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Simulation of body temperature and different gaseous phase on *in vitro* maturation and embryo development in goat

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ABSTRACT

The present study was carried out to study the effect of different simulation of body temperature during *in vitro* culture along with different gaseous phase on *in vitro* oocyte maturation, fertilization and embryo development in goat. Goat oocytes were aspirated from ovaries collected from a local slaughterhouse. The oocytes were matured in TCM-199 with HEPES, 10% FBS, 10% follicular fluid, 0.5 µg/ml FSH, 10 IU/ml LH, 0.5 µg/ml estradiol for 27h. The mature oocytes were fertilized with freshly ejaculated sperm in TALP media and cultured in mSOF media without any cell supplementation. The *in vitro* maturation, fertilization and culture (IVMFC) were done at different gas phases (5% CO_2- 20% O_2; 5% CO_2 - 10% O_2; 7.5 CO_2 - 20% O_2; 7.5% CO_2, - 10% O_2) at 38.5°C in CO_2 incubator with maximum humidity. The study indicated that comparatively better maturation rate., cleavage rate, development of 4–8 cell and morula stage embryos were observed at 39.5°C irrespective of gas phase. Similarly, the better maturation rate was observed in 5% CO_2 and 10% O_2, whereas better cleavage rate and morula were observed in 5% CO_2 and 20% as compared to other gas phases.

Key words: Body temperature, Gaseous phase, IVF, Goat

Normally IVMFC of goat oocytes are done at 37°C in a humidified CO_2 incubator at 20% CO_2. It is known that the average body temperature of goat is about 39°C and the fallopian temperature is about 0.5°C than body temperature. It is also known that different factors affect the *in vitro* development of embryos such as culture media, gas phases, i.e. O_2 and CO_2 tension, culture temperature etc. Several reports suggest that decreasing O_2 percentage and increasing CO_2 percentage improve the *in vitro* development of embryos in different species (Carney and Bavister 1987, Karja *et al.* 2004). So in the present study an experiment was conducted to observe the effect of the simulation of fallopian tube temperature and different gas phases on oocytes maturation, cleavage rate and embryo development in goat.

MATERIALS AND METHODS

Ovaries were collected from local slaughterhouse, Bareilly from goats of unknown reproductive status. They were carried to the laboratory in normal saline solution (0.85%) fortified with antibiotics (5µg/ml gentamicin) in a thermos flask within 2h of slaughter at 35–37°C. In the laboratory, ovaries were washed 5 times with normal saline solution fortified with antibiotics. Oocytes were aspirated from all visible non-atretic follicles with 18 gauge needle attached to 5 ml sterile plastic syringe containing oocyte collection medium (OCM). The cumulus oocytes complexes (COCs) along with follicular fluid were pooled into a 50 ml sterile plastic tube and were allowed to settle for 10 min. About 35 ml of supernatant was gently replaced with 35 ml of fresh OCM. This process was repeated two times. Finally, the sediments were taken in a large petri-dish (90 mm) and oocytes were searched under a sterio-zoom microscope. The cumulus oocyte complexes (COCs) were isolated, evaluated and graded. Only excellent (>5 layers of cumulus cells and evenly granulated cytoplasm) and good (>3 layers of cumulus cells and evenly granulated cytoplasm) COCs were collected and were washed in a sterile petri-dish containing 4 ml of OCM. The COCs were further drops washed 5–6 times with maturation medium. The washed COCs were cultured for 27 h in 50 µl droplets (15–20 oocytes/droplets) of maturation medium (CM-199 with HEPES, 10% FBS, 10% follicular fluid, 0.5 µg/ml FSH, 10 IU/ml LH, 0.5µg/ml estradiol) in a 35 mm sterile petri-dish in 38.5 and 39.5°C each having 5 and 7.5% CO_2 as well as 10 and 20% O_2 in water jacketed CO_2 incubator. The droplets were covered with warm non toxic Mineral oil and cultured at different culture conditions as above gas phases and temperatures. The evaluation of

