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EFFECTS OF COLLECTION TECHNIQUES AND CORPUS LUTEUM ON QUANTITY AND QUALITY OF GOAT OOCYTES FOR *IN VITRO* STUDIES

A.N.M.I. Rahman[1*], L. Asad[1], M.M. Hossain[1]
[1]*Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh*

Corresponding author:- anmirahman.angb@sau.edu.bd

The present study was undertaken to assess the relative effects of collection techniques and presence or absence of corpus luteum (CL) on recovery efficiency and oocyte quality for *in vitro* studies of goat oocytes. Cumulus-oocyte-complexes (COCs) were collected from slaughter house goat ovaries by aspiration, puncture and slicing methods. Following collection, oocyte qualities were classified into normal and abnormal group on the basis of cumulus attachment. The mean number of oocytes recovered per ovary was significantly (P<0.05) higher in slicing (5.34±0.35) than that of puncture (4.15±0.23) and aspiration (3.56±0.15) technique. The normal grade oocyte per ovary was significantly (P<0.05) higher in aspiration (54.78%) and in puncture (54.70%) method than slicing (30.71%)). Furthermore, the oocyte recovery was significantly lower (P<0.05) in CL containing ovaries (2.02±0.23) than that of ovaries without CL (4.92±0.27). It was observed that significantly (p<0.05) higher number of normal quality COCs per ovary were obtained from without CL ovaries compared to ovaries having CL with the mean of (2.47±0.12) and (0.98±0.14) respectively. Thus, it can be concluded that, slicing technique recovered more oocytes per ovary and without CL ovaries is a good source of normal grade oocytes for *in vitro* studies.

Key word: Goat; Corpus luteum; Aspiration; Puncture; Slicing.

The genetic improvement of goat especially in reproductive performance and quality of skin and meat is highly considered in livestock sector of Bangladesh. It is an economically important livestock species in Bangladesh like many South Asian countries. As a promising genetic resource of Bangladesh, it possesses about 25.66 million goats at present (DLS, 2014). Reproduction is the basic field of live stock production. Successful goat breeding highly depends on the genetic improvement that can be achieved through the application of reproductive biotechnologies. *In vitro* techniques are powerful tools for studying physiology of maturation, fertilization, development of pre-implantation embryos and increasing production as it gives access to micromanipulation of embryos. The recovery of large number of oocytes with high developmental competence remains an ultimate goal for the mass production of embryos in goat. For successful *in vitro* production (IVP) of goat embryos, the evaluation of ovaries, efficient collection and grading of oocytes is important. Cumulus cells concentration is very much dependent on the efficiency of oocyte harvesting. Several methods have been used for harvesting of oocytes from slaughterhouse ovaries. A number of research works have been conducted to compare the efficiency of the oocyte collection techniques in cattle (Katska, 1984; Lonergan *et al*., 1991), sheep (Wahid *et al*., 1992; Wani *et al.,* 2000) and goat (Mogas *et al.,* 1992; Wang *et al.,* 2007). In Bangladesh, few researches have performed in IVP of goat embryos, where COCs were collected only by aspiration of 2 to 6 mm diameter follicles (Ferdous, 2006, Islam *et al.,* 2007, Mondal *et al.,* 2008). However, no other technique was used for this purpose. Keeping the aforesaid reality in mind, the present research was undertaken to compare the effects of three oocyte collection techniques as aspiration, puncture and slicing on the recovery efficiency and

grading of oocytes for *in vitro* studies of goat oocytes.

MATERIALS AND METHODS

Experimental design: A total of 117 goat ovaries (4 replicates) were obtained from slaughtered house. To study the efficacy of three harvesting techniques on the oocyte yield and quality; oocytes were collected from individual ovaries by using aspiration, puncture and slicing methods. The total oocyte yield as well as different grades of COCs recovered from each ovary using three harvesting methods was recorded. Comparisons were made based on yield and quality of oocytes per ovary. The normal and abnormal grade oocytes are categorized on basis of surrounding cumulus cells layer of oocytes. To investigate the influence of corpus luteum on the quantity and quality and the developmental competence, the ovaries were divided into two groups. The number of ovaries with CL was (n=52) and without CL was (n=71). Oocytes were collected separately from each ovary from two groups and analyzed.

