Short Communication

COMPARATIVE EVALUATION OF OOCYTE RECOVERY METHODS IN GOATS

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ABSTRACT

The present study was aimed to assess the oocyte retrieval methods in goats using multifactorial approach (retrieval techniques, season, presence or absence of corpus luteum. The mean number of good and total oocytes recovered per ovary were significantly higher ($p<0.05$) for slicing method (2.94 ± 0.06, 6.46 ± 0.03) compared to puncture (1.23 ± 0.04, 4.12 ± 0.14) and aspiration (0.74 ± 0.06, 2.94 ± 0.13) techniques. The number of good and fair quality oocytes were also significantly higher during winter season than summer season. Furthermore ovaries without corpus luteum produced significantly higher ($p<0.05$) number of good quality oocytes compared to ovaries with corpus luteum. (5.78±0.11 vs 3.24±0.08). In conclusion, good quality oocytes could be recovered by slicing technique from ovaries without CL collected during winter season.

Keywords: Corpus luteum, follicular fluid, goat, oocyte

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INTRODUCTION

Goat husbandry presents a future hope for poverty alleviation, food and nutritional security to the millions of small and marginal farmers in the world and particularly in India. However, there is a need to develop a superior flock with desirable milk and meat traits and propagation of the high merit animals amongst the farmers. This can be achieved through application of assisted reproductive techniques like in vitro fertilization (IVF). To achieve greater fertilization rate, the immature oocytes must mature efficiently. Ovaries of slaughtered animals are the most common and inexpensive source of primary oocytes for large scale in vitro embryo production (Rajendar *et al.*, 2024). In goats, the primary oocytes undergo developmental arrest at diplotene stage of meiosis at birth, able to resume meiosis spontaneously during luteinizing hormone (LH) surge and are ovulated as Metaphase-II oocytes.

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However, such a stage is achieved under in vitro condition through in vitro maturation (IVM) (Figueiredo *et al.*, 2024). The method of oocyte harvest for IVM is the first and most important step toward effective in vitro embryo development (Hegab *et al*., 2018). The immature oocytes are retrieved by various techniques like slicing of follicles, puncture and follicle aspiration and results in variable number of good quality oocytes (Akter *et al*., 2022). In cyclic females, the presence of a functional corpus luteum (CL) in the ovary results in elevated secretion of progesterone. High progesterone concentrations exert a negative feedback effect on the anterior pituitary gland, suppressing the release of gonadotrophins, particularly LH and follicle-stimulating hormone (FSH). As a consequence, follicular growth is inhibited (Ozawa *et al*., 2005). In goats, heat stress leads to reduced plasma concentrations of oestradiol and lowers follicular oestradiol concentration, aromatase activity and LH receptor level, and delays ovulation (Melmed *et al*., 2015). The present experiment was aimed to study the effect of presence of oocyte retrieval techniques, CL and season on oocyte recovery rate.

MATERIALS AND METHODS

Study location

The present study was carried out in Assisted Reproductive Technology Lab at Division of Veterinary Gynaecology and Obstetrics, Faculty of Veterinary Sciences and Animal Husbandry, which is situated at R.S. Pura area of Jammu with latitude 32◦ 63′ North and longitude 74◦ 73′ east in summer and winter months of the year.

Collection and processing of ovaries

Goat ovaries (N=300) were collected from local municipal slaughterhouse, transported to the laboratory within 1-2 hours of slaughter in a thermos flask containing physiological saline supplemented with 100µg⁄ml streptomycin and 100 IU ⁄ ml penicillin (Gibco Life Technologies). From each ovary the surrounding tissue and overlying bursa were trimmed, then the ovaries were rinsed with 70% alcohol followed by Dulbecco's phosphate buffered saline (DPBS, Sigma Chemical Company St. Louis, MO, USA mention) with antibiotics to eliminate any contamination.

Harvesting of cumulous oocyte complexes

Ovaries were divided into two groups based on the presence or absence of CL. The diameter of follicles was measured with the help of Vernier calliper and accordingly classified into small (<2 mm), medium (2-4 mm), large (>4 mm) (Dar, 2014). The ovaries were then transferred to a beaker containing holding medium. From each ovary, cumulous oocyte complexes (COCs) were collected by aspiration, puncture and slicing methods. In aspiration technique, a 20-gauge needle was attached to a 5 ml sterile disposable plastic syringe containing 2 ml holding medium to aspirate all follicles visible on the ovary. Aspirated follicular fluid was then transferred to a sterile 90 mm

Petri dish (Fig. 1A). In puncture technique, a sterile 18-gauge hypodermic needle was used to puncture the whole ovarian surface while the ovary was held totally submerged in holding media in a 90 mm Petri dish (Fig.1B). In slicing technique, a hemostat was used to attach to the base of ovary to hold it firmly inside the holding media in a 90 mm Petri dish and 2-3 mm deep longitudinal incisions were made throughout the whole ovarian surface (Fig.1C). In all the three methods, the Petri dishes were left undisturbed for 5 minutes to allow the oocytes to settle. The COCS were observed under stereo zoom microscope and number of oocytes collected with each method was recorded. Based on the number of cumulus cell layers, their compactness and ooplasm granularity, COCS s were graded as good, fair, and poor as per the classification of Das *et al*. (1996) (Fig.2).

