IMPACT OF OVARIAN STATUS AND HARVESTING METHOD ON OOCYTE COLLECTION FROM BUFFALO OVARIES

C.F. CHAUDHARI[1]*, H.J. DERASHRI[2], L.C. MODI[1], N.F. CHAUDHARI[1], C.T. KHASATIYA[3] AND K.K. TYAGI[4]

Department of Veterinary Gynaecology and Obstetrics,
Navsari Agricultural University, Navsari, Gujarat - 396 450

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ABSTRACT

The impact of presence or absence of corpus luteum (CL) in a buffalo ovary and the oocyte harvesting methods viz., follicle aspiration and ovarian slicing on oocyte collection was investigated. The oocyte recovery rate as well as the per cent oocyte retrieved out of total visible follicles from ovaries with CL and without CL was higher ($p<0.05$) in slicing than aspiration. In brief, the ovarian slicing is superior over follicle aspiration method to obtain higher number of oocytes from buffalo ovaries and is even better than aspiration in ovaries without CL.

Keywords: Aspiration, Buffalo, Corpus luteum, Oocyte, Slicing

In vitro fertilization (IVF) studies in buffalo involve oocyte recovery from abattoir collected ovaries without considering the estrus cycle stage (Sahoo and Singla, 2013). The corpus luteum (CL) exerts negative impact on developmental competence of bovine oocytes depending upon the follicle size as well as has impact on the recovery of total oocytes (Singh *et al.,* 2001). Thus, the categorization of ovaries depending upon the presence or absence of CL and different follicle size would be helpful in commercial production of cattle laboratory embryos (Karami Shabankareh *et al.,* 2015). Therefore, the present work was designed to assess the impact of ovarian status on oocyte recovery and retrieval rates with different oocyte harvesting techniques from slaughterhouse derived ovaries.

Buffalo ovaries (n=202) of unknown reproductive status were collected from the local slaughterhouse in 37-38°C warm 0.9% normal saline solution (NSS) supplemented with penicillin G @ 100 IU/ml and streptomycin sulphate @ 100 µg/mL followed by transportation within 2h of slaughter to the laboratory. After removal of extraneous tissues and washing of ovaries with warm sterile NSS fortified with antibiotics, the visible follicles were identified and counted on each ovary. To investigate the effect of presence or absence of CL on oocyte recovery and retrieval rates by two oocyte harvesting techniques (slicing and aspiration), all the ovaries were classified as ovaries with CL (n=75) and ovaries without CL (n=127). To study the effectiveness of recovery method on oocyte retrieval and recovery rates on the basis of ovarian status, further the ovaries were randomly divided into aspiration (n=104, out of these with CL=39 and without CL=65) and slicing (n=98, out of these with CL=36 and without CL=62) methods for oocyte recovery. In aspiration method, oocytes from 2-8 mm visible non-atretic follicles were recovered with 18-guage needle attached to a 5 ml sterile plastic syringe containing 1 ml of pre-warmed oocyte collection medium (OCM). In slicing technique, the ovaries were chopped into small pieces with sterile surgical blades in 90 mm sterile petri dish containing 10 ml OCM to harvest the oocytes.

To calculate the oocyte recovery rate/ovary, the sum of average oocytes/ovary in batches was divided by total number of batches. While, to calculate oocyte retrieval rate (%), total number of oocytes collected from ovaries was divided by total number of visible

[1]Assistant Professor, [3]Associate Professor, [4]Assistant Research Scientist; [2]Ex-Director of Extension Education; *drcfchaudhari@yahoo.co.in

follicles counted on those ovaries. The data pertaining to various aspects were suitably analyzed using SPSS statistics (version 20) software. The differences among the parameter means were performed using t-test (oocyte recovery rate) and chi-square test (oocyte retrieval rate). The mean differences were considered significant at $p<0.05$.

When the CL was present on ovaries, the oocyte recovery rate by slicing method (2.37±0.19 oocytes/ovary) was higher ($p<0.0$) as compared to aspiration method (1.41±0.09 oocytes/ovary). Similar trend of oocyte recovery rate was observed when CL was absent on ovaries, where the recovery rate by slicing (3.74±0.19 oocytes/ovary) was higher ($p<0.05$) compared to aspiration method (2.11±0.14 oocytes/ovary). The present study revealed that the oocyte recovery rate from buffalo ovaries was better with slicing compared to aspiration. This is in agreement with earlier findings reporting greater number of COCs/ovary with the slicing method than aspiration (Das *et al.,* 1996; Khan *et al.,* 1997). As compared to slicing method, lower oocyte recovery rate by aspiration method might be due to fact that the oocytes were recovered from selected follicles (2-8 mm) from the ovarian surface, and they were limited in number on the surface. On the other hand, slicing the ovarian surface recovered COCs from follicles of every size, even from the follicles deep in the ovarian cortex (Arlotto *et al.,* 1990).

The percent oocyte retrieved out of total visible follicles was higher ($p<0.05$) from ovaries with CL in slicing method (57.9%) as compared to aspiration method (38.3%). Similar trend of oocyte retrieval rate was noticed from ovaries without CL, where the retrieval rate by slicing (72.2%) was higher ($p<0.05$) compared to aspiration method (43.0%). Earlier studies also reported 43.5% retrieval rate from bovine ovaries without CL in aspiration method (Boonkong *et al.,* 2012). However, they found higher retrieval rate by aspiration method (58.5%) from ovaries with CL compared to present study. Similarly, the oocytes were recovered via aspiration from 55% follicles and by slicing method from 78% follicles from buffalo ovaries (Khan *et al.,* 1997).

In conclusion, the slicing method is superior over aspiration method to obtain higher number of oocytes from buffalo ovaries and it is even better than aspiration when ovaries had no CL.

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