RESEARCH ARTICLE

Comparative Efficacy of Different Techniques of Oocyte Recovery from Slaughtered Goat Ovaries

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Abstract

The present study was conducted to compare the efficacy of the oocyte quality and recovery rate between three different retrieval methods from goat ovaries. Recovery of oocytes by three methods that is slicing, aspiration and dissection followed by grading into Grade I, Grade II and Grade III oocytes was carried out. On statistical analysis, the mean oocyte recovery rate per ovary and mean yield of Grade I and II oocyte was significantly ($p<0.05$) higher in slicing as compared to aspiration, and no significant difference ($p>0.05$) was seen with dissection method. However, there was no significant difference between aspiration and dissection methods. There was no significant difference in the mean yield of Grade III oocytes between slicing, aspiration and dissection methods. In conclusion, slicing was found to be the more effective method for oocyte recovery.

Key words: Aspiration, Dissection, Goat ovaries, Slicing.

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Introduction

Reproduction is the basic field of livestock production. The field of goat reproductive technology has advanced significantly with the first successful goat birth through *in vitro* fertilization in 1985 (Hanada, 1985). *In vitro* techniques are particularly valuable in small ruminants like goats due to their shorter generation intervals, higher reproductive rates, easier handling, and larger pronuclei, which are advantageous for biological research (Amin *et al.,* 2001). In many cases, infertility in female goats results from abnormalities in the reproductive organs, including the tract and gonads. Assisted reproductive technologies such as *in vitro* embryo production (IVEP) offer promising solutions to address fertility issues and improve goat production.

A critical factor for the success of IVEP is obtaining a sufficient number of high-quality oocytes. *In vivo*-matured oocytes were obtained either by surgical or laparoscopic methods during early years (Schellander *et al.,* 1989). As this method is expensive, and the number of oocytes recovered per female is less, an alternative and cheap source of immature oocytes from ovaries obtained from the slaughter house would greatly aid in research (Pawshe *et al.,* 1994). Large numbers of embryos can be produced from the ovaries of slaughtered donors when the female parental origin is not required to be known. Therefore, developing efficient oocyte collection and grading techniques is essential. This study was aimed to compare three *in vitro* oocyte retrieval methods - follicular slicing, aspiration, and dissection - in terms of their efficiency and the quality of oocytes recovered from abattoir ovaries for IVEP in goats.

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Materials and Methods

Location and Lab Preparation

The present study was conducted at the Department of Animal Reproduction, Gynaecology and Obstetrics, Veterinary College, Bidar, Karnataka (India). All the chemicals and media were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), all plastic wares were purchased from Hi-media Laboratories (Mumbai). Glass beaker (Borosil), Thermometer, Laminar air flow, 5% CO_2 incubator (Thermo Scientific), stage warmer, water bath, stereo-zoom microscope were procured from standard sources.

All media were sterilized through a 0.22 µm syringe-driven filter. The glass wares were sterilized in hot air oven at 160°C for 60 min and micropipette tips by autoclaving at 121°C for 45 min. The new sterilized disposable Petri dishes and syringes were used every time. All the equipments were exposed to UV light for 15 min before use. All the procedures,

starting from oocyte searching, and grading were carried out in highly sterile conditions under a laminar flow cabinet.

Source and Transportation of Ovaries

Goat ovaries were collected from local slaughter houses as soon as possible after the animals were slaughtered in and around Bidar district (Karnataka) with an undefined stage of the reproductive cycle. Ovaries were collected in a thermos flask containing warm Normal Saline Solution (0.9% sodium chloride, 35°-37°C) supplemented with 50 µg/mL gentamicin sulphate and transported to the laboratory within 2-4 h of slaughter.

Retrieval of Oocytes

In the laboratory before ooctye retrieval, the extraneous tissues from the ovaries were removed and they were washed 4-5 times with Normal Saline Solution containing antibiotic for further processing (Fig. 1). The ooctye retrieval was done with the following three techniques.

