Effect of different techniques for oocytes collection on retrieval and quality of oocytes from Iraqi buffalo ovaries

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Abstract

The aim of the present study was to investigate the effect of three techniques for oocytes collection on retrieval and quality of oocytes collected from local buffalo ovaries. 240 ovaries were collected from 120 non-pregnant buffaloes with 3-8 years old, slaughtered in the slaughterhouses in Babil Governorate for the period from September 2020 to February 2021. The oocytes were collected using ovarian slicing , ovarian puncture and follicle aspiration techniques, then the oocytes were classified according to the number of ovarian cumulus cells layers and cytoplasm homogeneity into three groups, first: good oocytes surrounded by more than three layers of cumulus cells and homogenous cytoplasm, second: medium oocytes surrounded by fewer than three layers of cumulus cells and homogenous cytoplasm, and third: poor oocytes denuded of cumulus cells with heterogeneous cytoplasm. The results indicated a significant increase (P <0.05) for the total number of retrieved oocytes and the average number of oocytes per ovary using the ovarian slicing technique compared with the ovary puncture and aspiration techniques. Results of this study was also recorded a significant increase (P <0.05) for the total number of oocytes, average number of oocytes per ovary, and the percentage of good oocytes obtained by ovarian dissection compared with ovarian puncture and follicle aspiration techniques. It could be concluded from this study that the ovarian slicing technique was the appropriate and good technique for oocytes retrieving in large numbers, with good morphology and quality.

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Key words: buffalo, oocyte, slicing, puncture, follicle aspiration

Introduction

The buffalo have low reproductive performance with inherent reproductive problems including silent estrus, seasonal anestrus, delayed each of the sexual puberty, first birth, postpartum pregnancy and length of calving interval (1). Additionally, female buffalo have fewer ovarian follicles, poor ovulation, and higher percentage of follicle atresia (2).Attention and improvement of breeding and reproductive means in order to develop this wealth and raise the level of reproductive performance through application of modern assisted reproductive technology programs, including superovulation, collection of immature oocytes, in vitro maturation, artificial insemination and in vitro fertilization to increase the number of offspring of the selected females, and to reduce the periods of reproduction in buffalo (3), and to work with these technologies, oocytes must be obtained at the low cost, thus slaughterhouses are the cheapest and most abundant primary sources of oocytes for the production of embryos on a large scale through in vitro maturation and in vitro fertilization (4). The quantity and quality of oocytes that can be retrieved from the ovaries plays a major role in the acquisition of developmental competence of oocytes in the laboratory (5).Modern laboratory techniques have been introduced to collect the oocytes from slaughterhouse ovaries, which include ovarian slicing, ovarian puncture, and follicle aspiration techniques, by which it is possible to obtain large numbers of oocytes with good quality (6) and (7). The purpose of the oocytes collection methods is to increase the number of collected oocytes (8). The current

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of Animal Production Techniques at the Technical Institute / Al-Musayyib, Al-Furat Al-Awsat Technical University for the period from September 2020 to February 2021. The female reproductive system was removed from 120 local buffalo females (240 ovaries) who were slaughtered in the slaughterhouses in Babil Governorate and examined visually. It was normal and free of congenital anomalies, and placed in a plastic bag containing normal physiological saline at a concentration of 0.9% with a temperature between (35-37 °C) (12), then transported to the laboratory in two hours. The ovaries were removed with sterile scissors and forceps and cleaned of the suspended tissues and ligaments and washed three times with normal physiological saline solution to remove the blood and debris of the attached tissues at a temperature of 37 ° C. The ovaries were divided randomly into three equal groups according to the oocytes collection technique (80 ovaries per method), and each ovary was treated separately. The oocytes were collected using the following techniques (13):

1- Collection with ovarian slicing technique:

The ovary was placed in a sterile Petri dish with a diameter of 90 mm and containing 5 ml of a phosphate buffer solution with heparin at a ratio of 25 IU / ml to prevent clotting of the follicular fluid, The base of the ovary was held with forceps and the whole surface of the ovary was dissected at a depth of 2-3 mm using a sterile surgical blade (14) (Figure 1).

