Research Article

AL- ANBAR JOURNAL OF VETERINARY SCIENCES

Vol. 17 Issue:1, (2024) ISSN: P-1999:6527 E-2707:0603

Role of Follicular Size on Oocyte Recovery Rate and in Vitro Embryo Production Stages in Local Iraqi Sheep

Mustafa Ghassan Sulaiman* and A. F. Majeed

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Fallujah, Iraq

*Corresponding Author**: mugh32042@gmail.com**

Doi: https://doi.org/10.37940/AJVS.2024.17.1.7

Received: 23/8/2023 Accepted: 22/1/2024

Abstract

In vitro embryo production (IVEP) encompasses a series of three basic steps, namely in vitro maturation (IVM), in vitro fertilisation (IVF), and in vitro embryo culture. IVEP approach has shown to be a valuable tool in advancing our comprehension of early mammalian development and expediting genetic enhancement in livestock. The aim of this research was to compare the impact of small and large follicles on the total number of oocytes recovered through the IVEP process. Therefore, 305 Ewe genitalia were collected at the Al-Fallujah abattoir in the Al-Anbar province. The follicles were identified, and their diameter was measured using an automated vernier calliper. Subsequently, the oocytes were classified into three distinct groups, namely excellent (A), acceptable (B), and poor (C). The results of maturation rate large follicles were 43.9% (51/116) and small follicles 30.7% (20/65). There was a statistical difference in maturation rate ($p<0.05$). Fertilization rate of matured oocytes collected from large follicles were 60.7% (31/51) and 50% (10/20) from small follicles. There was no statistical difference. The results of Blastocyst production rate. showed a higher percentage 58.06 (18/31) of Blastocyst produced from large follicles as compared a percentage of 30% (3/10) of Blastocyst production from small follicles. There was a statistical difference ($P<0.05$). It was concluded from current study that the size of follicle has an effect on IVM, IVF and IVC.

Keywards: Follicular size, IVF, IVM, IVC.

دور حجم الجريب في معدل استعادة البويضات ومراحل إنتاج الأجنة في الأغنام العراقية المحلية

الخلاصة

يشمل إنتاج الأجنة في المختبر (IVEP) سلسلة من ثلاث خطوات أساسية، وهي النضج في المختبر (IVM)، والتخصيب في المختبر (IVF)، وزراعة الأجنة في المختبر. وقد أثبت نهج IVEP أنه أداة قيمة في تعزيز فهمنا للنمو المبكر للثدييات وتسريع التحسين الوراثي في الثروة الحيوانية. هدفت هذه الدراسة إلى تأثير الجريبات الصغيرة والكبيرة على عدد البويضات المستعادة في مراحل IVEP. لذلك، تم جمع 305 جهاز تناسلي من النعاج من مسلخ الفلوجة في محافظة الأنبار. تم عد البصيلات وأخذ قطرها برنية أوتوماتيكية. بعد ذلك، تم تصنيف البويضات إلى ثلاث درجات: جيدة (A)، عادلة (B)، وسيئة (C). كانت نتائج معدل النضج للجريبات الكبيرة 43.9٪ (116/51) والجريبات الصغيرة 30.7٪ (65/20). كان هناك فرق إحصائي في معدل النضج ($p<0.05$). كان معدل إخصاب البويضات الناضجة التي تم جمعها من بصيلات كبيرة 60.7٪ (51/31) و50٪ (20/10) من بصيلات صغيرة. لم يكن هناك فرق إحصائي. نتائج معدل إنتاج الكيسة الأرمية. أظهرت نسبة أعلى 58.06 (31/18) من الكيسة الأرمية المنتجة من الجريبات الكبيرة مقارنة بنسبة 30٪ (10/3) من إنتاج الكيسة الأرمية من البصيلات الصغيرة. كان هناك فرق إحصائي ($P<0.05$). استنتج من الدراسة الحالية أن حجم الجريب له تأثير على IVM وIVF وIVC.

Introduction

The In Vitro Embryo Production (IVEP) technique has been established in previous periods with the aim of achieving rapid multiplication and obtaining bigger genetic variations. Several factors have been documented as having an impact on the outcomes of the in vitro embryo production (IVEP) technique. These factors encompass the source of the oocyte, whether taken from an abattoir or from living animals, as well as variables such as parity, stage of the oestrus cycle, season, size of the follicle, and the specific procedure employed (1).