Slicing Technique: The ovaries were held firmly with the help of forceps in a sterile petri dish containing 5 mL oocyte collection medium (OCM). Incisions were given along the whole ovarian surface using a surgical scalpel blade and sliced into possible thin sections, allowing oocytes to settle for 3-5 min and examined under a stereo-zoom microscope for the presence of oocytes (Fig. 2).

Aspiration Technique: The oocytes were aspirated from visible medium-size follicles present on the ovarian surface by puncturing at the base with a 20 gauge needle fixed to a 5 mL disposable syringe containing 1 mL of OCM. All the follicles along with follicular fluid pooled into a sterile petri dish containing an OCM allowing oocytes to settle for 3-5 min and examined under a stereo-zoom microscope for the presence of oocyte (Fig. 3).

Dissection Technique: The ovaries were placed in a sterile petri dish containing 2 mL of OCM. All the visible follicles were carefully subjected to blunt dissection with the help of forceps. Ruptured follicles and follicular fluid were allowed to flow into OCM to settle the oocytes for 3-5 min and examined under a stereo-zoom microscope for the presence of the oocyte (Fig. 4).

Searching and Grading of Oocytes

The cumulus-oocyte complexes (COCs) (Fig. 5A) were searched under a stereo-zoom microscope and collected by means of a 10 µL micropipette and transferred into a 35 mm petri dish containing fresh oocyte washing medium (OWM) maintained at 39^{0}C. The oocytes were then washed 4-5 times in the OWM. After washing, oocytes were graded based on the number of layers of cumulus cells and ooplasm character into Grade I, Grade II and Grade III (Fig. 5B,C,D) as described by Pawshe *et al.* (1994).

The oocyte recovery rate per ovary by slicing, aspiration and dissection method was calculated by dividing the total number of oocytes recovered by the total number of ovaries processed.

Statistical Analysis

The statistical analysis was carried out by using one-way ANOVA followed by Duncan's multiple comparisons test using SPSS software 16.0

Results and Discussion

Effect of Retrieval Techniques on Oocyte Yield per Ovary

A total of 287 ovaries processed for oocytes retrieval using three methods, *viz.,* slicing (104 ovaries, 809 oocytes),

Fig. 1: Processed ovaries (Washed with Normal saline containing antibiotic)

