

Effect of follicular size on recovery and *in vitro* maturation of black Iraqi goat Oocytes

A.F.Majeed*, M. N. Al-Saigh** and I. H. S. Al-Timimi**
 *College of Veterinary Medicine\ University of A Al-Anbar
 **College of Veterinary Medicine\ University of Baghdad

Abstract

The study was conducted on 599 genital system of Iraqi black goat, collected from local slaughter house in Al-Fallujah city and transported to the theriogenology Lab. within one hour of slaughtering, in normal saline in cool box, during the period from July 2010 to July 2011. The reproductive status of the animals were not known. The ovaries were cut by scissors and freed from the surrounding tissues and overlying bursa. The follicles was counted in each ovary, and the diameter of the follicle was measured by automatic vernier, and classified in to small follicle (2-4mm diameter) and large follicle (5-8mm diameter). Only good and fair quality oocytes were selected. The oocytes were washed two times in maturation medium (TCM_199, MEM and TALP), then incubated in appropriate maturation medium in 39°C temp., 5% CO₂ and 90% relative humidity for 24-26h. The presence of first polar body was the criteria for *in vitro* maturation (IVM) of oocytes. Chi square test were used for statistical analysis. The result showed there were a significant increase (P<0.05) in recovery rate and maturation rate of oocytes recovered from large follicles as compared with that of small follicles. The number of recovered oocytes from large follicles was 1046 out of 1377 follicles with a size between 5-8mm i.e. 75.9%, and the maturation rate was 45.9%, while the recovery rate and maturation rate of oocytes taken from small follicles were 61.06% (1043/1708) and 35.9% (375/1043) respectively. It was concluded from this study that follicular size could be affect the recovery rate and maturation rate of oocytes *in vitro*.

تأثير حجم الجريب على نسبة الاستحصال والإنتاج مختبرياً لبويضات الماعز العراقي الأسود

عبد الستار فرج مجيد*، مضفر نافع الصانع** وإحسان حمودي سعيد التميمي**

*كلية الطب البيطري/ جامعة الأنبار

**كلية الطب البيطري/ جامعة بغداد

الخلاصة

أجريت الدراسة على 599 جهاز تناسلي أنثوي للمعز العراقي الأسود، جمعت من مجزرة الفلوجة ونقلت إلى مختبر الخصوبة التابعة لكلية الطب البيطري/ جامعة الأنبار خلال ساعة من الذبح في حاوية مبردة حاوية على محلول الملح الفسلجي خلال الفترة من تموز 2010 لغاية تموز 2011. تم تحرير المبايض من الجهاز التناسلي والأجهزة المحيطة به بواسطة مقص معقم. تم حساب عدد الجريبات لكل مبيض وقياس قطر الجريب بواسطة مقياس أوتوماتيكي وصنفت الجريبات إلى جريبات صغيرة بقطر (2-4 ملم) وكبيرة بقطر (5-8 ملم). تم استحصال البويضات بواسطة السحب أو التقطيع واختيرت البويضات الجيدة لغرض إنتاجها في أحد الأوساط الزرعية (TCM-199, MEM and TALP) وحضنت في وسط مناسب في 39 م° و 5% CO₂ و 90 رطوبة نسبية لمدة 24-26 ساعة. أن وجود الجسم القطبي الأول يعتبر دليل على حدوث إنتاج للبويضات مختبرياً. أستخدم مربع كاي لإجراء التحليل الإحصائي للنتائج. أظهرت النتائج وجود فرق معنوي (>0.05) في

نسبة استحصـال وإنضـاج البويضـات المأخوذة من جريبات كبيرة عند مقارنتها من البويضـات المأخوذة من الجريبات الصغيرة. كانت نسبة الاستحصـال من الجريبات الكبيرة 75.9% بينما كانت نسبة الإنضـاج 45.9% بينما كانت نسبة الاستحصـال والإنضـاج المأخوذة من الجريبات الصغيرة 61.06% و 35.9% على التوالي. وقد أستنتج من الدراسة إن حجم الجريب له تأثير على نسبة استحصـال وإنضـاج البويضـات مختبريا.

