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Effect of season on oocyte maturation and embryo development in buffalo

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ABSTRACT

An attempt was made to find out the effect of season on oocyte maturation and embryo development in buffalo during low breeding season when environmental temperature was high. Oocytes were isolated from oocytes brought from local slaughter house during different months, viz. March (group 1), April (group 2) and May (group 3), when the environmental temperature was high. Isolated oocytes during different months were matured in TCM-199 with 10% FBS supplemented with follicular fluid and FSH (0.5ug/ml). Matured oocytes were fertilized by co incubating capacitated frozen thawed buffalo semen. After co incubation the oocytes were cultured in mSOF and cleavage rate and embryo development were noted. A total number of 1 340 buffalo ovaries were collected from local slaughterhouse, from which 1 080 culturable cumulus oocytes complexes (COCs) were recovered. The overall recovery rate of buffalo oocytes was 0.85 oocyte/ovary in the present study. The maturation rate of oocytes was 68.00 to 87.57%. The cleavage rate as well as morula stage embryo development in March (group 2), April (group 2) and May (group 3) were of 8.27 and 2.5, 5.6 and 3.1, 8.18 and 5.4% respectively. The average cleavage rate and morula stage embryo development were 9.60% and 4.60%.

Key words: Buffalo, Season, Oocyte maturation, Embryo development

There is increasing interest in optimizing embryo production in buffalo through combined techniques of *in-vitro* maturation, fertilization and culture (IVMFC). The development of a suitable IVMFC protocol is thus a paramount requirement. The IVMFC is influenced by several factors including environmental temperature. A high environmental temperature not only affects the quality of oocytes obtained from the animals but also decreases embryo production further (Nain *et al.* 2004). In the present study, the oocyte recovery, maturation and embryo development was studied during low breeding seasons.

MATERIALS AND METHODS

Collection of oocytes and maturation

Ovaries were collected from local slaughterhouse Mohanpur, Bareilly, from buffaloes of unknown reproductive status during March 2005 (group 1), April, 05 (group 2) and May 2005 (group 3). They were carried to the laboratory in normal saline solution (0.85 g %) fortified with antibiotics (5 µg/ml gentamycin) at 30–35°C in a thermos flask within 2 h of slaughter. In the laboratory, the surrounding tissues of ovaries were trimmed off and ovaries were washed several times with sterile normal saline solution fortified with antibiotics. The ovaries were then exposed to ethyl alcohol for 30 sec and finally they were washed 4–5 times with sterile normal saline solution. Oocytes were aspirated from all visible non-atretic follicles with 18-gauge needle attached to 5 ml sterile plastic syringe with OCM. The cumulus oocyte complexes (COCs) along with follicular fluid were pooled into a fresh 50 ml sterile plastic tube and allowed to settle for 20 min. About two-thirds of supernatant was gently replaced with two-third of fresh OCM. This process was repeated two times. Finally, the sediments were taken in a large sterile petri-dish (90 mm) and oocytes were searched under zoom stereomicroscope. The 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) quality COCs were taken and washed in sterile petri-dish containing 3–4 ml OCM. The COCs were further drop washed 4 to 5 times with OCM and finally with maturation medium in TCM-199. Washed 25–40 COCs were cultured in 60 µl drop of maturation media covered with sterile mineral oil in 35 mm culture dish at 38.5±1°C, 5% CO_2 and 15% oxygen under humidified air (95%) for 22–24 h in CO_2 incubator. The assessment of maturation of oocytes after *in-vitro* culture was done based on the visual assessment of degree of cumulus expansion under inverted microscope as described by Kobayashi *et al.* (1994).

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In-vitro *fertilization and embryo development*

One straw of frozen semen was thawed in warm (37°C) water for 1 min and was emptied into a 15 ml sterile graduated plastic tube. About 10 ml of TALP medium (Reed *et al.*1996) for sperm preparation was added and centrifuged at 800–1 000 rpm for 5 min. The supernatant was discarded. Washing of spermatozoa was again done by dissolving floppy sperm pellet in 10 ml of TALP. The supernatant was again discarded and the pellet was dissolved in 0.5 ml of FERT-TALP medium with fatty acid-free bovine serum albumin (BSA,6 mg/ml) and heparin (20 ug/ml). The sperm concentration was adjusted to 4–5×10^6/ml and kept in CO_2 incubator for about 45 min before inseminating matured oocytes.

After maturation, the oocytes (with degree-1 and degree-2 cumulus expansion) were washed several times in FERT-TALP media for removal of expanded cumulus cell mass. After washing, oocytes were transferred into 50 µl of FERT-TALP medium and were inseminated with 50 µl of processed spermatozoa. The droplets were covered with warm paraffin oil and placed in CO_2 incubator at 38.5°C, 5% CO_2, 15% O_2 level and 90% relative humidity. After 18 h of sperm oocytes co-incubation, the oocytes (presumptive zygotes) were washed 10–15 times in embryo development medium, viz. modified synthetic oviductal fluid (mSOF) and cultured in the same media supplemented with 0.8% BSA. The cleavage rate was recorded after 48–72 h of post insemination and further observations were made to monitor the development of embryos up to morula stage.

