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The Effect of Different Supplements to *in vitro* Maturation Medium on Nuclear Maturation Rate of Buffalo Oocytes Assessed based on Stage of Nucleus

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A B S T R A C T

For comparing the efficacy of serum gonadotropin @50 and 100IU/ml, estrodiol-17β @0.5 and 1µg/ml, EBS @5 and 10% and BFF @5 and 10% supplementation on *in vitro* nuclear maturation rate of buffalo oocytes. Ovaries were collected from buffaloes slaughtered at local abattoir. Oocytes were aspirated and good and excellent quality oocytes were matured in basic maturation media TCM199 supplementing above mentioned supplements separately. The recovery rate of culturable oocytes was 85.33%. The nuclear maturation rate (NMR) was highest in the oocytes supplemented with 10% EBS and lowest with estradiol-17β @1µg/ml. The statistical analysis revealed that NMR was significantly ($p \leq 0.05$) higher in the group supplemented with 10% EBS as compared to all other groups. It can be concluded that the supplementation of EBS to IVM medium has positive effect on nuclear maturation rate. Overall results suggest that serum gonadotrophin is less effective than EBS but more effective than estradiol-17β and BFF. Buffalo follicular fluid is more effective than estradiol-17β but less effective than EBS and serum gonodotrophin. Estrodiol-17β is the least effective supplement to increase *in vitro* nuclear maturation rate of buffalo oocytes.

Introduction

The buffalo forms the backbone of India's dairy industry and is considered as the 'bearer cheque' of the rural flock and India's milking machine (Balain, 1999). As per the 19th livestock census India has around 108.7 million of buffalo population and constitutes around 21.23% of total livestock population. Buffalo population of Karnataka is 33.06 lakhs. Buffalo contributes 51% (132430 tonnes) of total nation's milk production and around 1103.85 tonnes of meat production of total nation's meat production (Department of

Animal Husbandry Dairying and Fisheries, ministry of agriculture, New Delhi,2014).

The buffalo is the predominant domestic animal for milk and meat production. On average, buffalo is four times as productive as an average indigenous cow in India. India has world's best buffalo dairy breeds and provides superior buffalo germplasm to several countries of the world (Kaikini, 1992).The domestic water buffalo plays a key role in milk production in many Asian countries including India, and they are able to withstand the adverse environmental conditions of the tropics. However the buffalo are traditionally considered to have a low reproductive efficiency (Pankaj, 2015). The low reproductive efficiency in female buffalo can be attributed to delayed puberty, higher age at calving, long postpartum anoestrus period, long calving interval, lack of overt sign of heat, and low conception rate. In addition, female buffaloes have few primordial follicles and a high rate of follicular atresia. These factors are responsible for driving a large number of buffaloes to slaughter house either prior to maturity or after calving, which has resulted in enormous loss of genetic resources and significant decline in their number in recent years. Thus there is a need to improve reproductive performances of buffaloes.

In vitro maturation (IVM) of oocytes and *in vitro* fertilization (IVF), *in vitro* culture of embryos and embryo transfer technology appear to be useful techniques for the improvement of reproductive efficiency of buffaloes (Uma, 1997). Embryo transfer technology has made rapid strides in dairy cattle industry and has become viable commercially in some of advanced countries. Therefore, the emphasis has now shifted to *in vitro* embryo production (IVEP) as it can salvage the genetic potential from infertile female and can yield large number of embryos (Kumar and Anand, 2012).Ovaries of the slaughtered animals are the cheapest and the most abundant source of primary oocytes for large scale production of embryos through *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) (Agrawal *et al.,* 1995).

In vitro maturation (IVM) is the one of the essential step and starting point for lot of biotechnological applications in animals like *in vitro* fertilization (IVF), cloning, transgenic animal production and embryonic stem cell research. Oocytes maturation is the process of complex changes in the protein phosphorylation which transform the primary oocytes in to mature secondary oocytes. Maturation of the oocytes included two aspects viz., nuclear and cytoplasmic maturation.The nucleus and the cytoplasm of the oocytes undergo many changes during maturation, making it receptive to fertilization and competent to support embryonic development (Tomek *et al.,* 2002).

