

# Development of In vitro Maturation and In vitro Growth Culture Media Using Goat Colostrum

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**Abstract.** This research aims to develop a more optimal in vitro maturation (IVM) oocyte and in vitro growth (IVG) follicle culture medium through the utilization of goat colostrum as an additive to the standard IVM and IVG culture medium. This research was conducted at the Bioscience Laboratory of Brawijaya University, Malang, using goat ovary samples obtained from the Gadang Slaughterhouse (RPH), Malang Regency. This research was carried out in two stages, namely IVG and IVM, with the data obtained subsequently analyzed using ANOVA followed by LSD for significance testing. IVG was performed on goat follicles with an initial size of 2-3 mm, followed by culture for 6 days using media treated with the addition of goat colostrum from P0 to P4, respectively, 0%, 2.5%, 5%, 7.5%, and 10%. The IVG culture results obtained were observed in terms of follicle size increase, oocyte cell size, and cumulus cell expansion of the oocytes within the follicles. The results obtained were that IVG had no significant effect on follicle size growth ( $P>0.05$ ), but showed a significant effect on cumulus cell expansion ( $P<0.05$ ) with the highest value in P4=2.5 and the lowest in P1=1.545. Meanwhile, for oocyte cell size, it also showed a significant effect ( $P<0.05$ ) with the largest size being P4=195.0  $\mu\text{m}$  and the smallest being P0=168.7  $\mu\text{m}$ . For the next stage, IVM was performed on oocytes for 24 hours with media receiving the same treatments, namely P0 to P4 with goat colostrum concentrations respectively 0%, 2.5%, 5%, 7.5%, and 10%; the IVM culture results were observed in terms of oocyte cell size increase and cumulus cell expansion quality. The IVM oocyte results obtained showed a significant difference in oocyte size increase ( $P<0.05$ ), with the largest growth in P1 at 34.3  $\mu\text{m}$  and the smallest in P0 at 12.0  $\mu\text{m}$ . Meanwhile, for cumulus cell expansion, a significant difference was also obtained ( $P<0.05$ ) with the best average in P4=3.20 and the smallest in P0=1.66. Overall, goat colostrum has potential as an additive to culture media, particularly in improving cumulus cell expansion in IVG and IVM.

**Keywords:** goat colostrum, follicle, in vitro maturation, in vitro growth, oocyte

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## 1 Introduction

Advances in the field of Assisted Reproductive Technology (ART) play a vital role in efforts to accelerate the improvement of livestock genetic quality. One of the flagship techniques, Embryo Transfer (ET), unfortunately still faces serious challenges, particularly high operational costs and success rates that tend to be low, especially in the steps of In Vitro Maturation (IVM) and In Vitro Growth (IVG) [1, 2]. The high cost of in vitro embryo production is often caused by high failure rates in IVG and IVM; these failures can be caused by various factors, one of which is suboptimal culture media [3]. Therefore, the development of culture media that is not only effective is required. This research utilizes goat colostrum as an additive to the culture medium because it has complex contents and is suspected to improve the performance of the culture medium and increase the success of IVG and IVM [4]. Colostrum, which is the initial milk produced by dams that have just given birth, is rich in nutrients and bioactive factors. Contents such as Growth Factors (for example, IGF-1 and EGF), antioxidants, as well as immunoglobulins (IgG and Lactoferrin) are estimated to be able to optimally stimulate the development and maturation of oocytes [5, 6, 7]. The direct utilization of colostrum represents an important novelty, distinguishing it from conventional studies that often only isolate specific components [4]. Through this approach, this research aims to test and determine the optimal concentration of goat colostrum that can be added to the standard medium formulation. By improving that culture medium, it is hoped that the quality of the resulting oocytes and follicles can increase significantly, which in the end will enhance the overall efficiency of the ART program. The primary novelty of this study lies in the direct use of whole goat colostrum as a culture media supplement without any extraction or purification of its bioactive components, in contrast to most previous studies that typically utilize isolated fractions (such as purified IGF-1, processed bovine colostrum, or commercial serum).

## 2 Materials and methods

### 2.1 Study Location and Experimental Material

This experiment was conducted entirely at the Bioscience Laboratory, Brawijaya University, Malang. As the source of material, goat ovaries were collected from the Gadang Slaughterhouse (RPH) in Malang Regency, immediately after slaughter.