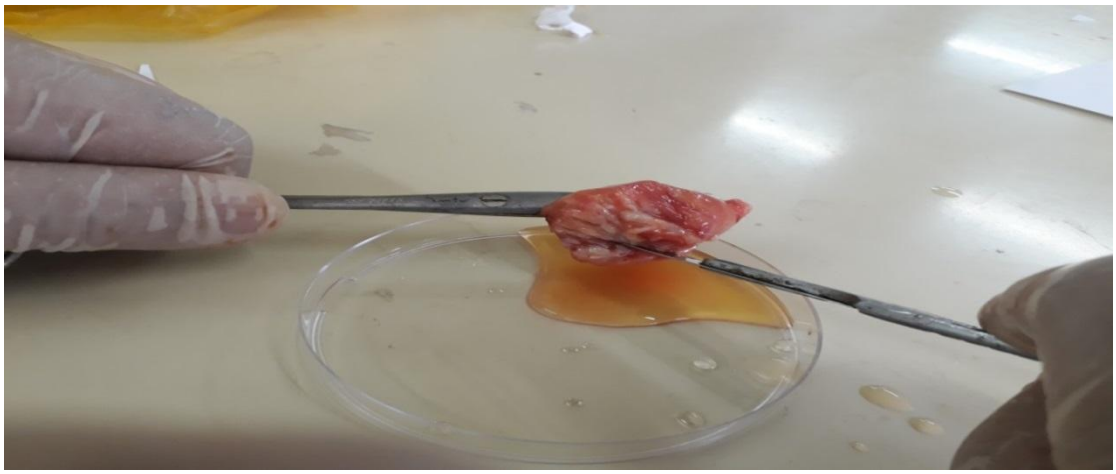

Figure (1) ovarian slicing technique

classification of oocytes is based on the morphology and number of ovarian cumulus cells layers and the external appearance of the cytoplasm (9). The presence of ovarian cumulus cells surrounding the ovum and cytoplasmic homogeneity are the most common morphological features used in the in vitro maturation of oocytes and development of embryos (10). The cumulus cells has effective role in nourishing, maturing and evaluation of oocytes (11), the oocytes can be evaluated depending on their quality and gradation which depending on the multiple layers of ovarian cumulus cells and the homogeneity of the cytoplasm, Accordingly, the current study aimed to evaluate the effect of three different oocytes collection techniques on the retrieval and quality of the oocytes and to choose the technique that gives oocytes with good morphology and quality at the lowest costs.

Materials and methods:

Experimental animals:

The study was carried out in the laboratory of Reproductive Physiology in the Department

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2- Collection with the ovarian puncture technique:

The ovary was placed in a petri dish with a diameter of 90 mm containing a phosphate buffer solution with heparin. The base of the ovary immersed in the medium was held by forceps and the whole surface of the ovary was punctured with a sterile, wine-sized needle of 18 gauge (15) (Figure 2).

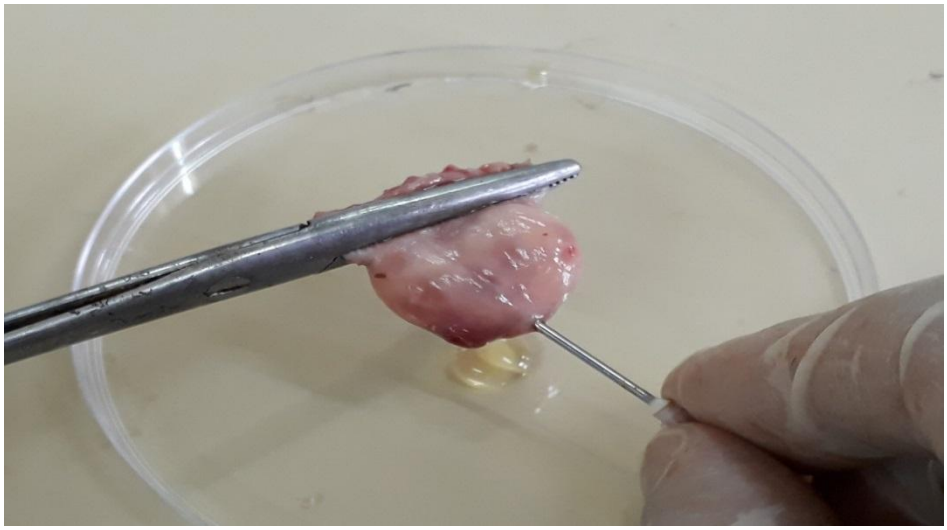

Figure (2) ovarian puncture technique

3- Collection with follicle aspiration technique:

