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Effect of Season on Average Number of Culturable Oocytes Recovered From Cattle and Buffalo Ovaries

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Abstract.- In this experiment average number of culturable oocytes recovered from buffalo (*Bubalus bubalus*) and cattle ovaries during different seasons were determined. Cattle and buffalo ovaries were collected from local slaughter house and transported to the laboratory at 28°C in phosphate buffer saline (PBS) solution within two hours of slaughtering. The ovaries were sliced with a ten unit blade slicer in PBS supplemented with 0.4% bovine serum albumin (BSA) and 250 iu/L heparin. Average number of good quality cumulus oocyte complexes (COCs) recovered in cattle and buffalo were 3.9±0.21 and 1.9±0.14 per ovary, respectively. A significant difference has been observed between average number of good quality oocytes in cattle and buffalo. The number of COCs recovered in different seasons were significantly ($P<0.05$) lower in summer in cattle (3.41±0.15 vs 4.4±0.07) and buffalo (1.6±0.16 vs 2.2±0.1). The average number of culturable oocytes in cattle were higher than that of buffalo, moreover both are affected by the season.

Key words: Average number of oocyte, culturable oocyte, cattle and buffalo,

INTRODUCTION

The production performance of livestock breeds reflects the level of social; economic, scientific and technological development of the country. Although, there are plenty of bovine genetic resources in Pakistan, most bovine breeds are native breeds with lower productivity. As a result, the issue of improving bovine breeds has assumed considerable importance in Pakistan agricultural circles. In this respect the use of *in vitro* matured oocytes in combination with *in vitro* fertilization for the production of livestock embryos in the laboratory is rapidly increasing (Hasler *et al.,* 1995).

Different methods are used for the recovery of oocytes form the ovaries for *in vitro* maturation *i.e.* trans-illumination (Arav, 2001), aspiration (Khan *et al.,* 1997) and slicing method (Kumar *et al*., 1997). Considering the available resources and harvesting a large number of oocytes at a time from ovarian follicles, slicing method is the best one (Hamano and Kuwayama, 1993). The slicing method is not in common use in Pakistan. No data is available on the recovery rate using this method in our local breeds. Moreover, the effect of season is also to be investigated, both in endemic as well as exotic breeds in this subtropical environment. The current study has been designed to investigate the number of good quality oocytes recovered by slicing from cow and buffalo ovaries with key emphasis on variation (in number) of good quality oocytes with respect to season.

MATERIALS AND METHODS

Phosphate buffer saline (PBS) solution; $MgCl_2.6H_2O$, 0.1g; KCl, 0.2g; KH_2PO_4, 0.2g; NaCl, 8.0g; Na_3PO_4 (anhydrous), 1.15g; D-(+)-Glucose, 1.0g; Streptomycin sulphate, 0.05g; $CaCl_2.2H_2O$, 0.133g; Pyruvic acid, 0.036g; Penicillin G. Na $1x10^6$ units mixed in one titer distilled water, pH adjusted at 7.2 filtered by a membrane of 0.22 micron pore size was prepared one day before the collection of ovaries from the slaughter house.

Ovaries were collected within 45 minutes after the slaughter of the animals, and kept in a container having PBS, at a temperature ranging from 28-30°C (Otoi *et al.,* 1993). Cattle and buffalo ovaries were collected in separate container. The further processing was carried out in Animal Reproduction Laboratory, National Agriculture Research Center, Islamabad.

Ovaries were washed two times with fresh

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PBS. Large follicles (in the size range of 2-6 mm diameter) and corpora leutea were removed in order to get the cumulus oocyte complexes (COCs) of almost the same sizes prior to slicing. Ovaries were sliced with the help of a forceps and ten unit blade slicer, in a condition that they were half dipped in the BS solution having 0.4% BSA and 250 iu/L heparin at 28°C. All the ovaries were sliced one by one in this way. Prior to this, the number of ovaries of each group (cattle and buffalo) was counted.

All the liquid containing COCs was put into a 100 ml glass beaker carefully and was allowed to settle down for 15 minutes. The upper surplus liquid was sucked till it reduced to 25 ml, more PBS was added and allowed again to settle. In this way, they were washed two to three times till the solution became clear. Finally, it was filtered with common mesh of pore size 1 mm in order to remove any surplus tissue. All the material was put into a Petri dish and searched for COCs under stereomicroscope. Oocytes having following characters were collected: (a) surrounded by more than six layers, (b) evenly granulated, (c) round in shape, (d) no apparent sign of degeneration and (e) no injury on the ooplasm membrane as done by Hamano and Kuwayama (1993).