Only IVEP accounts for about 20–40% of embryos produced from oocytes recovered from slaughterhouse ovaries. Some believe that a follicular size-dependent oocyte's ability to fertilize and progress in vitro to an advanced stage of embryonic development is a matter of fact (2, 3, 4). The authors, Sarwar et al. (5) Reportedly, oocyte recovery, equality, and IVF development in cows with follicles that are 6 mm or larger will be better than those with follicles that are less than 6 mm in size.

Moreover, oocytes used in IVEP are collected from developing follicles and arrested in prophase 1 of meiosis. After they are sucked from the suppressive environment of the follicle. Restoration of meiosis and progression to metaphase II are considered to be the fundamental components of IM (6). The low activity of in vitro created embryos and the wide range in oocyte quality and quantity limit the widespread application of IVF technique (7 ,8).

Importantly, Oocytes obtained from larger-diameter follicles are more likely to have progressed to the blastocyst stage than those retrieved from smaller follicles, according to multiple research (8, 9).

Overall, this study aimed to evaluate the relative effects of small and large follicles on the total number of oocytes recovered with IVEP.

Materials and Methods

This study was done in Al-Fallujah, Al-Anbar. Female genital systems 305 of the ewes (610 ovaries) were obtained from the Al-Fallujah abattoir. The genital systems were transferred in one hour using normal saline at 33–35 °C. This transit was done in a cool box to preserve the specimens. Using sterile scissors, the ovaries were carefully removed from the surrounding tissue and bursa. Each ovary received three standard saline washes.

Follicles were counted and measured with an automated vernier calliper. Two- to eight-mm follicles were aspirated using a sterile, disposable syringe filled with three mm of collection medium and an 18-gauge needle. The aspirated oocytes were placed in a 16-well petri dish under UV light. Oocytes were examined under an inverted microscope to determine quality. Criteria were used to classify these oocytes as good (A), acceptable (B), or bad (C) (10). The presence of cumulus cells and cytoplasmic homogeneity dictated categorization.

In vitro Maturation:

Only oocytes of high quality and fairness were chosen. The oocytes underwent two rounds of washing in a mature medium, specifically either TCM199 or MEM. The specimens were subjected to incubation in a suitable maturation medium under controlled conditions of 38.5 °C, 5% CO2, and 90% relative humidity for a duration of 24 hours. The petri dish that had been placed in an incubator was seen using an inverted microscope. The identification of the 1st polar body served as a reliable indicator for the attainment of oocyte maturation in the context of in vitro maturation (IVM) (11). The maturation oocytes were calculated.

Semen collection:

The electro-ejaculator was employed in this study for the collection of semen samples that were obtained from two fertile rams. Subsequently, the seminal fluid was expeditiously conveyed to the Theriogenology laboratory, maintaining a temperature range of 30–35°C within a time frame of 5 minutes. The semen samples were subjected to microscopic analysis using a light microscope in order to assess the quality of the semen. The measurement of mass and the evaluation of individual motility were conducted. The semen samples were subjected to warming in a water bath maintained at a temperature of 35 °C. The semen samples were diluted at a ratio of 1:20 using either MEM or TCM199 medium. Heparin was added as a capacitating factor at a rate of 10 pg/ml.

In vitro fertilization:

Heparin at a concentration of 10 picograms per milliliter (pg/mL) was introduced to both the semen and mature oocytes. Subsequently, the mixture of semen and oocytes was subjected to incubation conditions of 38.5 °C, 5% carbon dioxide (CO2), and a humidity level of 90% for a duration of 24 hours. Fertilization was indicated by the appearance of a second polar body. The quantification of fertilized oocytes, also known as zygotes, was performed.

In vitro culture of fertilized oocytes (zygote):

The fertilized oocytes were subjected to culture in either TCM-199 or MEM culture media and thereafter incubated under specific conditions of 38.5 °C, 5% carbon dioxide (CO2), and a relative humidity of 90%. Approximately 50% of the media underwent replacement within a 24-hour timeframe. Development of the different stages of division was observed every 24 hours until the formation of the blastocyst stage after 168 hours and the hatched blastocyst stage after 216 hours after fertilization.