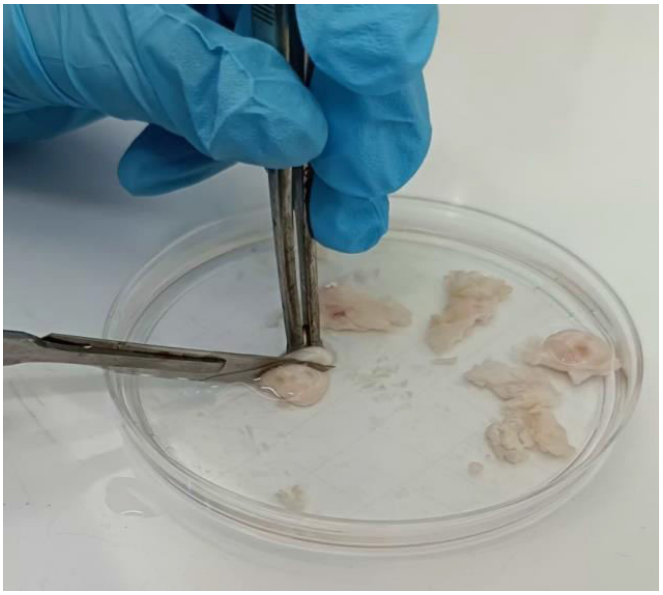

Fig. 2: Slicing technique

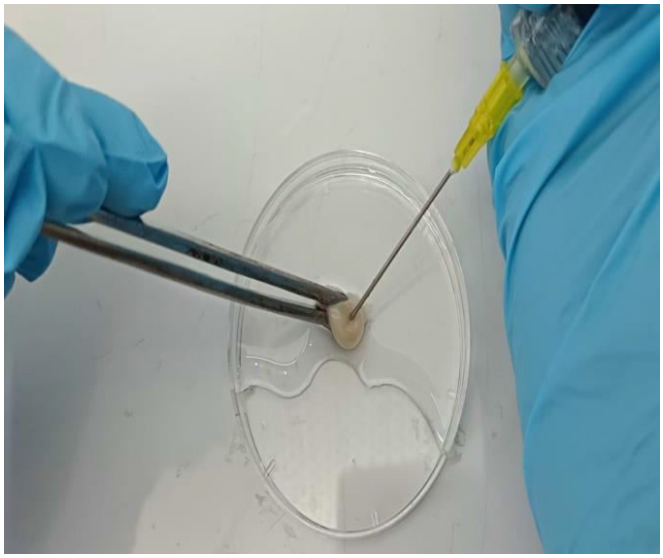

Fig. 3: Aspiration technique

Fig. 4: Dissection technique

aspiration (87 ovaries, 272 oocytes) and dissection (98 ovaries, 303 oocytes) yielded 1384 oocytes. The mean oocyte yield per ovary was highest in the slicing method (8.29 ± 0.90), followed by dissection (5.62 ± 1.96), and lowest in aspiration (4.01 ± 0.62) (Table 1). The slicing method had significantly higher ($p<0.05$) mean yield of oocytes recovery than aspiration method. However, there was no significant difference between aspiration and dissection methods. The mean oocyte recovery per ovary by slicing was consistent with findings of Rameez *et al.* (2017) and Naik *et al.* (2023), who observed 7.03 ± 0.21 and 8.55 ± 0.47 oocytes in goat ovaries. In contrast, the current findings revealed higher mean oocyte recovery rate per ovary compared to Lima *et al.* (2016) and Al-Nuaimi *et al.* (2020), who observed 5.06 and 6.80±0.11 oocytes per ovary in goats. The oocyte yield observed by Kale *et al.* (2020) from goat ovaries was 10.79 ± 1.00, which is greater than the results of the current investigation.

The high COC yield per ovary after slicing is due to its ability to release oocytes from follicles on the surface and within the ovarian cortex, whereas aspiration approach is limited to superficial follicles (Pawshe *et al.,* 1994; Bohlooli *et al.,* 2015). Furthermore, some oocytes might be lost during follicle aspiration, which could be avoided by slicing (Wani *et al.,* 2000). However, slicing produces more cell debris due to fragmentation of the ovary, which makes it difficult to locate oocytes and could harm them during the process (Akter *et al.,* 2022).

The mean oocyte recovery per ovary by aspiration was comparable to findings of Rahman *et al.* (2016), and Naik *et al.* (2023), who reported 3.93 ± 0.11 and 3.56 ± 0.15 oocytes per ovary, respectively, in goats. Lower yields (1.35±0.18 to 2.84±0.03 oocytes per ovary) were reported by Pawshe *et al.* (1994), Ramsingh *et al.* (2013) and Al-Nuaimi *et al.* (2020) in goats, while higher yields were reported in goats (6.54 ± 0.39) by Mahesh *et al.* (2013) and in Deccani ewes (9.33 ± 0.76) by Kumar *et al.* (2023). The lower oocyte yield from aspiration may result from its selective focus on visible surface follicles (2-6 mm), potentially missing smaller, deeper follicles (Akter *et al.,* 2022). Additionally, aspiration can damage cumulus cells and cause oocyte loss during the procedure (Nowshari, 2004; Al-Nuaimi *et al.,* 2020).

The mean oocyte recovery of 4.01 ± 0.62 per ovary by dissection in current study was similar to the result (4.66 ± 0.34) of Yadav *et al.* (2007). Other studies in goats by Ramsingh *et al.* (2013) and Akter *et al.* (2022) reported lower counts of 2.52 ± 0.28 and 2.89 ± 0.22 indicating a higher yield in the current study. On the contrary, the present findings showed a lower count in comparison to 9.41 ± 0.36 oocyte per ovary in ewes by Kumar *et al.* (2023) and 6.8 ± 1.0 in cows by Khandoker *et al.* (2017). Blunt dissection technique was found more

