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Assessment of ova collection with or without centrifugation after ovarian slicing for In vitro fertilization of slaughterhouse specimens of Iraqi Awassi ewes

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Abstract

This study aims to determine the most appropriate method for oocyte collection based on their quality and quantity by slicing with or without centrifugation and testify its ability for in vitro embryo production (IVP). The ovum was collected in the Artificial Insemination Laboratory, College of Veterinary Medicine, University of Mosul, Mosul city between 1/9/2020 to 1/11/2020. A total twenty females Genitalia slaughtered at local abattoir were collected. *In vitro* fertilization was done according to private lab protocols (Soran laboratory related to Soran Private Hospital for fertility disease and embryo transfer) in Irbil city/Iraq. Results of the study show no significant changes between the two methods in the extraction of Grade A oocytes. However, the slicing method, which was 33.60% of ova recovery than centrifugation ORC techniques 22.73%, ORCs method in presence Grade C oocyte higher than ORCs method which were 29.09 and 20.00%, respectively also results of in vitro fertilization show that slicing method produces embryo in high percentage 45.4% than ORCs method 25.0%. We can conclude that the best method for oocyte collection was the slicing method and this method results in the best embryo production during *in vitro* fertilization compared with the ORCs method.

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Introduction

Artificial insemination was the first major biotechnology used to improve the reproductive and genetics of farm animals, and it paved the way for later innovations such as cryopreservation, sperm sexing, estrus cycle management and ovum synchronization, embryo harvesting, and cloning (1,2). Oocytes generated from slaughterhouse ovaries are a low-cost source of animal gametes that can be used to develop embryos on a large scale (3). When animal gametes are unavailable, like when genetically important livestock animals or endangered wild species suddenly die, needing to postmortem oocyte utilization is vital (4) also animals with serious illnesses include fractures, subfertility, and reproductive system surgery, as well as seasonal sterility and physiological disturbance like abnormalities of genital track (5), ovulation time (6), public health issues (7), which represented another type of gamete collecting challenges (8,9). Ovum pick-up (OPU) or ova collection in sheep was done in a variety of ways, although slicing was the most effective approach for collecting oocytes from abattoir samples (10). Several factors influence the estimation of collected ovum, including oocyte quantities, quality, time after slaughter, presence of corpus luteum CL, and in vitro maturation index (IVI). Previous research has shown that the slicing procedure produces more oocytes with better quality when compared to other methods (11). Animal fertility status, exogenous gonadotropin stimulation, collection equipment, culture media for collection and maturation during in vitro handling, and additives to handling media

such as hormones or proteins, embryo cell division were all important factors that influenced oocyte collection methods and embryo production (12,13), as a result, some researchers believed that centrifugation after slicing leads to the best quality ovum with effective rate of cleavage and blastocysts development and while no differences were recorded in case of meiotic resumption rate (14). Another researcher that backed with this notion suggests centrifugation after slicing when collecting ovum from sheep in the off-season give best results for ova gathering (15).

The aim of this study is to determine the most appropriate method for oocytes collection depending up on their quality and quantity by slicing method with or without centrifugation and testify its ability for in vitro embryo production (IVP).

Materials and methods

Location of the study

The ovum was collected and treated in the Artificial Insemination Laboratory, College of Veterinary Medicine, University of Mosul, Mosul city (the city's coordinates span between 2°36′ longitude and 43°7′ latitude) between 1/9/2020 and 1/11/2020, Ewes genitalia slaughtered at a local abattoir were collected within two hours of slaughter, the samples were washed with normal saline and antibiotics and transferred in a refrigerated box. *In vitro* fertilization was performed in Irbil city/Iraq, according to private lab guidelines (Soran laboratory at Soran Private Hospital for fertility disease and embryo transfer).

Oocytes collection

Clean scissors that had been rinsed many times with dis. Water and then normal saline was used to firmly and smoothly remove ovarian samples from surrounding tissues, as described in Abdul hafedh (15). Before processing, samples were separated into two sets of 20 ovaries, with one oocyte collection process performed under aseptic circumstances in a Becker containing phosphate buffer saline (PBS) with an antibiotic solution for 5-10 minutes at room temperature (RT). It was carried out under aseptic settings in a well-sterilized hood cabinet to avoid air pollution or other polluted environments that could have influenced the final results. The following procedure was used to retrieve oocytes.

Ovarian slicing for oocytes collection

The ovarian sample was cleaned with distilled water and normal saline before being suspended in a Becker containing PBS medium with antifungal and antibiotic treatments at RT. Each ovary was gripped or snatched with artery forceps slightly above the Becker solution preparation. The kidnapped ovaries would be cut multiple times, primarily over the follicles, to include oocytes in the follicular fluid, and then soaked in Becker to ensure that all of the material was dumped down in the medium (16).

