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## Effect of Aspiration Technique on Oocyte Yield and Quality in Crossbred Cattle of Kerala

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### Abstract

Successful oocyte recovery technique which yields higher quality and quantity of cumulus oocyte complexes play a key role for success in *in vitro* embryo production. In the present study a total of 231 abattoir ovaries were obtained from local slaughter house. COCs were retrieved from visible surface follicle of 2-8mm diameter by aspiration technique using oocyte collection medium. Mean  $\pm$  S.E of number of follicles observed, number of oocytes recovered per ovary and recovery rate of oocytes were  $5.58 \pm 0.23$ ,  $4.83 \pm 0.23$  and  $86.44$  per cent. The mean yield of grade A, B, C and D oocytes obtained in the present study were  $38.71 \pm 2.15$ ,  $39.56 \pm 1.49$ ,  $17.33 \pm 1.62$  and  $4.59 \pm 0.87$ . The yield of culture grade oocytes in the present study by aspiration technique was  $78.27 \pm 2.08$ . The result indicates that aspiration technique is an efficient method to retrieve good quality COCs from crossbred cattle of Kerala.

**Keywords:** In Vitro embryo production, Abattoir Ovaries, Aspiration Technique, Culture grade Oocytes, Recovery rate

### 1. Introduction

*In vitro* embryo production in crossbred cattle begins with recovery of oocyte from live or slaughter animals. The oocytes can be recovered from abattoir ovaries in large number which constitute cheapest and economical source of oocytes. This paves the way for large scale and economic embryo production (Sianturi *et al.*, 2002) [1]. Developmental competence of oocytes to develop into embryo under *in vitro* conditions begins with quality and quantity of oocytes obtained. Selection of oocyte retrieval method aims at obtaining maximum number of good quality cumulus oocyte complexes (COCs) per ovary in short duration of time with minimum contamination. Oocytes have been retrieved from slaughter ovaries by aspiration of oocytes from follicles, slicing of ovary for oocyte collection, puncture of visible surface follicles and aspiration followed by slicing (Farahavar and Shahne, 2010) [2]. Even though slicing method yields more number of good quality oocytes, aspiration method is being widely used because of ease of procedure and speed of recovery (Hammad *et al.*, 2014) [3]. The aim of this study is to assess the yield and quality of oocytes recovered through aspiration method from slaughter ovaries of crossbred cattle of Kerala.

### 2. Materials and Methods

Abattoir ovaries from various crossbred cattle were obtained from Thrissur corporation slaughter house, collected and transported within 2 h of slaughter in Normal Saline (NS) fortified with penicillin and streptomycin at 35 to 37 °C. In the laboratory, ovaries were cleaned to remove blood by rinsing thrice in NS prior and post removal of extra ovarian ligaments. Ovaries were placed in a beaker containing warm NS at 35 to 37 °C. Aspiration technique was followed for oocyte retrieval in the present study (Fig 1). Ovarian surface follicles of 2 to 8 mm in diameter were aspirated with 10 ml disposable syringe fitted with 18 gauge needle primed with 0.5 ml of Oocyte Collection Medium (Bavister *et al.*, 1983) [4] supplemented with bovine serum albumin 3 mg/ml and gentamycin 50µg/ml.

All visible follicles were threaded and aspirated, the contents in the syringe were poured into a glass test tube and kept in the incubator and the oocytes were allowed to settle down for a 10 minutes. The sediment was taken and transferred into a 90mm petri dish and added with TCM-199 and 10 per cent fetal bovine serum. COCs were searched under 40X magnification of zoom stereomicroscope, the oocytes were pooled into a 35-mm petri dish and washed three times in same medium. Oocytes were graded based on cumulus cell layer before transferring into maturation medium (Gordon, 2003) [5].

### Grading of COCs

Cumulus oocyte complexes were graded based on compactness, number of layers of cumulus cells and homogeneity of cytoplasm (Cetica *et al.*, 1999) [6]. COCs with more than five layers of unexpanded cumulus and homogenous ooplasm were considered as Grade A. Those with 3-5 layers of compact cumulus and evenly granular ooplasm were considered as Grade B, COCs with 1-2 layers of cumulus of partially denuded COCs with irregular ooplasm and completely denuded oocyte were considered as Grade D (Fig 2).



