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# Influence of Effciency of Different Oocyes Harvesting Methods on Oocyte Retrieval and Quality in Local Ewes (*Ovis Aries*)

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***Abstract.*** *The aimed of present study to determine the influence of different oocytes harvesting methods on oocyte retrieval and quality in Local ewes (Ovis aries). The genital organs of 225 (450 ovaries) for adult non - gravid ewes slaughtered at the slaughterhouses of Babylon provice for duration from September 2020 to January 2021. The oocytes were obtained from ovaries by three methods include ovarian slicing, ovarian puncture and aspiration of follicle methods. The oocytes were graded as good, fair and poor oocytes on basis of cumulus cells, homogeneity of ooplasm and shape of oocyte. The results showed a significant increase ($p \leq 0.05$) in the total number of oocytes and the average number of oocytes per each ovary by using ovarian slicing method and amounted to 1032 and 6.88±0.06 respectively compared with ovarian puncture method and reached to 741 and 4.94±0.11 respectively and aspiration of follicle method and reached to 465 and 3.10±0.08 respectively. A significant increase ($p \leq 0.05$) in the average number of oocytes per each ovary (3.12±0.13) and the percentage of good oocytes 45.35% obtained by the ovarian slicing method observed compared to the ovarian puncture method (1.87±0.03 and 37.80 % respectively) and aspiration of follicle method (0.88±0.06 and 28.22% respectively) . According to this results can be concluded that the ovarian slicing method was found the suitable and effective method for obtained a larger number and good quality of oocytes and least number of poor oocytes compared with ovarian puncture method and aspiration of follicle method.*

***Keywords:*** *local ewes; oocytes; slicing; puncture; aspiration.*

## 1. INTRODUCTION

The oocytes obtained from ovaries of live animals by means of ovum pick up (OPU) process for use in various scientific researches require experience in this field and are expensive [1]. The ovaries from slaughtered animals can be used as an alternative source for oocytes collection [2]. In order to improve the reproductive Characteristics of local ewes , assisted reproductive technologies (ART) necessitate introduced and applied, such as collecting of oocytes from ovaries by new laboratory techniques, *in vitro* maturation (IVM) and *in vitro* fertilization (IFV) of oocytes and embryos transfer (ET) [3]. To apply this technologies, oocytes must be gained at low prices and at lower costs. So, the ovaries attained from slaughter houses are available, cheap and important source for oocytes collection [4]. New laboratory techniques have been introduced to collect oocytes from ovaries of ewes slaughtered in slaughterhouses , which include slicing of ovary, puncture of ovary and aspiration of follicle. However, with these techniques we can obtain good quality oocytes in many numbers and using in ART [5]. The morphological traits of oocyte, which includes cumulus cells , ooplasmic state and zona pellucida are used

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in the classification and grading of oocytes and they are more commonly used as criterion *in vitro* maturation of oocytes and development of embryos [2]. The aim of this study was determine possible influence of different oocytes harvesting methods on oocyte retrieval and quality from the ovaries of local ewes slaughtered in the abattoirs.

## 2. MATERIALS AND METHODS

### *2.1. Study area*

The study was conducted in reproductive physiology and artificial insemination lab. Technical Animal Production Department, Al-Musaib Technical College, Al-Furat Al-Awsat Technical University (ATU) from September 2000 to January 2021.

### *2.2. Collection of genital organs*

A total 225 non - pregnant adult with unknown reproductive histories ewes in good health and with normal reproductive tracts upon macroscopical examination after slaughtered at abattoirs of Babylon province were collected half an hour after slaughter. The genital organ was placed for each animal in a plastic bag containing physiological saline (0.9% NaCl) ,then all these bags put in chilled thermos flask and transferred to laboratory with an 2 hours after slaughter [6].

### *2.3. Collection of ovaries*

In the laboratory the ovaries removed with sterile scissors and forceps and ovaries were washed with normal physiological saline to remove the excess blood and tissue debris. The excess tissues were removed with a sterile scissors, then the ovaries were washed twice with phosphate buffer saline (PBS) with pH 7.3 [7]. The ovaries were divided evenly randomly for each day of samples collection between three methods of oocytes retrieval, at a rate of 150 ovaries for each method, and then the oocyte. were obtained from each ovary separately by the following method [8].

- Aspiration of follicle: The follicular fluid (FF) was aspirated from the visible follicles (2-6 mm diameter) on the surface of the ovary , using a 20 - gauge needle attached to 2 or 5 ml sterile disposable Syringe containing 2 ml of PBS, The collected FF was then transferred to a 35 mm petri dish (Fig.1/a)
- Puncture of ovary: The ovary was held tightly by artery forceps and put in a petri dish contains 5 ml of PBS and the whole surface of ovary was punctured by using 18 - gauge hypodermic needle (Fig. 1/b)
- Slicing of ovary. The base of the ovary hold using artery forceps to hold it in place . Two to three mm deep incisions by sterile surgical blade were made on each ovaries surface for ovary placed in petri dish that contains 5 ml of PBS (Fig.1/c).