Ova recovery by centrifugation (ORC) after slicing

Ovaries were sliced, and additional and deeper slicing was performed to acquire more oocytes (post slicing) (14). The total fluid in a petri dish was then filtered through a polypropylene filter and centrifuged at 1500 C/ minute for two minutes, discarding the supernatant and examining the precipitate for eggs (17).

Oocytes evaluation

In terms of cumulus cell arrangement surrounding oocytes and cytoplasm state, the approaches for collecting oocytes were subjected to quality control as described by Rahman *et al.* (18). The oocytes receive an excellent grade or grade A when they have numerous layers of cumulus cells and a translucent, homogenous, and uniform cytoplasm. The oocytes are given a fair or Grade B when they are less compact cumulus cells that are transparent, less homogeneous (some granules may be present), and uniform. Oocytes with a mild or absent cumulus (denuded) and black, granular cytoplasm are classified as poor or grade C. After grading and quality assessing the collected oocytes, oocytes were transferred to another Petri-dish containing PBS medium as prepared, re-examining the dishes after these ova transports, and confirming that all selective ova were transported by aspiration with an automated micropipette. Ova was grading papers and keeping track of the numbers (Figure 1).

Oocytes maturation

The maturation procedure was carried out on only good (grade A) oocytes, and the maturation medium was created according to lab protocols. The maturation media (MM) was equilibrated in a CO_2 incubator for two hours before adding the oocytes; 5-6 ml of the earlier solution was placed in a glass petri dish, and the oocytes were added later. The matured oocytes were viewed under an inverted microscope, and the degree of maturation was measured as reported by Wani *et al.* (19). The same maturation media was used to wash the graded and chosen oocytes many times, and the number of matured oocytes was tallied and recorded.

In vitro fertilization and embryo production

The initial combination of the capacitated epididymal ram spermatozoa from slaughtered samples with the Petri dish containing the developed oocytes was handled and capacitated two hours before using heparin in a special petri dish. The diluted spermatozoa must yield $1\text{-}2*10^6$ spermatozoa (17). The gametes were mixed and infected. The samples were incubated for 28-30 hours at 5 percent CO_2 at 38.5°C and 90% relative humidity. The fertilization media included LH, FSH, BSA, antibiotics, and antifungal

preparations. Developed embryos must be inspected and reviewed every 24 hours until 72 hours of incubation; embryos that show no signs of progress must be removed, and all developed embryos must be evaluated, with all results recorded.

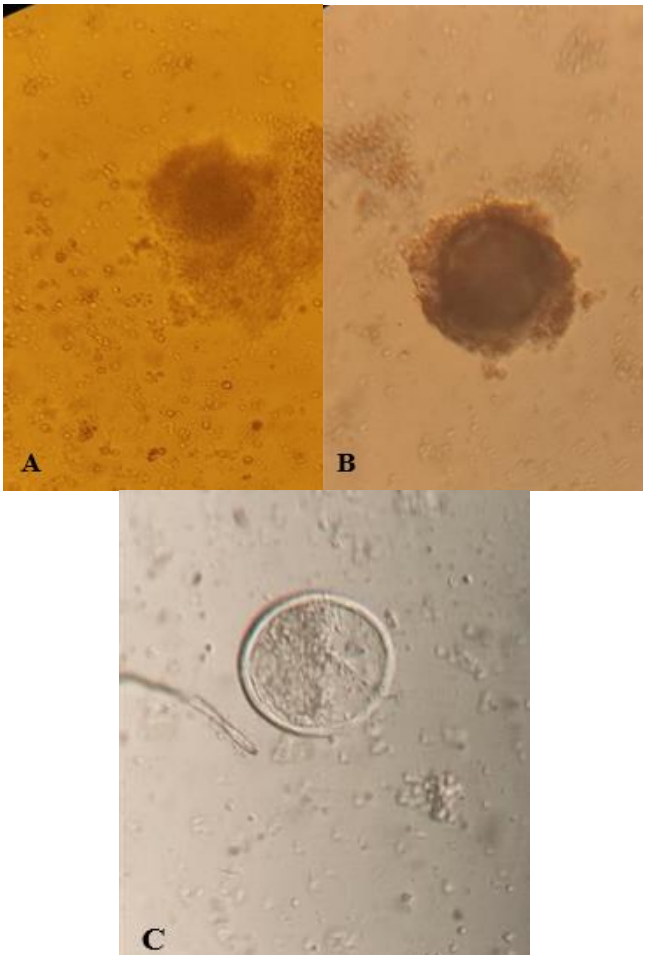

Figure 1: (A) oocyte with good Grade (Grade A); (B) oocyte with fair Grade (grade B); (C) oocyte with poor Grade (grade C) under ICSI inverted microscope X40.

