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Comparative studies on effect of BSA vs FCS as a supplement in TCM-199 on *in vitro* maturation rate of buffalo oocytes

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Summary: Availability of developmentally competent buffalo oocytes is critical for *in vitro* embryo production and application of related biothecniques. The objective of the present study was to assess the effect of BSA in place of FCS as maturation media supplement on *in vitro* maturation buffalo oocytes, Oocytes were aspirated from abattoir ovarian follicles of 2-8mm diameter followed by maturation in TCM-199 supplemented with hCG, PMSG and containing either 0.4% BSA (group-I) or 10% FCS (group-II). Based on cumulus expansion maturation rate, it was assessed that among the two groups, group II showing a significant higher percentage values (89.1±3.5%) as compare to the group I (73.9±4.2%).

Key Words: Buffalo oocytes, *in vitro* maturation, BSA, FCS

Introduction

In buffalo embryo transfer has had a limited success as compared to other livestock species. Buffalo has low productive capacity evidenced by less number of follicles in the ovary (Agrawal and Tomar, 1998), high percentage of atretic follicles, change in acrosomal protein and membrane damage during freezing, because freezing buffalo semen results in acrosomal damage mediated leakage of enzymes, alteration of pH, complete withdrawal of the hydration shell of protein in solution and loss of sperm motility (Nandi *et al*., 2003).

In vitro maturation (IVM) of unfertilized oocytes have great potential for cattle breeding especially when combined with *in vitro* fertilization (IVF) and *in vitro* culture (IVC) and cryopreservation techniques . The culture medium employed for IVM is important in view of its effect on the maturation rate of follicular oocytes and also on embryonic development following IVF (Bavister *et al*., 1992). The commonly used media are complex buffered with bicarbonate or HEPES and supplemented with various sera and /or gonadotrophin (FSH/LH) and/or steroid (estradiol 17ß) hormones. It is known that the culture conditions employed for IVM of mammalian oocytes can significantly influence IVF rates and subsequent embryonic development (Brackett *et al*., 1989; Abdoon *et al*., 2001). Various types of medium viz., TCM-199 (Singh *et al*., 1989; Totey *et al*., 1993; Madan *et al*., 1994; Nandi *et al*.,2000), Ham's F-10 (Singh *et al*., 1989; Totey *et al*., 1993), MEM (Abdoon *et al*., 2001) have been commonly used for IVM-IVF studies in buffaloes. Among them, TCM-199 is the most commonly used medium . These complex medium cannot support oocyte maturation on their own and are usually supplemented with hormones (Totey *et al*., 1993; Nandi *et al*., 2000), sera (Bacci*et al*., 1991; Lu and Hsu, 1991; Madan *et al*., 1994; Totey *et al*., 1996) or follicular fluid (Chauhan *et al*., 1997) which introduce many known and unknown substances to the IVM medium for proper maturation. In the present studies we have evaluated the effect of Bovine serum albumin (BSA) and Fetal calf serum (FCS) as a supplements in TCM-199 as a basic *in vitro* maturation medium for buffalo oocytes.

Materials and Methods

Collection of ovaries

Buffalo ovaries were collected from Delhi abattoir in sterile normal saline solution (NSS) supplemented with antibiotics (Penicillin 100 IU/ml, streptomycin 50µg/ml, Hi-Media, India) at 30-35 ºC in a thermos flask and transported to the laboratory within 4h of slaughter.

Retrieval of oocytes

In the laboratory, the surrounding tissues were trimmed off and the ovaries were washed 4 to 5 times with sterile and warm (30-35 ºC) NSS. The ovaries were then exposed to 70% ethyl alcohol for 2 seconds and finally washed with phosphate buffered saline and immediately soaked with paper towel (Hurtt et al., 2000). Oocytes from ovarian follicles of 2 to 8 mm in diameter were aspirated using 18G needle attached to 5ml sterile disposable syringe (Dispovan, India) containing 0.5ml aspiration media.