Several workers have studied different aspects of IVM in mammalian oocytes. In most of the studies revealed that media designed originally for cell culture, are not capable of supporting high levels of oocytes maturation and, therefore, are commonly supplemented with sera and hormones, growth factors and antioxidants to improve the maturation rates at high level (Kumar and Anand, 2012). The maturation medium with the selection of protein supplements, hormones for IVM play an important role in subsequent IVF and *in vitro* development.

The oocytes maturation process involves the activation and inhibition of enzymes, hormones and growth factors, which results in nuclear and cytoplasmic maturation (Gilchrist *et al.*, 2007). Nuclear maturation occurs spontaneously, and mechanical removal of the oocytes from the follicle is capable of triggering the process, but cytoplasmic

maturation occurs more gradually (Brevini *et al.,* 2007). Hormones like luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogens are frequently used as additives in maturation media to enhance the quality of the maturation process.

The identification of substances capable of delaying the nuclear maturation time and thus allowing cytoplasmic and nuclear changes to occur synchronously has been the subject of several studies. Follicular fluid (FF), consisting of electrolytes, hormones, amino acids, growth factors, among other components, has been used as a natural substance for blocking the meiosis (Aguilar *et al.,* 2001). Supplementation of serum in media had favourable effect on maturation. The serum contains a number of known growth factors that have an important role in the regulation of oocyte maturation, particularly via cumulus cells, it also prevents the hardening of the zona pellucida; moreover, the beneficial action of serum may be due to its antioxidant properties (Mahmoud and Nawito, 2003). Estrus buffalo serum (EBS), a rich source of hormones and growth factors, could be used to improve the developmental competence of buffalo oocytes culture *in vitro* (Abid *et al.,* 2008).

An attempt to induce *in vitro* maturation can only be successful in conditions that are similar to those existing *in vivo*. Therefore, the biochemical composition of the oocyte culture media has great influence on the success rate of *in vitro* maturation. Since, under *in vivo* conditions the oocytes maturation is influenced by various steroid and gonadotropic hormones, it is necessary to supplement the steroid and hormones to the medium in order to sustain the growth and development of oocytes either by directly or through the hormone rich serum medium. Therefore the development of a suitable culture system and components of maturation media for *in vitro* maturation of oocytes is a major component of the *in vitro* embryo production procedures.

Perusal of literature indicates less number of works on optimizing the *in vitro* maturation of buffalo oocytes as compared to the other species. Considering all these points present study was designed to investigate the effect serum gonadotrophin, estradiol-17β, estrous buffalo serum (EBS) and buffalo follicular fluid (BFF) supplementation on *in vitro* nuclear maturation rate.

Materials and Methods

All the media and chemicals were procured from Himedia Laboratories, Mumbai unless otherwise stated. Folligon® (Pregnant Mare Serum Gonadotrophin, 1000IU/vial) and Chorulon® (Human Chorionic Gonadotrophin, 1500IU/vial) were purchased from Intervet International B.V., Boxmeer, Netherland. The disposable plastic wares used in this experiment were obtained either from Tarsons Products Private Limited, Kolkota or from Himedia Laboratories, Mumbai.

All the media were prepared by using sterile tissue culture grade water. All the working solutions/media excluding OCM were kept for at least 3-4hr in CO_2 incubator at 38°C, 5%CO_2 and 95 % relative humidity for quenching before use. Prepared OCM was kept in BOD at 37°C. The stocks of media were stored at 4°C and used within one month.

Sterilization procedures

Sterilization of all media was done by filtering through 0.2µm syringe driven filters. The glassware and micropipette tips were sterilized by autoclaving at 121ºC for 30min. Fresh sterilized and disposable culture bottles, petridishes, tubes and syringe were used every

time. All the equipments were exposed to UV light for 15 minutes before use. In order to avoid bacterial and fungal contamination all the procedure except the aspiration of oocytes, starting from oocyte searching to *in vitro* culture works and media preparation were carried out in highly sterile condition under laminar flow cabinet.

Preparation of buffalo serum and estrous buffalo serum (EBS)

The blood was collected from jugular vein of normal cycling buffaloes that are not in estrus as well as from the buffaloes that are in estrus for the separation of buffalo serum and estrus buffalo serum respectively. The collected blood was kept in slant position and allowed to clot, later blood was centrifuged at 3000rpm for 15min for serum separation. The separated serum was heat inactivated at 56°C for 30minutes, filtered through 0.45µm filter and stored at -20°C in 2.0ml micro centrifuge tubes as aliquots for future use. Same batch of pooled serum was used for all the trials. The buffalo serum was used in the preparation of working oocyte collection medium (OCM) whereas EBS was used as supplement in T5 and T6 groups.