Statistical analysis

The results of the experiments were expressed as mean + percentage. ANOVA was used to compare distributed data (One-Way Analysis of Variance). Duncan's Multiple Range Test was used to find significant variances. Sigma Stat was used to do the statistical analysis (18).

Results

Ova collection methods

Data of comparison between Ova methods extraction, which were Slicing and ORCs methods (Table 1). The data of the study show that no significant changes between the two methods in extraction of Grade A oocytes, but the slicing method recorded a higher percentage of data which were 33.60% than ORCs techniques 22.73%, ORCs Grade C oocytes higher than slicing method which were 29.09 and 20.00% respectively.

***In vitro* fertilization**

A total (n=11) matured ova were chosen according to their degree and well maturation (Grade A) from the slicing method, and eight ova chosen from ORCs (n=8) matured underwent fertilization. The results of in vitro fertilization were summarized in table 2, which declares that the slicing method produces embryos in a high percentage of 45.4% than the ORCs method, 25% (Figure 2).

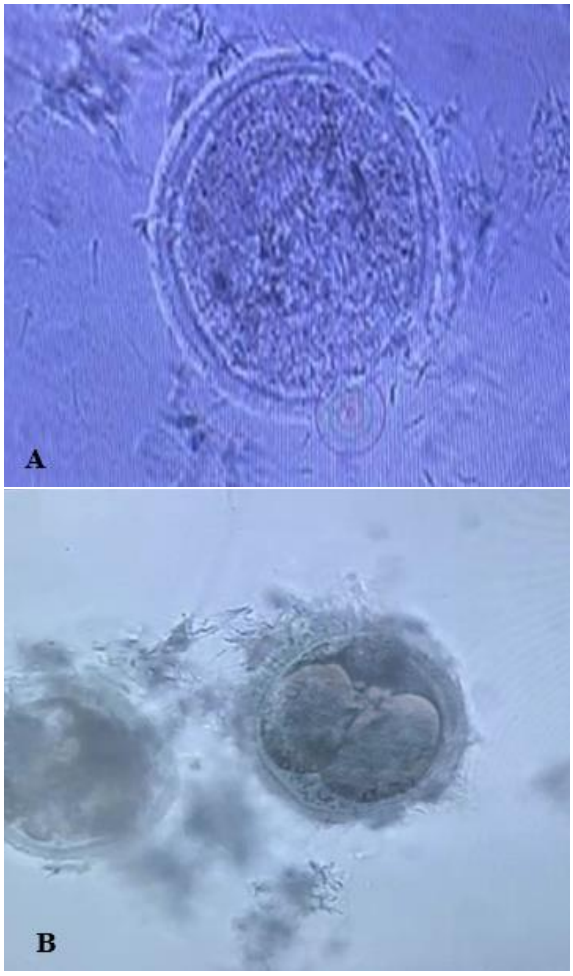

Figure 2: Retrospective of oocyte handling and maturation: (A) surrounding ova by spermatozoa; (B) embryo after 30 hours followed maturation show first meiosis division and presence to blastocysts cells.

Table 1: Grading of the two ova collection methods

Treatments		Ovum grading					
Method	Total ovum numbers	Grade A	%	Grade B	%	Grade C	%
Slicing	155	52	33.60[a]	72	46.45[a]	31	20.00[a]
ORCs	220	50	22.73[a]	106	48.18[a]	64	29.09[b]

Different letters in the same columns refer to significant differences at (P<0.005).

Table 2: Shows the percentage of embryo developments after ova fertilized and maturated ovum with two collection methods

Treatments			Embryo development 72 of incubation (Grades)					
Method	evaluated ovum (n)	fertilized & maturated ovum (n)	A (n)	%	B (n)	%	C (n)	%
Slicing	22	11	5	45.4	0	0	0	0
ORCs	20	8	2	25.0	0	0	1	12.5

Discussion

The main goal of this study was to evaluate the Slicing method and ova collection after slicing by centrifugation (ORCs) and compare them in order to determine the best method for in vitro fertilization of slaughterhouse specimens for Iraqi Awassi ewes based on simple strategies for fertilization, maturation, and embryo production. The results of this investigation demonstrated that slicing, rather than ORCs, was the optimal approach for obtaining good oocyte quality (grade A). The reason why the slicing method was superior may be related to its easy technique, which did not lead to a false final judgment of collected ova (3,19-21).