Collection and processing of ovaries

Goat ovaries were collected from slaughterhouse and were kept in collection vial containing 0.9% physiological saline solution in a thermo flask at 25˚C to 30˚C and transported to the laboratory within 5 to 6 hours of slaughter. The ovaries were rinsed thoroughly by physiological saline solution for two times at 25˚C. In the laboratory each ovary was trimmed to remove the surrounding tissues and overlying bursa. Each ovary was treated to three washings in D-PBS and two washings in oocyte harvesting medium (D-PBS+4 mg/ml BSA+1.50 IU/ml Penicillin) as described by Wani *et al.* (2000). After collection and trimming, ovaries were evaluated on the basis of presence and absence of CL.

Oocyte recovery- After collection and trimming, the oocytes were collected aseptically from the ovaries by three methods:

a) Aspiration- After washing 2 to 3 times in saline solution, ovaries will be placed in a beaker and kept in a water bath at 30 ^{0}C. The 10ml syringe will be loaded with DPBS(1-1.5ml),and the needles (19G) is put in the ovary parenchyma near the vesicular follicles (2 to 6mm diameter) and follicles aspirated near the point at the same time. After aspiration of the follicles from one ovary, the aspirated follicular materials are transferred slowly into a 90-mm petri dish, avoiding damage to the cumulus cells and the COCs will search and graded under microscope at low magnification.

b) Puncture- The whole ovarian surface was punctured by a sterile 18 gauze hypodermic needle while the ovary is held completely sub merged in oocyte collection media (OCM) in a 30 mm Petri-dish.

c) Slicing- The ovaries were held firmly with the help of forceps in a sterile glass petri dish containing 2 ml of DPBS. The ovaries were sliced into possible thin sections with a blade fixed to the artery forceps. The oocytes containing DPBS media were transferred to the petri dish and observed under microscope to grade the oocytes. The petri dish was observed under microscope and the oocytes were transferred to a searching dish containing DPBS for grading.

Grading of cumulus-oocyte-complexes (COCs)- The COCs which are obtained from three different collection method and with or without CL group ovaries, will be classified into 4 grades as described by Khandoker *et al*. (2001). Briefly, grade A: oocyte completely surrounded by cumulus cells; grade B: oocytes partially surrounded by cumulus cells; grade C: oocytes not surrounded by cumulus cells and grade D: degeneration observed both in oocytes and cumulus cells, where grade A and B will be considered as normal COCs and grade C and D as abnormal.

Statistical analysis

Oocyte recovery rates using different collection techniques were analyzed by analysis of variance (ANOVA) and effect of presence or absence of CL on oocyte recovery was analyzed using Student's t-test. Comparison of means Duncan's multiple range test (DMRT) was applied with the help of statistical analysis system (SAS,

1998). Differences in means were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The collected number of oocyte was significantly higher in slicing method (5.34±0.35), followed by puncture (4.15±0.23) and aspiration (3.56±0.15) (Table 1). Wani *et al.* (2000) and Shirazi *et al.* (2005) were also applied similar techniques in small ruminants and retrieved different number or percent of oocytes. Among the three harvesting techniques, the slicing method appeared to be superior in terms of total recovery. This method yielded a significantly ($P<0.05$) higher number of oocytes per ovary compared to the puncture and aspiration methods. Higher oocyte recovery in ovarian slicing may be due to their release from both surface follicles as well as from deeper cortex (Das *et al.*, 1996). In case of slicing, incisions were given along the whole ovarian surface using a scalpel blade; that is, all sizes of surface follicles were harvested. However, in case of puncture, the whole ovarian surfaces were punctured by hypodermic needle, and in the aspiration technique, COCs were collected from 2 to 6 mm diameter of surface follicles using a hypodermic needle with 10 ml syringe. The oocytes remain firmly attached to the small and medium sized follicles before cumulus expansion and cannot be aspired, but can be easily recovered from the small follicles when the slicing method is employed. Thus, the lower number of COCs recovered by the aspiration method in this experiment may be attributed to the presence of some follicles released by puncture or slicing of the ovary. The result of this study was comparable with the observation of Wang *et al*. (2007) who harvested oocytes from ovary of Boer goat by one of the four collection techniques (slicing, puncture, aspiration I and aspiration II). They reported that, slicing and puncture of the ovaries yielded a higher ($p < 0.05$) number of oocytes per ovary (6.3 and 5.8, respectively) when compared to aspiration I (2.9) and aspiration II (3.1). This result also supports the findings of Singh *et al*. (2013) in goat. Various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yielded significantly higher number of oocytes compared to luteal phase ovaries. Also a greater number of usable oocytes could be obtained from ovaries not bearing CL (Table 1). Most of the ovaries used in slicing technique bearing little number of CL. This might be a cause for obtaining large number of oocyte in slicing technique. Age, season, nutritional status (body

Table-1: Effects of collection techniques and CL presence or absence on the quantity and quality of goat oocytes recovery.

