Effect of the Presence of Corpus Luteum on Oocyte Recovery and Subsequent *in vitro* Maturation and Fertilization in Buffaloes

Sajjan Singh*, O. P. Dhanda and R. K. Malik
Central Institute for Research on Buffaloes, Sirsa Road, Hisar-125001, India

ABSTRACT : The effect of the presence or absence of corpus luteum in the ovaries of slaughtered buffaloes was studied for the oocytes recovery and their subsequent maturation and fertilization *in vitro*. On an average, 0.41 and 0.67 oocytes per ovary were recovered from ovaries with and without corpus luteum, respectively. Immature oocytes were matured in TCM-199 medium. Significant difference was observed in maturation rate between good (74%) and fair (37%) oocytes. However, there was no significant difference in cleavage rate between the two types. The results of this study show that although the presence of corpus luteum in the ovary at the time of recovery significantly affected availability of total oocytes and in-vitro maturation, but fertilization and cleavage remained unaffected under *in vitro* conditions. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1675-1677)***

Key Words : Buffalo, Ovary, Corpus Luteum, Oocytes Recovery, *In vitro* Maturation, Fertilzation

INTRODUCTION

For *in vitro* maturation (IVM) and fertilization (IVF), a large number of oocytes must be recovered from ovaries of slaughtered/live animals. These oocytes are recovered from both pregnant and non-pregnant females. Studies have been conducted in bovines to investigate the effect of the presence of corpus luteum on recovery rate of oocytes, IVM and IVF (Fukui and Sakuma, 1980, Tan and Lu, 1990; Behboodi et al., 1992; Moreno et al., 1993). However, the results from some of these studies are contradictory (Tan and Lu, 1990; Moreno et al., 1993) and hence needs further investigation. This paper, therefore, discusses the effect of the presence or absence of corpus luteum on recovery of oocytes in buffaloes which are mainstay of rural economy of the country. The relation between initial quality of oocytes and their subsequent maturation and fertilization under *in vitro* conditions was also examined.

MATERIALS AND METHODS

All chemicals and media required were acquired from Sigma Chemical Co., USA unless otherwise indicated. Paraffin oil and micro filters were purchased from Squibb and Fons (USA) and Millipore (France), respectively.

Oocyte recovery and maturation

Two independent experiments were conducted. In the first experiment, the effect of the presence or absence of corpus luteum on oocytes recovery and *in vitro* maturation was assessed while in the second experiment the relation between initial quality of oocytes and *in vitro* maturation and fertilization was investigated. For realizing these objectives, buffalo ovaries obtained from slaughter house at Delhi were brought to laboratory in Dulbecco's phosphate buffered saline (PBS) with pH 7.2 supplemented with 100 μg/ml streptomycin and 100 I. U./ml benzyl penicillin at 32-37°C within 5 h. Oocytes were aspirated from 2-6 mm follicles in ovaries with or without corpus luteum by using a 19 gauze needle attached to 1.5 ml glass syringe. These were classified as good and fair quality depending upon the number of cumulus cells surrounding them. Oocytes having 5 or more layers of cumulus cells were graded as good and below 5 as fair. They were transferred to 35 mm petri dish and then washed with TCM-199 containing 10% estrus buffalo serum. A minimum of 10-12 COCs from both good and fair categories were introduced in a drop (50-60 μl) of TCM-199 medium fortified with FSH (0.5 μg/ml), 17β-estradiol (10 μg/ml), 10% BSA and antibiotics as indicated above and the drop was covered with paraffin oil in a 35 mm petri dish.The COCs were cultured for 24 hours in a CO_2 incubator having 5% CO_2 in air and 95% humidity at 38.5°C. Each COC was examined under stereomicroscope and evaluated for maturation based upon cumulus cell expansion and/or extrusion of the first polar body.

Sperm preparation

Two straws (0.25 ml) of frozen-thawed buffalo semen were emptied into 15 ml tube and washed with Bracket and Oliphant's (BO) medium. This medium consisted of 76 ml BO (A) and 24 ml of BO (B), respectively and was placed in an incubator at 38°C. To this 500 I.U. heparin, sodium pyruvate (0.027 g per 100 ml of BO medium), caffeine, sodium benzoate (0.0388 g per 100 ml of BO medium), antibiotics (streptomycin 1 mg/100 ml medium) were added and pH adjusted to 7.2. The whole solution was then filtered through a millipore filter and after that straw of semen were emptied in a 5 ml tube. It was centrifuged at

* Corresponding Author: Sajjan Singh. Tel: +91-1662-76739, Fax: +91-1662-75004, E-mail: sajjansingh@lycos.com
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Table 1. The effect of presence of corpus luteum on oocytes recovery and maturation

Type of ovaries	No. of ovaries	No. of oocytes recovered	Avg. no. of oocytes/ ovary	No. of oocytes selected for maturation	No. of oocytes matured
With CL	943	392	0.41[a]	250	69 (38.40%)[c]
Without CL	1,136	768	0.67[b]	450	180 (40.00%)[c]

[a,b,c] The values carrying dissimilar superscript between rows differ from each other ($p<0.05$).

