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Influence of Oocyte Collection Techniques on Oocyte Retrieval and Quality from Ovaries of Iraqi Goats (*Capra Hircus*)

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ABSTRACT

The objective of this current study to determine the influence of different three retrieval techniques which included slicing of ovary, puncture of ovary and aspiration of visible surface follicles on oocytes quantity and quality from goat ovaries. The ovaries were recovered from local slaughterhouse and oocyte was retrieval by using slicing, puncture and aspiration techniques. The harvested oocytes were classified according to cumulus cells surrounding them and homogenous of ooplasm into three grades, oocytes with at least three layers of cumulus cells oocytes with less than three layers of cumulus cells and denuded oocytes. The results showed that the average total number of oocytes retrieved per ovary was significantly increased (P<0.05) in slicing (6.8±0.11) than punctures (4.14±0.06) and aspiration (2.80±0.03) techniques. The rate of oocytes collected per ovary were 4.58±008 of which 2.01±0.12 (40.07%), 1.20±0.03 (28.93%) and 1.29±0.02(30.99%) were flood, fair and poor oocytes respectively. The average number and percentage of good grade oocytes were significantly increased (P<0.05) by slicing 3.56±0.04 (52.35%) than puncture 1.75±0.15 (42.55%) and the aspiration 0.72±0.08 (25.32%) techniques. Current results led to the conclusion that slicing technique was more efficient than puncture and aspiration techniques as oocytes retrieval technique on oocytes yielded and quality for goat oocytes.

Keywords: Goats ovaries, follicle, oocyte, slicing, puncture, aspiration.

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INTRODUCTION

Self-sufficiency in animals' agricultural products is considered an important strategic goal at the present time for the national economy (Atsan *et al*.,2007), and for the purpose of improving the productive and reproductive characteristics of goats the means of breeding and reproduction must be promoted through the application of assisted reproductive techniques (ART) (Rahman *et al*., 2008). The assisted reproductive techniques, such as *in vitro* maturation (IVM) and fertilization (IVF), were introduced and application as a substitute to beat on the reproductive inefficiency in goats (Madan *et al*.,1994). In order to work with these technologies, it is necessary to obtain the primary oocytes at the lowest costs, and the slaughterhouse are considered a cheap and important source to retrieval these oocytes and applying and working with these techniques
(Asad *et al*.,2016). Modern laboratory methods have been introduced to collect oocytes from the ovaries of slaughtered animals, which include slicing, puncture of ovary and aspiration of follicle methods, by which it is possible to obtain good quality oocytes and many numbers (Wani *et al*.,2000). In Iraq, until now, few and very limited studies have been conducted on goats, so the present research was designed for differentiation among three techniques for obtaining oocytes on the productivity and quality of goat oocytes taken from slaughterhouse ovaries.

MATERIAL AND METHODS

This study was conducted at reproductive physiology and artificial insemination laboratory, Technical Animal Production Department, AL-Musaib Technical college (50kg south of Baghdad), AL-Furat AL-Awsat Technical University (ATU) from September 2018 to May 2019. A total of 300 genital system (600 ovaries) for adult non-pregnant local goats with unknown reproductive history slaughtered at abattoirs of Babylon province were collected directly after slaughter. The ovaries were put in 0.9% physical saline in thermo flask at 37Cº and transported to the laboratory within 1-2 hours of slaughter. In the laboratory, the ovaries were washed in normal saline solution to remove excess blood and tissue debris. The ovaries after that kept in petri dish and extraneous tissues were trimmed by clean scissor, and the ovaries were washed treble by phosphate buffer saline (PBS) with pH7.3 (Gordan, 1994). The visible follicles with diameters of (2-6 mm) were counted and recorded. Following then the ovaries put in completely soaked in a cup contains (0.09%) saline and kept at 38.5Cº until times of retrieval of oocytes (Shahid *et al.,* 2013). Each ovary was processed individually, and the oocytes were retrieval by one of the following techniques.