RESULTS AND DISCUSSION

The overall recovery rate of buffalo oocytes was 0.85 oocyte/ovary (Table 1). The maturation rates of oocytes ranged from 68.00 to 87.57% (Table 1). The cleavage rate as well as morula stage embryo development in March 2005 (group 2), April (group 2) and May 2005 (group 3) were 8.27 and 2.5%, 5.6 and 3.1%, 8.18 and 5.4% respectively. The average cleavage rate as well as morula stage embryo development were 9.6% and 4.6% respectively (Table 1).

The average maturation rate varied from 66 to 87% (Table 1). In March (group 1), the maturation rate was 68%, in April (group 2) 85% and in May (group 3) 87% respectively. In these months, cleavage rate as well as morula stage embryo development were 8.27 and 2.5% in group 1, 5.6 and 3.1% in group 2, and 8.18% and 5.4% in group 3 respectively. The average rate and morula stage embryo development were 9.1 and 4.6% (Table 2) respectively. In all groups the same maturation media were used, i.e. TCM-199 supplemented with 10% FBS, 10% buffalo follicular fluid and FSH-P (0.5 µg/ml). In buffalo oocyte culture, the maturation rate varies from 70 to 90% (Totey *et al.* 1992, Chauhan *et al.* 1999, Nandi *et al.* 2002 and Gupta *et al.* 2002). The TCM-199 supplemented with 10% FBS and FSH-P (0.02U/ml) resulted in 74% maturation in buffalo oocytes (Chauhan *et al.* 1997). Replacement of FBS with buffalo follicular fluid (20% or 40%) resulted similar rate of maturation (Chauhan *et al.* 1997). FSH in the maturation medium also has an important role in regulation of oocyte maturation via cumulus cells. Buffalo follicular fluid (BFF) contains steroid hormones, gonadotropins and growth factors like IGF-1 and EGF, which have stimulatory effect on oocyte maturation and these factors acting synergistically with FSH as autocrine and paracrine modulator of granulose cells, therefore promoting mitosis, steroidogenesis and protein synthesis (Gasparrini 2002).

In this experiment, cleavage and development of morulae stage embryo under 15% oxygen tension in March 2005 was 8.27% and 2.4% respectively. In April 2005, the cleavage rate was 5.6% and morula was 3.1%. Similarly in May 2005, cleavage rate was 8.18% and morulae were 5.4%. When all the data were pooled throughout the study, the overall cleavage rate was 9.1% and development of morulae (up to 16 cell stages) was 4.6%. Experiment in this study was conducted from March to May, and this higher environmental temperature during this period may be one of the causes of low cleavage rate. A high environmental temperature not only affects the quality of oocytes obtained from the animals but also decreases embryo production further (Nain 2004). In the present study, the maturation of oocytes was considered on the basis of cumulus expansion, which does not indicate real nuclear maturation of oocytes. However proper maturation of oocytes is a prerequisite for successful fertilization leading to embryo development (Thibault *et al.* 1997). In the present study, in spite of proper oocyte maturation, the cleavage rate was not up to the mark. *In-vitro* culture of buffalo embryos in modified synthetic oviductal fluid (mSOF) resulted in an average cleavage rate of 42% with concomitant development of 42% morula stage (Kalleshwarappa 2002) and 48% transferable embryo production (Arathy 2003). Gasparrini (2002) reported higher cleavage rate and blastocyst development in mSOF. But in

Table 1. *In-vitro* oocyte maturation, cleavage rate and embryo development in buffalo during different months of non-breeding season

Month	Number of oocyte cultured	Number of oocyte matured (%)	Number of oocyte cleaved (%)	Number of morula (%)
March 2005	342	234 (68.00)[b]	29 (8.27)	9 (2.5)
April 2005	408	329 (80.63)[a]	23 (5.6)	13 (3.1)
May 2005	320	289 (87.57)[a]	27 (8.18)	18 (5.4)
Overall	1070	852 (79.62)	79 (7.38)	30 (3.51)

his study, when mSOF was used, the incubation environment was adjusted by lowering the oxygen tension to 7%. In the present experiment, the oxygen level was maintained at 15%. Higher oxygen tension may damage the embryo development by increasing the level of reactive oxygen species (ROS) in the cells (Parchment *et al*. 1993) and this may be one of the reasons for lower transferable embryo production in mSOF medium.

Further, in the present experiment, semen from different bulls was used. Considerable variations were reported among different buffalo bulls in terms of ability of their spermatozoa to fertilize oocytes *in vitro* (Totey *et al.* 1996, Yadav *et al.* 2001). Studies confirmed that better climatic condition increases the rate of fertility. In the present study, FBS used was also of different source; this might be one of the causes for affecting the result as cleavage rate varied with the change of serum also. Further, water quality has also been observed to affect the rate of fertilization and embryo development, though in the present study, water used was from different milli-Q water system (reverse osmosis). In conclusion, the result indicated that IVMFC of buffalo oocytes were affected by the environmental conditions in buffalo.

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