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In Vitro Maturation of Bubaline Oocytes in Three Different Culture Media (TCM 199, Ham's F 10 and Way mouth MB medium)

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ABSTRACT

In order to evaluate the effect of media on *in vitro* maturation, culturable grade buffalo oocytes were matured *in vitro* in three different media (Ham's F-10, Waymouth MB and TCM-199) with same supplements (5µg/ml FSH, 5µg/ml LH, 1µg/ml estradiol, 25 mM Hepes, 0.25 mM pyruvate and antibiotics). The overall mean culturable grade oocyte recovery was 3.12±0.20. At the end of experiment all oocytes in all groups were fixed and stained to evaluate the nuclear status. Oocytes were considered mature if they were at metaphase II. Significantly higher ($P<0.01$) proportion of oocytes were matured *in vitro* in Waymouth medium compared to Ham's F-10. In TCM-199, IVM rates were non-significantly higher compared to Ham's F-10 and non-significantly lower compared to Waymouth MB medium. It was concluded that Way mouth MB medium is the most appropriate medium for *in vitro* maturation of buffalo oocytes followed by TCM-199 and Ham's F 10.

Keywords: *In vitro* maturation, buffalo oocytes, follicles, *in vitro* embryo production

Superovulatory responses and subsequent embryo recoveries in buffalo had been low due to some inherent problems like low number of primordial follicles, deep atresia of antral follicles, poor palpable characteristics of the follicular structures and seasonal variation in fertility (Purohit *et al*., 2003; Abd-Allah *et al.,* 2013). Such limitations have led to increased interest in the *in vitro* production of embryos employing *in vitro* maturation, fertilization and culture (Abd-Allah, 2003; 2011 and Gasparrini, 2007). Buffalo *in vitro* maturation rates were low, but improved with the addition of buffalo serum (Chauhan *et al*., 1998; Jamil *et al*., 2007; Hammam *et al*., 2010) or hormones (Totey *et al*., 1993; Jamil *et al*., 2007; Hammam *et al*., 2010) in the culture medium. However, addition of cumulus cells alone did not improve the oocyte maturation rate (Das *et al*., 1997). Expensive components of *in vitro* maturation (IVM) medium, such as fetal calf serum and hormones, were successfully replaced by steer serum and follicular fluid (Nandi *et al*., 2002). The effect of supplements in the medium such as cysteamine (Gasparrini *et al*., 2000), growth factors (Purohit *et al*., 2005) and PMSG (Gupta *et al*., 2001) were observed to promote *in vitro* maturation and subsequent fertilization of buffalo oocytes. The commonly used media for IVM of buffalo oocytes is TCM 199 (Purohit and Sharma, 2002; Mehmood *et al*., 2011; Deneke *et al*., 2012; Deneke *et al*., 2013) however since this media is complex other culturable media such as Ham's F 10 (Totey *et al*., 1992, Hammam *et al*., 2010) DMEM (Kumar and Purohit, 2004; Hegab *et al*., 2009) Ferti Cult medium (Hegab *et al*., 2009), Ham's F 12 (Jamil *et al*., 2007) have been experimented with variable results. The objective of this study was to compare the in vitro maturation of buffalo follicular oocytes in three different media TCM-199, Hams F-10 and Waymouth MB medium.

Materials and Methods

Buffalo ovaries (n= 166) were collected from local abattoir in warm (37-39˚C) Dulbecco's PBS containing 0.1% antibiotics and transported to laboratory within an hour. In the laboratory, ovaries were rinsed with warm (32-37˚C) 0.9% NaCl. Surface follicles from the ovaries were aspirated as per previously described procedures (Kumar and Purohit, 2004) to collect the oocytes. Recovered oocytes were examined under a microscope and considered culturable grade if they had evenly granular cytoplasm and 2-3 or more layers of cumulus cells attached to them. Culturable grade oocytes were matured in three different media TCM 199, Way mouth MB medium and Ham's F 10 (Sigma chemical co, USA) under three different groups, each with 15 replicates. Each medium was supplemented with 5

µg/ml FSH, 5 µg/ml LH and 1 µg/ml estradiol. Oocytes were randomly allotted to the above treatment and after serial washings in washing and maturation media, were matured in 50-100 µl drops of maturation media under paraffin oil at 38.5 ± 1°C temperatures, 5% CO_2 in an incubator for 24 hours. On completion of *in vitro* maturation all the oocytes were fixed and assessed for nuclear maturation as per previously described procedures (Kumar and Purohit, 2004; Purohit *et al.,* 2005). Briefly oocytes were considered mature if they were at the metaphase-II stage. Comparison of oocytes reaching M-II or other stages was done by F – test. The percentage of oocytes matured for 3 groups was also carried out by F–test. Arc sine transformed data of the proportion of oocytes reaching each stage were analyzed by ANOVA.