Introduction

Ovarian follicles has been developed either during embryonic period or after birth, during which a layer of follicular cells surrounding primitive oogonia at birth as a source of follicular development (1). Oocytes growth inside the follicle in a slow process that last about six month in cattle (2). During this period, the oocytes acquires the competence to undergo meiotic maturation by an interaction between the oocytes and the theca and granulosa cells (3) and accumulates transcripts and proteins that will guide the maturation fertilization and initiate embryo development (4). Hendriksen et al.(5) reported that oocytes competence increased as the follicular size increase. Large follicles showed high maturation rate than small one (1). The present study has been undertaken to show the effect of follicular size on recovery rate and maturation rate of oocytes in black Iraqi goat.

Materials and Methods

The study was conducted on 599 genital system of Iraqi black goat, collected from local slaughter house in Al-Fallujah city and transported to the theriogenology Lab. within one hour of slaughtering, in normal saline in cool box, during the period from July 2010 to July 2011. The reproductive status of the animals were not known. The ovaries were cut by scissors and freed from the surrounding tissues and overlying bursa. Each ovary was washed three times in normal saline and two times in collecting media (TCM_199, MEM and TALP). The follicles was counted in each ovary, and the diameter of the follicle was measured by automatic vernier. The follicles were classified in to small follicle (2-4mm diameter) and large follicle (5-8mm diameter) according to Crozet et al. (6) and Cognie et al.(7). Oocytes were collected either by aspiration techniques using 18 gauge needle attached with a sterile disposable syringe containing 2ml of collecting media. The media with collected oocytes was transferred to one well out of 24 wells petridish by slicing of the ovaries in sterile petridish containing 10ml of collected media with a scalpel blade-in both techniques the petridishes were kept undisturbed for five minutes allowing the oocytes to settle down. The wells of the petridish were examined under an inverted microscope and then the total numbers of collected oocytes were counted. The collected oocytes were graded according to Wani et al.(8) as good (A), fair (B) and poor (C) on the basis of cumulus cell and cytoplasm.

- **In vitro maturation:** Only good and fair quality oocytes were selected. The oocytes were washed two times in maturation medium (TCM-199, MEM and TALP), then incubated in appropriate maturation medium in 39°C temp., 5% CO₂ and 90% relative humidity for 24-26h. The presence of first polar body was the criteria for in vitro maturation (IVM) of oocytes. Chi square test were used for statistical analysis.

Results and Discussion

The result showed in Table-1, a significant increase ($P<0.05$) in recovery rate and maturation rate of oocytes recovered from large follicles as compared with that of small follicles. The number of recovered oocytes from large follicles was 1176 out of 1377 follicles with a size between 5-8mm i.e. 87%, while the recovery rate and maturation rate of oocytes taken from small follicles were 64% (1109/1708) and 31.05% (241/776) respectively. Similar observation have been made by Crozet et al.(6), Cognie et al. (7) and Kharche et al.(9) in goats and Cognie et al.(10) in sheep and Hendriksen et al. (5),

Pavlok et al. (11), Lonergan et al. (12) and Lequarre et al. (13) in cattle. The result also agreed with other workers Pavlok et al. (11); Torner et al.(14); Majeed et al.(15). This result might be due to fact that ovarian follicle is a balanced physiological unit whose structure or function depend on extra cellular factors such as gonadotropins and complex system of intrafollicular relationship. There was a difference between different breeds due to extended times during which recruitment takes place. The low incidence of selection, and the ability of fully grown follicles to wait few LH peak, In large antral follicles (but not in small follicles), the follicular fluid contains remarkable high level of 17B-estradiol in the follicular phase and progesterone as ovulation approaches, viable ovarian follicles also accumulated and secrete several physiologically active non steroid such as oocyte maturation inhibitor, lutenization inhibitors, inhibitory protein, relaxin and inhibin (1). The low developmental rates of oocytes from small follicles maybe because they still do not reach complete meiotic and /or cytoplasmic competence, or because they are from follicles already undergoing atresia. Therefore, follicle health seems to be important in this process since oocyte from follicles. In the advanced stage of the atresia are more compromised atresia(5,16). It was concluded from this study that follicular size could be affect the recovery rate and maturation rate of oocytes in vitro.

Table (1) Effect of follicular size on recovery and maturation rate of oocytes in black Iraqi goat in vitro

Size off follicles	No. of follicles	No. of Recovered oocytes	Recovery rat%	Cultured Grade A + B	No. of Matured	Maturation rate %
Small (2-4mm)	1708	1109	64%	776	241	31.05% ^b
Large (5-8mm)	1377	1176	87%	1030	484	46.99% ^a

There was a significant difference ($P<0.05$) between different superscripts.

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