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Effect of Ovarian Status on Oocyte Quality and Recovery Rate Retrieved by Aspiration Method in Buffalo Ovaries

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A B S T R A C T

A study was taken up to know the effect of corpus luteum (CL) on the oocytes retrieval, quality in buffalo ovaries. Ovaries were collected from apparent healthy slaughtered buffalo during breeding season and categorized based on the presence (or) absence of CL. The cumulus oocyte complexes (COCs) were aspirated aseptically from the follicles of >6 mm diameter present on the surface of the ovary by using 18 G needle attached to 5 ml disposable syringe containing 2 ml of collection media. The oocytes were graded based on the presence of the cumulus cells complex around the oocytes. The mean A grade (3-5 cumulus cell layer) oocyte recovery rate per ovary was 1.35 from the ovaries with CL, while those without CL were 1.97, and the mean oocyte recovery rate per ovary was 1.78. In conclusion, an ovary without CL was found to be more suitable for harvesting higher number and superior quality of COCs.

Introduction

Buffaloes contribute about 60% of the total milk production of India. Buffaloes in India are spread over almost all parts of the country with varying population density, majority (72%) being concentrated in the north and western states. According to the 19th livestock census (2012), total buffalo population in India is 108.7 million (NDDB, 2015-16), ranking number one in the world with huge genetic diversity. Buffaloes are preferred over cattle in India, because of their distinctive qualities such as better feed conversion efficiency, more resistance to diseases and higher milk fat percentage than in cows (Banerjee, 1998). However, they suffer from many reproductive issues like delayed puberty, postpartum ovarian inactivity and seasonality which cause great economic loss to the farmer and are the main obstacles in rearing this species.

Low reproductive efficiency characterized by low conception rate and high embryonic mortality is a major problem in buffaloes.

Therefore, there is an imminent need to utilize recent advances in the field of reproduction to overcome these problems.

Assisted reproductive techniques (ART) were helpful in producing offspring from valuable farm animals that were considered less fertile using standard breeding techniques. ART include artificial insemination, embryo transfer, *In Vitro* fertilization, embryo cryopreservation, sexing of semen and embryos, cloning, transgenic technology, stem cell technology, embryo-genomics, micro and nanotechnologies. The recent scientific developments in ART have made it possible to manipulate the reproductive processes in many ways to revolutionize world animal agriculture. Efforts have been made to enrich the knowledge about various recent assisted reproductive techniques which may be helpful for improving the current status of livestock reproduction (Chakravarthi and Sri Balaji, 2010; Verma *et al.*, 2012 and Hansen, 2014).

In Vitro maturation is a method in which immature oocytes are retrieved from the ovary and are allowed to mature in the laboratory which is a crucial step for fertilization and subsequent embryonic development (Meirelles *et al.,* 2004 and Dadarwal *et al.,* 2015). However, IVM of buffalo oocytes obtained poor recovery of good quality immature oocytes (Palta and Chauhan, 1998). So, the present study was taken up to assess the number of oocytes recovered per ovary with CL and without CL recovered from abattoir-collected buffalo ovaries.

Materials and Methods

Chemicals and media

All media, hormones and chemicals were sourced from Sigma Chemical Co., USA, and plastic ware was from Nunc, Denmark, HEPES buffered tissue culture medium 199 supplemented with 10% PBS (Handling medium) was used for washing and handling of oocytes. Heparin (25 IU/ml) was additionally added to the handling medium for collection of oocytes. Bicarbonate buffered tissue culture medium 199 (TCM199B) supplemented with gentamicin (50 µg/ml) was used as control medium for maturation of oocytes. The media used for transport and washing of ovaries, collection, handling, and maturation of oocytes were supplemented with gentamicin (50 µg/ml) and filter sterilized (0.22 µm) before use. Handling, collection, and maturation media were equilibrated with 5% carbon dioxide in air, in a humidified atmosphere at 38.5°C for at least 2 h before use.

Ovaries collection and oocytes collection

Collection and processing of ovaries for aspiration of cumulus oocyte complexes (COCs) were carried out as described by Shahid *et al.,* (2014). The ovaries were washed with phosphate buffered saline (PBS P 4417, Sigma USA) and kept in sterile polythene sachets containing warm (37°C) PBS. These sachets were transported to the laboratory within 1-2 hr after collection in a thermos flask containing warm water (37°C). External surface of the each ovary was sterilized by rinsing once in 70% alcohol and thrice in D-PBS. The COCs were aspirated aseptically from the follicles of >6 mm diameter present on the surface of the ovary by using 18 G needle attached to 5 ml disposable syringe containing 2 ml of collection medium. The COCs having homogenous cytoplasm and surrounded by more than three layers of compact cumulus cells were considered as good quality oocytes.

Statistical analysis

Oocyte recovery rate was analysed by Two-Sample t-Test by assuming Equal Variances

Results and Discussion

The present study was carried out with a total number of 1371 ovaries that were collected from the local slaughter house in Hyderabad during January-April 2017, of which 1010 ovaries were without corpus luteum (CL) (73.67%) and 361 ovaries were with CL (26.33%). A total of 2400 good quality (those oocytes having more than three layers of cumulus cells) oocytes were recovered from the ovaries out of which 1909 oocytes were recovered from the ovaries without CL and 491 oocytes were recovered from the ovaries with CL. The oocyte recovery rate per ovary was 1.35 from the ovaries with CL, while those without CL were 1.97, and the mean oocyte recovery rate per ovary was 1.78 (Table 1).

