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Evaluation of sequential changes on 1st meiotic division of goat oocytes in vitro

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In this study, the nuclear events of 1st meiosis of goat oocytes in vitro was determined. Immature COCs were cultured in two maturation medium (eg., TCM-199 medium; mSOF medium) at different time points with 3 hr interval of up to 30 hr. Sequential changes observed in the chromosome configurations were evaluated and described.In both maturation conditions, 95.4% ofoocytes were at GV stage at 0 hr. GVBD was occasionally seen starting at 3 hr (19.1%) of culture but most prominent at 6 hr (81.9%). From 9 hr -15 hr of culture, most of the oocytes were at M-1 stage. In mSOF, A-1 toT-1 stage oocytes were occasionally seen at 15 – 18 hr whereas in TCM-199, A-1 to T-1 stage oocytes presence extend up to 21 hr of culture. From 18 hr to 24 hr of culture, oocytes that completed the 1st meiosis (M-2 stage) increased progressively butthe proportion of M-2 oocytes were higher in mSOF medium (85.7 %) than in TCM-199 medium (68.9 %). The completion of 1st meiosis in both maturation conditions reached its peak at 27 hr of culture. The results provided basic informations on the completion of goat oocytes 1st meiotic division in vitro, thus facilitating the needed timing of insemination for successful fertilization of in vitro matured oocytes to occur.

Key words: Oocytes, 1st meiosis, goat, maturation, in vitro

Introduction

In vitro maturation is a valuable artificial reproductive technology that must deal with a mixed population of oocytes collected from follicles of varying stages of development, dominance and atresia. It has the potential to capture the vast supply of oocytes within an ovary for eventual production of embryos after in vitro fertilization. However, the efficiency of oocyte maturation technology is limited by the oocytes intrinsic developmental competence that allows it to be fertilized normally and develop to an embryo, which upon

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transfer will enable to produce offsprings. Thus, understanding what constitutes oocyte developmental competence, including the role of culture time in achieving both nuclear and cytoplasmic maturity of the oocytes in vitro is important. For successful fertilization to occur, the oocytes should have achieved complete maturation, since abnormalities in fertilization occur especially when the oocytes are exposed to sperm too early (still immature oocytes) or too late (aged oocytes).

In vitro embryo production in goat has not been well established due to limited informations on goat oocytes acquisition for developmental competence. Moreover, the timing of oocyte maturation and insemination in vitro is not accurate. In this study, the sequential changes occurring during the maturation of immature goat oocytes in vitro was determined to facilitate the correct timing for sperm-oocyte coincubation.

Materials and Methods

Oocyte collection and maturation

Ovaries of adult goatswere collected immediately postmortem at local abattoirs and transported to the laboratory in 0.9 % saline solution at 30 - 35°C within 4 - 6 hr. The ovaries were pooled irrespective of the donors estrus cycle. Cumulus-oocyte complexes (COCs) were aspirated from antral follicles (2 - 5 mm in diameter) by using 21- gauge needle attached to a 5- ml sterile plastic syringe, washed in Dulbecco's phosphate buffered saline (PBS) medium before selecting using a stereomicroscope based on the criteria described by Rajikin et al., (1994). The final two washings were done in the maturation medium (TCM-199 medium + 10% fetal calf serum (FCS) + 10 µg/ml FSH, LH + 1 µg/ml estradiol-17ß + or mSOF medium + 4 mg/ml BSA + 10µg/ml FSH/LH + 1 µg/ml estradiol-17-ß +). A group of 10 - 15 COCs were transferred into 50 µl droplets of maturation medium under mineral oil (Sigma Chem Co., St. Louis, USA) in a 35 x 10 mm Falcon polysterene culture dish (Becton and Dickinson Labware, N.J., USA) which had been previously pre-incubated to equilibrate for at least 2 hr in a CO_2 incubator. COCs were cultured at 39°C under an atmosphere of 5 % CO_2 and 95 % air with high humidity.