Statistical analysis

The Data were analyzed by Chi-square test by using spss program

Results and Discussion

Effect of side of the ovary on recovery rate

The number of follicles observed in 305 ovaries is 2302. The study revealed that the right ovary had 1205 follicles, accounting for 52.34% of the total follicles, while the left ovary had 1097 follicles, representing 47.65% of the total follicles.

Our results of the recovery rate of the oocyte revealed 10.45% (126/1205) from the right ovary, while it was 8.84% (97/1097) from the left one. There was no statistical difference in the oocyte recovery rate between the right and left ovaries (Table 1).

These findings displayed a similarity to the outcomes documented in other studies (12,13). Furthermore, the findings presented a contrasting perspective to the research (14), wherein they observed a higher level of activity in the left ovaries (59.4%) compared to the right ovaries (40.6%).

Table.1 Effect of side ovary on recovery rate.

Ovary side	No. of follicles (rate %)	No. of oocyte recovered	Recovery Rate (%)
Right ovary	1205 (52.34%)	126	10.45% **a**
Left ovary	1097 (47.65%)	97	8.84% **a**
Total	**2302**	**223**	**9.68%**

Effect of size of the follicle on the recovery rate

A total of 457 large follicles measuring between 5-8mm were seen, accounting for 30.41% of the total follicle count. These large

follicles had a mean size of 5.21 ± 0.053. Additionally,1845 follicles measuring between 2-4mm were detected as small follicles. The mean size of these small follicles was 1.84 ± 0.022. A statistically significant difference ($p<0.05$) was seen in the size of follicles when comparing large and small follicles (Table. 2).

Previous studies (14,15) have reported comparable findings. These results might be due to the fact that one or more follicles may reach normal physiological conditions for growth and become gradian (dominant), which plays a role in the secretion of oestrogen in contrast to small follicle numbers (16). It has been investigated that the size of the follicle is affected by several factors, such as breed variations, seasonal influences, reproductive status, ages, hormone stimulation, and the nutritional condition of the animals. (12,13).

Table.2. The recovery of oocytes from follicles of varying sizes and quality.

Follicular Size	No. of follicles	Mean Size ± SE	Recovery (%)	Grade A	Grade B	Grade C
(2-4mm)	1845	1.84±0.022	80 **a** 4.33%	30 35.2%	35 41.6%	15 17.8%
(5-8mm)	457	5.21±0.053	143 **b** 31.29%	55 39.5%	61 43.8%	27 19.4%
Total	**2302**	**2.51±0.034**	**223 9.70%**	**85 38.1 %**	**96 43.04 %**	**42 18.8%**

Different superscripts showed significant difference ($p<0.05$).

Effect of follicular size on maturation rate, fertilization rate and Blastocyst Production.

The percentage of oocytes that reached maturity, as indicated by cumulus cell growth, was 43.9% (51/116) for oocytes extracted from large follicles and 30.7% (20/65) for oocytes retrieved from small follicles. Several writers have observed similar findings (8,9,12). A statistically significant difference ($p<0.05$) was seen in the maturation rate of oocytes obtained from large follicles compared to those obtained from small follicles. Previous research has indicated that oocytes obtained from larger follicles exhibit a greater number of cumulus cell layers. These cumulus cells serve as a crucial interface between the oocytes and the external environment, facilitating enhanced contact between the oocytes and the culture medium. This increased contact enables the transfer of essential nutrient materials required for the optimal growth and development of the oocytes (17).

The fertilization rates of mature oocytes obtained from large follicles were found to be 60.7% (31 out of 51), while those obtained from tiny follicles had a rate of 50% (10 out of 20) (see Table 3). There was no statistically significant difference observed in the fertilization rate between oocytes obtained from large follicles and those obtained from small follicles. (9,14) have reported analogous findings. There is evidence to suggest that larger follicles are associated with a higher rate of fertilization.

According to reports, the observed disparity in the quantity and quality of oocytes obtained from large follicles compared to small follicles may be attributed to the superior developmental potential of oocytes originating from larger follicles. The oocyte's developmental competence is influenced by the presence of a high concentration of 17-B estradiol in large follicles, as well as the presence of an impacted factor within the cytoplasm of the aspirated oocyte from said follicles (9).

