

In Vitro Maturation Rate of Bligon Goat Oocytes Supplemented With Gonadotropin

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ABSTRACT: Research to determine in vitro maturation rate of Bligon Goat supplemented with gonadotrophin (human chorionic gonadotrophin = hCG) in maturation medium. Cumulus-oocyte complexes of Bligon Goat were in vitro matured for 24 h in TCM 199 supplemented with 10 IU and without hCG. Oocytes were then stained with 1% aceto orcein to examine changes in the configuration of chromosomes and nuclear membrane. Oocytes were considered mature when reached metaphase II (MII), characterized by first polar body (PB1) extrusion and arrangement chromosome similar to first metaphase stage I (M1). The results demonstrated that oocytes will efficiently undergo IVM under hCG supplementation. hCG supplementation significantly increased proportion oocyte underwent maturation (69.1 ± 1.471 versus 44.2 ± 2.5 %) as also indicated by nuclear maturation and MII. Also lower rate of oocytes degeneration were observed in the medium with hCG supplementation (2.1 ± 0.3 versus 10.7 ± 2.3 %). It could be concluded that gonadotrophin supplementation was effective to improve oocytes maturation in vitro and yielding more mature oocytes for future in vitro fertilization.

Keywords: Bligon Goat Oocytes, Human Chorionic Gonadotrophin, In vitro maturation rate, metaphase II

INTRODUCTION

Bligon goat is a natively adapted goat in Indonesia. This goat breed is an established crossbreed between Etawah and local goat. The use of assisted reproductive technology to improve its reproductive efficiency is important. In vitro embryo production is alternatives technologies that need to be developed to increase its population. Obtaining oocyte from ovaries followed by in vitro maturation prior to fertilization is the initial steps of ART. A study is required to determine efficacy of ART in Bligon goat.

Ovaries were by-products of slaughterhouses were used as a source of oocytes. Oocytes obtained from those ovaries have diverse and immature stages, and required to be matured in vitro. However, the results of in vitro oocyte maturation were not always satisfactory. Follicle size, hormone, serum and growth factors in vitro maturation medium and culture conditions greatly affect the success of oocyte maturation (Velilla *et al.*, 2002 *cit.* Rahman *et al.*, 2008; Widayati *et al.*, 2014). Improvement of developmental competence of mammalian oocytes by supplementation of in vitro maturation (IVM) media with hormone and serum supplements has been the subject of many investigations. Supplementation of the IVM media with gonadotropins and estradiol has been found to be essential for acquisition of developmental capacity of oocytes in cattle (Fukushima and Fukui, 1985; Brackett *et al.*, 1989). The successful of oocytes to achieve metaphase II were

absolutely necessary for successful fertilization and pregnancy. The ability of oocytes to reach metaphase II also influenced by internal factors such as the quality of the oocyte itself and genetic. This study was conducted to determine the effect of gonadotropins on different oocyte quality of Bligon goat on their ability to reach metaphase II.

MATERIAL AND METHODS

The research was conducted in October 2014 until March 2015 at the Laboratory of Animal Physiology and Reproduction, Faculty of Animal Husbandry, Gadjah Mada University, Yogyakarta. Materials used in this study were obtained from ovarian goat abattoir (slaughterhouse) specialized sheep and goats New Babadan, located on Jl. Kaliurang KM. 7, Sleman, Yogyakarta; tissue culture medium (TCM) -199 (Gibco, USA) supplemented with penicillin, streptomycin, and bovine serum albumin (BSA); Dulbeccos' phosphate buffered saline (DPBS) (Gibco, USA); fetal calf serum (FCS); human chorionic gonadotropin (Teikoku Zouki, Japan), mineral oil; penicillin (Meiji, Indonesia); streptomycin (Meiji, Indonesia); distilled water; ; and 70% alcohol. Research equipment for IVF such as CO₂ incubator (Cole Parmer, USA), stereo microscope (Cole Parmer, USA), disposable tissue culture dish (TCD) (Falcon, USA), tube micro hematocrit non-heparin (Brand, Wertheim).

Aspiration of oocytes. Goat ovaries obtained from slaughterhouses immediately after being taken from the body and put into 31-34°C normal saline solution then taken to the laboratory. Goat oocytes were collected by aspiration of follicles using 18 G needle attached to 3 mL disposable syringe. Only cumulus-oocyte complexes grade A, B and C were used in this research.

In vitro maturation. Oocytes were cultured for 24 h in TCM 199 with hCG, 20 IU/10 mL (treatment group) or without (0 IU or control group)) in an incubator at 39 C and 5% CO₂ with humidified air. In vitro maturation rate were determined using staining with 1% aceto orcein to examine stage of oocytes maturation by changes in chromosome configuration and nuclear membrane (Karja *et al.*, 2010). Maturation oocytes status was determined based on changes in the configuration of chromosomes and nuclear membrane. Germinal vesicle (GV) characterized by nuclear membrane and nucleus clearly visible on the edges; germinal vesicle break down (GVBD) characterized by nuclear membrane rupture and nucleolus were not clearly visible; metaphase I (MI) characterized by homologous chromosome pairing and lined in equator, anaphase I (AI) characterized by the centromere toward the opposite pole and attracted homologous chromosomes into two parts, telophase I (TI) characterized by homologous chromosomes perfectly collected on each pole, and metaphase II (MII) characterized by polar body I and arrangement chromosome similar to metaphase stage I.