Analysis of nuclear status

During IVM, representative goatoocytes from each trial were cultured and fixed at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 hr. At the end of culture for each time point, the adhering cumulus cells were removed by pipetting and the denuded oocytes were mounted and fixed in aceto-ethanol solution (1:3 v/v), stained

with 1% aceto-orcein and examined by phase contrast microscopy (200 – 400x). The oocytes were classified accordingly based on the chronological changes in the nuclear stages namely; germinal vesicle (GV) characterized by having a single large nucleus with fine filaments of chromatin and/or condensed chromatin forming a ring around the compact nucleolus; germinal vesicle breakdown (GVBD) stage distinguished by the disappearance of a compact nucleolus, nuclear membrane and gradual condensation of chromatin material; metaphase 1 (M-1) stage, formation of individual bivalents is completed; anaphase 1 (A-1) stage, characterized by the elongation and separation of two chromosome sets towards opposite poles; telophase 1 (T-1) stage, separation of two chromosome sets is completed and metaphase 2 (M-2) stage, chromosomal alignment at the equatorial plate and emission of 1st polar body (Ocampo *et al.*, 1991). The results wereexpressed as percentages of oocytes observed at each stage of 1st meiois for each time point.

Results and Discussion

In this study, only oocytes that appear golden, golden-yellow or brownish in color with even granulation of the ooplasm that fills the entire part of the vitteline space and with a diameter of 115-125 µm(Fig. 1) were selected and used in the study. The total number of COCs cultured and fixed at each time points from three to four replicates ranged from 30 to 68. The changes in the nuclear status of oocytes cultured in TCM-199 medium is shown in Table 1. From the time of aspiration (0 hr) to 3 hr of culture, 68/69 (98.5 %) were at GV stage. Oocytes at GVBD stage were oftenly seen at 6 hr (84.6 %). M-1 stage oocytes were most common from 9 hr to 12 hr (68/74, 91.9 %) and start to disappear starting from 15 hr of culture. At this point, A-1 to T-1 oocytes were observed occasionally (14/38, 36.8 %) until 24 hr (6/53, 11.3 %). Also, M-2 stage oocytes could already be seen at 15 hr (21.1 %) and peaked at 27 hr (94.7 %) of culture.

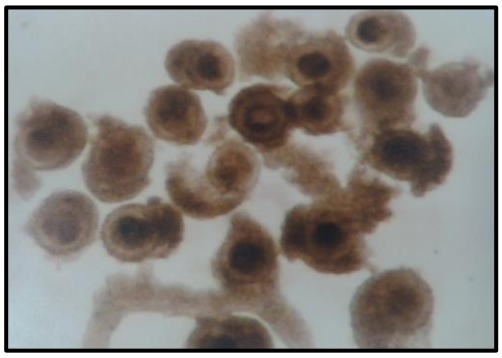

Figure 1. Good quality goat oocytes

Table 1. Sequential changes on the nuclear status of oocytes cultured in TCM-199 medium.

Culture time (hr)	Number of COCs	Stage of 1st meiosis (%)						
		GV	GVBD	M-1	A-1	T-1	Others M-2 (%)	
0	34	34 (100.0)	-	-	-	-	-	
3	35	34 (97.1)	1 (2.8)	-	-	-	-	
6	39	-	33 (84.6)	5 (12.8)	-	-	-	1 (p)
9	37	-	3 (8.1)	34 (91.9)	-	-	-	
12	37	-	-	34 (91.9)	3 (8.1)	-	-	
15	38	-	-	16 (42.1)	6 (15.7)	8 (21.1)	8 (21.1)	
18	48	-	-	12 (25.0)	2 (4.2)	9 (18.8)	25 (52.0)	
21	43	-	-	-	2 (4.7)	8 (18.6)	33 (76.7)	
24	53	-	-	6 (11.3)	1 (1.9)	5 (9.4)	41 (77.4)	
27	38	-	-	-	-	2 (5.3)	36 (94.7)	
30	52	-	-	2 (3.8)	-	2 (3.8)	48 (92.4)	

