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# Effect of collection methods on oocyte recovery rate in sheep

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***Abstract***
*This study was carried out on 45 genital system of Iraqi ewes collected from slaughterhouses located at Baghdad city, during the period November 2016 to February 2017. The genitalia samples were transferred to gynecology lab within 2 hrs in normal saline by a cool box. Many techniques were used for collecting Oocytes including slicing, aspiration and puncture. The number of ova were counted.*

*The results revealed a statistical difference (P<0.05) in different ovaries (left and right) in recovery rate of different methods of oocytes recovery. It has been found that the big follicles give a high recovery rate. The results illustrated statistical differences (P<0.05), among the big and small follicles. The recovery rate of the oocyte by using aspiration and puncture methods increased statistically more than the ova recovered via slicing. The results showed that the higher recovery rate was obtained with grade B (Medist) oocyte in all collection methods as compared with other grades. It was concluded from this study that collection method of oocytes from slaughtered animals might affect the recovery rate of oocytes.*

**Keywords:** Sheep, Oocyte recovery, aspiration, slicing and puncture.

## Introduction

Ovaries obtained from slaughtered animals are available and low-cost sources for in vitro embryo production (IVEP).[4] The use of abattoir offal (Ovaries) has been considered as an invaluable source of Oocytes for *in vitro* fertilization. There are several techniques used for harvesting the oocytes from ovaries taken from the abattoir. These techniques include aspiration, slicing and puncture.[3,8,14,15,21,23] Each method has advantages and disadvantages.[14] The work aimed to compare different procedures of oocytes recovered from ovaries of slaughtered sheep.

## Material and Methods

The study was conducted on 45 genital system of Iraqi ewes collected from slaughter houses located at Baghdad city during the period from November 2016 to February 2017 (Outside the breeding season). The samples were transferred to the gynecology lab. within 2 hrs in normal saline by a cool box. Ova were recovered via three methods:

1) Aspiration with 18- gauge needle: The fluid of follicles was aspirated through sterile 18-gauge needle attached to a 5 ml syringe containing a harvested medium (follicular fluid).

Aspirated contents were expelled into a fresh Petri dish containing the medium and ova with cumulus cells were chosen.

2) Ovarian slicing was put in a Petri dish that includes collected media or normal saline and was cut into small parts by a blade.[20,21] Ova that surrounded by cumulus cells was selected from collected media.

3) Puncture visible follicles on the surface of the ovaries ranging from 2-6 mm in diameter with 18- gauge syringe needle. Cumulus cells were chosen from the fluid of follicles.[8,20,21] Oocytes collected from slaughtered ewes by aspiration or slicing or puncture were examined under the microscope and graded according to Wani et al[21] as a good = grade A, medist = grade B and poor = grade C according to the presence of the ova cells and uniform cytoplasm.

**Good:** Oocytes with numerous layers of cumulative cells and uniform cytoplasm.
**Medist:** Oocytes with thin or incomplete layers of cumulative cells and uniform cytoplasm.
**Poor**: Oocytes with few or no cumulative cells.

The values were recorded as means ±S.E. and subjected to analysis via T-test and analysis of variance.

## Results and Discussion

Ovarian type's effect on the count of follicles and ova recovery through different methods of collection were illustrated in the table 1. The results showed that the left ovary is littler active than the right in the presence of follicles and oocytes recovery rate. The results revealed a statistical difference (P<0.05) in different ovaries (right vs left) in the number of follicles and oocytes recovery. The similar observation has been made by several investigators[6,9,22] while Al-Jumaily[2] showed no significant differences. This might be due to breed difference and/or seasonality.[5]

The influence of follicle size on recovery rate has been illustrated in table 2. The result showed that big follicles give high recovery rates. There was a significant difference (P<0.05) in recovery rate between the large and small follicles. Similar observation has been made by several

workers.[5,13] This might be due to large follicles containing well-developed oocytes as compared with smaller follicles.[7,22] The size of follicular may be impacted by age, reproductive performance, season of breeding and nutritional levels.[7]

Other researchers detected that the follicular size affected the oocytes quality. The involved proteins (mRNA) play a role in ova maturation.[11] It has been observed in cows that follicles more than 6mm in diameter produce more statically ova with different cell layers (granulosa cells). These ova produced a large number of *in vitro* blastocyst. It is believed that large size follicles might have GF (growth factor) promoting function and shape of the cumulus oocyte complexes (COCs) and embryo produced.[1,12,16]

The effect of collection methods on the recovery rate of oocyte is shown in table 3. Recovered ova via aspiration and puncture are statistically different than ova recovered by slicing. Similar observation was shown by Sogorescu et al[17] while Majeed et al[13] investigated in the goats. The results disagreed with Wani et al[21] and Wang et al[19] in sheep and goat respectively. The aspiration and puncture methods recorded a high recovery rate due to the aspiration and puncture considered as the applicable technique for obtaining perfect oocytes production (quality and quantity), while the presence of the ovarian tissue debris in the slicing due to destruction the ova during the examination[10]. Furthermore, it needed extra-cleaning as comparatively used with other methods, table 4 showed the type of collection methods on the grade of the ova recovered.

**Table 1**
**Effect of ovarian location on the number of follicles and oocytes recovery in diff. methods**

| Ovarian location | No. | No. of follicles | No. of oocytes recovery |
|---|---|---|---|
| **Right** | 45 | 1.111±0.096$^{a}$ | 1.111±0.096$^{a}$ |
| **Left** | 45 | 0.844±0.089$^{b}$ | 0.844±0.089$^{b}$ |

Values: Mean ± SE. Different lower letters reveal a significant difference (P<0.05)

**Table 2**
**Effect follicular size on the recovery rate**

| Follicle size | Recovery rate |
|---|---|
| Small follicle (2-4mm) | 62%$^{b}$ |
| Large follicle (5-8mm) | 85%$^{a}$ |

Different lower letters reveal a significant difference (P<0.05).

Results showed that higher recovery rates were obtained with grade B (Medist) oocyte in all collection methods as compared with good grade (A) or poor grade (C). Statistical variations were present (P< 0.05) in quality of ova among aspiration and puncture as compared with slicing method. Wani et al[21] have made similar observation in sheep and Rahman et al[15] made in goats. The observation of low-quality grade oocyte recovered could be due to slaughters of low-quality ewes.

**Table 3**
**Effect of collection methods on recovery rate of ova**

| Methods | Ovaries numbers | Ova numbers |
|---|---|---|
| Aspiration | 30 | 0.966±0.139$^{a}$ |
| Slicing | 30 | 0.571±0.320$^{b}$ |
| Perforation(puncture) | 30 | 0.966±0664$^{a}$ |
| **Total** | **90** | **0.822 ± 0.666** |

Values: Mean ± SE. Different lower letters reveal a significant differences (P<0.05).

**Table 4**
**Effect of collection methods on the grade of oocytes (oocyte quality).**

| Type of collection method | No. of ovaries | Good (A) % | Medist (B)% | Poor (C)% |
|---|---|---|---|---|
| Aspiration | 30 | 34$^{a}$ | 59$^{a}$ | 7$^{b}$ |
| Slicing | 30 | 22$^{b}$ | 40$^{b}$ | 38$^{a}$ |
| Puncture | 30 | 30$^{a}$ | 54$^{a}$ | 16$^{b}$ |

Different lower letters reveal a statistical variation (P<0.05).

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