Follicular fluid was aspirated from the visible follicles on the surface of the ovary with a diameter of 2-8 mm by means of a 20-gauge needle connected to a 2 and 5-ml plastic v-tube containing a phosphate buffer solution with heparin (16), the medium and contents were placed in a 35 mm petri dish and left for 5 minutes to allow oocytes to settle and settle at the bottom. The number of oocytes was examined and recorded (Figure 3).

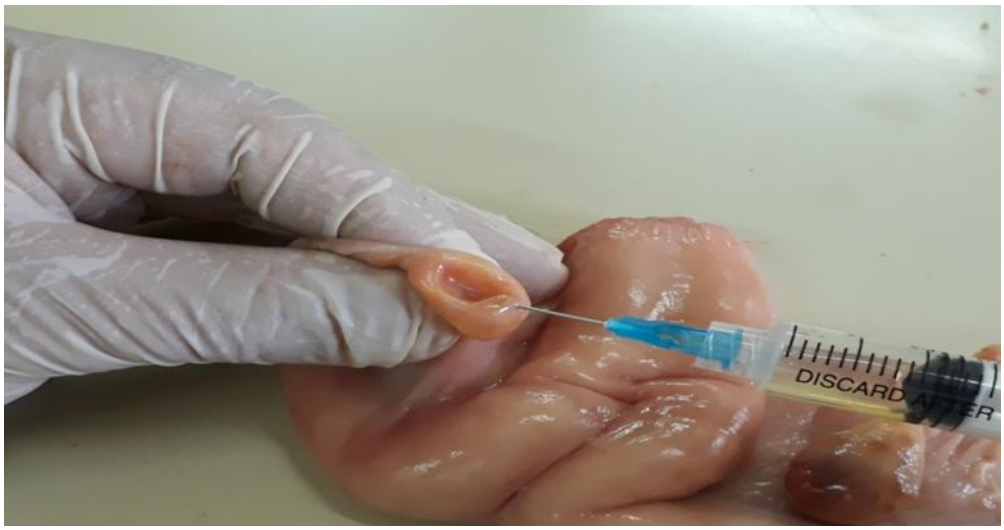

Figure (3) Follicle aspiration technique

Oocyte grading :

A Petri dish was left in all samples for 5 minutes to allow the oocytes and settle at the bottom. The oocytes were examined under inverted microscope and the number of oocytes was recorded . The oocytes were grading as good , fair and poor oocytes on basis of cumulus layer granuli of ooplasm and shape of oocyte (24).

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1- Good Oocytes: The oocyte was surrounded by more than three layers of ovarian cumulus cell with cytoplasmic homogeneity (Figure 4).
2- Fair Oocytes: The oocyte was surrounded by fewer than three layers of ovarian cumulus cell with cytoplasmic homogeneity (Figure 5).
3- Poor Oocytes): The oocyte was denuded from ovarian cumulus cell with cytoplasmic heterogeneity (Figure 6). The oocytes were photographed with the ovarian cumulus cell by means of a camera (Sawyer microscope company) linked to a computer.

