequent embryonic development in immature oocytes derived from Korean native black goats.

MATERIALS AND METHODS

In Vitro Maturation of Oocytes

Ovaries from Korean native black goats (*Capra hircus coreanae*) were collected at a local slaughterhouse in Anseong, Korea, and transported to the laboratory within 2 hours in 0.9% physiological saline maintained at 35–37°C. The animals were all non-pregnant adult females between 2–4 years of age, and samples were collected during the breeding season (October to December).

Donors were randomly selected from a local slaughterhouse and were not hormonally synchronized prior to ovary collection. Information on estrous cycle stage, parity, or health status was not available. This variability may have influenced oocyte quality, but consistent follicle size (2–6 mm) and age range were used to reduce physiological differences as much as possible.

Follicular fluid was aspirated from 2–6 mm diameter follicles using an 18-gauge needle attached to a 10 mL syringe. Immature oocytes with more than three compact layers of cumulus cells and a homogeneous ooplasm, measuring approximately 120–135 µm in diameter under 40× magnification were chosen for maturation. Selected oocytes were cultured under four in vitro maturation (IVM) conditions, designated G1, G2, T1, and T2 (Table 1).

Table 1: In vitro maturation (IVM) media composition and hormone supplementation for each treatment group.

Name	G1 Phase 1	G1 Phase 2	G2 Phase 1	G2 Phase 2	T1	T2
Media	450 µl	450µl	465 µl	490 µl	448 µl	448 µl
E2	-	-	-	-	1 µg/ml	-
EGF	30ng/ml	30ng/ml	30ng/ml	30ng/ml	10ng/ml	10ng/ml
GTH	2.5 IU/mL	-	2.5 IU/mL	-	-	-
FSH	5µg/ml	-	-	-	0.5µg/ml	0.5µg/ml
LH	1 µg/ml	5 µg/ml	-	-	5 µg/ml	5 µg/ml
Time	22hr	After 22hr	22hr	After 22hr	27hr	27hr

IU values used where manufacturer units were provided only in IU (e.g., GTH).

In G1, the base medium was TCM-199 (31100-027; Gibco, Grand Island, NY, USA) supplemented with sodium bicarbonate (S5761; Sigma-Aldrich, St. Louis, MO, USA) at 2.2 g/L, cysteamine (M9768; Sigma-Aldrich, St. Louis, MO, USA) at 0.2 mM, and 15% fetal bovine serum (FBS; 16000-044; Gibco, Grand Island, NY, USA). Hormonal supplementation included epidermal growth factor (EGF; E9644; Sigma-Aldrich, St. Louis, MO, USA) at 30 ng/mL, follicle-stimulating hormone (FSH; F2293; Sigma-Aldrich, St. Louis, MO, USA) at 5 µg/mL, gonadotropic hormone (GTH; Denka Seiken, Tokyo, Japan) at 2.5 IU/mL, and luteinizing hormone (LH; L5269; Sigma-Aldrich, St. Louis, MO, USA) at 1 µg/mL for the first 22 hours. After replacement with fresh medium with the same composition, the LH concentration was increased to 5 µg/mL and oocytes were incubated for an additional 22 hours. The G2 group used the same base medium as G1, but hormonal supplementation included EGF at 30 ng/mL, GTH at 2.5 IU/mL, and LH at 1 µg/mL during the first 22 hours, followed by replacement with the same medium supplemented with 5 µg/mL LH for another 22 hours. For the T1 group, the maturation medium was based on TCM-199 modified by the addition of L-glutamine (25030-081; Gibco, Grand Island, NY, USA) at 0.68 mM, sodium pyruvate (P5280; Sigma-Aldrich, St. Louis, MO, USA) at 0.8 mM, gentamycin (15710-064; Gibco, Grand Island, NY, USA) at 50 µg/mL, and 10% FBS. This was supplemented with EGF at 10 ng/mL, FSH at 0.5 µg/mL, and LH at 5 µg/mL and cultured for 27 hours. The T2 condition was identical to T1, except that estradiol (E2; E8875; Sigma-Aldrich, St. Louis, MO, USA) was additionally included at 1 µg/mL. All oocytes were cultured in a humidified incubator at 38.5°C under 5% CO_2, 5% O_2, and 90% N_2 gas conditions. Approximately 10–15 oocytes were placed in 35 µL droplets of maturation medium under mineral oil (M8410; Sigma-Aldrich, St. Louis, MO, USA).