Colection of buffalo follicular fluid (BFF)

Ovaries were collected immediately after slaughter of buffaloes of unknown reproductive status at the local abattoir. The ovaries were maintained in a thermos flask containing warm (35-37°C) normal saline (0.9% NaCl) fortified with 50µg/ml gentamycin sulphate (Gentalab). The ovaries were transported to the laboratory within 2 hrs after slaughter of animals. At the laboratory, the buffalo ovaries obtained from the abattoir were rinsed thoroughly with fresh sterile normal saline supplemented with gentamycin @50µg/ml 5-6 times and final wash was done with Phosphate buffered saline (PBS). Follicular fluid was aspirated from non atretic, surface follicles (>3mm diameter) of the ovaries using 5ml syringe attached with 18-gauge needle. The pooled follicular fluid was allowed to settle for 10mins and the supernatant was collected. The collected follicular fluid was sterilized by filtering through 0.22µm syringe driven filter and stored in sterile micro centrifuge tubes of 2.0ml capacity at -20°C for subsequent use for IVM as supplement in T7 and T8 groups.

Collection of ovaries and semen sample

Buffalo ovaries were collected immediately after slaughter of the animals of unknown reproductive status at the local abattoir. The ovaries were maintained in a thermos flask containing warm (35-37°C) normal saline (0.9% NaCl) fortified with 50µg/ml gentamycin sulphate (Gentalab). The ovaries were transported to the laboratory within 2 hrs after slaughter of animals.

The frozen buffalo bull semen straws supplied by Department of Animal Husbandry and Veterinary Services, Government of Karnataka to the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Bidar were utilized for *in vitro* fertilization.

Methods

Oocytes collection, processing and grading

Ovaries were collected immediately after slaughter of adult female buffaloes of unknown reproductive status at the local abattoir. The ovaries were maintained in a thermos flask containing warm (35-37°C) normal saline (0.9% NaCl) fortified with 50µg/ml gentamycin sulphate. The ovaries were transported to the laboratory within 2hrs after slaughter of animals. In the laboratory, ovaries were washed 6-7 times in warm

normal saline solution (37°C) fortified with antibiotic and final two washings with PBS. Washed ovaries were submerged in PBS solution in sterile beaker and were used for oocyte collection. Oocytes were aspirated from all visible non atretic follicles (2-8mm in diameter) by an 18gauge needle attached to 5ml sterile disposable syringe (Dispovan, India) containing 0.5ml oocyte collection media (OCM) (Appendix-I). The cumulus oocyte complexes (COC) along with follicular fluid was pooled into 50ml sterile plastic tube containing 1-2ml OCM at 37°C and were allowed to settle for 10minutes.

Finally the sediments were taken in large petridish (90mm) and oocytes were searched under zoom stereo microscope (Motic, Germany). The cumulus oocyte complexes (COC) 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) COC were collected and washed several times in OCM followed by maturation media (MM) (Appendix II). The recovery rate of oocyte was calculated by dividing the sum of excellent and good quality oocytes recovered with total number of ovaries aspirated.

$$\text{Ooctye recovery rate (\%)} = \frac{\text{Total no of excellent and good quality oocytes}}{\text{Number of ovaries aspirated}} \times 100$$

In vitro maturation of oocytes

Graded oocytes were washed in respective maturation media for 4-5 times. After washing, 15-20 oocytes were cultured in 50µl droplets of respective maturation media in 35mm sterile petridish. The droplet was covered with warm, non toxic mineral oil and incubated at 38°C, 5% CO_2, 95% relative humidity for 24hrs in CO_2 incubator (Nuaire, USA). The experiment was repeated 6 times for each group as replicates.

The selected oocytes were cultured in TCM 199 medium containing 10%FCS, 0.3% BSA and 10IU/ml of hCG with different supplements as shown in Table No 1.