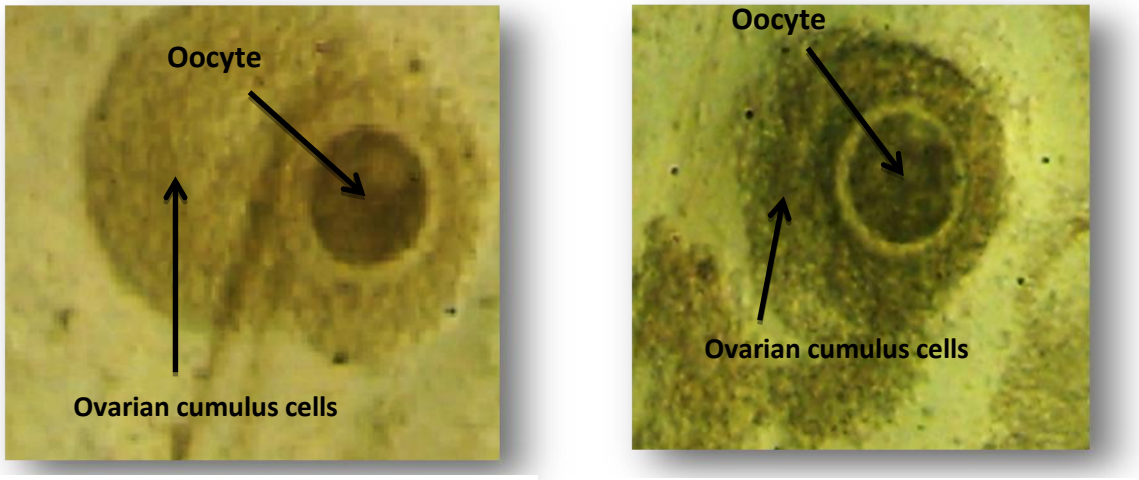

Figure (4) oocyte surrounded by more than three layers of ovarian cumulus cells

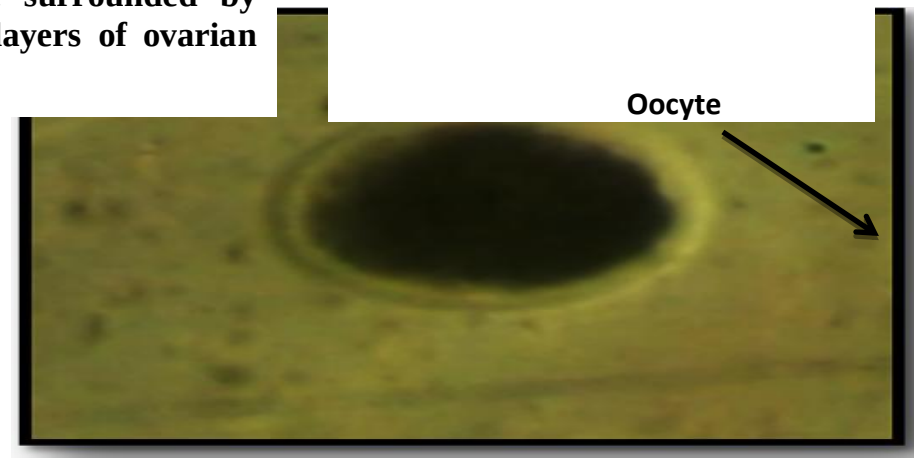

Figure (6) denuded oocyte devoid of cumulus ovarian cells

technique and follicle aspiration technique in the number of retrieved oocytes , mean number of oocytes collected per ovary and percentages of retrieved oocytes which were 445, 300 and 236 for the number of retrieved oocytes respectively and 5.57 ± 0.09, 3.74 ± 0.09 and 2.95 ± 0.08 for mean number of oocytes collected per ovary respectively and 45.432 % , 30.505% and 24.061% for percentages of retrieved oocytes respectively.

The superiority of the ovarian slicing technique in the total and mean number of oocytes per ovary may be due to the dissection process of the entire ovarian surface, including all small and medium

Statistical analysis:

The statistical program SAS (Statistical Analysis System) (18) was used in data analysis to study the effect of different techniques on the studied traits according to a complete random design (CRD). The significant differences between the means were compared with the Duncan [19] polynomial test, and the chi-square test was used to compare the significant differences between Percentages.

Results and discussion

The results of table (1) showed a significant increase (P <0.05) for the ovarian slicing technique than ovarian puncture

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aspiration technique in the total and number of oocytes per ovary. On the other hand, (25) explained that the number of oocytes per ovary for dissection and aspiration does not differ significantly in ewes, while (26) stated that there is no effect for dissection and aspiration techniques on the retrieval of oocytes in buffalo. The conflicting results in this regard may be attributed to the effect of the interaction between the collection technique and the reproductive status. The results of this study differed than (27) as they found a significant increase for method of follicle aspiration in obtaining the largest number of oocytes and producing good quality oocytes compared with the ovarian dissection method, as well as disagreeing with [28] as they noticed that the oocytes obtained by the aspiration method were more than those obtained by the dissection method. In the end, the dissection method was found to be a fairly appropriate technique for retrieval of total and usable oocytes in programs of assisted reproductive techniques.

follicles, before the ovarian cumulus cells expanded (20). The high number of oocytes resulting from the ovarian dissection technique, or perhaps the reason is due to the release of oocytes from all the surface of the ovary and from the depth of the ovarian cortex, and that the excess high pressure in holding the ovaries may release the oocytes from these follicles (21).