Statistical analysis

The number of oocytes recovered in cattle and buffalo and the variations in their number with respect to season were analyzed by applying t-test using Systat Statistical Package (10.2 version, America).

RESULTS AND DISCUSSION

During these experiments, 172 ovaries of cattle were collected. The average number of good quality oocytes based on their morphology, recovered per ovary were 3.9±0.21 (Table I). Good quality oocytes were preferred for in vitro culture as suggested by Hamano and Kuwayama (1993). Similarly, 134 ovaries of buffalo were collected, the average number of culturable oocytes recovered per ovary were 1.9±0.14 (Table I). A significant difference ($P<0.05$) was found between the mean number of oocytes recovered per ovary between cattle and buffalo (Fig. 1).

Table I.- Average number of culturable oocytes recovered in cattle and buffalo.

	Cattle	Buffalo
Total no. of ovaries sliced	172	134
Total No. of oocytes recovered	654	272
No. of oocytes recovered per ovary (Mean±SE)	3.9±0.21	1.9±0.14

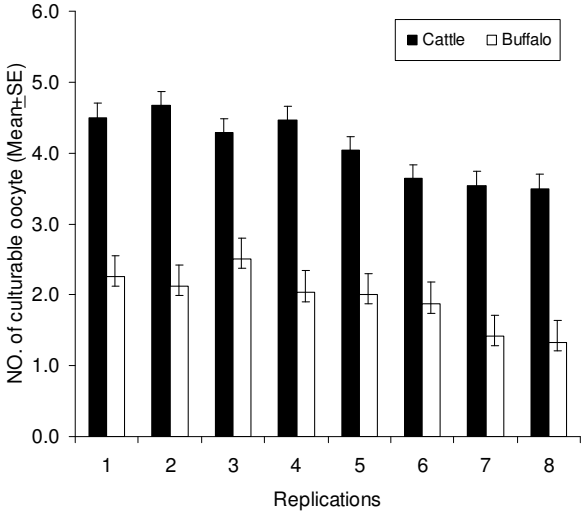

Fig. 1. Comparison of culturable oocytes recovered from buffalo and cattle ovaries.

These results are also comparable with Arav (2001) who obtainded 4.9 oocytes per ovary by transillumination (as an improved method). The number of oocytes recovered in cattle by Hamano and Kawayama (1993) were 8.8 oocytes per ovary, as 3.9±0.2 oocytes per ovary in this study. This difference may be due to climatic changes or type of breeds as discussed by Hafez (1993). In buffalo, the average number of good quality oocytes per ovary (1.9±0.1) was similar to that (1.8) reported by Khan *et al*. (1997).

The ovaries were sliced in two seasons i.e. summer and winter. The number of oocytes recovered in buffalo showed significant variation ($P<0.05$) in summer (1.6±0.16) and winter (2.2±0.1). In summer the decline in number of oocytes (Fig. 2) may be due to decrease in available

follicles, which are greater in winter, being the breeding season (Hafez, 1993).

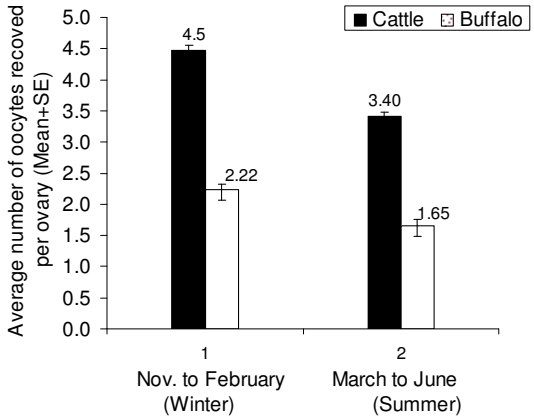

Fig. 2. Variation of culturable oocytes with respect to season in cattle and buffalo.

In case of cattle, the number of oocytes remained high in winter (4.5±0.07), while decreased in summer (3.4±0.15) (Fig. 2). The reason underlying this observation may be the nature of cattle, which were mostly cross bread and have a summer stress for follicular production (Valle, 1996). In conclusion, cattle yield more harvestable good quality oocytes than buffalo. However, both species face a remarkable effect of season on follicular number of ovaries.

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