Assessment of nuclear maturation of oocytes

After 24h of culture in maturation medium, oocytes were denuded by treating with TCM-199 containing 0.1% hyaluronidase and passing them through a fine pipette. Denuded oocytes were washed twice with WM (2% FCS) and mounted on slides in a microdroplet (10-20µl) between 2 parallel lines of wax:vaseline (1:20). A cover slip was placed on the lines and pressed gently using a needle until it touched the microdroplet containing the oocytes. Gentle pressure was applied to anchor the oocytes between the slide and cover slip. Oocytes were fixed for 24h in a mixture of acetic acid and alcohol at 1:3 ratios at room temperature. Then fixed oocytes were stained for 10min with 1% (w/v) orcein in 45% acetic acid. After 3-5min, destaining solution (acetic acid:distilled water:glycerol: 1:3:1) was passed thoroughly to remove excess stain, making sure that the oocytes were not washed away. The cover slip was sealed with DPX mountant and stained oocytes were examined under a light microscope (400×) and phase contrast microscope to determine the stages of nuclear maturation, i.e. germinal vesicle (GV) and germinal vesicle breakdown. Oocytes with a distinct nuclear membrane and no detection of chromatin material were categorized as GV stage. Germinal vesicle breakdown (GVBD) was detected by either deeply stained chromatin at the mitotic plate (M I) or deeply stained chromatin with the presence of 1 or 2 polar bodies (M II). Nuclear maturation was

calculated by dividing number of GVBD (M-I and M-II stage) oocytes with total number of matured oocytes stained and expressed in percent nuclear maturation.

$$\text{Nuclear maturation (\%)} = \frac{\text{Number of GVBD+M-I and M-II stage oocytes}}{\text{Total number of matured oocytes stained}} \times 100$$

Results and Discussion

Nuclear maturation rate

Nuclear maturation rate was assessed as GV, GVBD, MI and MII stages. Oocyte with a distinct nuclear membrane and no detection of chromatin material were categorized as GV stage. Germinal vesicle breakdown (GVBD) was detected by either deeply stained chromatin at the mitotic plate (M I) or deeply stained chromatin with the presence of 1 or 2 polar bodies (M II) (Plate 6; Table 4 and 5).

Out of 24 oocytes assessed for nuclear maturation in each group, mean percentage of oocytes in GV stage for serum gonadotrophin @50IU/ml, serum gonadotrophin @100 IU/ml, estradiol-17β @0.5µg/ml, estradiol-17β @1µg/ml, 5% estrus buffalo serum, 10% estrus buffalo serum, 5% buffalo follicular fluid and 10% buffalo follicular fluid supplementation were 33.33±8.33, 25.00±9.12, 41.67±5.27, 54.17±10.03, 29.17±7.68, 12.50±5.59, 45.83±7.68 and 41.67±5.27 respectively. GVBD stage was observed in 8.33±5.27, 29.17±7.68, 20.83±7.68, 12.50±5.59, 8.33±5.27, 20.83±7.68, 12.50±5.59 and 16.67±5.27 percentage of oocytes respectively in T1, T2, T3, T4, T5, T6, T7 and T8 groups. The percentage of oocytes reaching MI stage of nuclear maturation were 12.50±5.59, 20.83±7.68, 16.67±8.33, 12.50±5.59, 25.00±9.12, 25.00±6.45, 8.83±5.27 and 12.50±5.59 in T1, T2, T3, T4, T5, T6, T7 and T8 groups respectively. The mean percentage values of oocytes showing MII stage with polar body were 45.83±7.68, 25.00±6.45, 20.83±7.68, 20.83±7.68, 37.50±10.70, 41.67±5.17, 33.33±5.27 and 29.17±4.16 for T1, T2, T3, T4, T5, T6, T7 and T8 groups respectively.

For the comparison among the groups, the nuclear maturation rate was assessed as the total number of oocytes showing GVBD, MI and MII with polar body stages. The mean percentage nuclear maturation rate in serum gonadotrophin @50IU/ml, serum gonadotrophin @100 IU/ml, estradiol-17β @0.5µg/ml, estradiol-17β @1µg/ml, 5% estrus buffalo serum, 10% estrus buffalo serum, 5% buffalo follicular fluid and 10% buffalo follicular fluid supplemented groups were 66.66±8.33, 75.00±9.12, 58.33±5.27, 45.83±10.03, 70.83±7.68, 87.50±5.59, 54.16±7.68 and 58.33±5.27 respectively (Table 4; Plate 9).