The findings from the analysis of blastocyst production rate, as presented in Table 3, indicate that a greater proportion of blastocysts, specifically 58.06% (18 out of 31), were created from large follicles. In contrast, a lower percentage of blastocyst production, specifically 30% (3 out of 10), was observed

from tiny follicles. A statistically significant difference (P<0.05) was seen in the generation of blastocysts between follicles of larger and smaller sizes. Multiple researchers have reported comparable findings. According to previous studies (8,12,14). Previous studies have indicated that oocytes obtained from follicles with larger diameters exhibit a higher likelihood of progressing to the blastocyst stage in comparison to oocytes retrieved from smaller follicles (8,9,14). The present investigation yielded the finding that the size of the follicle exerts an influence on in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC).

Table 3. Effect of follicular size on maturation rate, fertilization rate and blastocyst production.

Follicular size	No.	Maturation %(n/n)	Fertilization % (n/n)	Blastocyst production%(n/n)
Small follicle (2_4)	65	30.7% (20/65) **a**	50% (10/20) **a**	30% (3/10) **a**
Large follicle (5_8)	116	43.9% (51/116) **b**	60.7% (31/51) **a**	58.06% (18/31) **b**
Total	**181**	**39.2% (71/181)**	**57.7% (41/71)**	**51.2% (21/41)**

Different superscripts showed significant difference (p<0.05).

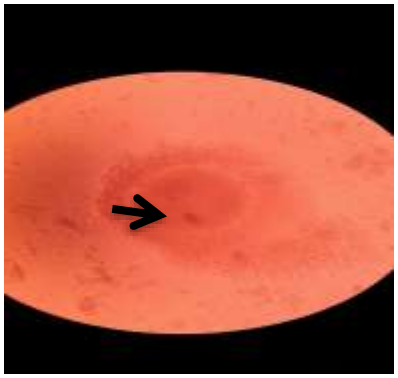

Figure 1: Matured oocyte. Arrow showed first polar body.

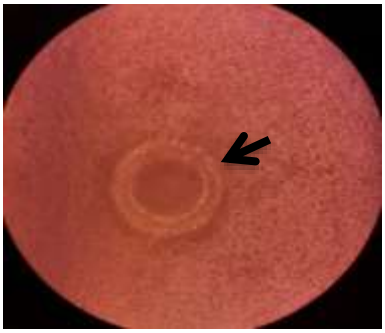

Figure 2: Fertilized oocyte Arrow showed second polar body.

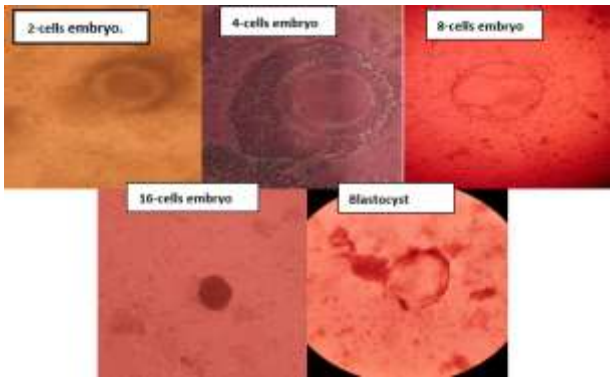

Figure 3: Blastocyst formation.

Conclusion

The current study revealed that the size of the follicle plays an important role in (IVEP). So, we concluded that the number of oocytes recovered from the large follicle was higher than the small follicle. Also, it has a high effect on IVM, IVF, and IVC.