Statistical analysis

To compare the three oocyte recovery techniques, the data generated was analysed by Paired-T test and Chi-Square test with significance determined by Wilcoxon Signed Ranks test at 'p' value less than 0.05 (Software SPSS Version-13). The percentage data was analysed by simple proportion test at 'p' value less than 0.05.

RESULTS AND DISCUSSION

The present study was conducted to know the effect of oocyte harvesting techniques on recovery rate of oocytes retrieved from abattoir derived goat oocytes. The mean number of good, fair and total oocytes recovered per ovary using different (aspiration, puncture, slicing) methods, winter vs summer season; left vs right ovary, and from ovaries with and without CL is presented in table 1. Comparatively, the recovery of good, fair, and total oocytes was significantly higher ($p<0.05$) for slicing than puncture and aspiration technique. Significantly, a greater number of oocytes were recovered in winter than summer season. The recovery of fair and poorquality oocytes was significantly higher ($P<0.05$) as compared to good quality oocytes in ovaries having CL. The mean number of good and fair quality oocytes recovered from ovaries without CL was significantly higher ($p<0.05$) as compared to poor-quality oocytes.

The important aim of oocyte collection method from ovaries is to maximize the quality oocyte yield at low cost, which can be used forin vitro embryo production. Comparatively, the recovery of good, fair, and total oocytes was significantly higher ($p<0.05$) for slicing than puncture and aspiration technique. Similar findings were reported by Ashedkar *et al*., (2025), AL-Nuaimi *et al*., 2020, Singh *et al*., (2013) and Wang *et al*., (2007) in goats. Higher oocyte recovery in slicing technique might be due to release of oocytes from both surfaces as well as from deeper cortex (Das *et al*., 1996). Further more, in case of slicing, incisions were given along the whole ovarian surface using a scalpel blade in which all sizes of surface follicles were harvested (Rahman *et al*., 2016). The higher recovery rate of good, fair and total oocytes obtained by puncture method than aspiration technique in the present study concurred with the findings of Rahman *et*

al., (2016). This might be due to the release of oocytes from small and medium sized follicles caused by the additional pressure applied during the puncture method (Rao and Mahesh, 2012). The lower recovery rate of good, fair and total oocytes by aspiration might be because of the fact that oocytes remain firmly attached to the small and medium sized follicles before cumulus expansion and cannot be aspired, but could be easily recovered from the small follicles through slicing method (Wani *et al*., 2000).

The present results with respect to season were comparable to the findings of Shukla *et al.*, 2021; EnayetKabir *et al.*, 2021; Soliman *et al.*, 2016; Zoheir *et al.*, (2007); El-Naby *et al*., (2013) and Raj *et al*., (2016) in buffalo and Dode *et al*., (2001) and Hussain *et al*., (2005) in cattle. Summer has a detrimental effect on both recovery and quality of oocytes (Samad and Raza, 1999; Jamil *et al*., 2008; Manjunatha *et al.*, 2009; Davachi *et al.*, 2014) as oocyte function was compromised during oogenesis by heat stress leading to recovery of more poor and fair quality oocytes in summer season.

The oocyte recovery with respect to effect of CLwere in accordance with the studies of Sidi *et al*., 2025 in cattle,Talukder *et al*., (2011) in sheep; Jamil *et al*., (2008) and Mahesh *et al.,* (2014) in buffalo; Rahman *et al*., 2016, Islam *et al*., (2007), Mahesh *et al.*, (2013) and Dar (2014) in goat who observed significantly higher number of good, fair and usable oocyte in without CL ovary than with CL ovary. Wani *et al*., (1999) indicated that the significantly lower yield of oocytes from ovary with a CL than ovary without CL ($p<0.05$) might be due the possibility of animal being pregnant, infertile or in diestrus phase in which corpus luteum covers the major portion of ovary. Hafez (1993) reported that a CL inhibits the growth of follicles and increases their atresia which might be a secondary factor responsible for lower oocyte recovery from ovaries with CL, since significantly reduced follicular population in ovaries with CL.

