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Research Article

Effect of Oocyte Diameter on *In Vitro* Fertilization in Iraqi local Goats

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Abstract | This study was conducted to investigate the association between the in vitro fertilization and the diameter of goat's oocytes. Ovaries collected from a local abattoir and transported in normal saline within 1-2 hours after slaughter to the Theriogenology lab/College of Veterinary Medicine. The oocytes were collected by slicing method and classified on the basis of the nature of cumulus cells envelop oocytes. The selected oocytes (n = 534) were classified according to oocyte diameter into4 categories: <110, 110-125, 125-135 and >135µm.Each oocytes measured after excluding the zona pellucida and subjected to culture in TCM-199 improved with 10 IU/ml FSH, 10 IU/ml hCG and 1 µg/ml E2.Each category was cultured in TCM-199 to 24-28 hrs at 38°C. After the incubation period, these mature oocytes in each experimental group were assessed by protruding the first polar body. The percentage of oocytes that reached the MII stage was 7.5, 42.02,65.78 and 68.75%for the4 categories diameters of <110, 110-125, 125-135, >135µm respectively. The result of oocyte maturation was low in less diameter oocyte and high in large diameter oocyte. The mature oocyte fertilized by epididymal sperms after maturation and capacitation with heparin, after 24 hrs of incubation.The fertilization rates were estimated by protruding the second polar body or penetration sperms zona pellucid. The fertilization rate for each category was 0, 20.25, 34.66 and 40.25%respectively. In conclusion, there is a positive relationship between oocyte diameter and their maturation and fertilization.

Keywords | Goat, Oocyte diameter, *In vitro* maturation, *In vitro* fertilization.

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INTRODUCTION

The technique of *in vitro* fertilization of goat oocyte was a potential for production of a great number of embryos and then transfer to another recipient to production of normal offspring (Cognie et al., 2003).The procedure of *in vitro* embryo production begins with harvest oocyte from donor's ovaries either from live or slaughtered animal. The most communal source of oocytes is ovaries collected from the slaughter house, it's an economical source of oocytes, this allows for huge scale and inexpensive production of embryos. However, the quality of these oocytes is highly variable (Gandolfi et al., 1997).Slaughterhouse fresh genetalia could be regarded as a highly compliant male (sperms) and female (oocyte), in which this sample careful as are spectable reservoir for male and female gametes that could be contributed to the progressive reproductive technique (Saleh and Al-Timimi, 2016). Oocyte maturation is the first and important critical step toward successful *in vitro* embryo production Mahmoud et al, (2013).Protocols of IVF of the embryo have been used in goats; however oocyte quality is the highest factor for embryos attainment blast ocyst stage for IVM/IVF / IVC oocytes (Paramio, 2010).The quality of the immature oocytes is determined by th equality of cumulus oocyte complex and oocyte diameter (Ali et al, 2014). Numerous studies in different species have determined that oocyte diameter is directly related to follicle diameter. The increasing in follicle size and oocyte diameter improves embryo development (Gandolifi et al., 2005). So the present study aimed throws some lights on the factor of diameter oocytes on *in vitro* fertilization in local Iraqi goats.

MATERIALS AND METHODS

Male and female genitalia specimens were collected from AL-shulla abattoir in Baghdad, fresh genitalia of buck and local bread doe were collected 15 minutes after slaughtering, then separated the ovaries and testes from genital and cleaned in normal saline then be located in a container for transporting them to the laboratory of Theriogenology in the College of Veterinary Medicine in about 1-2 hrs. Ovaries and testis were washed in a saline solution containing antibiotic (100 IU/mlpenicillin and 100µg/ mlstreptomycin).

Oocytes Collection

In the laboratory ovaries separated from all surrounded tissue than washing again with 0.9 normal saline solution to make sure they are clean All collected ovaries had been gathered in a glass Petri dishes comprise 5ml of TCM-199 with antibiotics, using a sterilized scalpel blade, ovaries were incised more and more to very small pieces. Petri dishes of the both techniques were left to a settlement at apartment temperature for 15 minutes and then examined under inverted microscope searching for the oocytes (Wani et al., 2013).The COCs were then categorized into 4 grades as defined by Saha et al, (2014). The grades: grade A: oocytes totally surrounded by cumulus cells; grade B: oocytes partly surrounded by cumulus cells; grade C: oocytes not surrounded by cumulus cells and grade D: degeneration detected both in oocytes and cumulus cells. The grade A and B were carefulas normal and grade C and D as abnormal and rejected.

OOCYTES MATURATION

Normal quality COCs (A and B grade) were washed three times distinctly in the maturation media (TCM-199). then normal oocytes divided into 4 categories dependent on their diameter, the diameter of the oocyte was measured with video micrometer on ascreen connected to a camera on stereo microscope, the diameter of oocyte was measured excluding the zona pellucida at 4 different sizes (<110, 110-125, 125-135 and>135µm) the measured COCs were transferred into different 4 well petri dishes contained of 1mlof TCM-199 media provided with 27µg/ml sodium pyruvate, 50µg/ml gentamycin, 10 % fetal calf serum, 10µg/ml FSH ,10µg/ml LH and 1µg/ml estradiol, incubated in 5% CO_2 incubator at 38°C for 24-28hrs, after incubation period, oocytes were subjected to microscopically examination by inverted microscope in 4 Petri dishes to differentiate between mature and immature oocyte according the existence of first polar body in the prevetlin space of mature oocyte (MII), while all immature oocyte were rejected.