Statistical analysis. Data were analyzed using one way analysis of variance (ANOVA) with one way randomized completely design.

RESULT AND DISCUSSION

The cumulus-oocytes complexes recoveries were distributed in micro drops of petri dishes with maturation media (TCM 199 without (0IU/10mL) and with 20IU/10mL hCG). After 24 hours of oocytes maturation in incubator with a 5% CO₂ humidified air atmosphere, the oocytes were evaluated on the basis of cytological and morphological criteria. The mature oocytes characterized expansion of cumulus cells surrounding oocytes and the extrusion of first polar body. Gordon (2003) reported that mature oocytes indicated the expansion of cumulus cells, the germinal

vesicle breaks down and the first polar body extrusion. Expansion of cumulus cells was the most easily seen as a sign of mature oocytes. Expansion of cumulus cells was essential for successful fertilization because it can help the migration of spermatozoa between cumulus cells (Widayati *et al.*, 2013). The presence of this gonadotropin in the *in vitro* maturation medium enhances expansion of the cumulus cells surrounding the oocyte, which in terms enhances sperm capacitation and the fertilization process. Abdoon *et al.* (2001) found that FSH or eCG supplementation to the IVM medium significantly increased cleavage rate and development of buffalo embryos up to the blastocyst stage when compared with negative control medium.

After 24 hours IVM, control (0IU) showed 10.7±2.3 % degeneration and 2.1±0.3 % in treatment group (20IU/10mL). The rate of oocytes reached metaphase II in control and treatment group were 44.2±2.5 and 69.1±1.4 % respectively (Table 1).

Table 1. Effect of gonadotrophin supplementation on Bligon oocytes maturation after 24 hours in culture (%)

Stage of oocytes	Control group (hCG 0IU/10 ml)	Treatment group (hCG 20IU/10 ml)
Germinal vesicle (GV)	8.0 ± 1.1	6.0 ± 0.3
Germinal vesicle break down (GVBD)	4.8 ± 0.7	3.3 ± 0.5
Metaphase I (MI)	20.3 ± 2.6	14.5 ± 2.0
Metaphase II (MII)	44.2 ± 2.5a	69.1 ± 1.4b
Degenerate	10.7± 2.3 a	2.1 ± 0.3 b

Supplementation gonadotrophin into IVM medium was effective in stimulating the development of oocytes to mature *in vitro*. More MII oocytes were collected from treatment group than controls ($P < 0.05$). This finding was similar with the previous research that canine oocytes reached metaphase II after culture in 10 IU/mL hCG (De Los Reyes *et al.*, 2005). Enhanced cytoplasmic maturation of oocytes was obtained in some studies when gonadotropins were included in IVM media. Akçay *et al.* (2008) reported that addition of gonadotropins and E2 to a maturation medium would be necessary to improve developmental and fertilization ability of bovine oocytes *in vitro*. An increase of gonadotropin concentration in the culture medium resulted in an increase in the the percentage of oocytes reaching metaphase II, normal configuration of the spindle, normal chromosomal alignment, cortical granule migration, and mitochondrial aggregation (Sha *et al.*, 2010).

The less oocytes degeneration presented in the oocytes with hCG supplementation. The development of oocytes strongly influenced by the activity of hormone gonadotropin, namely FSH and LH (Hafez, 2000; Wattimena *et al.*, 2006). Absence of hormones resulted failure of oocytes to develop further and degenerates.

Human chorionic gonadotropin is a hormones secreted by human placenta during its pregnancy and have similarity or contains Luteinizing hormones (LH). Balasch *et al.* (1995) observed the role of luteinizing hormone in human follicle development and oocyte fertility in a woman with long-standing hypogonadotropic hypogonadism and using recombinant human follicle stimulating hormone. The results showed a direct primary role of LH in complete maturation of the follicle. *In vivo* administration prior to oocyte pick up on IVF patient showed that administration of r-LH produce more oocyte that yielded in grade 1 and grade 2 embryos. Meanwhile recombinant FSH only injection produce higher number oocyte, also when its combined with r-LH, but all treatments

produce similar number of embryos available for embryo transfer (Lisi *et al.*, 2012).

Animal study by Lu *et al.* (2014) showed that gonadotropin was widely used in in vitro oocyte maturation. Their group used bovine as model and showed no difference by adding 7.5 IU/mL and 75 IU/mL. It showed that the dose does not improve maturation rate but the presence of gonadotropin is required. In human clinical practices, gonadotropin also been used in clinical in vitro maturation a dose of 0.5 IU/mL human Chorionic Gonadotropin combined with 75mIU/mL FSH in TCM-199 supplemented with 20% FBS give a better maturation rate compare to the absence of hCG in the media (Ge, *et al.*, 2008). Sha *et al.* (2010) studied the effect of FSH and LH and hCG on porcine oocyte. The results showed that FSH and LH also hCG can significantly improve cytoplasmic and nuclear maturation in porcine oocyte, but there was a dose dependency among those hormones. The results showed combining two gonadotropin vary the maturation and ration LH:hCG may play important role.

CONCLUSION

It could be concluded that gonadotrophin supplementation was effective to improve oocytes maturation in vitro and yielding more mature oocytes for future in vitro fertilization.

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