The FSH concentrations used in G1 (5 µg/mL) and T1 (0.5 µg/mL) were chosen based on earlier goat studies showing that higher doses improve cumulus expansion and MII progression (Kharche *et al*., 2013; Singh *et al*., 2015). The low-dose T1 condition was designed to test whether reduced hormonal input could still support meiotic maturation under simplified protocols.

Parthenogenetic Activation

After IVM, cumulus cells were removed by gentle pipetting in HEPES-buffered TCM-199 containing 0.1% hyaluronidase (H4272; Sigma-Aldrich, St. Louis, MO, USA). Oocytes at the MII stage were activated using 7% ethanol (459836; Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes at 38.5°C. Activated oocytes were washed three times in HEPES-buffered TCM-199 and transferred to modified synthetic oviductal fluid (mSOF) based on the formulation of Tiwari *et al*. (2022), consisting of sodium chloride, potassium chloride, sodium bicarbonate, calcium lactate, sodium lactate, magnesium sulfate, and essential amino acids, for embryo culture.

Culture was conducted in a humidified incubator at 38.5°C with 5% CO_2, 5% O_2, and 90% N_2 for 7 days. Medium was refreshed every two days. Cleavage was assessed on Day 2 and defined as the presence of at least two blastomeres. Blastocyst formation was evaluated morphologically on Day 7.

Fluorescence Staining and Nuclear Analysis

For nuclear status evaluation, oocytes were fixed in 4% paraformaldehyde (PFA; P6148; Sigma-Aldrich, St. Louis, MO, USA) for 30 minutes at room temperature and washed three times in phosphate-buffered saline (PBS; P4417; Sigma-Aldrich, St. Louis, MO, USA). Oocytes were stained with Hoechst 33258 (H3569; Thermo Fisher Scientific, Waltham, MA, USA) at 10 µg/mL for 10 minutes. Stained oocytes were mounted on slides using ProLong™ Gold antifade reagent (Thermo Fisher Scientif-

ic) and covered with coverslips. Fluorescent images were captured using a Ti2-U inverted fluorescence microscope (Nikon, Tokyo, Japan) under a UV channel (Excitation 352 nm / Emission 461 nm). At least 30 oocytes per group were evaluated for MII stage, abnormal nuclear morphology, and cleavage. Cumulus expansion was scored on a 4-point scale modified from Vanderhyden *et al.* (1990), based on how widely the cumulus cells spread and how clearly the corona radiata remained defined. Scores ranged from 1 (no visible expansion) to 4 (fully dispersed outer layers). Each experiment was independently replicated three times.

Ethics Statement

All experimental procedures using goat ovaries were approved by the Institutional Animal Care and Use Committee (IACUC) of Hankyong National University (Approval No. HKNUIACUC-2024-01). Ovaries were obtained post-mortem from animals slaughtered for commercial meat purposes and were not euthanized or treated for research. No additional procedures were conducted on the animals before slaughter.

Statistical Analysis

Statistical analysis was conducted using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). All data are presented as mean ± standard error of the mean (SEM). Differences among the four groups (G1, G2, T1, T2) were tested using a general linear model (GLM). When a significant main effect was detected, Duncan's multiple range test was applied for post hoc comparisons. For key comparisons, exact p-values were calculated and are reported in the Results section (e.g., G1 vs. T1 MII rate, $p = 0.021$). No effect sizes (e.g., η^2) were computed in this study, but their inclusion may be useful in future experiments. Significance was accepted at $p < 0.05$.