P = parthenote

Similarly, the changes on the nuclear status of oocytes cultured in mSOF medium is presented in Table 2. At 0 hr, 3 hr and 6 hr of culture, 28/31 (90.3 %), 28/36 (77.8%) and 10/39 (25.6 %) were at GV stage, respectively. GVBD stage oocytes were commonly seen at 6 hr (25/39, 64.1 %), M-1 stage oocytes at 9 hr – 15 hr (77/94, 81.9 %) and A-1 to T-1 stage oocytes from 15 hr to 18 hr (11/67, 16.4 %). Also, few M-2 stage oocytes occurred at 15 hr (13.3 %) and gradually increased to 95.6 % on the 27 hr of culture.

Table 2. Sequential changes on the nuclear status of oocytes cultured in mSOF medium.

Culture time (hr)	Number of COCs	Stage of 1st meiosis						
		GV	GVBD	M-1	A-1	T-1	Others M-2	
0	31	28 (90.3)	1 (3.2)	2 (6.4)	-	-	-	
3	36	28 (77.8)	6 (16.7)	-	2 (5.5)	-	-	
6	39	10 (25.6)	25 (64.1)	4 (10.3)	-	-	-	
9	32	-	4 (12.5)	28 (87.5)	-	-	-	
12	32	-	-	30 (93.7)	2 (6.3)	-	-	
15	30	1 (3.3)	-	19 (63.3)	4 (13.3)	2 (6.6)	4 (13.3)	
18	37	2 (5.4)	-	1 (2.7)	1 (2.7)	4 (10.8)	29 (78.4)	
21	39	1 (2.6)	2 (5.2)	2 (5.2)	2 (5.2)	-	32 (82.1)	
24	64	2 (3.1)	-	6 (9.4)	-	-	55 (86.0)	1 (p) (1.5)
27	45	-	-	2 (4.4)	-	-	43 (95.6)	
30 (4.9)	41	2	-	- (95.1)	-	-	39	

(p) – parthenote

The results presented showed the changes on the nuclear status of goat oocytes cultured in different maturation (eg.,TCM-199 and mSOF) medium during the 1st meiotic division (Fig. 2). In both condition, most of the oocytes at 0 hr of maturation were found at GV stage. Also, majority of GVBD stage oocytes showed at 6 hr of culture. These observations are comparable to those reported in sheep (Moor and Crosby, 1986), cattle (Sirard *et al.*, 1989), goat (Gal *et al.*, 1992) and buffalo (Totey *et al*., 1993). M-1 was observed between 9 hr – 15 hr, a bit earlier compared in buffalo (Totey et al., 1993; Ocampo et al., 2001) and cattle (Sirard *et al.*, 1989) at 12 hr – 15 hr. Also, we have demonstrated that the 1st meiotic division in goat could be completed as early as15 hr of culture and reached its peak just before or after 24 hr. This observation was comparable in buffalo reporting that most of the oocytes at 15 hr – 16 hr reached M-2 stage (70.0%) and at 19 hr had 87.0 % maturation rate (Neglia *et al*., 2001).