The mean values of nuclear maturation rate was highest for the oocytes cultured in IVM medium supplemented with 10% estrus buffalo serum and the lowest nuclear maturation rate was observed with supplementation of estradiol-17β @1µg/ml among all the experimental groups. The statistical analysis of the data revealed that cytoplasmic maturation rate was significantly ($p \leq 0.05$) higher in the group supplemented with 10% estrus buffalo serum as compared to all other groups of the experiment. There was no significant ($p \leq 0.05$) difference in the nuclear maturation rate among the groups supplemented with serum gonadotrophin @50 IU/ml, serum gonadotrophin @100IU/ml, estradiol-17β @0.5µg/ml, estradiol-17β @1µg/ml, 5% estrus buffalo serum, 5% buffalo follicular fluid and 10% buffalo follicular fluid.

The comparison of mean values of nuclear maturation rate between two different concentrations of each supplement suggested that there was no significant ($p \leq 0.05$) difference between serum gonadotrophin @50IU/ml and @100IU/ml, estradiol-17β @ 0.5µg/ml and @1µg/ml, 5% and 10% buffalo follicular fluid. Whereas, IVM medium supplemented with 10% estrus buffalo serum showed significantly ($p \leq 0.05$) higher nuclear maturation rate than that supplemented with 5% estrus buffalo serum.

In sexually matured females, after the leutinizing hormone surge, the fully grown oocytes in graffian follicles tends to initiate and complete the first meiotic division which leads to meiotic metaphase II. On the other hand, oocytes isolated mechanically from the follicles are found to resume meiosis spontaneously *in vitro.* Oocyte maturation is the process associated with the initiation of germinal vesicle breakdown and completion of first meiotic division and entering into second meiotic division and reaching metaphase II. Recent studies indicate that there are some growth factors which initiate the meiotic maturation *in vitro*. These growth factors are capable of promoting maturation of bovine cumulus oocyte complexes. Hormone and serum supplementations are also essential for promoting maturation of bovine oocytes complexes (Totey *et al.,* 1992). In the present study serum gonadotrophin, estrodiol-17β, estrous buffalo serum and buffalo follicular fluid supplementation are used to compare their efficacy on *in vitro* nuclear maturation of buffalo oocytes

In vitro maturation

Serum gonadotropin

Supplementation of serum gonadotrophin @100IU/ml to IVM medium showed no significant effect in terms of nuclear maturation where as supplementation of the same @50IU/ml did not show much beneficial effects. Tsafriri *et al.,* (2005) stated that in many mammalian species, gonadotrophins are found to stimulate cumulus cells to synthesize molecules able to drive germinal vesicle breakdown GVBD as meiosis- activating sterols. It is suggested that the presence of gonadotrophins in the maturation media increases the level of intracellular cAMP, the activity of the hyaluronic acid synthesis enzyme system and induced cumulus expansion complexes. The results of the present work are in agreement with the findings of several researchers in different mammalian species. Mattioli *et al.,* (1991) concluded from their study that gonadotrophins accelerated and facilitated meiotic progression selectively improved cytoplasmic maturation in porcine oocytes which is required to promote the formation of a female pronucleus. Momena *et al.,* (2010) reported that IVM of goat oocytes is influenced by the supplementation of gonadotrophin in TCM-199 medium. Farag *et al.,* (2013) showed that the supplementation of gonadotrophin (PMSG-hCG) to culture media significantly ($P < 0.05$) improved meiotic maturation rate of camel denuded oocytes than that cultured in hormone-free media. Elkhadrawy *et al.,* (2014) examined maturation rate of buffalo oocytes in the medium supplemented with different doses of purified FSH. Their results indicated that the maturation rate of buffalo oocytes were significantly ($P<0.001$) higher in TCM-199 supplemented with 6.25, 12.5 and 25 µg/ml of purified FSH than the control and highest maturation rate obtained by addition of 25µg/ml purified FSH.