### 2.2 Treatment Design

This research was designed as a two-stage study (IVG and IVM) with a single treatment consisting of the addition of goat colostrum to the basal culture medium. We divided the samples into five treatment groups based on the volume percentage of colostrum added:

P0: 0% (Control Group, standard medium only)

P1: 2.5% Colostrum

P2: 5% Colostrum

P3: 7.5% Colostrum

P4: 10% Colostrum

The standard medium used has the following composition:

1. Tissue Culture Medium (TCM) 199
2. Fetal Bovine Serum (FBS) 10%
3. L-Glutamine 0.6 g/L
4. FSH 0.1 IU/mL

5. HEPES 10 mM

6. Gentamicin 50 µg/mL.

Whereas the colostrum milk used originates from goat dams that have given birth less than 24 hours ago; before use, the colostrum was filtered with a pore size of 0.45 µm and subsequently 0.22 µm. Ovaries from goats were collected from the Gadang Slaughterhouse, Malang Regency, and used as the research material. A total of 20 follicles per treatment were utilized for the in vitro follicle growth (IVG) procedure, while 15 oocytes per treatment were used for the in vitro oocyte maturation (IVM) procedure.

### **2.3 Implementation of In Vitro Growth (IVG)**

Goat follicles with initial dimensions of approximately 2 to 3 mm were isolated and cultured for six days. During this period, we recorded and compared three main indicators: the increase in follicle diameter, the actual size of the oocyte cells, and the degree of expansion of the cumulus oocyte cells. The success of the in vitro growth (IVG) procedure was evaluated based on three main parameters: measurement of follicle diameter increase after 6 days of incubation, measurement of oocyte diameter within the follicle at the end of the incubation period, and assessment of cumulus cell expansion score based on the degree of expansion and the density level of the cumulus cells.

### **2.4 Implementation of In Vitro Maturation (IVM)**

Oocytes were obtained from the ovaries through aspiration of follicles measuring 3-4 mm. Subsequently, the oocytes were cultured with the same colostrum treatments (P0 to P4) and incubated for 24 hours. At this critical stage, the focus of observation was directed toward the increase in oocyte size as well as the qualitative evaluation of cumulus cell expansion, which is highly determinative of maturation success.

### **2.5 Data Analysis**

All collected quantitative data were analyzed statistically using Analysis of Variance (ANOVA). If the ANOVA results indicated significant differences, the analysis was then continued with the Least Significant Difference (LSD) test to identify which treatment was the most superior [4].

## **3 Results and discussion**

### **3.1 In Vitro Follicle Growth (IVG)**

#### **3.1.1 Follicle Size**

Follicle Size: The analysis results indicate that the addition of goat colostrum did not produce a statistically different impact on the overall increase in follicle diameter ( $P>0.05$ ); from literature studies, many previous IVG research efforts also failed to significantly increase follicle size but were still able to achieve good oocyte quality. The follicle growth produced was very small compared to in vivo conditions, which can reach 1-1.5 cm; this is due to the fact that in vitro culture has many limitations and cannot perfectly mimic the in vivo process, including plasma transudation, physical paracrine interactions between tissues, vascular

support and dynamic oxygen supply, hydrostatic and osmotic pressure from blood vessels [8].

### 3.1.2 Cumulus Cell Expansion

Cumulus Cell Expansion: This quality was proven to increase significantly ( $P < 0.05$ ). The P4 group (10%) recorded the best expansion score with an average of 2.5, indicating that the bioactive factors in colostrum effectively stimulated the cumulus cells [9]. This expansion quality is actually still considered less than maximal because, on a scale of 1-4, the average of the best group is still at 2.5. This is due to the follicle growth also showing unsatisfactory results. With minimal follicle growth, the amount of culture medium that can penetrate the follicle tissue is also limited, thereby the amount of nutrients from the culture medium that can be accessed by the oocyte cells is certainly very restricted. Nevertheless, there is still a consistent increasing trend from P1 to P4, and this increasing pattern has not yet reached its peak, so further exploration can still be conducted.

### 3.1.3 Oocyte Cell Size

Oocyte Cell Size: On this variable, the data show a significant effect ( $P < 0.05$ ) on the enlargement of oocyte cells. The largest oocytes were produced by the P4 treatment (195.0  $\mu\text{m}$ ), far exceeding the P0 control group (168.7  $\mu\text{m}$ ). This size increase is crucial because larger oocytes generally have greater energy potential and more adequate cytoplasm to support post-fertilization embryo development [10, 9]. These results are consistent with the proposed hypothesis, where nutrients from goat colostrum milk can enhance the performance of the culture medium, thereby enabling an improvement in oocyte quality.