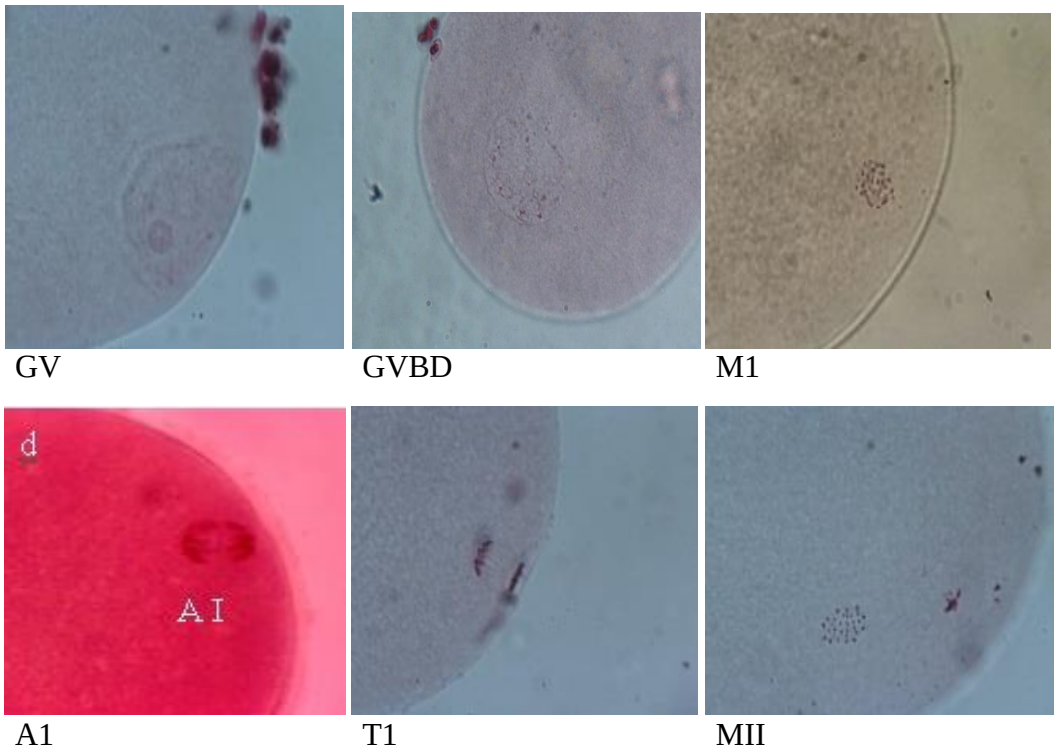

Figure 2. Nuclear stages of goat oocytes

In this study, the use of mSOF medium appeared to support the maturation of goat oocytes better than TCM-199 medium. From 18 hr to 24 hr of culture, the proportion of M-2 stage oocytes were higher when using mSOF medium.mSOF medium with vitamin addition was reported to significantly improved the maturation and subsequent developmental potential of goat oocytes in vitro (Bormann *et al.*, 2003). Incontrast, goat oocytes cultured in TCM-199 medium with hormones and serum was reported to be the most effective medium for IVM of goat oocytes (Pawshe et al., 1996; Tajik et al., 2003). In another study, using TCM-199 medium for IVM, oocytes were fixed at 4 hr intervals andhad M-2 rate of only 55.0 % at 24 hr and 73.0 % upon reaching 27 hr of culture (Rho *et al*., 2001). Other factors that could have contributed to these variabilities include the size of the follicles (De Smedt et al., 1992; 1994, Crozet et al., 1995) where oocytes were aspirated, the degree of presence or absence of cumulus cells (Han *et al*., 2008; Rahman *et al*., 2008), source of the oocytes (Izquierdo *et al*., 1999; Koeman *et al*., 2003) either from prepubertal or adult goats and size of the oocyte (Martino *et al.*, 1994; Ma *et al*., 2003, Anguita *et al*., 2007). Such variabilities could significantly influence

the outcome of embryo production from in vitro matured and fertilized oocytes. For instance, oocytes derived from small antral follicles (< 2mm) could developed up to 8-16 cell stage but do not form morula/blastocyst embryos (Han et al., 2008) or oocytes derived by aspiration had a higher potential of developing to morula/blastocyst than those derived by mincing of the ovaries (Keskintepe et al., 1994).

Overall, the results presented in this study have clarified the time required for an immature goat oocytes (115-125 µm in diameter) to complete the 1st meiotic division. That,considerable variations already exist in goat oocytes even at the start of maturation. The acquisition of goat oocytes for meiotic competence is determined by multifactor interactions that requires considerations in predicting the quality of oocytes to be used for embryo production to term.Moreover, the optimum maturation time required for efficient insemination to achieve normal fertilization has to be determined considering the observations on the length of gap on the completion of 1st meiosis in vitro in both maturation conditions used. Meiotic competence of goat oocytes can be acquired in both TCM-199 and mSOF medium.

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