Table 1: Recovery rate of oocyte per ovary of goats by using different retrieval technique (Mean ± SE)

Retrieval techniques	Total no. of ovaries	Total no. of observations	Total no. of oocytes recovered	Recovery rate of oocytes/ ovary
Aspiration	87	12	272	4.01 ± 0.62^{b}
Dissection	98	12	303	5.62 ± 1.96^{ab}
Slicing	104	17	809	8.29 ± 0.90^{a}

Means with different superscripts within a column vary significantly ($p < 0.05$).

efficient for COCs retrieval with higher number and better quality of recovered COCs (Khandoker *et al.,* 2017).

In dissection technique, all surface follicle sizes, even those deeply embedded in the cortex, were readily released with intact cumulus cells and less remnant tissues after the follicles were split open with a scalpel blade (Akter *et al.,* 2022).

Effect of Retrieval Techniques on the Quality of COCs

The slicing method yielded the highest mean of Grade I and Grade II oocytes (2.71 ± 0.49 and 2.85 ± 0.43), significantly more than aspiration (0.73 ± 0.13 and 1.17 ± 0.18; $p<0.05$), and the yield with dissection technique (1.43 ± 0.36 and 2.08 ± 0.77; $p>0.05$) was found to be intermediate between aspiration and dissection technique ($p>0.05$) (Table 2).

Grade III oocyte yields were comparable across slicing (2.73 ± 0.40), aspiration (2.11 ± 0.41), and dissection (2.10 ± 0.88) methods, with no significant difference ($p>0.05$) (Table 2). This is in agreement with those recorded by Ramsingh *et al.* (2013), who mentioned that the percentage of good grade oocyte in goats was significantly higher in slicing followed by dissection and aspiration techniques. In contrast, Hoque *et al.* (2011) showed that the number of the good oocytes per ovary by aspiration was significantly ($p<0.05$) greater than the slicing. The current observation aligned with Akter *et al.* (2022), who also recovered greater percentage of grades A oocytes through dissection technique when compared with aspiration and slicing. This may be due to the reason that in the dissection technique,

Table 2: Oocyte recovery rate per ovary of different grades retrieved by different techniques (Mean ±SE)

Retrieval techniques	Recovery rate of oocytes/ovary	Grade I (Good)		Grade II (Fair)		Grade III (Poor)	
		Mean ±SE	Yield (%)	Mean ±SE	Yield (%)	Mean ±SE	Yield (%)
Aspiration	4.01 ± 0.62^{b}	0.73 ± 0.13^{b}	20.95	1.17 ± 0.18^{b}	30.15	2.11±0.41	48.90
Dissection	5.62 ± 1.96^{ab}	1.43 ± 0.36^{ab}	29.70	2.08 ± 0.77^{ab}	31.02	2.10±0.88	39.27
Slicing	8.29 ± 0.90^{a}	2.71 ± 0.49^{a}	29.66	2.85 ± 0.43^{a}	36.14	2.73±0.40	34.28

Means with different superscripts within a column vary significantly ($p < 0.05$).