RESULTS

Nuclear Maturation of Oocytes Under Different IVM Conditions

The distribution of nuclear maturation stages (GV, GVBD, MI, and MII) in immature oocytes from Korean native black goats was analyzed after in vitro maturation under four experimental conditions (G1, G2, T1, and T2) (Table 2). The G1 group had the highest rate of MII-stage oocytes (54.83%), while the proportions of GV (12.12 ± 1.3%), GVBD (10.52 ± 1.3%), and MI (13.78 ± 3.2%) stages were relatively lower. In the G2 group, the MII rate decreased to 35.48%, with higher proportions of GVBD (15.22 ± 1.2%) and MI (17.88 ± 2.7%) compared to G1.

In the T1 and T2 groups, MII rates were 28.13% and 32.26%, respectively. Notably, oocytes remained at the GV stage at significantly higher proportions in T1 (25.13 ± 2.5%) and T2 (27.14 ± 0.5%) compared to the G groups, indicating delayed or incomplete meiotic progression. The addition of estradiol in T2 slightly improved MII rates compared to T1.

Table 2: Nuclear maturation status of in vitro matured oocytes derived from Korean native black goats under four different culture conditions.

Group	No. of oocytes	GV (%)	GVBD (%)	MI (%)	MII (%)
G-1	200	12.12±1.30%	10.52±1.30%	13.78±3.20%	54.83%**
G-2	200	10.25±2.40%	15.22±1.20%	17.88±2.70%	35.48%*
T-1	200	25.13±1.50%	18.13±0.50%	16.05±3.20%	28.13%
T-2	200	27.14±1.50%*	20.11±2.20%	18.12±2.80%	32.26%

Data are presented as the percentage of oocytes at each maturation stage: germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), and metaphase II (MII). Values are expressed as mean ± SEM. Different superscript letters within the same column indicate significant differences ($p < 0.05$).

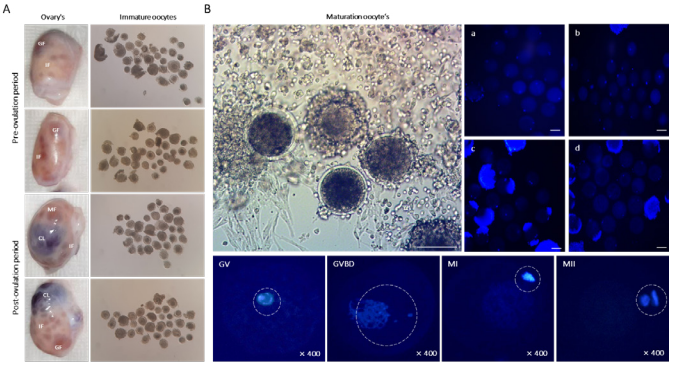

Figure 1: Morphological classification of ovaries and nuclear maturation status of in vitro matured oocytes under different hormonal treatments. **A:** Gross anatomical images of caprine ovaries and corresponding cumulus–oocyte complexes (COCs) collected from follicles at different developmental stages: GF (Graafian follicle), IF (immature follicle), MF (mid-sized follicle), and CL (corpus luteum). Despite morphological differences among follicular types, no substantial variation in oocyte quality was observed across ovarian stages. **B:** Morphological and fluorescence-based evaluation of oocytes after in vitro maturation under four different culture conditions: G1 (a), G2 (b), T1 (c), and T2 (d). Central images represent COCs with varying degrees of cumulus expansion, while nuclear status was assessed via Hoechst 33258 staining. Representative nuclear stages include GV (germinal vesicle), GVBD (germinal vesicle breakdown), MI (metaphase I), and MII (metaphase II). Oocytes from G1 and G2 groups exhibited higher rates of MII progression and more prominent cumulus expansion compared to T1 and T2 groups. Upper panels pictures are at magnification ×200. Lower panels pictures are at magnification ×400. Scale bars = 100 µm.