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matured oocytes after *in vitro* culture for 27 h was based on the visual assessment of degree of cumulus expansion under inverted microscope. The semen was freshly collected from adult buck of proven fertility maintained in the Divisional Animal shed. After collection and transportation to the laboratory at 38°C within 10 minutes, the semen quality were assessed by the gross motility and wave like motion. The semen with >70% initial motility and ++++ motion were used for IVF. About 20 µl of ejaculated semen was taken in a centrifuge tube. About 5 ml of fertilization TALP was added to it and centrifuged at 1000 rpm for 5 minutes. Supernatant was discarded. The sperm pellet was reconstituted with 5 ml of fresh medium and centrifuged at the same rate. The supernatant was discarded again and sperm pellet was then reconstituted to 2×10^6 spermatozoa/ml. The sperm number was counted by Neubaurs counting chamber. Semen sample was then kept at 38.5°C and 5% CO_2 in humified air until co-incubation with oocytes. After maturation, the oocytes (degree 1 and 2 of cumulus expansion) were washed several times in fertilization TALP media for removal of expanded cumulus mass. After washing, oocytes from different groups were transferred into 50µl of fertilization-Talp medium and were inseminated with 50µl of processed spermatozoa. The droplets were covered with warm paraffin oil and they were kept in CO_2 incubator as above (2.5). The co-incubation was done for 18 h. After 18 h of sperm-oocyte co-incubation, the oocytes (presumptive zygotes) were separated and cultured in modified synthetic oviductal fluid (mSOF) supplemented with 0.8% BSA. The cleavage rate was observed after 42–44 h of post insemination. The cleaved embryos (2–4 cell stage) were separated and put for culture in the same media and same environment from which they were separated as detailed out in the experimental design for a period of 7 days. Every alternate day, the embryos were examined under an inverted compound microscope to evaluate the development stages.

Analyses of the data were done as per the standard procedure (Snedecor and Cochran 1989). The percentage proportion of agreement was compared statistically through Normal deviate test.

RESULTS AND DISCUSSION

Oocytes were cultured in 5% CO_2 and 20% O_2 at 38.5°C showed the oocyte maturation rate was 82.77%, 81.33% respectively (Table 1). The maturation rate was higher at 38.5°C as compared to 39.5°C although not significanty. The maturation rate was higher at 38.5°C as compared to 39.5°C as compared to 38.5°C (<0.05). The number of 4–8 cell and morula stage embryos were 5.00% and 4.4% and 12.00%. 1.27% at 38.5°C, 39.5°C, respectively. The development of morula was significantly higher at 39.5°C as compared to 38.5°C (P<0.05). When oocytes were cultured in 5% CO_2 and 10% O_2 at 38.5°C, 39.5°C, the oocyte maturation rate was 87.57%, 86.18%, respectively (Table 1). There was no significant difference in maturation rate of oocytes between 38.5°C and 39.5°C. Here the cleavage rate was 7.90%, respectively. Similarly, the number of 4–8 cell and morula stage embryos were 3.95% and 1.13% and 2.44%, 0.79%, respectively. The cleavage rate was higher (7.90%) at 38.5°C as compared to 39.5°C. When CO_2 level was 7.5% and 20%

Table 1. Effect of different temperature levels on *in vitro* oocyte maturation and embryo development in goat

Culture condition		Total no. of culturable oocytes	Oocyte maturation and embryo development		No of 4–8 cell stage embryos	No of morula
Gasseous phase	Temp		Total number of matured oocyte	No of oocytes cleaved		
CO_2=5% O_2=20%	T1	180	149^a (82.77%)	17^b (9.44%)	9 (5.00%)	8^b (4.44%)
	T2	75	61^a (81.33%)	15^a (20.00%)	3 (4.00%)	9^a (12.00%)
CO_2=5%, O_2 = 10%	T1	177	155a (87.57%)	14 (7.90%)	7 3.95%	2 (1.13%)
	T2	123	106^a (86.18%)	5 (4.07%)	-	3 (2.44%)
CO_2=–7.5% O_2=20%	T1	140	106^a (75.71%)	9^0 (6.43%)	5^{ab} (3.57%)	4^a (2.86%)
	T2	120	9^a (80.00%)	96^a (17.50%)	21^a (7.50%)	9^a (7.50%)
CO_2= 7.5%	T1	115	87^a (75.65%)	7 (6.09%)	2 (1.74%)	4 (3.48%)
O_2=10%	T2	125	103^a (82.40%)	9 (7.21%)	3 (2.4%)	6 (4.8%)

Values with common superscript in column are not significantly different to each other.