CONCLUSION

From the study, it was concluded that Slicing technique was the best method in terms of oocytes retrieved per ovary but puncture technique could also be utilized for collection of goat oocytes.Winter season was better for collection of good quality oocytes than summer season and ovaries without corpus luteum were better for collection of oocytes from goat ovaries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table 1: Oocyte recovery in different techniques

	No. of ovaries	No. of oocytes recovered	Different average grades of oocytes per ovary			
			Good	Fair	Poor	Total
Technique						
Aspiration	100	294	0.74 ± 0.06^{aA}	0.85 ± 0.05^{aA}	$1.35\pm0.03^{b}B$	2.94 ± 0.13^{a}
Puncture	100	412	1.23 ± 0.04^{bA}	1.64 ± 0.07^{bB}	$1.25\pm0.02^{b}A$	4.12 ± 0.14^{b}
Slicing	100	646	$2.94\pm0.06^{c}B$	2.85 ± 0.03^{cB}	$0.67\pm0.04^{a}A$	6.46 ± 0.03^{c}
Season						
Winter	143	737	1.76 ± 0.05^{bB}	$2.14\pm0.03^{b}C$	$1.26\pm0.05^{a}A$	5.16 ± 0.15^{b}
Summer	157	615	1.04 ± 0.02^{aA}	1.32 ± 0.04^{aB}	$1.51\pm0.04^{b}C$	3.87 ± 0.11^{a}
Ovary Characteristic						
With CL	113	366	0.79 ± 0.05^{aA}	1.26 ± 0.06^{aB}	1.19 ± 0.04^{aB}	3.24 ± 0.08^{a}
Without CL	187	986	2.11 ± 0.05^{bB}	$2.05\pm0.06^{b}B$	1.62 ± 0.07^{aA}	5.78 ± 0.11^{b}

Means with superscripts a, b, c within a column differs significantly at P<0.05
Means with superscripts A, B, C differ significantly row-wise at P<0.05

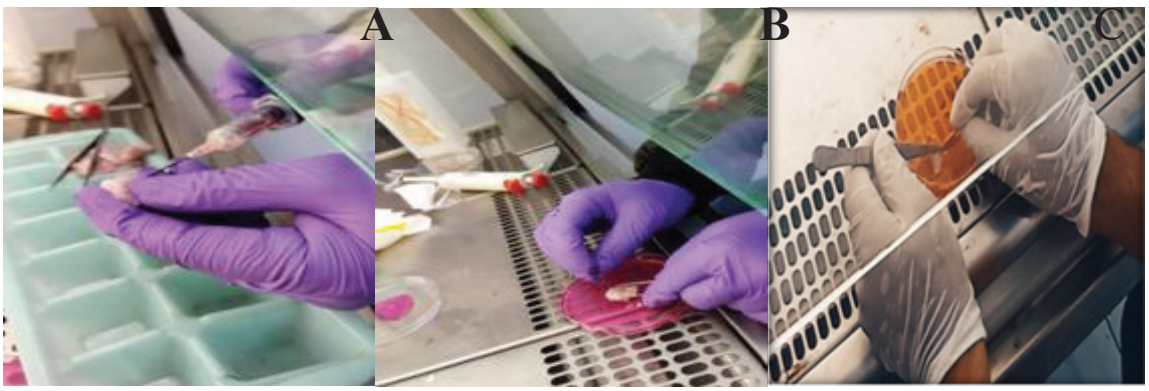

Fig.1. Different oocyte collection techniques. A) Aspiration technique, B) Puncture technique, C) Slicing technique

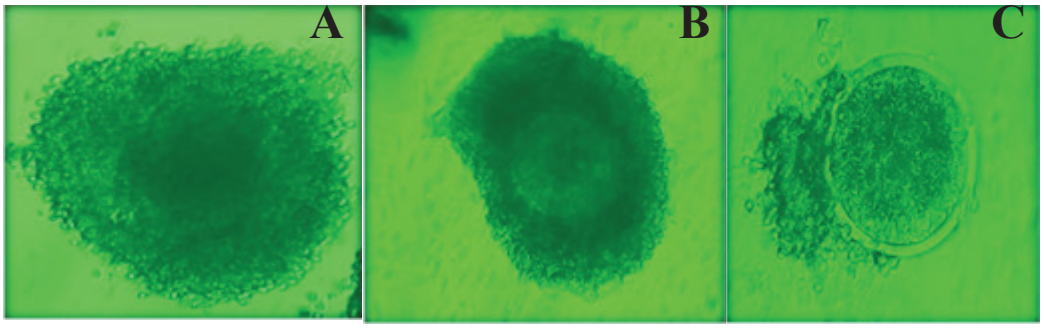

Fig. 2: Grading of oocytes. A) Good quality oocytes with many layers of compact cumulus cells, B) Fair quality oocytes with less than three layers of cumulus cells, C) Poor quality oocytes with denuded cumulus cells

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