**Table 1.** Folikel growth, cell cummulus expansion and oocyte size of IVG.

| Treatment | IVG follicle size ( $\Delta\text{h}$ , mm) | IVG cumulus expansion | IVG oocyte size (x100 microns) |
|-----------|--------------------------------------------|-----------------------|--------------------------------|
| P0        | 0.212                                      | 1.833 <sup>b</sup>    | 1.687 <sup>c</sup>             |
| P1        | 0.237                                      | 1.545 <sup>b</sup>    | 1.754 <sup>b<sup>c</sup></sup> |
| P2        | 0.337                                      | 1.556 <sup>b</sup>    | 1.805 <sup>ab</sup>            |
| P3        | 0.212                                      | 1.833 <sup>b</sup>    | 1.916 <sup>a</sup>             |
| P4        | 0.187                                      | 2.500 <sup>a</sup>    | 1.950 <sup>a</sup>             |

### 3.2 In Vitro Oocyte Maturation (IVM)

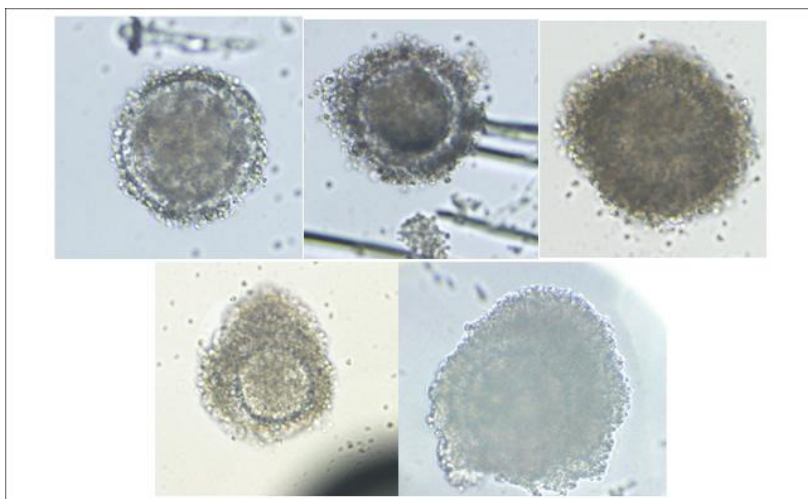
Oocyte Cell Size Growth: In the in vitro maturation of oocyte cells, the analysis results indicate that the increase in oocyte size differs significantly among treatments ( $P < 0.05$ ). If observed further from this data, it is also known that only P1 shows a significant difference, whereas the other data are relatively stable or exhibit a flat trend. This is caused by the fact that the oocyte size for all treatments has reached the maximum size and is at the ideal size.

The achievement of this ideal size is also influenced by the osmolality and viscosity values of the culture medium that are appropriate to the tolerance limits of the oocyte cells [11].

**Table 1.** Cellcumulus expansion and oocyte growth of IVM

| Treatment | Cumulus cell expansion | Oocyte growth ( $\Delta h$ , x100 $\mu m$ ) |
|-----------|------------------------|---------------------------------------------|
| P0        | 1.66 <sup>c</sup>      | 0.120 <sup>bc</sup>                         |
| P1        | 2.06 <sup>bc</sup>     | 0.343 <sup>a</sup>                          |
| P2        | 2.60 <sup>b</sup>      | 0.163 <sup>b</sup>                          |
| P3        | 2.80 <sup>a</sup>      | 0.147 <sup>bc</sup>                         |
| P4        | 3.20 <sup>a</sup>      | 0.147 <sup>bc</sup>                         |

Cumulus Cell Expansion Quality: Cumulus cell expansion at the maturation stage shows a highly significant difference ( $P < 0.05$ ). The 10% treatment (P4) once again proves its superiority, achieving the highest average expansion score (3.20), far better than the control group (1.66). The superior expansion at the IVM stage constitutes strong evidence that the oocyte has reached optimal nuclear maturation (Metaphase II phase), an absolute prerequisite for fertilization success [1]. The results of this study demonstrate an improvement in culture media performance compared to the use of standard media alone. For comparison, according to various review studies on in vitro maturation (IVM) using only standard media (TCM-199 + serum + gonadotropin), the percentage of oocytes successfully reaching maturation (metaphase II) generally falls within the range of 55–75%. Meanwhile, the percentage of embryo development to the blastocyst stage is typically much lower and is strongly influenced by the initial quality of the oocytes as well as the culture conditions [12].



**Fig. 1.** Oocyte cell after culture IVM

Overall, it is evident that the addition of colostrum, particularly at the highest concentration, not only merely provides nutrients but also serves as a source of natural growth factors that promote the enhancement of oocyte quality.

## 4 Conclusion

Based on the experimental results, it can be concluded that goat colostrum milk has great potential and is effective as a natural supplement in oocyte culture media. Colostrum has been proven to provide a significant positive impact on crucial oocyte quality parameters, namely increasing the size of mature oocyte cells at the In Vitro Growth (IVG) stage. Increasing the quality of cumulus cell expansion in both stages, namely In Vitro Growth (IVG) and In Vitro Maturation (IVM).

The goat colostrum concentration of 10% (P4) demonstrates the most consistent and optimal results in improving the quality of the resulting oocytes. This innovation paves the way toward more affordable, efficient, and sustainable culture media for applications in assisted reproductive technology.

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