As shown in Figure 1A, gross morphological differences were observed between ovarian stages (Graafian follicles, mid-sized follicles, corpora lutea), but oocyte quality, as evaluated by nuclear status (GV, GVBD, MI, MII), did not show substantial variation. No significant difference in oocyte quality was associated with corpus luteum status, possibly due to consistent follicle size selection (2–6 mm) and uniform donor age range. This observation aligns with studies reporting follicular stage as a more critical determinant than ovulatory status per se.

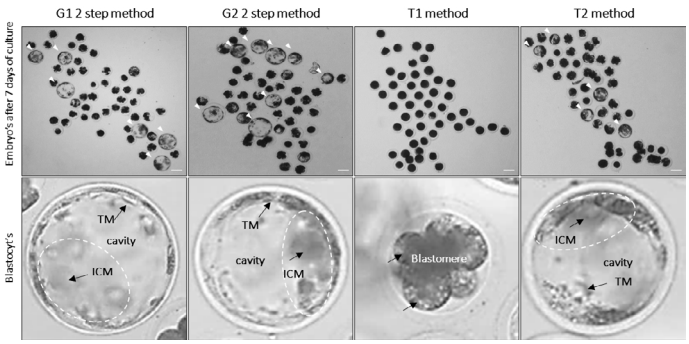

Figure 2: Comparative morphology of parthenogenetically derived embryos and blastocysts under four IVM conditions. Upper panels: Representative images of embryos after 7 days of culture. Expanded blastocysts are indicated by white arrows. Lower panels: High-magnification views (×400) of blastocysts, with black arrows pointing to the inner cell mass (ICM), trophectoderm (TM), and blastocoelic cavity. Dashed lines further outline the ICM region where applicable. Blastocysts from G1 and G2 showed greater expansion and well-defined ICM structures, while those from T1 and T2 exhibited limited development and abnormal morphology. Scale bars = 100 µm.

Morphological classification of matured oocytes under each condition (Figure 2B) revealed distinct differences. In G1 and G2, cumulus cell expansion was complete, and the extrusion of the first polar body was distinctly observed, correlating with high MII rates. In contrast, T1 and T2 oocytes often exhibited insufficient cumulus expansion and poorly defined polar bodies, resulting in lower MII progression. This suggests that both cumulus morphology and visible nuclear features are closely associated with the extent of nuclear maturation.

Embryonic Development and Blastocyst Morphology Following Parthenogenetic Activation

Following chemical activation with 7% ethanol, oocytes were cultured in mSOF medium for seven days. Embryo cleavage and developmental stages (2-cell, 4-cell, 8-cell) were assessed alongside cell death rates across all groups (Table 3).

The G1 and G2 groups showed superior developmental competence, with cleavage rates of 82% and 84%, respectively, while the T1 and T2 groups showed reduced cleavage (63% and 74%). In the G1 group, embryos were evenly distributed across developmental stages: 2-cell (20%), 4-cell (28%), and 8-cell (34%), with a relatively low cell death rate of 18%. The G2 group also exhibited balanced cleavage and an 8-cell rate of 28%, slightly lower than G1 but remained robust, indicating robust development.

Table 3: Embryo cleavage patterns and cell death rates following parthenogenetic activation of in vitro matured oocytes in Korean native black goats.

	No. of oocytes used	No. Of embryo cleaved(%)	Embryo development (%)			Death (%)
			2cell	4cell	8cell <	
G-1	200	164(82%)	40(20%)	56(28%)*	68(34%)*	18%
G-2	198	166(84%)	60(30%)*	50(25%)	56(28%)	17%
T-1	200	126(63%)	40(20%)	8(4%)	0(0%)	76%*
T-2	197	146(74%)	58(29%)	40(20%)	48(24%)	27%

Data represent the percentage of embryos at the 2-cell, 4-cell, and 8-cell stages, along with the percentage of cell death after 7 days of culture. Values are presented as mean ± SEM. Different superscript letters within the same column indicate significant differences ($p < 0.05$).

