American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Needle Gauge Affecting Recovery and in Vitro Maturation Rates of She-Camel Oocytes

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Received: 2024, 15, Dec
Accepted: 2025, 21, Jan
Published: 2025, 28, Feb

Annotation: In this study, she-camel oocytes were obtained from the slaughterhouse in order to determine the impact of needle gauge size on recovery and in vitro maturation rate (IVM). 110 oocytes were collected by aspiration method using two gauges size of needle (18g and 22g). The recovery rate (RR) and the in vitro maturation (IVM) rate were studied. The results showed a superiority of 22g vs the 18g in recovery rate 65/85(76.47%) vs 45/89(51.5%), with a reverse result were noticed in the IVM rate 29/41(70.73%) vs 36/58(62.07%) for 18g and 22g respectively. In conclusion, the use of needle with 18g is the best to use for in vitro maturation (IVM) purposes in she-camel, while when choosing the grade A oocytes only, it is best to use 22g for oocytes recovery.

Keywords: Needle gauge, she-camel, recovery, oocytes.

Introduction

Alternatives to conventional IVF, such as advanced reproductive technology (ART), are becoming increasingly popular (1,2). In assisted reproductive technologies, in-vitro maturation (IVM) of oocytes collected after ovarian stimulation has emerged as a possible substitute for conventional IVF. Despite advancements in related techniques like IVF and somatic cell nuclear transfer, which have resulted in live births, in-vitro embryo production (IVP) in camels has not progressed at the same step (4). One of the most important prognostic markers in IVF is the total number of oocytes retrieved (5). There are no noticeable differences in nuclear maturation, fertilization, or cleavage rates between oocytes matured in vivo and in vitro, but rather in the oocytes' developmental competence, as seen by the poor embryonic growth performance and pregnancy rate (6). The needle gauge must also be taken into account when discussing the parameters that influence the aspiration technique (1). The most common approach for collecting

bovine cumulus-oocyte complexes from slaughterhouse ovaries is aspiration (7). The first and most crucial stage in the in-vitro production of oocytes in terms of quality and quantity is to recover oocytes using the right procedure (8). The ovum must complete two stages of development in order to be successful: nuclear maturing through meiosis I and II, and cytoplasmic developing (6). Following artificial insemination and multiple ovulation and embryo transfer (MOET), in vitro production of embryos (IVP) is the third generation of animal reproduction techniques that includes in vitro maturation as an essential phase of successful reproduction (9,10). Cumulus oophorus cells are a collection of cells that surround the oocytes (8). Also, these particular kind of granulosa cell, are essential for the development and maturation of oocytes (11). The release of a mature egg from the ovary, a process known as ovulation, depends on the action of cumulus cells. These cells play a critical role in detaching the egg from the follicle wall, expanding and changing in texture as the follicle and egg reach final maturation (12). Successful pregnancies have been achieved in dromedary camels using embryos created in the lab from eggs that were matured and fertilized outside the body (13).

Materials and methods

One hundred and tenth of she camel oocytes were improved from 28 ovaries from the slaughterhouse by aspiration method using two needle gauges (18g and 22g). In less than 2 hours, all ovaries were transferred to the laboratory in a box containing 0.9 percent of warmed normal saline augmented with 100 g/ml streptomycin and 100 IU penicillin (14). Under microscope, the recovered oocytes were examined and evaluated, High-quality oocytes showed a uniformly dark granular cytoplasm and were surrounded by a thick layer of cells. Whereas, medium-quality oocytes also were displayed uniformly dark granular cytoplasm, but were surrounded by one to three layers of cells. Low-quality oocytes whole or partial shed with granulated ooplasm or dispersed cytoplasm.

IVM of oocytes

Only type A and B oocytes were chosen and washed three times in PBS before being transferred to maturation medium for 24 hours at 90% humidity, 5% CO2, and 38.5°C. Under a light microscope, the incubated oocytes were examined. The development of the first polar body and the expansion of the morphological cumulus are reliable indicators of oocyte maturation in vitro (16).

Statistical analysis

The data were presented as numbers and percentages, and the statistical analysis was done using SPSS version 27. The $\chi 2$ -test was applied, and a P value of less than 0.05 was deemed significant (17).

Results

Results revealed a significant superiority (P<0.01) of 22g needle in recovering camel oocytes from slaughterhouse specimens compared with 18g (76.47%) and 51.5%) respectively especially in grade A oocyte recovery (70.8%) vs (60%) **(Table 1), Fig.(1&2).**

Table 1. Ratio of recovered camel oocytes collected by Aspiration method by two types of needle gauge

Needle gauge	No. follicles	Recovered oocytes	Recovering %	Grade A %	Grade B %	Grade C %	P value
18 g	89	45	51.5%	27(60%)	14(31.1%)	4(8.9%)	0**
22 g	85	65	76.47%	46(70.8%)	12(18.4%)	7(10.8%)	0**
Total	174	110	63.2%	73	26	11	

The results showed no significant difference (P > 0.05), but a highly significant difference (P < 0.01).