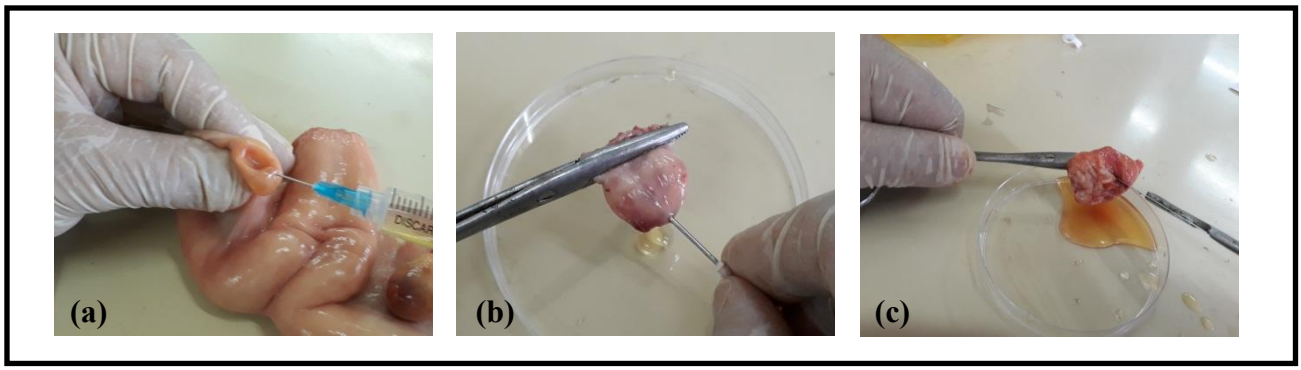

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**Fig. 1. Methods of harvesting oocytes from ovaries**
(a) Aspiration of follicle (b) Puncture of ovary (c) Slicing of ovary

*2. 4. Oocytes Examination and graded*

After completing each oocyte retrieval method, the contents were preserved quiet in a petri dish for 5 minutes, so that the oocytes could settle at bottom. The contents of petri dish were then examined under inverted microscope and oocytes were investigated and counted. The investigated oocytes were grading as good, fair and poor oocytes on basis of cumulus cells, ooplasm granulity and shape of oocyte [9] in to:

- Good oocyte: The oocyte is surrounded by more than three layers of cumulus cells with homogeneous ooplasm and normal shape of oocyte (Fig.2/a)
- Fair oocyte: The oocyte is surrounded by less than three layers of cumulus cells with homogeneous ooplasm and normal shape of oocyte (Fig. 2/b)
- Poor oocyte: The oocyte with absent of cumulus cells (denuded oocyte) with heterogeneity of ooplasm and small or dead oocyte (Fig. 2/c),
- The number of retrieval oocytes for each ovary were counted and recorded. The number of good, fair and poor oocytes obtained were counted and recorded for each ovary.

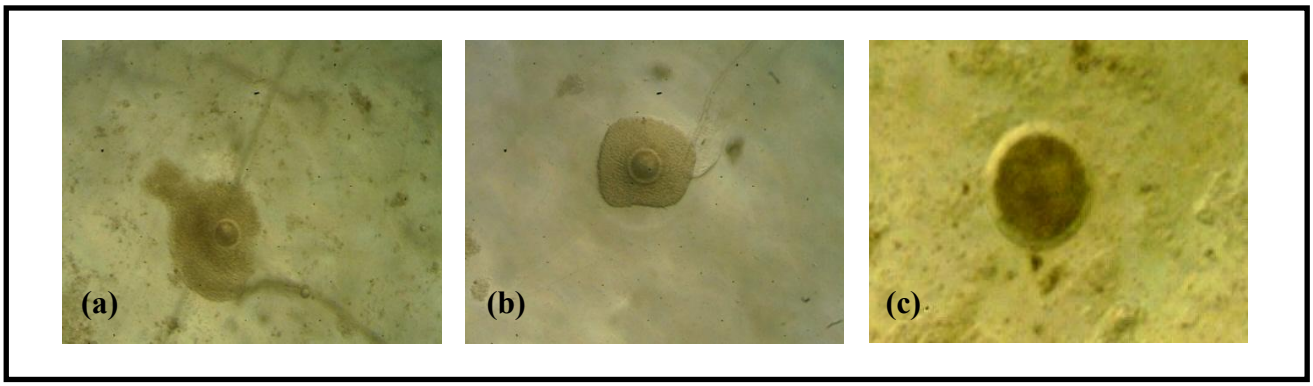


**Fig. 2. Freshly retrieval ewes oocytes**
(a) Good oocyte (b) Fair oocyte (c) Denuded oocyte

*2.5. Statistical analysis*

The data were analysed using statistical analysis system (SAS) [10] program. The significant difference were through using Duncan's multiple range test [11]. The ($p \leq 0.05$) considered significant difference.