In the present study, a total of 1371 ovaries were collected during the experimental period from which 2400 good quality oocytes were recovered with a mean of 1.78 oocytes per ovary. These results were in agreement with that reported by Das *et al.*, 1996 and Samad *et al.*, 1998, who reported mean recovery rates of 1.7 and 1.76 respectively in buffaloes. However, higher oocyte recovery rates 2.2, 2.65 and 3.12 in buffaloes were reported by Tasripoo and Kamonpatana (1997), Shahid *et al.*, (2014) and Ruhil and Purohit (2016) respectively. On the contrary, lower mean oocyte recovery values in buffaloes were reported by Totey *et al.*, (1992), Madan *et al.*, (1994) and Singh *et al.*, (2001) which are 0.7, 0.42, and 0.54 respectively. The reasons for these differences could be due to the reproductive status of the donor animal from which the oocytes were recovered (Madan and Raina, 1984; Totey *et al.*, 1992 and Mehmood *et al.*, 2011), the season of recovery along with the recovery procedure adopted (Sharma and Loganathaswamy, 2007 and Mehmood *et al.*, 2011), the geography, the number of ovaries processed and the method of selecting ovaries from the slaughter house (Sharma and Loganathaswamy, 2007).

Buffaloes are usually more sensitive to environmental changes when compared to other livestock owing to poor thermo-regulation. The degree of seasonal variation (particularly ambient temperature and relative humidity) on the breeding efficiency of buffaloes is well pronounced (Mahmoud and El-Naby, 2013).

The collection of majority of ovaries was carried out during relatively hot season (March-April), and this may be the reason behind lower mean oocyte recovery rates achieved here, when compared to the higher recovery rates reported by other workers.

Table.1 Number of oocytes and oocyte recovery rate

	No. of ovaries	No. of oocytes	Oocyte recovery rate (per ovary)
Ovaries with CL	361	491	1.35
Ovaries without CL	1010	1909	1.97
Total No. of ovaries	1371	2400	1.78

('P' value at 48 degrees of freedom and 5% level of significance is zero *i.e.*, oocyte recovery rates of ovaries with CL and without CL differ significantly)

Fig-A Collection of ovaries

Fig-B Trimming of ovaries

Fig-C Ovary with CL

Fig-C Ovary without CL

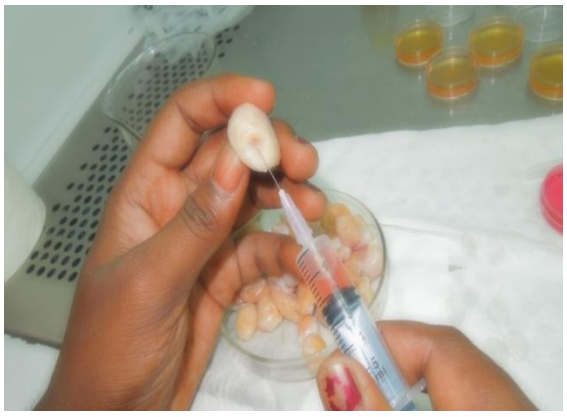

Fig-E Oocytes retrieved by Aspiration Method

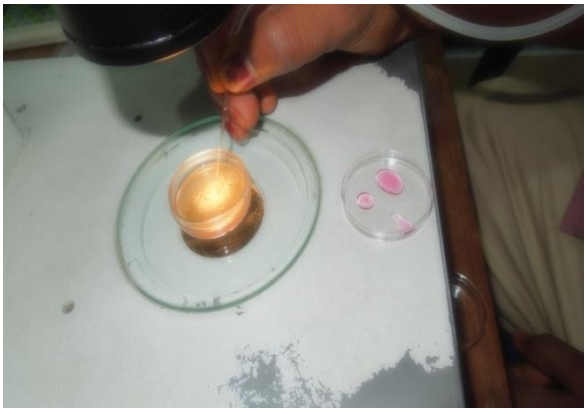

Fig- F Oocytes collected from Handling Medium

The mean oocyte recovery rate obtained from the ovaries with CL (1.35) was significantly ($P \leq 0.05$) lower than that obtained from the ovaries without CL (1.97). Similar trend was reported by Das *et al.*, (1996) and Raza *et al.*, (2001) recorded that the presence of a CL significantly reduces the number of ovarian follicles as well as the quality of oocytes in buffaloes. This is because follicular development is restricted, as lutein cells

occupy most of the ovary (Kumar *et al.*, 1997).

Oocytes can be retrieved by three methods *viz.* slicing, puncture and aspiration (Pawshe *et al.*, 1994). Mehmood *et al.*, (2011) and Shahid *et al.*, (2014) reported that aspiration method is better than others for IVM studies as we can get developmentally competent oocytes from the follicles on the surface rather than from the cortex. Moreover, slicing method results in large amount of debris in the culture medium which adversely affects the IVM of buffalo oocytes (Shahid *et al.*, 2014). Hence, aspiration method was chosen for oocyte recovery in the present study.

Only good quality oocytes (possessing more than 3 layers of granulosa cells) were chosen for *In Vitro* maturation in this experiment, as increase in the percentage of good quality oocytes increases the cumulus expansion and maturation rates. The oocyte quality is also determined by its ability to mature, get fertilized and give rise to normal offspring (Mahmoud and El-Naby, 2013).

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