The aspiration media consisted of phosphate buffered saline (Gibco, USA) supplemented with 0.4% fatty acid free embryo tested bovine serum albumin (BSA)

(Sigma, USA) and antibiotics. The aspirated suspension were poured into a sterile 90mm petri dish (Greiner, Germany) having 4 to 6 ml aspiration media. The dishes were searched under stereo zoom microscope (Olympus, Japan).Cumulus-oocytes complexes (COCs) were located and picked up from the petridish. These oocytes were then washed serially three times with TCM-199 (Gibco, USA), supplemented with 0.4% BSA or 10% fetal calf serum (FCS) (Gibco, Mexico) as per requirement in 35mm petri dish (Greiner, Germany) containing 2 ml of the maturation media and finally transferred into washing drops of 100µl in a 60mm petri dish for a serial wash in 3 drops horizontally. The COCs were graded as described by Nandi *et al.* (1998):

Grade-I: Compact cumulus oocyte complexes with unexpanded cumulus mass having □ 5 layers of cumulus cells and homogenous evenly granular ooplasm.

Grade-II: COCs similar to Grade-I but with 2-4 layers of cumulus cells.

Grade-III: Oocytes with partially denuded or completely devoid of cumulus cells and having an irregular dark ooplasm.

Grade-IV: Oocytes with highly expanded or scattered cumulus cells and an irregular dark ooplasm.

The oocytes of Grade I and II were used for *in vitro* maturation within 2 hours of their removal from the follicles. Oocytes were matured in two groups. Group-I represents oocytes matured in TCM-199 supplemented with 0.4% BSA and Group-II those matured with10% FCS supplementation.

In vitro **maturation of oocytes**

The maturation media included TCM-199 that was supplemented with either (a) 0.4% BSA or (b) 10% FCS (heat inactivated in water bath at 56 ºC for 30 min) and streptomycin (100 µg/ml, Penicillin 100 IU/ml, Sigma, USA), L-Glutamine (200mM, Sigma, USA) and hormones PMSG (10 IU/ml) and hCG (10 IU/ml) (Sigma, USA). After 2 times washing with 2 ml maturation media (without hormone supplement) in 35mm sterile petri dish 15-20 COCs were randomly placed in 100µl of maturation drops (media + hormone) (Shamsuddin *et al*., 1993) in 35mm sterile petri dish. The maturation drops were covered with warm (35-37 ºC) light weight mineral oil (Sigma, USA) and kept for 24h in CO_2 incubator at 38.5 °C under a condition of 5% CO_2 in air with a relative humidity of 90 to 95%. Maturation of the oocytes were evaluated after 24h of culture to access the degree of cumulus cell expansion under stereo zoom microscope and also the appearance of the polar body using the methods described by Kobayashi *et al*. (1994) and Nandi *et al.* (1998).

Degree -0 (slight or no expansion of cumulus cells): oocytes having cumulus cells tightly adherent to the zona pellucida,

Degree -1 (moderate cumulus cell expansion): oocytes having expansion of the cumulus cell mass to 2-diameter away from zona pellucida, cells were homogenously spread and cluster cells were still observed.

Degree-2 (full cumulus cell expansion): oocytes showed enlargement of cumulus cell mass to at least 3-diameter away from the zona pellucida, cells were homogenously spread and clustered cells were no longer present.

Results

A total number of 1954 oocytes were cultured for 24 h in a CO_2 incubator with 5% CO_2 in air under humidified conditions at 38.5 °C and their maturation was assessed by the expansion of their cumulus cells and formation of polar body (Fig.1). Based on cumulus expansion maturation rate was assessed in two groups which resulted in 819 matured out of 1108 oocytes cultured in TCM-199 supplemented with 0.4% BSA (group-I) and 754 oocytes matured out of 846 cultured in TCM-199 with 10% FCS (group-II), showing 73.9±4.2% and 89.1±3.5 maturation rates in both groups, respectively (Table 1).