**Fig 1:** Aspiration technique of oocyte retrieval



**Fig 2:** Culture grade oocytes

### 3. Results and Discussion

A total of 231 slaughter ovaries were subjected to study, out of which 1286 follicles were aspirated. The mean number of follicles observed per ovary in the present study was  $5.58 \pm 0.23$  (Table 1). The result of the present investigation is in accordance with the findings of Boonkong *et al.* (2012) [7] who recorded 5.6 follicles per ovary (without CL) and 2.4 follicles per ovary (with CL) from cross bred cattle. Priscilla (2001) [8] obtained 5.31 follicles per ovary similar to those obtained in the present study.

The oocytes recovered per ovary in the present study were  $4.83 \pm 0.23$  (Table 1), which was in agreement with findings of Hammad *et al.* (2014) [3], who recovered  $4.5 \pm 0.5$  oocytes per ovary.

**Table 1:** Assessment of yield and quality of oocytes in crossbred cattle of Kerala

Sl.No.	Parameters	Number/Percent (Mean $\pm$ SE)
1	Number of follicles per ovary	$5.58 \pm 0.23$
2	Yield of Cumulus Oocyte Complexes (COCs) per ovary	$4.83 \pm 0.23$
3	Oocyte recovery rate	86.44 %
4	Yield of morphologically different grade oocytes (%)	
	Grade A	$38.71 \pm 2.15$
	Grade B	$39.56 \pm 1.49$
	Grade C	$17.33 \pm 1.62$
	Grade D	$4.59 \pm 0.87$
5	Yield of culture grade oocyte per ovary	$3.83 \pm 0.23$
6	Per cent yield of culture grade oocyte	$78.27 \pm 2.08$

A higher oocyte yield of  $5.8 \pm 0.3$ ,  $11.59 \pm 0.10$  and  $12.02 \pm 0.85$  per ovary was observed by different researchers (Wang *et al.*, 2007 [9], Binoy, 2009 [10] and Sianturi *et al.*, 2012 [11]). However, Magnus (2005) [11], Rao and Mahesh (2012) [12], Bohlooli *et al.* (2015) [13] and Kuriakose (2016) [14] obtained a lower oocyte yield per ovary of  $3.70 \pm 0.43$ ,  $2.38 \pm 0.19$ ,  $2.31 \pm 0.21$  and  $3.35$  respectively. Oocyte recovery rate obtained in the present study was  $86.44 \pm 0.01$  (Table 1). The result of the present investigation is in agreement with the findings of Hammad *et al.* (2014) [3] who observed a recovery rate of 81.3 per cent. However researchers like Singh *et al.* (2001) [15] and Boonkong *et al.* (2012) [7] obtained a lower oocyte recovery rate of 67 and 58.6 per cent. The variations in oocyte recovered per ovary and recovery rate observed in the present investigation might be due to difference in retrieval technique, skill of the person, pressure exerted and gauge of the needle used during aspiration.

The mean yield of grade A, B, C and D oocytes obtained in the present study were  $38.71 \pm 2.15$ ,  $39.56 \pm 1.49$ ,  $17.33 \pm 1.62$  and  $4.59 \pm 0.87$ . The per cent yield of culture grade oocytes obtained in the present study was  $78.27 \pm 2.08$ . Similar

observation was reported by Magnus (2005) [11] who observed 76.22 per cent of culture grade oocytes by aspiration technique. This finding is comparable with the results of Binoy (2009) [10], Rao and Mahesh (2012) [12] and Sianturi *et al.*, (2012) [11] who observed a slightly lower yield of culture grade oocytes as 63.90, 67 and 68 per cent respectively by aspiration technique. Sianturi *et al.* (2012) [11] reported a similar culture grade oocyte by slicing technique (69%) which was similar to yield of culture grade oocytes by aspiration technique in the present the study (78%). Wang *et al.* (2007) [9] obtained lower per cent of culture grade oocytes (66.5%) by slicing technique.

### 4. Conclusion

It can be concluded from the present investigation that aspiration technique is an efficient method to retrieve optimum number of good quality oocytes from crossbred cattle of Kerala.

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