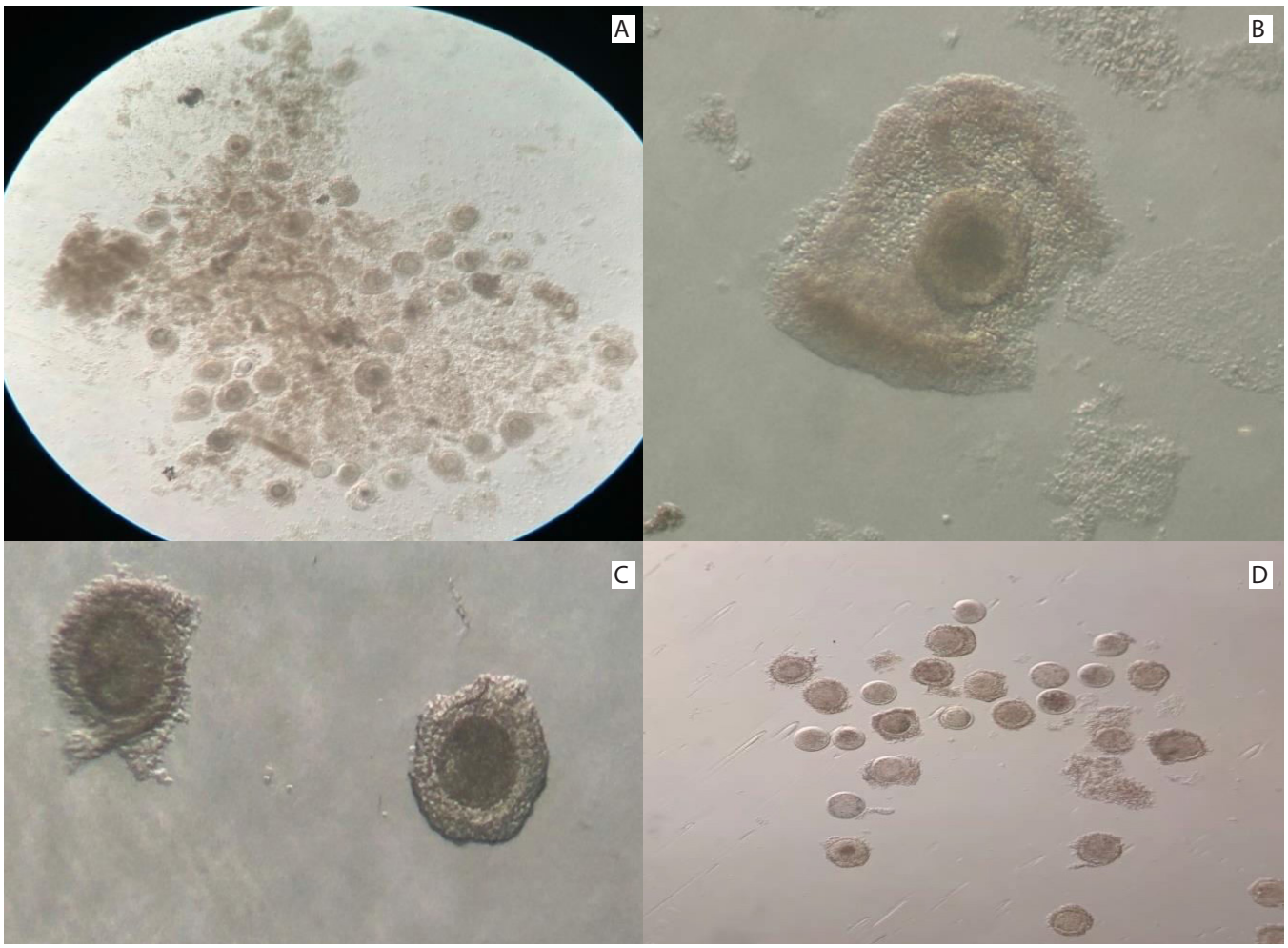

Fig 5: A -Pool of oocytes with debris; B -Grade-I oocyte; C -Grade-II oocytes; D -Grade -III oocytes

follicles were opened with a scalpel blade and the granulosa cell layer was scraped carefully with a needle.

Variations in season and reproductive status of the slaughtered goats may be linked to the observed variation resulting from the various techniques utilized for COC recovery (Greve and Madison, 1991). An additional explanation could be attributed to the animal's age, the condition of its ovaries and the surrounding environment at the time of collection (Hammam *et al.,* 1997), or season, body condition, nutritional status and cyclicity at the time of slaughter, as well as the size and functional status of its follicles and the technique used to retrieve the oocytes (Das *et al*., 1996). Furthermore, the effectiveness of oocyte retrieval may have been impacted by the person's experience processing ovaries as well as the strain of managing many ovaries each day (Al-Nuaimi *et al.,* 2020).

Conclusion

Slicing method as compared to aspiration and dissection methods of oocyte retrieval is simple and effective method for recovering good quality and quantity of oocytes from the goat ovaries.

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