Results and Discussion

A total of 607 oocytes were recovered by aspiration of follicles from 166 buffalo ovaries obtained from an abattoir. Out of these only 517 oocytes were of culturable grade and were subsequently used for *in vitro* maturation using three different media TCM 199, Way mouth MB medium and Ham's F 10 medium. The overall oocyte recovery was 3.65± 0.24 and the mean culturable grade oocytes recovery was 3.11 ± 0.20. The total number of oocytes that were fixed and stained on completion of *in vitro* maturation was 175, 156 and 186 for Ham's F 10, Way mouth MB (WMB) and TCM 199 media respectively. The oocytes evidenced cumulus expansion at completion of IVM. Nuclear status evaluation of oocytes revealed that the number and proportions of oocytes that were arrested at GV for Ham's F 10 media was highest followed by TCM 199 and was lowest for Way mouth MB media (Table 1). The number and proportion of oocytes that were arrested was highest for TCM 199 followed by Ham's F 10 and lowest for Way mouth MB media. Analysis of variance revealed that significantly higher ($P<0.01$) number of oocytes were arrested at GV stage in the Ham's F 10 and TCM 199 media compared to the Way mouth MB media.

Analysis of variance revealed that significantly higher ($P<0.01$) number of oocytes matured *in vitro* (reached M-II stage) in Way mouth MB media compared to Ham's F 10 media. The number of oocytes that matured *in vitro* was non significantly higher ($P>0.01$) in Way mouth MB media compared to TCM 199 suggesting better performance of oocytes in Way mouth MB media.

Table 1. Number and proportion of buffalo oocytes that did not regain meiosis (GV), were arrested (MI and AT-I) or were matured *in vitro* (M-II) in three media.

Medium	Number of oocytes	Number of replicates	Mean number of oocytes at different stages		
			GV	M-I + AT-I (Arrested)	M-II
Ham's F-10	175	15	1.66 ± 0.27^{b} (14.29%)	2.46 ± 0.38^{a} (21.14%)	7.53 ± 0.66^{a} (64.57%)
Waymouth	156	15	0.20 ± 0.106^{a} (1.92%)	1.86 ± 0.25^{a} (17.95%)	8.33 ± 0.35^{b} (80.13%)
TCM-199	186	15	1.20 ± 0.10^{b} (9.67%)	2.93 ± 0.28^{a} (23.65%)	8.26 ± 0.62^{ab} (66.70%)

Values in parenthesis represent percentage

Values with a, b superscript within column are significantly different ($P < 0.01$) whereas values with ab superscript are non-significantly different from a and b ($P > 0.01$).

The overall mean total and culturable oocytes recovered from buffalo ovaries during the present study was 3.65 ± 0.24 and 3.11 ± 0.20 oocytes respectively. Similar recovery rates were observed in a few studies on buffalo (Kumar *et al*., 1997; Samad and Raza, 1999; Hammam *et al*., 2001; Mistry and Dhami, 2009). However a large number of previous studies had recorded a lower culturable grade oocyte recovery rates varying from 0.4-2.17 (Totey *et al*., 1992; Das *et al*., 2005; Gupta *et al*., 2006; Mehmood *et al*., 2011). The reasons for differences in the oocyte recovery rates are diverse and include reproductive status of the animal from which they are retrieved, presence or absence of CL, season of recovery and recovery procedure adopted (Das *et al*., 1996; Gasparrini, 2007; Mehmood *et al*., 2011). Palta and Chauhan (1998) had mentioned that a lower number of primordial follicles, a low population of antral follicles and a high incidence of deep atresia contribute to a lower and variable oocyte recovery in buffalo.

During the present study the proportion of oocytes that matured *in vitro* (reached M-II stage) was significantly ($P<0.01$) higher for Waymouth MB medium (80.13%) compared to Hams F-10 (64.57%) and non-significantly higher compared to TCM-199(66.70%). The overall maturation rates (all three media) obtained during the present study were 70.01 percent. Similar maturation rates were recorded in many previous studies on buffalo oocytes matured *in vitro* (Nandi *et al*., 2002; Suresh

and Maurya, 2005; Ullah *et al*., 2006; Jamil *et al*., 2007; Sadhan *et al*., 2010; Leal *et al*., 2010).

Previous studies on buffalo oocyte maturation *in vitro* have shown TCM-199 to be better over Hams F-10 (Totey *et al*., 1993; Hegab *et al*., 2009; Jamil *et al*., 2007; Hammam *et al*., 2010). The beneficial effect of TCM-199 on IVM may be related to some factors in its composition such as essential amino acids and glutamine that stimulate DNA and RNA synthesis and enhance cell division (Pawshe *et al*., 1996; Mahmoud and Naby, 2013).

Waymouth medium was found to support *in vitro* maturation of buffalo oocytes even better to TCM-199. Xu *et al*. (1992) have previously shown that Waymouth medium yielded better cleavage rates compared to TCM-199 during bovine *in vitro* embryo development.

A previous study on buffalo oocytes (Purohit *et al*., 2005) had recorded comparable *invitro* maturation rates in both TCM-199 and Waymouth medium.

The proportion of oocytes not resuming meiosis (GV stage) during the present study varied between 1.92 to 14.3%. Madan *et al*. (1994a) had similarly observed that between 9.23-17.8% of the buffalo oocytes do not resume meiosis in media with different supplements. Purohit *et al*., (2003) also recorded that 2-9% of buffalo oocytes do not regain meiosis. Leibfried Rutledge (1999) have explained that oocytes selected for IVM either have or have not acquired a complete program for development at the time of their recovery from ovarian follicles. This biological program is either intact or has undergone decay, and hence IVM protocols can barely improve the oocytes developmental competency if their developmental programs have suffered decay. It was concluded that Way mouth MB medium is the most appropriate medium for *in vitro* maturation of buffalo oocytes followed by TCM-199 and Ham's F 10.

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