Estrodiol-17β

The mean values of nuclear maturation rate in the groups supplemented with estradiol-17β

@ 0.5µg/ml and 1µg/ml concentration were 58.33±5.27 and 45.83±10.03 respectively. The supplementation of estradiol-17β @1µg/ml showed the lowest nuclear maturation rate among all the experimental groups. This inhibitory effect of Estradiol-17β on oocyte maturation is observed by McGaughey (1977) in pigs, Eppig and Koide (1978) and Dianne and Tenney (1980) in mouse. Estradiol-17β inhibited the cAMPphosphordiesterase activity of mouse oocyte in a concentration-dependent manner (Kaji, 1987). This finding provides an explanation for the inhibitory effect of steroid hormones on germinal vesicle breakdown (GVBD) of mouse oocytes *in vitro*. Similar to the present results Tesarik *et al.,* (1995) reported that in humans the addition of E2 to oocyte maturation medium did not produce any apparent effects on either germinal vesicle breakdown or further progression of meiosis, but it did increase the fertilization and cleavage rates of the *in vitro* matured oocytes. Beker *et al.,* (2002) matured bovine oocytes in TCM199 in the presence of 1µg/ml E2 with or without 0.05 IU/ml recombinant hFSH. They concluded that supplementation of 1µg/ml E2 to a serum free maturation medium negatively affects bovine oocyte nuclear maturation and subsequent embryo development and these negative effects of E2 could be attenuated in the presence of FSH. Anna *et al.,* (2004) have also observed a significant decrease in the percentage of oocytes that reached MII stage when COCs of bovine oocytes were cultured in the presence of E2 or E2- BSA. Estadiol 17-β supplementation also delayed or inhibited oocyte meiotic maturation, such as chromosome alignment on the metaphase plate and extrusion of the first polar body (Wataru *et al.,* 2014).In contrast Fukui *et al.,* (1982) have reported increased *in vitro* maturation rate of bovine oocytes in the presence of estrodiol in culture medium. Similarly, Zheng *et al.,* (2007) have reported improved oocyte developmental competence in rhesus monkey oocytes when they were cultured in *in vitro* maturation medium supplemented with estradiol.

Estrus buffalo serum

the nuclear maturation rates in the groups supplemented with estrus buffalo serum @ 5% and 10% concentrations were 70.83±7.68 and 87.50±5.59 respectively. Among the eight experimental groups under study the mean values of nuclear maturation rate was highest in the oocytes cultured in IVM medium supplemented with 10% estrus buffalo serum. The beneficial effect of supplementation of estrus buffalo serum to IVM medium on oocyte maturation and subsequent better cleavage rate could be attributed to the fact that estrus buffalo serum contains various hormones like FSH, LH and E2. Biological role of estrus serum is to compensate for whatever essential elements are missing from the medium by serving as a reservoir for many of the beneficial components, such as different energy substrates, steroids, amino acids, fatty acids, vitamins and growth factors. Serum also serves as a protective compound against scavenging ions and small molecules secreted from the developing embryo. Samad *et al.,* (1998) evaluated four types of serum supplements viz., estrus cow serum (ECS), estrus buffalo serum (EBS), pro estrus buffalo serum (PrBS) and post estrus buffalo serum (PtBS) added to TCM-199 for *in vitro* maturation of buffalo follicular oocytes. They recorded oocytes maturation rate of 80.00, 82.08, 78.77 and 66.23 % respectively and concluded significantly high maturation rate in EBS supplemented group followed by ECS, PrBS and PtBS supplemented groups. Suresh and Maurya (2000) observed the maturation rate of buffalo oocytes in TCM-199 containing foetal calf serum, bubaline estrus serum and buffalo calf serum and concluded that the supplementation

of bubaline estrus serum can enhance the buffalo oocyte maturation *in vitro*. Anthony *et al.,* (2013) examined the maturation capacity of buffalo, sheep and goat oocytes in the media containing sera of three different groups of buffaloes (regularly cycling, pregnant and repeat breeding). The oocytes maturation rate of buffalo oocytes cultured in media containing sera of the control group and regularly cycling group were not significantly different. However when oocytes cultured in the media containing sera of pregnant buffaloes the maturation rate were significantly declined. Further significant declined in maturation rate were observed when oocytes cultured in media containing sera of repeat breeding buffaloes.

Buffalo follicular fluid

The mean nuclear maturations in groups supplemented with buffalo follicular fluid @ 5% and 10% concentrations were 54.16±7.68 and 58.33±5.27 respectively. The nuclear maturation rate of oocytes supplemented with buffalo follicular fluid was significantly lower than that of estrus buffalo serum supplemented groups.