## 3. RESULTS AND DISCUSSION

The results of the table 1 showed that a significant increased ($P \leq 0.05$) in the slicing of ovary method in the number of oocytes (1032) and mean number of oocyte for each ovary (6.88±0.06) and at percentage of 46.11% compared to the ovarian puncture method, which amounted 741, 4.94± 0.11 and 33.11% respectively, and the aspiration of follicle method which reached 465, 3.10 ± 0.08 and 20.78% respectively. The increase in the number of oocytes resulting from the method of ovarian slicing attributed the release of oocytes from all superficial follicles and from follicles in the ovarian cortex, and the excessive high pressure in holding of the ovaries lead to release oocytes from these follicles [6]. The method of aspiration of follicles include large follicles, leaving the small follicles buried and cannot be

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obtained, perhaps the reason for the lack of production of oocytes in this method [12]. The oocyte remains firmaly attached to the small and medium follicles before the expansion of the cumulus cells, and cannot be aspirated by the method of aspiration of follicles but the oocytes can be easily released from small follicles by using slicing and puncture method of ovary [8]. The results of our study are almost similar to previous studies in ewes by [8], [13] ,[14] they stated that the ovarian slicing method was significantly higher ($p<0.05$) compared to the ovarian puncture method in obtaining the larger number of oocytes from ewes ovaries. The results were in agreement with previous reports in goats by [15] and [16] demonstrated slicing yielded a significantly increased number of total oocytes for each ovary than that of aspiration of follicle method.

**Table 1. Influence of oocyte collection methods on harvesting of oocyte in local ewe (Ovis aries)**

| Harvesting methods | Number of ovaries | Number of harvested oocytes | Number of oocyte per ovary (Means ±S.E.) |
|---|---|---|---|
| Slicing | 150 | 1032 (46.11%) | 6.88±0.06 a |
| Puncture | 150 | 741 (33.11%) | 4.94±0.11 b |
| Aspiration | 150 | 465 (20.78%) | 3.10±0.08 c |

a,b,c Mean the averages with different letters in the same column differ significantly ($P\leq0.05$)
Figures in parentheses show percentages.

The results of the study showed a significant increased ($p\leq0.05$) of the ovarian slicing method in mean number of oocytes for each ovary for good oocyte (3.12±0.13) and fair oocyte (2.35±0.01) compared with the ovarian puncture method and the aspiration of follicle method (Table 2).The results of table also showed the percentage of poor oocyte by slicing method (20.46%) was less than of the ovarian puncture method (34.70%) and the aspiration of follicle method (45.60%). The production of good and fair oocyte by ovarian slicing may be due to the release of oocytes with many numbers of surface follicles and follicles in the ovarian cortex, while in aspiration of follicle method, the oocyte can be obtained from visible superficial follicles in which the cumulus cells probably firmly attached to layers of granulose cells [17],[18]. Similar results were obtained by researchers in previous studies as a studies [19], [20] and [14] they stated that the ovarian slicing method was significantly higher compared with aspiration of follicle method and ovarian puncture method to obtained a larger number of good and fair oocyte from the ewes ovaries. However, our results are in contrast with the finding by [21] they demonstrated that the slicing and puncture of ovaries methods produce ovarian tissue residues which interfere with finding the oocyte during the search under the microscope, and also these oocytes to be washed than once compared with aspiration of follicle method, and as a results, the oocytes are stripped from cumulus cells and finally we get lower good oocytes when compared to the aspiration of follicle method. The results of our study also differ from finding by [6] in sheep and [15] in goats, they reported that the percentage of good oocytes were higher in the aspiration method compared with slicing method. The reason for this differences in oocyte quality may be due to breed, species, age, season, type of oocyte collection and size and functional state of follicle [22],[23],[24] .

## 4. CONCLUSIONS

According to this results can be concluded that the ovarian slicing was found the suitable and effective method for obtained a larger number , good quality and normal shape of oocytes and in lower

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cost , with many oocytes capable of producing embryos , and least of poor oocytes compared with ovarian puncture method and aspiration of follicle method .

**Table 2. Influence of oocyte harvesting methods on quality of oocyte in local ewes (*Ovis aries*)**

| Harvesting methods | Means ( S.E.) oocyte quality | | | |
|---|---|---|---|---|
| | Total | Good oocyte | Fair oocyte | Poor oocyte |
| Slicing (150 ovaries ) | 6.88 ± 0.06 a [1032] | 3.12 ± 0.13 a (45.35 %) | 2.35 ± 0.01 a (34.19 %) | 1.41 ± 0.06 a (20.46 %) |
| Puncture (150 ovaries ) | 4.94 ± 0.11 b [741] | 1.87 ±0.03 b (37.80 % ) | 1.36 ±0.07 b (27.50 % ) | 1.71 ± 0.09 b (34.70%) |
| Aspiration ( 150 ovaries ) | 3.10 ± 0.08 c [465] | 0.88 ± 0.06 c (28.22 %) | 0.81 ± 0.02 c (26.18 %) | 1.41 ± 0.07 c ( 45.60 % ) |
| Overall ( 450 ovaries ) | 4.07± 0.13 [2238] | 1.96 ±0.06 (37.12 %) | 1.51 ± 0.01 (29.29 %) | 1.51 ± 0.04 (33.59 %) |

a,b, Mean the averages with different letters in the same column differ significantly ($p<0.05$).
( ) : Figures in parentheses show percentages .
[ ] : Figures indicate number of oocyte .

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