In contrast, the T1 group exhibited limited cleavage progression: 2-cell (20%), 4-cell (4%), and no embryos reaching the 8-cell stage. Additionally, the cell death rate was markedly high at 76%, suggesting that simplified hormonal conditions significantly impaired embryo viability. T2 embryos, cultured with estradiol supplementation, achieved higher cleavage rates (74%) and more advanced development (2-cell: 29%, 4-cell: 20%, 8-cell: 24%) than T1, though the cell death rate remained relatively high (27%) compared to the G groups.

Morphological assessment of blastocysts (Figure 2) indicated well-expanded structures in the G1 and G2 groups, with clearly defined inner cell mass and trophectoderm layers. In contrast, blastocysts from the T1 and T2 groups exhibited poor compaction, lower cell counts, and indistinct cell boundaries. In the T1 group, frequent developmental arrest, nuclear fragmentation, and cytoplasmic shrinkage were observed.

Together, these findings show that comprehensive hormonal supplementation during IVM (G1 and G2) supports nuclear maturation and parthenogenetic embryo development, while simplified or low-dose protocols (T1 and T2) are associated with increased cell death and impaired blastocyst formation.

DISCUSSION

This study quantitatively and morphologically evaluated the effects of in vitro maturation (IVM) conditions on the

nuclear maturation and parthenogenetic embryo development of immature oocytes derived from Korean native black goats. Four experimental groups (G1, G2, T1, and T2) showed significant differences in maturation rates and embryonic development, offering insights aligned with and extending previous studies.

The G1 condition yielded the highest maturation to MII stage (54.83%) and blastocyst formation rate (45%), indicating that the combination of FSH, LH, GTH, and EGF was the most effective among the tested IVM conditions. These findings are consistent with prior reports by Souza-Fabjan *et al.* (2021), who emphasized the beneficial effect of combined hormone and growth factor supplementation on IVM and embryo development in small ruminants. The observed difference in MII progression between G1 and G2 groups where FSH was omitted in G2 (MII: 35.48%) underscores the critical role of FSH in supporting ooplasmic maturation (Kharche *et al.*, 2013).

In contrast, T1 and T2 groups, which were cultured in a simplified medium with low-dose hormone supplementation, exhibited markedly reduced nuclear maturation and developmental outcomes. This supports prior assertions that not only hormone concentrations but also their combinations and timing are pivotal in regulating oocyte competence and subsequent development (Fernandes *et al.*, 2014). Notably, the T1 group showed no embryos reaching the 8-cell stage and a high cell death rate, suggesting that minimal hormonal stimulation is insufficient to sustain developmental potential.

Interestingly, the T2 group, supplemented with estradiol, demonstrated improved cleavage and 8-cell development rates (24%), compared to T1. However, the relatively high cell death rate (27%) in T2 suggests that while early cleavage was stimulated by estradiol, later-stage development may have been affected by cytoplasmic instability. Estradiol is known to promote meiotic resumption and spindle formation (Choi *et al.*, 2021), but excessive or prolonged exposure can disrupt redox balance and induce oxidative stress, which may explain the increased degeneration observed in T2. Steroid hormones may modulate transcriptional profiles during meiotic progression and early embryogenesis (Alves *et al.*, 2021; Choi *et al.*, 2021). Nonetheless, the blastocyst formation rate in T2 remained inferior to that of the G groups, potentially due to insufficient growth factor support and limited cumulus–oocyte interaction.

Morphological analysis of blastocysts (Figure 2) revealed well-structured inner cell mass and trophectoderm layers in the G groups, indicating a higher likelihood of implantation and post-transfer viability (Menéndez-Blanco *et al.*, 2020).