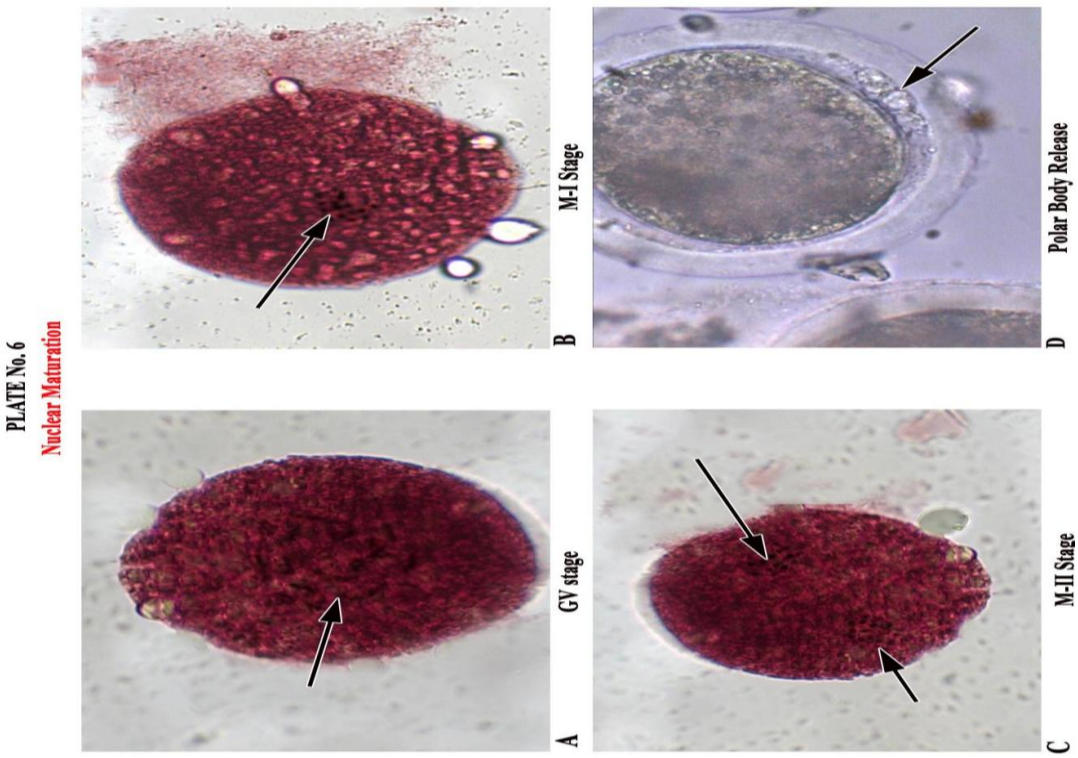
Table.1 Composition and supplements of the maturation media in different groups

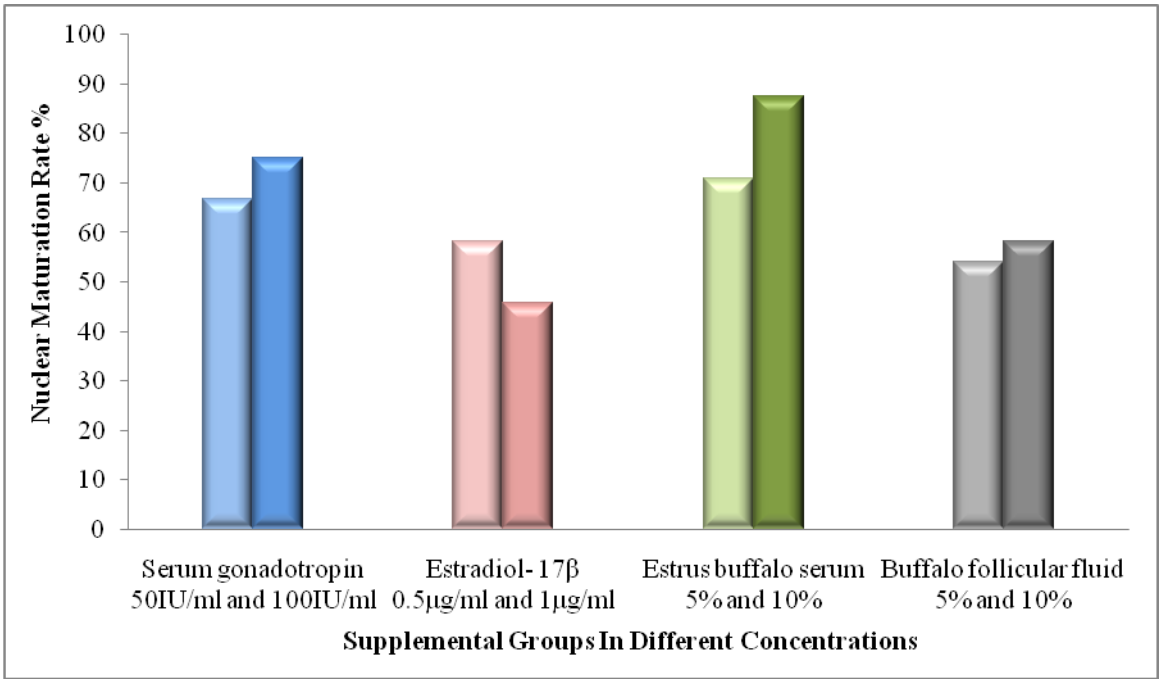
Groups	Supplements	Detailed Composition of maturation medium
T1	Serum Gonadotrophin @50 IU/ml	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + serum gonadotrophin @50IU/ml
T2	Serum Gonadotrophin @100 IU/ml	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + serum gonadotrophin @100IU/ml
T3	Estradiol-17β @0.5µg/ml	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + Estradiol-17β @0.5µg/ml
T4	Estradiol-17β @1µg/ml	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + Estradiol-17β @1µg/ml
T5	5% Estrous buffalo serum	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + 5%EBS
T6	10% Estrous buffalo serum	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + 10%EBS
T7	5% Buffalo follicular fluid	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + 5%BFF
T8	10% Buffalo follicular fluid	TCM 199+10% FCS+ 0.3% BSA+ hCG @10IU/ml + 10%BFF