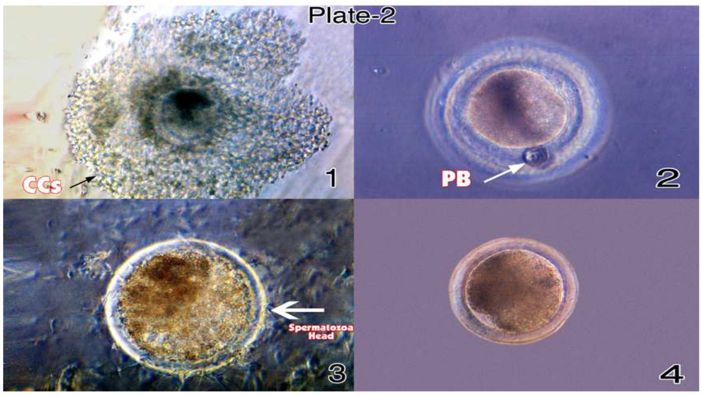

Fig.1. Expansion of cumulus cells.

Table 1. Oocytes maturation rates in TCM-199 with supplements 0.4% BSA vs 10% FCS

TCM-199 Supplements	**No. of oocytes used**	**No. of oocytes matured**	**% Maturation rate**
0.4% BSA	1108	819 (±6.6)	73.9 (± 4.2)
10% FCS	846	754 (± 4.2)	89.1 (± 3.5)

Discussion

It has been accepted that the expansion of cumulus cells to be important to achieve complete oocyte maturation since it was correlated to the fertilization rate and developmental potential in ovine and bovine oocytes (Saigmiller and Moor, 1984; Cox *et al.,* 1993). Based on cumulus-cell expansion, in the present study, about 73.9± 4.2% in group-I and 89.1± 3.5% in group-II were considered to mature after 24h of *in vitro* maturation.

In buffalo oocytes 80 per cent maturation has been reported by Totey *et al.* (1993b) in TCM-199 supplemented with FSH, LH and estradiol. Thus, the result in the present study are comparable with the one observed by Totey *et al.* (1992, 1993a), Singh *et al*. (1989), Chauhan *et al.* (1991), Madan et al. (1994 b) and Das *et al.* (1996b). Brackett *et al.* (1989) reported 95- 100 per cent maturation of cow oocytes in the above medium. When the medium is supplemented with other supplements like hormones and follicular fluid the maturation rate becomes higher 95-100% (Chauhan *et al.*, 1997). The difference in success rate of maturation rates may be due to a number of factors like health of oocytes at the time of collection, physiological and nutritional status of slaughtered animals, time taken during transportation of ovaries and composition of the medium. Das *et al.* (1996b) and Chauhan *et al.* (1997) did not have desired maturation rates from buffalo oocytes in TCM-199 with FCS and FSH.

Fukuda *et al.* (1990) reported 74 per cent maturation rate for bovine oocytes cultured in TCM-199 supplemented with 10% bovine estrus serum (BES). Totey *et al.* (1993b) also reported maturation rate of 76% when oocytes were matured in TCM-199 supplemented with hormones and BES.

Cumulus cells are important during oocytes maturation and contribute to the production of cytoplasmic maturation factor (Vanderhyden and Armstorng, 1989) and prevention of hardening of zona pellucida (De Felici and Siracusa, 1982). Additionally, the cumulus cells secret the non-sulfated glycosaminoglycan and hyaluronic acid (Ball *et al*., 1983), when stimulated by FSH, which causes their dispersion forming a mucous matrix between and around cumulus cells. Hyaluronic acid promotes the acrosome reaction of epidydimal bovine spermatozoa (Handrow *et al.*, 1982) and the dispersed cumulus cells make ease for sperm cells to reach the oocytes, although oocytes without expanded cumulus cells have been fertilized *in vitro* with high frequency (Schroeder and Eppig, 1994; Sirard *et al.*, 1988).

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