1. Slicing technique: The base of the ovary was attached with help of forceps in petri dish containing 4ml of PBS. The oocyte was retrieved by slicing the surface of ovary into deep incisions with a single sterile surgical blade (Fig. 1/A).

2. Puncture technique: The base of the ovary was attached with help of forceps in petri dish containing 4ml of PBS. The complete surface of ovary was carefully punctured by using 18 gage hypodermic needles (Fig. 1/B).

3. Aspiration techniques: The oocytes from the follicles with a diameter of 2-6mm on the surface of ovaries were aspirated using 23-gauge needle attached with a sterile 5ml disposable syringe containing 1-2 ml of PBS. The oocytes with PBS medium were put slowly into petri dish (Fig.1/C).

After these processes, the petri dishes were preserved serene for 10 minutes in order for oocyte to become stable at the bottom. The petri dishes were than observed under an inverted microscope and the oocytes were scanned. The scanned oocytes were examined for grading and assessed as good oocyte, fair oocyte and poor oocyte depending to their morphological shape (Rahman *et al.* 2008) Into:

1.Good grade: the oocyte with at least three layers of cumulus cells and transparent, homogeneous and uniformly ooplasm (Fig. 2 /A).

2.Fair grade: The oocyte contains as few as three layers from cumulus oophrous cells and transparent, less homogeneous and uniformly ooplasm (Fig. 2 /B).

3.Poor grade: The oocyte with mild or absent cumulus cells (Denuded oocyte) (Fig. 2 /C).
The total number of recovered oocytes were counted.

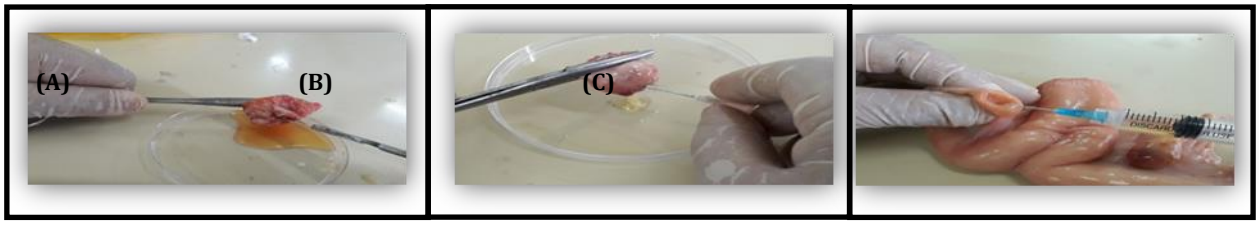

Fig. 1. Retrieval oocytes techniques: (A: Slicing, B: Puncture and C: Aspiration)

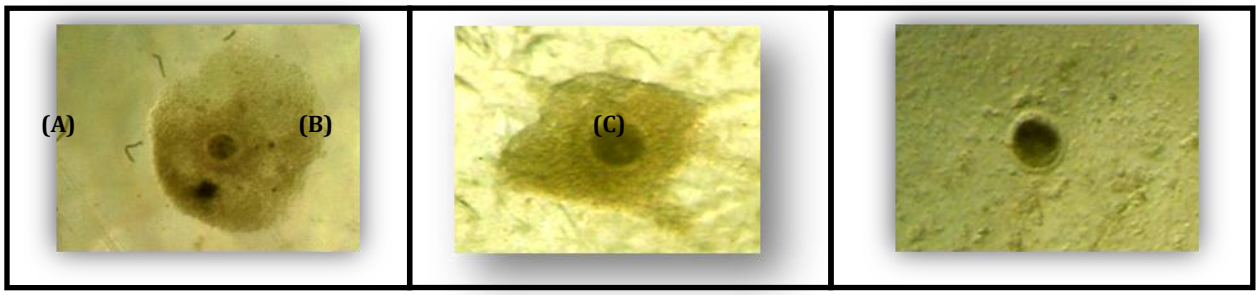

Fig. 2. Different grade of retrieved oocytes: (A: Good oocyte, B: Fair oocyte and C: Poor oocyte)