Table.4 The effect of different supplements to IVM medium on nuclear maturation rate (%) of buffalo oocytes assessed based on stage of nucleus (Mean±SE)

Groups	No. of replicate	Number of oocytes assessed	Stage of nuclear maturation				Nuclear maturation rate
			GV	GVBD	M-I	M-II with PB	
T1	6	24	33.33±8.33	8.33±5.27	12.50±5.59	45.83±7.68	66.66±8.33^{bc} (16)
T2	6	24	25.00±9.12	29.17±7.68	20.83±7.68	25.00±6.45	75.00±9.12^{b} (18)
T3	6	24	41.67±5.27	20.83±7.68	16.67±8.33	20.83±7.68	58.33±5.27^{bc} (14)
T4	6	24	54.17±10.03	12.50±5.59	12.50±5.59	20.83±7.68	45.83±10.03^{bc} (11)
T5	6	24	29.17±7.68	8.33±5.27	25.00±9.12	37.50±10.70	70.83±7.68^{bc} (17)
T6	6	24	12.50±5.59	20.83±7.68	25.00±6.45	41.67±5.17	87.50±5.59^{a} (21)
T7	6	24	45.83±7.68	12.50±5.59	8.83±5.27	33.33±5.27	54.16±7.68^{bc} (13)
T8	6	24	41.67±5.27	16.67±5.27	12.50±5.59	29.17±4.16	58.33±5.27^{bc} (14)

a,b,c: Mean values of nuclear maturation rate with different superscripts differ significantly (p≤0.05)





The observation of Stephen *et al.*, (1984) that the follicular fluid transiently suppresses the germinal vesicle breakdown (GVBD) in mouse oocytes by elevating cAMP in the oocyte cumulus cell complex supports the present findings. Mammy *et al.*, (2012) conducted *in vitro* maturation of buffalo oocytes in TCM 199 supplemented with 10% bovine follicular fluid (BFF), 5% bovine serum albumin (BSA) or without supplementation. They concluded that supplementation 10% BFF and 5% BSA significantly increased the maturation rate than the control group. The discrepancy in the results of present experiment with others may be because of variations in the size and maturational status of the follicles from which the follicular fluid was collected. Because, Ayoub and Hunter (1993) collected bovine follicular fluid at different stages of the estrous cycle from small, medium, and large follicles. Follicular fluid from both small and medium follicles at estrus had the greatest ability to prevent germinal vesicle breakdown but became less potent at postestrus. Follicular fluid from large follicles at estrus had less ability to inhibit germinal vesicle breakdown than fluid from small and medium follicles. However, follicular fluid from large follicles had less germinal vesicle breakdown inhibiting activity at proestrus than follicular fluid from large follicles at late metestrus, early diestrus, and mid diestrus.

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