Table 2. The effect of initial quality of oocytes on subsequent IVM and IVF in buffaloes

Group	No. of oocytes cultured	No. of oocytes matured	No. of oocytes cleaved
Good quality	282	210 (74.48%)[a]	125 (59.52%)[c]
Fair quality	375	140 (37.35%)[b]	80 (57.12%)[c]

[a,b,c] The values carrying dissimilar superscript between rows differ from each other ($p<0.05$).

700 g for 5 minutes. The sperm pellet formed was again washed with the same medium by centrifugation at 700 g for 5 minutes. The pellet of spermatozoa formed were resuspended in 1 ml of BSA free BO medium supplemented with all other additives. This was diluted with 1 ml of BO medium contatining 20 mg/ml BSA supplemented with heparin. Then 100 µl sperm suspension drops (10-12 million sperm/ml) were covered with light paraffin oil in culture dishes for *in vitro* capacitation at 38.5°C, 5% CO_2 in air and 95% humidity for 1 h. Capacitation was judged by whiplash motion of sperm tail.

***In vitro* fertilizatiion**

All oocytes with expanded cumulus complexes were washed with BO medium and were then introduced into 10 µl drop of precapacitated sperm suspension. At least 10-12 matured oocytes per drop were introduced at 38°C, 5% CO_2 in air and 95% humidity for *in vitro* fertilization. After 24 hrs. of incubation, the oocytes were examined under stereomicroscope for cleavage (2-4 cells).

RESULTS AND DISCUSSION

The number of oocytes recovered from the ovaries with and without corpora lutea and their effect on subsequent in-vitro maturation is given in table 1. On an average, 0.41 and 0.67 oocytes were recovered from ovaries having corpora lutea and without corpora lutea, respectively. Totey et al. (1992) and Das et al. (1994) have reported similar results when oocytes were recovered by aspiration. However, Tasripoo and Kamonpatana (1997) harvested more oocytes per ovary (2.2) in swamp buffalo in comparison to the number of oocytes recovered in the present study, although they observed similar pattern of oocytes recovery as observed in our experiment from buffalo ovaries with and without C. L. Our observations have also been supported by Jain et al. (1995) who found a significant difference ($p<0.05$) in average number of oocytes obtained from ovary with and without corpus luteum. On the contrary, Moreno et al. (1993) reported significantly higher recovery rate from pregnant cows in comparison to non-pregnant one.

Although the effect of presence or absence of corpora lutea was reflected in number of oocytes retrieved, no significant difference ($p<0.05$) was observed in maturation in both groups. In fact, the maturation rates of oocytes recovered from ovaries with and without C. L. were almost identical suggesting thereby that the source of oocytes, obtained either from pregnant (with C. L.) or non pregnant (without C. L.) female does not influence their subsequent maturation. Such observation has been recorded by Fukui and Sakuma (1980), Tan and Lu (1990), Behboodi et al. (1992) and Vajta et al. (1992) in cattle indicating thereby that pregnancy does not affect the meiotic competence of bovine oocytes. However, Taripoo and Kamonpatana (1997) recorded significantly higher maturation rate of oocytes recovered from ovaries having corpora lutea in comparison to those from ovaries without corpora lutea, is in disagreement with our observations in riverine buffalo and other studies in cattle.

The data on the relationship between initial quality of oocytes and their subsequent *in vitro* maturation and fertilization are presented in table 2. Out of 282 good quality oocytes selected on the basis of the presence of the cumulus cell layers, 210 were maturated while only 140 out of 375 fair quality oocytes which had less than five cumulus cells layers were matured. Significant difference ($p<0.05$) was observed in the maturation rate (74% vs. 37%) between these two groups. Higher maturation rate from expanded oocytes in comparison to compact and denuded oocytes has been reported by Tasripoo and Kamonpatana (1997) in Swamp buffaloes suggesting thereby presence of promoting factors in the cumulus cells surrounding the expanded oocytes which may form the future area for research. However, no significant difference was found in the fertilization rate between good and fair quality oocytes. It appeared thus that cleavage may not be correlated with initial quality of oocytes once maturation has occurred.

Two deductions can be made from the present study; that the presence of corpus luteum in the ovaries did decrease the average number of oocytes availability in buffaloes and good oocytes retrieved from ovaries in this

species is considerably low in comparision to cow which might be due to 20% less follicles present in the buffalo ovaries during preovulatory follicular phase (Ty et al., 1988). Maturation is correlated with initial quality of oocytes to a great extent. However, fertilization under *in vitro* conditions does not appear to be influenced by initial quality of the oocytes once maturation has been accomplished.

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