Collection of Spermatozoa

Testicle transported to the lab of theriogenology, washing with normal saline, epididymis was separated, and then cauda separated from theepididymis and the surrounding tissue, washed thoroughly with distal water then hold in Becker containing TCM-199 medium with antibiotic preparation. Cauda samples were injected with 5-7 ml of the medium put in glass Petri dishes and sliced into small pieces by sterile blade, spermatozoae valuated and stained smear, dead and alive were examined and recorded, Petri dishes preserved at refrigerator temperature for periods of time till using (Al-Timmi, 2013).

Spermatozoa and Capacitation

Caudal spermatozoa were evaluated under a light microscope, individual motility lower than 60% were rejected. Spermatozoa incubated in CO_2 at 38°C for 6hrsfor sperm maturation, the presence of distal protoplasmic droplet was an indicator of sperm maturation Omar, (2015). Samples left for 30 minutes at room temperature, then 1ml of the sample add to 3ml of TCM-199 containing antibiotics, washed twice by centrifugation at 2000 rpm for10 min, supernatant were discard, spermatozoa pellet was suspended incapacitated medium and washed again, then second spermatozoa pellet re-suspended in TCM-199 containing50 IU/ml heparin, penicillin and streptomycin, incubated in CO_2 incubator (38 °C, 90% humidity)at sloped positioning for 1-2hr (Wani, 2013).

In Vitro Fertilization

Matured oocytes of fourty pes were washed twice with medium supplied with antibiotics before transferred to a glass Petri dishes containing medium with the same supplementation. Capacitated spermatozoa sample were prepared after diluted to yield 1×10^6/ml sperms (Wani, 2013). The mixture of gametes was incubated in theCO_2 incubator at 38C^0 and 90% relative humidity for 24-28hrs. Fertilized oocytes were removed from cultured medium after diagnostic by inverted microscope. Fertilization rate was evaluated based on pro-nucleus formation, presence either sperm head in the vitelline space, emission of second polar body. The evaluation was performed according to Angela (2006).

STATISTICAL ANALYSIS

Statistical analysis was accomplished using SAS (Statistical Analysis System - version 9.1). Proportions were compared by chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The oocytes of Iraqi goat (n= 534) were collected by a slicing method and classified into4categories depending to their diameters (<110, 110-125, 125-135, >135µm) after

maturation by incubation for 24-28hrs in TCM-199 tissue culture media, the maturation percentage was 7.50, 42.02,65.78 and 68.75% respectively (Table 1), this result confirm that the maturation rate increase when the diameter of oocyte increase, oocytes smaller than110µm corresponded to incompetent maturation rate, from 110 to 125 µm they corresponded to moderately competent oocytes and the oocytes larger than 125 µm had full maturation competence, these results are agreed with Fair et al, (1995) he explained that by the limited proportion of small diameter oocytes which were capable to perform the protein synthesis and activation required for further progress of oocyte maturation to MII, it would appear that oocytes in general develop the full capacity for transcription, translation, and post-translational modifications required to develop to MII *in vitro* at a large diameter oocytes.

Table 1: Effect of oocytes diameter on maturation and fertilization rates.

Diameter of oocytes	No of oocytes collected	No of matured oocytes (MII)	Maturation rate %	No of fertilized oocyte	Fertilization rate
<110	120	11	9.16	0	0% c
110-125	188	79	42.02 b	16	20.25% b
125-135	114	75	65.78 a	26	34.66% a
>135	112	77	68.75 a	31	40.25% a

Means in the same columns with different superscripts differ significantly ($P < 0.05$).

The maturation oocyte incubation with epididymis sperm capacitation after 24-28 hrs determinate the fertilization rate by intruded second polar body or penetration sperm by zona pellucida. The fertilization rate 0,20.25, 34.66 and 40.25 to4 category <110, 110-125, 125-135,>135µm respectively (Table 1).This result confirm the importance of diameter oocyte for fertilization because oocyte with small diameter were not able to be fertilized after incubation with sperm,the other category (110-125µmdiameter) fertilized in low ratio but oocyte greater than 125µm were with high ratio fertilization, this result confirm the progressive association between oocyte diameter and oocyte fertilization. The results are in agree with results obtained by Lechniak et al, (2002) who showed that oocytes with a diameter of less than 110 µm could still be in the growth phase .These oocytes are less able progress to fertilization. Such small oocytes are also prone to undergo certain chromosome alterations during maturation, which impairs further development. However our results disagreed with Ototi et al, (2000), who reported that is a clear association between oocyte diameter and maturation rate, but no association between oocyte diameter and sperm penetration was found. Canine oocytes may have acquired maturation once they reach at a diameter of 120 µm, but the oocytes may allow the penetration of spermatozoa into the ooplasm unrelated of oocyte diameter.

In conclusion: the oocyte diameter could consider on of the important parameters that should be taken in our consideration during oocyte collection and evaluation for fertilization. The present study confirmed the association between the oocyte diameter and oocyte maturation and fertilization. Hence the selected oocyte must be larger than 125 µm in order to be able for *in vitro* fertilization in Iraqi local goats.

AUTHORS CONTRIBUTION

Research work was done by Imad M.AL-Meeni. F.R.Al-Samarai conducted the data analysis. The author read and approved the final manuscript.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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