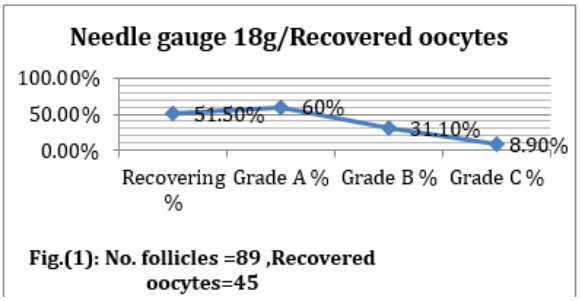

Fig.(1): No. follicles =89 ,Recovered oocytes=45

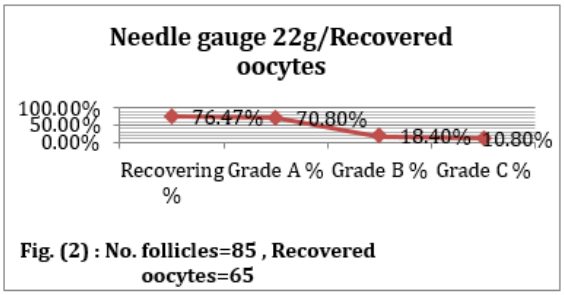

Fig. (2) : No. follicles=85 , Recovered oocytes=65

On the level of maturation rate of camel oocytes in vitro, there was a superiority for the oocytes collected by 18g needle **(70.73%)** compared with that collected by 22g needle **(62.06%)**, but the case is reversed in the maturation of grade A in both gauges (72.41%) vs (83.33%) **(Table 2), Fig.(3&4)**. The first polar body (F.P.B) also seen in grade A matured oocytes collected by 22g needle higher than that collected by 18g **(Table 2)**. **Table 2. Ratio of in vitro maturation of camel oocytes collected by aspiration method with two types of needle gauge**

Needle gauge	Cultured oocytes	Total Matured oocytes	Matured oocytes			Matured oocytes			P value
			Grade A	F.P.B.	other	Grade B	F.P.B.	other	
18 g	41	29(70.73%)	21(72.41%)	17	6	8(27.59%)	5	6	0.001**
22 g	58	36(62.06%)	30(83.33%)	24	16	6(16.67%)	4	6	0**
Total	99	65(65.65%)	51(78.46%)	41	22	14(21.54%)	9	12	

*** No significant difference at P<0.05 ** Highly significant difference at P<0.01**

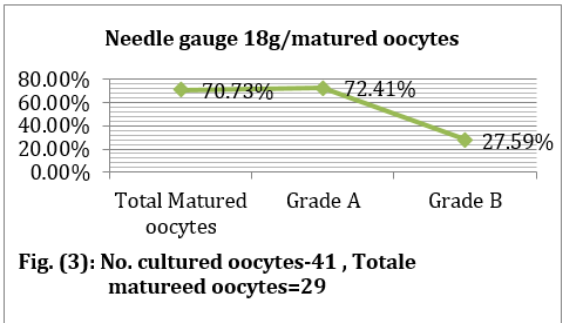

Fig. (3): No. cultured oocytes-41 , Totale matureed oocytes=29

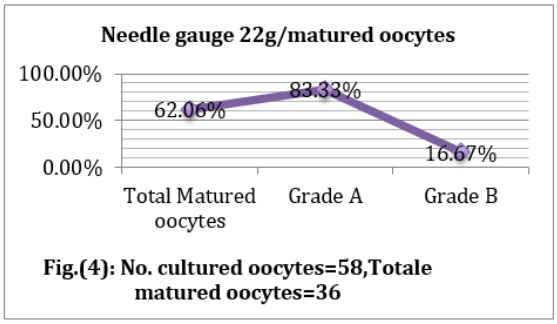

Fig.(4): No. cultured oocytes=58,Totale matured oocytes=36

Discussion

The first and most crucial stage in the IVP of oocytes in terms of quality and quantity is to recover oocytes using the right procedure (8). Follicular aspiration is widely recognized as the most effective technique for oocyte retrieval in dromedary camel (4). Early research in the history of IVF revealed that using larger gauge needles resulted in better oocyte recovery (1) . According to (8), the best procedure for recovering oocytes is aspiration with a needle 18g. The rate of oocyte recovery and developmental competency in bovine eggs have been shown to be influenced by the needle's diameter (18). The identification of a polar body at the completion of the incubation period was used to define maturation (19). Nuclear and cytoplasmic maturation are required for IVM to be successful, and the quantity of cumulus layers gathered has an impact on IVM's maturation and success rates (6). The polar body's expulsion, an enlarged perivitelline gap, and cumulus cell enlargement are indirect morphological indicators of cytoplasmic maturation (20). Our results revealed a recovery ratio of (70.8%) vs (60%) and in vitro maturation ratio of (70.73%) when using 18g needle compared with that collected by 22g needle **(62.06%)**, with a maturation rate of grade A in both gauges of (72.41%) vs (83.33%) respectively. This result is relatively similar to that of (8,21,22) who get a recovery rate of A grade oocytes of 62.27% ±1.60 , 60.00±2.1 and 55% respectively when they using aspiration method to collect oocytes. Our results of in vitro maturation is relatively similar to that of (23) who recorded a percentage of mature oocytes 86.5% in the 17g needle and 91.7% in the 19g needle by using OPU in human. While its greater than the results of (19) who recorded a ratio of in vitro maturation in Alpaca equal to 36% (49/136).

Conclusions

To get the best in vitro maturation (IVM) rates, it is best to use 18g needle in she camel, while when choosing the grade A oocytes only, it is best to use 22g for oocytes recovery.

Ethical Approval

Our investigations followed to all current guidelines regarding animal care and use, at all applicable levels of international, national, and institutional implementation. Actions concerning animals complied with all ethical standards of the institution where the studies were conducted. Activities consider ethical implications for our animal subjects in detail along with references in the Materials and Methods section.

Conflict of interest statement

No potential conflicts of interest were declared by the authors.

Funding

Self-funding

Acknowledgements

The author would like to acknowledge the technical support received from the College of Veterinary Medicine, University of Al-Qadisiyah, Iraq.

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