Statistical analysis
The statistical computation was performed using SAS program (SAS, 2012). The difference between means was detected by Duncan's multiple range test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

Influence of oocyte collection techniques on oocyte quantity.
The average total number of oocytes retrieved per ovary in the present study were 4.58±0.08 of which 6.80±0.11 by slicing technique was significantly higher ($P<0.05$) than achieved by puncture (4.12±0.06) and aspiration (2.84±0.03) techniques (Table 1). The same observation was previously reported on goat by Martino *et al.* (1994), Pawsh *et al.* (1994), Wang *et al.* (2007), Ramsingh *et al.* (2013), John *et al.* (2015) and Rameez *et al.* (2017). They showed that slicing technique yield more oocytes per ovary than puncture and aspiration techniques. In contrast Wani *et al.* (2000) in sheep and Hoque *et al.* (2011) in goat did not find any differ between slicing and puncture techniques when using both in retrieval of oocytes, always both produce similar quantity of oocyte. The reason for more oocytes yields per ovary in slicing technique could be attributed that by slicing technique, oocytes from follicles as well as from deeper stroma are released, whereas by puncture and aspiration techniques oocytes from surface alone released (Pawshe *et al.*, 1994; Das *et al.*, 1996). The lower number of oocytes retrieved by aspiration technique in this study may be attributed to the reason that only surface follicles are accessible and deep-set follicles cannot be reached, whereas in slicing technique the oocytes from both superficial follicle and those in the deeper cortical stroma can be accessed (Das *et al.*, 1996). Moreover, those oocytes which are not free floating and remain firmly attached in small and medium follicle could not be aspirated before onset of cumulus cells expansion but could easily be retrieval by slicing technique (Ball *et al.*,1983).
Less number of oocyte retrieval by aspiration technique might be because aspiration disrupts cumulus damage the whole oocytes (Nowshari, 2004), and some oocyte maybe lost during process of aspiration of follicles techniques, but this process does not occur during the use of ovarian slicing technique (Hammad *et al.*, 2014). The variation in oocytes recovered by the same technique in different studies recorded by several research might be attributing the age, season, body conditions, and the state of estrus cycle and follicles at the time of animal's slaughter (Zoheir *et al.*, 2007; Amer *et al.*, 2008). Furthermore, the expertise of the individual in processing the ovaries and stress of handling large number of ovaries per day could be also have affected the efficiency of oocyte retrieval, and also other variables like breed character, nutritional status of animals and agro climatic condition (Arul, 2017).

Table 1. Influence of oocyte retrieval techniques on quantity of oocyte collected from ovaries of Iraqi goats

Retrieval techniques	No. of ovaries	No. of retrieval oocytes	No. of oocytes per ovary (mean±S.E.)
Slicing	200	1360 (49.41%)	6.80±0.11 a
Puncture	200	824 (29.94%)	4.12±0.06 b
Aspiration	200	568 (20.63%)	2.84±0.03 c
Total	600	2752	4.58±0.08*

a.b.c mean in column with different literals by factor are different at (P<0.05).
Figures parentheses indicate percentage.
* Indicate average

Influence of oocyte collection techniques on oocyte quality.

In the current study each the oocytes retrieved by three techniques were graded according on oocyte morphology, like number of layers of cumulus oophorus cells surrounded of zona pellucida and traits of ooplasm and then classified as grades good fair and poor oocytes with special features as described by Rahman *et al*. (2008). The rate of oocyte retrieved per ovary in present study were 4.58±0.08 poor oocytes of which 2.01±0.12 (40.07%) good oocytes, 1.29±0.03 (28.93%) fair oocytes and 1.28±0.02 (30.99%) good oocytes (Table 2). The good grade oocytes were retrieved greatly from slicing technique (3.56±0.14) compared to puncture (1.75±0.15) and aspiration (0.72±0.08) techniques (Table 2). In agreement with our study Rameez *et al.* (2017) showed that the total number of good grade oocytes per ovary retrieved by slicing technique was 2.84±0.16 (40.43%) compared with puncture technique 1.63±0.12 (27.00%) and aspiration technique 0.61±0.13 (33.19%) similar finding by Pawshe *et al.* (1994).