O_2, the oocyte maturation rate was higher at 39.5°C as compared to 38.5°C, however, there was no statistically significant variation of oocyte maturation rate between 38.5°C and 39.5°C (Table 1). The cleavage rate was 6.4%, 17.50% at 38.5°C, 39.5°C. The cleavage rate was significantly ($P<0.01$) higher at 39.5°C, as compared to 38.5°C. The development of 4–8 cell and morula stage embryos were higher at 39.5°C as compared to 38.5°C (Table 3). When CO_2 was increased and O_2 level was reduced (10%) the maturation rate was higher at 39.5°C as compared to 38.5°C, however, the difference was not significantly different (Table 1). The cleavage rate was 6.09%, 7.21%, respectively 38.5°C and 39.5°C. There was no significant difference of cleavage rate among the three different temperatures. Similarly, the number of 4–8 cell and morula stage embryos development was not significantly different among the three temperatures. However, the cleavage rate and further embryo development was comparatively better at 39.5°C as compared to 38.5°C as compared to 38.5°C (Table 1)

In the present study, the oocyte maturation in terms of cumulus cell expansion was better at 39.5°C as compared to 38.5°C. However the maturation rate between 38.5°C and 39.5° C was not significantly different. The cause of higher oocyte maturation rate in case of 39.5°C might be due to mimicking the higher core body temperature of goat. The reason of higher oocyte maturation rate in case of 39.5°C might be due to mimicking the higher core body temperature of goat. Further, the cleavage rate and development of 4–8 cell and morulae stage embryos were higher at 39.5°C as compared to 38.5°C. It may be due to simulation of *in vivo* condition of temperature of caprine oviduct during IVMFC. In the present experiment the better maturation rate was observed at 5% CO_2 and 10% as compared to other gas phases, which might be due to maintenance of proper pH (~7.4) in the culture media (Rivera and Hansen 2001) in this gaseous phases. Similarly, in the present experiment the better cleavage rate and percentage of morula was observed at 39.5°C in 5% CO_2 and 20% O_2. The 7.5% CO_2 level might be causing more acidity to medium as compared to 5% CO_2. The 5% CO_2 level was better for maintenance of proper pH of culture medium as compared to 7.5% (Rivera and Hansen 2001). The higher oxygen tension (20% vs 10%) during culture alters the redox state of embryos and helped in energy production via glycolysis/oxidative phosphorylation pathways (Park *et al*. 2005). In the present experiment higher oxygen tension (20% Vs 10%) improves the cleavage rate and percentage of morula as reported by Park *et al*. (2005). Yuan *et al*. (2003) observed the highest rate of bovine 8-cell embryos at 20% as compared to 2% and 5%. They reported that development to the 8-cell stage embryo was not negatively affected by high oxygen tensions (20%) but that further development to the (hatched) blastocyst stage is severely jeopardrzed.

Several reports suggest the decreasing O_2 percentage and increasing CO_2 percentage improve the *in vitro* development of embryos in different species (Carney and Bavister 1987, Karja *et al*. 2004). Brinster (1971) found no significant differences in development when mouse embryos were cultured under 1%, 5% or 10% CO_2 however, Carney and Bavister (1987) reported an approximate doubling in the percentage of embryos developed to the blastocyst stage upon raising the concentration of CO_2 gas phase from 5% to 10% CO_2 at 37°C. Increased partial pressure of oxygen generates excessive amounts of cytotoxic reactive oxygen species (ROS) and may directly affect the viability of gametes (Johnson and Nasr-Esfahani 1994). On the other hand, oxidative stress, depending on its severity, could lead to either cell necrosis or apoptosis. The hypoxic oxygen tensions (below the physiological level of 5%) do not seem to harm the embryo as shown in mice (Umaoka *et al.* 1991) and rabbits (Li and Foote 1993). In contrast, several studies have shown that the efficiency of oocyte or embryo culture could be improved by decreasing oxygen tension (Berthelot and Terqui 1996, Izquierdo *et al*. 1998, Izquierdo *et al*. 1999), whereas others were contradictory (Machaty *et al*. 1998, Khurana and Niemann 2000, Hashimoto *et al*. 2000, de Matos *et al*. 2002).

In summary, the study indicated that comparatively better maturation rate, cleavage rate, development of 4–8 cell and morula stage embryos were observed at 39.5°C irrespective of gas phase. Similarly, the better maturation rate was observed in 5% CO_2 and 10% O_2, where as better cleavage rate and morula were observed in 5% CO_2 and 20% O_2 as compared to other gas phases.

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