Collection methods	Total number of ovaries	Total number of oocytes recovered	Mean number of oocytes recovered	Oocytes per ovary Mean ± SEM, (%)	
				Normal	Abnormal
Aspiration	59	210	3.56±0.15[a]	1.95±0.12[a] (54.78)	1.61±0.18[a] (45.22)
Puncture	59	245	4.15±0.23[b]	2.27 ± 0.26[a] (54.70)	1.88±0.21[a] (45.30)
Slicing	59	315	5.34±0.35[c]	1.64 ± 0.44 [b] (30.71)	3.70±0.34[c] (69.28)
Total	177	770	4.35±0.29	1.95± 0.35(44.83)	2.40±0.22 (55.17)
Presence or absence of CL					
With CL	52	105	2.02±0.23[a]	0.98± 0.14[a] (48.51)	1.04 ± 0.06[a] (51.49)
Without CL	71	350	4.92±0.27[b]	2.47±0.12[b] (50.20)	2.45 ± 0.05[b] (49.80)

Mean values in the same column with different superscripts differ significantly at $p < 0.05$

condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval etc. are some of the factors that might contribute to recorded variation in oocyte quality (Nandi *et al*., 2001; Zoheir *et al.*, 2007; Amer *et al*., 2008). In terms of quality of oocytes, Ferdous (2006) reported that the numbers of normal COCs were found to be significantly higher ($p < 0.05$) in 2 to 6 mm diameter follicles than others. Moreover, puncture and slicing techniques produce more debris which might interfere with the searching of oocytes under the microscope and also required more washing when compared to aspiration. As a result, a number of COCs were denuded from cumulus cells due to repeated washing and ultimately resulted in a lower number of normal COCs when compared to aspiration at the final observation. In this study slicing technique obtain significantly ($P<0.05$) lower number (1.64±0.44**)** of normal oocyte than puncture and aspiration techniques. These results also support the results of Masud *et al*., (2011) in case of goat. When focus accounted on CL ovaries, significantly higher ($p<0.05$) number of collected COCs obtained from CL-absent ovaries than CL-containing ones (Table 1) with the mean of 4.92 and 2.02 COCs per ovary, respectively. Significantly higher ($p<0.05$) number of normal COCs were found in CL-absent ovaries than that of CL-containing ovaries. The presence of CL in cyclic female's ovary produces a higher level of progesterone hormone that signals a negative response to anterior pituitary gland for the restriction of gonadotrophin secretion and ultimately follicular degeneration occurs (Webb *et al.*, 1999). The cause of low number of oocytes per ovary with a CL is likely because of the follicular development is restricted, as lutein cells occupy a great portion of the ovary and also attributed that CL may inhibits the growth of follicles and increase their atresia (Hafez., 1993). In this study, the average number of collected COCs and normal COCs per ovary were significantly higher ($p<0.05$) in CL-absent ovaries due to the absence of corpus luteum in non-cyclic female. The goat destined slaughtering were usually less reproductive performer and most of them might be non-cyclic. So there had been the possibility to get more non-cyclic ovaries from the slaughterhouse during random sampling. The higher number of COCs in CL-absent ovaries than that of CL present group was found in this study explains the role of hormonal balance (FSH and LH) on goat folliculogenesis. The negative effect of progesterone might not be functional and estrogen-progesterone remains in balanced level which allows follicular growth and oocytes maturation. This results support the previous report of (Khandoker *et al.*, 2011) who found that the presence of a CL significantly reduced the recovery rate as well as the quality of the oocytes.

CONCLUSION

From the results of the present study, we could conclude that, slicing is the most suitable technique for oocyte collection in terms of number of oocytes than puncture and aspiration. The aspiration technique helps to get the maximum number of normal grade oocytes. Considering the effects of CL on ovaries, highest number and normal grade oocyte would be collected from without CL containg ovaries. Moreover, this result creates a great opportunity of conducting further research on goat embryo production in Bangladesh.

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