Acknowledgments

Thanks to the Deanship of the College of Veterinary Medicine, University of Fallujah, for providing all the necessary requirements. My deepest respect to the members and Head Dept. of Surgery and Obstetrics, College of Veterinary Medicine, University of Fallujah, for their support and help during this study.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Moore SG, Hasler JF. A 100-Year Review: Reproductive technologies in dairy science. Journal of Dairy Science [Internet]. 2017 Dec 1;100(12):10314–31. Available from: https://doi.org/10.3168/jds.2017-13138
2. Camargo LD, Viana JH, Sá WF, Ferreira AD, Ramos AD, Vale Filho VR. Factors influencing in vitro embryo production. Animal Reproduction (AR) [Internet]. 2018 Jul 26;3(1):19-28.
3. Majeed AF, Omar AA, Alrawi ST, Al-Maadhidy AH, Ahmed KD, Mohamed K. Effect of collection methods on oocyte recovery rate in sheep. Research Journal of Biotechnology [Internet]. 2022;14:262-4.
4. Munther, A.A, Mohammed, T.R. and Majeed, A.F. Effect of addition of natural and synthetic antioxidant for maturation and in vitro fertilization media of Iraqi ewes oocytes and the level of malondialdehyd. 2023 (under publication).
5. Sarwar Z, Saad M, Saleem MH, Husnain A, Riaz A, Ahmad N. Effect of follicle size on oocytes recovery rate, quality, and in-vitro developmental competence in Bos indicus cows. Animal Reproduction [Internet]. 2020 Jan 1;17(3). Available from: https://doi.org/10.1590/1984-3143-ar2020-0011
6. Gilchrist RB, Thompson JG. Oocyte maturation: Emerging concepts and technologies to improve developmental potential in vitro. Theriogenology [Internet]. 2007 Jan 1;67(1):6–15. Available from: https://doi.org/10.1016/j.theriogenology.2006.09.027
7. Mermillod P, Locatelli Y, Tran RD, Uzbekova S, Baril G, Guignot F, et al. In vitro production of ruminant embryos: results, limits and perspectives. HAL (Le Centre Pour La Communication Scientifique Directe) [Internet]. 2006 Nov 7; Available from: https://hal.inrae.fr/hal-02753187
8. Wani AR, Khan MZ, Sofi KA, Malik AA, Lone FA, Bhat FA. Effect of follicular size on in vitro maturation, fertilization and culture of sheep embryos. Iranian Journal of Veterinary Research [Internet]. 2013 Dec 30;14(4):299–304. Available from: https://www.cabdirect.org/abstracts/20143024628.html
9. Alsaadoon AA, Atiyah SS, Shanshoul NJ. Effect of Follicle Diameter and Culture Medium on The in Vitro Maturation of Sheep Oocytes Using Culture Media Supplemented With Different Concentrations of Sucrose in Local Sheep (Ovis Aries). IOP Conference Series [Internet]. 2021 Nov 1;923(1):012038. Available from: https://doi.org/10.1088/1755-1315/923/1/012038
10. Wani NA, Wani GM, Khan MZ, Salahudin S. Effect of oocyte harvesting techniques on in vitro maturation and in vitro fertilization in sheep. Small Ruminant Research [Internet]. 2000 Apr 1;36(1):63–7. Available from: https://doi.org/10.1016/s0921-4488(99)00097-8
11. Al-Malikey K, Al-Delemi DHJ. Effect of Vitamin C on In Vitro Maturation of Iraqi She-Camel Oocytes. MağAllaẗ Al-anbāR Li-l-ʻulūM Al-bayṭAriyyaẗ [Internet]. 2021 Jun 30;14(1). Available from: https://doi.org/10.37940/ajvs.2021.14.1.8 .

12. Majeed AF, Al-Timimi IH, Al Saigh MN. In vitro embryo production from oocyte recovered from live and dead Iraqi black goat: A preliminary study. Research Journal of Biotechnology [Internet]. 2019;14:226-33.
13. Munther A, Mohammed T, Majeed A. Effect of Some Months on Follicles and Oocytes Recovered from Iraqi Ewes. MağAllaẗ Al-anbāR Li-l-ʻulūM Al-bayṭAriyyaẗ [Internet]. 2021 Dec 30;14(2):73–7. Available from: https://doi.org/10.37940/ajvs.2021.14.2.9
14. Omar AA., Ahmed MN, Asker AS, Majeed AF, Faraj TM, Rejah SJ. In vitro production of ovine embryo in non-breeding season. Indian Journal of Forensic Medicine & Toxicology [Internet]. 2021; doi:10.37506/ijfmt.v15i1.13709.
15. Kharche SD, Goel P, Jha BK, Goel A, Jindal S. Factors influencing in-vitro embryo production efficiency of caprine oocytes: A review. Indian Journal of Animal Sciences [Internet]. 2011 Jan 1;81(4). Available from: http://agris.fao.org/openagris/search.do?recordID=IN2013000336.
16. Garg D, Berga SL. Neuroendocrine mechanisms of reproduction. Handbook of Clinical Neurology [Internet]. 2020 Jan 1;171:3-23.
17. Mohammed MA, Abdulridha WM, Abd AN. Thickness effect on some physical properties of the Ag thin films prepared by thermal evaporation technique. Journal of Global Pharma Technology [Internet]. 2018;10(3):613-9.