The technique of follicle aspiration includes the large follicles, leaving the small follicles Embedded and cannot be obtained, Perhaps this is the reason for the lack of production of oocytes in this technique (22). results of current study agreed with what was stated by (23), who showed the superiority of the ovarian dissection technique obtained from the oocytes (7.88), than the ovarian puncture (3.59) and follicle aspiration technique (2.50) in the mean number of oocytes per ovary respectively, and also agreed with (6) ,(13) and (24) who demonstrated the superiority of the ovarian dissection technique, followed by the ovarian puncture technique, and then follicle

Table (1) :The effect of three techniques for oocytes retrieval on the number of oocytes and the mean of oocytes production per ovary in local Iraq buffaloes (Mean ± S.E.)

Collection techniques	number of ovaries	number of obtained ocytes	mean number of oocytes	percentage %
Ovarian slicing	**80**	**445**	**5.57± 0.09 A**	**45.432**
Ovarian puncture	**80**	**300**	**3.74± 0.09 B**	**30.506**
Follicle aspiration	**80**	**236**	**2.95± 0.08 C**	**24.062**
Total	**240**	**991**	**0.26 ± 12.26**	**100**

dissection technique than ovarian puncture and follicle aspiration methods, the results also showed a significant superiority (P <0.05) for the number and rate of oocytes per ovary and poor oocyte ratios (Fig. 6) by follicle aspiration method compared with both ovarian dissection and puncture methods.

The production of good and fair oocytes by the ovarian dissection method may be due to the release of oocytes with high numbers of superficial follicles as well as the follicles in the ovarian cortex (29) while the method of follicle aspiration can obtain oocytes from visible follicles on the surface of the ovary in

Means with different letters differ significantly (P <0.05).

The results of table 2 showed a significant increase (P <0.05) for the ovarian slicing method in the total number of good oocytes (227) (picture 4), the mean number of good oocytes per ovary (0.09 ± 2.84), and the percentage of good oocytes (51.079%) compared to both ovarian puncture and follicle aspiration techniques , the results of Table (2) also indicated a significant increase (P <0.05) for the number and average number of oocytes per ovary and the percentage of fair oocytes (picture 5) obtained by ovarian

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results of this study differed with (33), was explained that the technique of follicle aspiration is useful because it produces complete, non-expanded ovarian cumulus cells from visible follicles with a diameter of 2-8 mm with a high percentage of good oocytes gradation and a small percentage of tissue debris and requires a small number of washings.

which the ovarian cumulus cells can be strongly attached to the layers of granulose cells (30), the age, season, reproductive differences of the animal, nutritional status, estrus cycle, size, functional state of the follicles, and the technique of retrieval of oocytes are among factors affecting oocyte quality [9], (5) and (31). The results of this study agreed with (13), (20) and. (32). The

Table (2) Effect of collection methods on oocyte gradation, quality and percentages of oocytes (Mean± S.E)

Collection techniques	number of ovaries	grading, numbers and percentages of obtained oocytes					
		good	%	fair	%	poor	%
Ovarian slicing	80	2.84± 0.09 A (227)	51.079	1.55± 0.10 A (124)	27.877	1.17± 0.07 A(94)	21.043
Ovarian puncture	80	1.80± 0.10 B (144)	47.872	0.93± 0.08 B(74)	24.734	1.03± 0.06 B(82)	27.393
Follicle aspiration	80	1.42± 0.09 B(113)	46.864	0.81± 0.08 B(64)	26.732	0.80± 0.07 C(64)	26.402

Means with different letters differ significantly (P <0.05).

It can be concluded from the current study that the ovarian slicing technique is the appropriate and good technique for retrieving oocytes in large numbers with good morphology and quality.

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