In contrast, blastocysts in the T groups displayed reduced cell numbers and poor expansion. This outcome may result from altered mitochondrial function or disrupted epigenetic regulation, as reported in similar caprine studies. Investigating mitochondrial distribution and DNA methylation in future experiments could clarify these developmental defects. While these factors were not directly assessed in the present study, their role has been emphasized in previous research (Sharma *et al.*, 2022), and future work will aim to incorporate direct analysis of these determinants. Assessing blastocyst quality based only on morphology is a limitation. Cell counting, ICM/TE ratio, or marker staining (e.g., CDX2, OCT4) will be added in future studies to validate structural observations with objective data.

The parthenogenetic activation in this study was performed using 7% ethanol, a protocol reported to be effective for oocyte activation in caprine species (Kouamo *et al.*, 2015; Goel and Kharche, 2016). The activation efficiency and blastocyst rates observed here are comparable or superior to previous reports in goats (Pathak *et al.*, 2013). This study did not include a comparison with other activation methods such as ionomycin plus 6-DMAP. Including such controls would clarify whether ethanol-based activation is equally effective, as suggested in prior goat studies (Kouamo *et al.*, 2015).

The high performance of G1 supports its potential for improving the goat IVP platform. According to Souza-Fabjan *et al.* (2023), the quality of oocytes and their IVM environment are critical bottlenecks in caprine IVP systems. Our findings reinforce the importance of IVM conditions in shaping early embryonic development.

Moreover, this study helps establish a systematic embryo production strategy in Korean black goats, a breed valued for its medicinal and nutritional properties in Korea. The development of an efficient IVM–PA system based on high-quality oocytes could enhance reproductive efficiency and genetic resource preservation. The results presented here provide valuable data for the industrial application of IVP and for validating the feasibility of parthenogenesis as a reproductive tool in caprine biotechnology. Future research should focus on optimizing culture systems by incorporating epigenetic reprogramming factors, regulating oxidative stress, and improving mitochondrial function. This study offers a solid basis for such expanded approaches.

CONCLUSIONS AND RECOMMENDATIONS

We compared the effects of four different in vitro maturation (IVM) conditions (G1, G2, T1, and T2) on the

nuclear maturation and parthenogenetic developmental potential of immature oocytes derived from Korean native black goats. The sequential hormonal stimulation in the G1 group, including EGF, FSH, GTH, and LH, resulted in the highest MII progression and blastocyst formation rates, indicating that comprehensive hormonal supplementation positively influences the developmental competence of oocytes in this species. In contrast, the G2 group, which excluded FSH, and the T1 and T2 groups, which utilized low-dose hormones in a single-phase culture, showed significantly reduced maturation and developmental rates, highlighting the critical role of hormone composition and treatment regimen during IVM. Morphological classification further demonstrated that cumulus cell expansion and polar body visibility were closely associated with nuclear maturation. Our results offer practical data for the development of a species-specific IVM and embryo production system tailored to Korean native black goats. Furthermore, this study supports the potential application of biotechnological tools in improving reproductive efficiency, genetic conservation, and industrial embryo production for this economically important breed. While G1 resulted in the highest blastocyst rates, its use of multiple high-cost hormones may limit scalability in commercial settings. In contrast, the T2 protocol, though less efficient, offers a simpler and more economical alternative that could be adapted for routine use where cost and accessibility are limiting factors.

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NOVELTY STATEMENTS

This study is a basic study that suggests a new direction for in-vitro embryo production and a plan to improve production rate in order to increase the efficiency of improvement and reproduction of Korean black goats.

AUTHOR'S CONTRIBUTIONS

The author was responsible for the study conception and design, experimental execution, data acquisition and analysis, and drafting and revising the manuscript. All aspects of this work were carried out independently by the Sang Hwan Kim.

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Conflict of Interest

The authors declare no conflicts of interest.

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