This investigation showed that the ova recovery by centrifugation ORCs methods shows lower numbers of Grade A oocytes and high numbers with grade B and Grade C oocytes. These data were in agreement with previous studies (12,22), while the present study was in disagreement with another study Dadashpour *et al.* (23) Who suggests that the ORCs method can collect the best Ova number and grades, especially in out of those seasonal animals. This incongruity may be because the ORCs technique contains centrifugation. This process leads to harmful effects on the ovum. It also leads to the loss of granulosa cells surrounding the ova which is regarded as the best guide for good ova (grade A) and also the process leads to elongated the ova cell and deformity of its standard shape. These two main changes affect ovum efficacy and influence the ability of ova for ovum meiosis during in vitro fertilization (24). On the other hand, another paper (12) suggested that the centrifugation force can be controlled to minimize the harmful effect of ORCs (800 cycles/minute for three minutes in cooling centrifugation.

Data of the present study refers to the slicing methods' significantly higher percentage of embryo production after 30-72 hours of incubation. These results were in agreement with previous reports (25) and this agreement may be related to similarities of *In vitro* conditions, like procedures, seasonality and incubation circumstances, but we disagreed with other papers (26). Efficacy of IVF related to Oocytes collection techniques, in case of ORC, according to Rahman *et al.* (27) who explain that even though ORCs we could contain some disadvantages like the removal of columned cells around the ova and oocytes injuries regarding *in vitro* fertilization, only Grade A oocyte which is taken for fertilization. We disagree with these explorations because the ORCs technique causes severe damage at cellular level or shock to oocytes and elongated ova cells. The fact that they are not noticeable under a microscope does not mean they are not there, in the other hand: the action of metabolism and growth factors released by cumulus cells have a significant impact on oocytes and their ability to develop into later embryonic stages. Additionally, the fertilization process between ova and sperm arranges cellular or attraction for each other Abdulhafedh (15), Hashimoto *et al.* (28)

Conclusion

In Awassi sheep, the best method for oocyte collection was the slicing method and this method results in the best embryo production during *in vitro fertilization* compared with the ORCs method.

Acknowledgment

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Conflict of Interest

There was no conflict of interest.

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تقييم جمع البويضات مع الطرد المركزي أو بدونه بعد تقطيع المبيض لأغراض التلقيح في المختبر لعينات المجزرة المذبوحة للنعاج العواسية العراقية

فاتن فاضل مصطفى وعدي طلعت نعمان

فرع الجراحة وعلم تناسل الحيوان، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

الهدف من هذه الدراسة هو تحديد الطريقة الأنسب لجمع البويضات اعتمادا على نوعيتها وكميتها عن طريق طريقة التقطيع مع أو بدون الطرد المركزي وإثبات قدرتها على إنتاج الأجنة في المختبر. تم جمع البويضات في مختبر التلقيح الصناعي بكلية الطب البيطري جامعة الموصل مدينة الموصل خلال الفترة من ٢٠٢٠/٩/١ الى ٢٠٢٠/١١/١. تم جمع ما مجموعه عشرين جهاز تناسلي انثوي ذبحت في مسلخ محلي وتم إجراء الإخصاب خارج الجسم وفق بروتوكولات المختبر الخاص (مختبر سوران التابع لمستشفى سوران الخاص لأمراض الخصوبة ونقل الأجنة) في مدينة أربيل. العراق. أظهرت نتائج الدراسة عدم وجود تغييرات معنوية بين طريقتين في استخراج البويضات من الدرجة أ ولكن طريقة التقطيع كانت اعلى ٣٣,٦٠٪ من استخلاص البويضات بالطرد

المركزي ٢٢,٧٣٪، البويضات من الدرجة ج كانت أعلى في طريقة جمع البويضات بالطرد المركزي من طريقة التقطيع حيث كانت ٢٩,٥٩ و ٢٠,٠٠٪ على التوالي ووجدت الدراسة أيضا فيما يخص إنتاج الأجنة في المختبر أن طريقة التقطيع تنتج أجنة بنسبة اعلى ٤٥,٤٪ من طريقة جمع البويضات بالطرد المركزي والتي بلغت ٢٥,٠% ١٢,٥% على التوالي. يمكن أن نستنتج ان أفضل طرق جمع البويضات هي طريقة التقطيع وهذه الطريقة تؤدي إلى إنتاج أفضل للأجنة أثناء الإخصاب في المختبر عند مقارنتها بطريقة استحصال البيوض بالطرد المركزي.