Wang *et al* (2007) reported that the number of good grade oocytes per ovary collected by the slicing (3.9) and puncture (3.2) methods were significantly increased (P<0.05) than that collected by aspiration I (1.4) and aspiration II (1.8). However, the results of the present study different from those reported by Hoque *et al.* (2011) who demonstrated that the number of the good oocytes per ovary recovered by aspiration (2.29±0.08) significantly (P<0.05) higher than slicing technique (1.24±0.07) and puncture (1.20±0.09) technique. Who showed that both techniques slicing and puncture were increasing debris which might impede the search for oocyte with the microscope and the oocytes need more washing comparison with aspiration technique?

Hence, because of re-washing, a number of oocytes were lost cumulus cells and finally resulted decreasing of number of good and fair grades oocytes comparison with aspiration technique. The slicing technique of ovaries is easy and effective method for recovery good quality oocytes, but the aspiration technique is laborious and time consuming (Pawshe *et al*., 1994; Ramsingh *et al*., 2013). The results of the present study also indicated the aspiration of follicles appears less effective in both retrieval of oocytes and yielded of normal good oocytes. Similar finding has been recorded by Pawshe *et al.* (1994), Ramsingh *et al.* (2013). Different observation was made by Hoque *et al.* (2011) demonstrated that the number of normal (grade A + B) oocytes per ovary were significantly increased (P<0.05) in aspiration technique reached (2.48±0.10) than by slicing technique of ovary (1.91±0.10) and puncture techniques (1.85±0.09).

Concerning the percentage of good grade oocytes was higher in slicing technique (52.35%) when compared with puncture (42.55%) and aspiration (25.327) techniques (Table 2). The higher percentage of good grade oocytes observed in the present study were in agreement to those reported for goats by Ramsingh *et al.* (2013) mentioned that the percentage of good grade oocyte in goats was significantly higher in slicing (50.57%) followed by dissection (37.52%) and aspiration (20.07%) techniques. Similar finding has been reported by Rameez *et al.* (2017) who showed that percentage of good oocytes in goats was higher in slicing (40.43%) comparable with the puncture (27.00%) and aspiration (23.19%) techniques.

Similar observation was made in goat by John *et al.* (2015). However, our results are in contrast with the finding of Wang *et al.* (2007) who demonstrated that the percentage of good grade oocytes per ovary for slicing (61.9%) and puncture (55.2%) did not differ significantly compared with aspiration (48.3%) techniques. In conclusion, based on these data we can inferred that slicing technique increased yielded of oocytes and higher percentage of good grade oocytes. Such results indicate that slicing technique was more efficient than puncture and aspiration techniques as oocytes retrieval technique on quantity and quality of goat oocytes.

Table 2. Influence of oocyte retrieval techniques on quality of oocyte collected from ovaries of Iraqi goats (mean±S.E.)

Retrieval techniques	Oocyte quality			
	Good oocytes	Fair oocytes	Poor oocytes	Total
Slicing 200 ovaries	3.56±0.14a (52.35%)	1.78±0.22a (26.24%)	1.45±0.24a (21.41%)	6.80±0.11a
Puncture 200 ovaries	1.75±0.15b (42.55%)	1.24±0.13b (30.18%)	0.12±0.13b (27.27%)	4.12±0.06b
Aspiration 200 ovaries	0.72±0.08c (25.32%)	0.86±0.03c (30.38%)	1.26±0.02c (44.30%)	2.84±0.03c
Overall 600 ovaries	2.01±0.12 (40.07%)	1.29±0.03 (28.93%)	1.28±30.99 (30.99%)	4.58±0.08

a.b.c mean in column with different literals by factor are different at (P<0.05).
Figures parentheses indicate percentage.

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