

Effect of collection techniques on recovery, and *in vitro* maturation of Black Iraqi Goats oocytes

A.F. Majeed*, I. H. Saied** and M. N. Al-Saigh**

* College of Veterinary Medicine/ University of Anbar

** College of Veterinary Medicine/ University of Baghdad

Abstract

The study was conducted to know the effect of collection techniques on recovery and on *in vitro* maturation of goat oocytes. Oocytes were collected by two techniques aspiration and slicing of goat ovaries obtained from slaughter house. It was observed that aspiration showed a recovery rate of 76.94% (1401/1821) while slicing method showed a recovery rate of 69.93% (884/1264). There was a significant difference ($P < 0.05$) between the two methods. Aspiration method showed a maturation rate of 40.68% of the recovered oocytes of good quality (1297/1401), While the slicing method showed a maturation rate of 30.84% of the recovered oocytes (198/642). There was a significant difference ($P < 0.05$) in maturation rate between the aspiration and slicing methods.

It was concluded from this study that the method of collection oocytes from ovaries in slaughter house effect on recovery and the maturation of oocyte in Black Iraqi Goat.

تأثير طريقة جمع البويض على نسبة إستحصال وإنضاج البويضات مختبرياً في الماعز الأسود العراقي

عبد الستار فرج مجيد*، إحسان حمودي سعيد** ومظفر نافع الصائغ**

*كلية الطب البيطري/ جامعة الأنبار

**كلية الطب البيطري/ جامعة بغداد

الخلاصة

أجريت الدراسة لمعرفة تأثير طريقة جمع البويض على نسبة استحصال وإنضاج البويضات مختبرياً في الماعز الأسود العراقي. جمعت البويضات بواسطة طريقتين هما السحب والنقطيع لمبايض الماعز التي تم الحصول عليها من المجزرة. لوحظ أن نسبة استحصال البويض بطريقة السحب كانت 76.94% (1401\1821) بينما كانت 69.93% (884\1264) بطريقة النقطيع. لوحظ وجود فرق معنوي ($0.05 >$) بين الطريقتين في استحصال البويض. كما لوحظ ان نسبة الإنضاج للبويض المستحصلة بطريقة السحب كان 40.68% (للبيوض المستحصلة الجيدة) (1297\1401) بينما كانت بطريقة النقطيع 30.84% (642\198). لوحظ وجود فرق معنوي في نسبة الإنضاج ($0.05 >$) بين الطريقتين.

وقد استنتج من الدراسة ان لطريقة جمع البويضات من مبايض مأخوذة من المجزرة تأثيراً على نسبة استحصال البويض وإنضاجها مختبرياً للماعز العراقي الأسود.

Introduction

Slaughter house ovaries are attractive alternative source for retrieval as they are less expensive and most abundant source of immature oocytes for large scale production of Caprine embryo *in vitro* because the goat is the main meat source in many countries (1).

The obvious advantage of oocyte recovery technique is speed of operation, quality of oocytes and quantity of oocytes (2). There are several techniques used for harvesting the oocytes from ovaries taken from slaughter house. These techniques includes aspiration, slicing and puncture (2, 3, 4, 5, 6).

The objective of this study was to evaluate two techniques of oocyte collection from slaughtered Iraqi Black Goats ovaries and the effect on *in vitro* maturation.

Materials and Methods

The study was conducted on 599 genital system of Iraqi Black Goats, collected from a local slaughter house in Fallouja city and transported to the Theriogenology Lab within one hour slaughter in normal saline in cool box, during the period from July 2010 to July 2011. The reproductive status of animals were not known. The ovaries were cut by sterile seizer and freed from the surrounding tissues and overlying bursa. Each ovary was treated to three washings in normal saline and two washings in collecting media (TCM-199, MEM and TALP). Oocytes were collected by one of the following two techniques:

1. Aspiration: 2- 8 mm size follicles were aspirated using a 18 gauge needle attached with a sterile disposable syringe containing 2 ml of collecting medium. The media with harvested oocytes was transferred to one well of 24 wells Petri dish.
2. Slicing: ovaries were placed in a Petri dish containing 10 ml of collecting medium, held with the forceps and the ovarian surface were incised with a scalpel blade.

Then in the both techniques the Petri dishes were kept undisturbed for five minutes allowing the oocytes to settle down. The wells of Petri dish were examined under an inverted microscope and then the total number of collected oocytes were counted. The collected oocytes were graded according to Wani et al. (3) as good (A), fair (B) and poor (C) on the basis of cumulus cells and cytoplasm.

Good: oocytes with many complete layers of cumulus cells and uniform cytoplasm.

Fair: oocytes with thin or incomplete layers of cumulus cells and uniform cytoplasm.

Poor: oocytes with few or no cumulus cells.

The number of good, fair and poor oocytes obtained were recorded.

In vitro maturation: only good and fair quality oocytes were selected. The oocytes were washed twice in maturation medium either TCM-199 supplemented with FBS or MEM or TALP and then incubated in the appropriate maturation medium in 39 °C temp, 5% CO₂ and 90% relative humidity for 24-26 hr.

Presence of first polar body was a good criteria for *in vitro* maturation (IVM).

Chi- square test were used for statistical analysis.

Results and Discussion

Table -1- showed the effects of collection techniques on recovery rates and Grade of oocytes recovered. The results showed a significant difference (P<0.05) between collecting methods. Aspiration showed 76.93% recovery rate while slicing showed 69.93%. similar observation have been made by Sogorescu et al (7) in goat and sheep. The results disagreed with the found of other workers in goats (8) and sheep (9). They indicated that slicing and puncture yielded significantly more oocytes per ovary than aspiration. High recovery rate with aspiration might be due to aspiration was the most common techniques for obtaining good quality and quantity of oocytes and the presence of ovarian tissue debris in slicing or puncture technique that interfere with searching of oocytes under the microscope (1) and also required more washing when compared to aspiration (6). As a result, number of cumulus oocyte cells (COCs) were denuded from cumulus cells due to repeated washing and ultimately resulted in lower number of

normal COCs when compared to aspiration at the final observation. Visual assessment of morphological features remains the most important vehicle for selection of oocytes before maturation during oocytes recovery. The results showed that higher recovery rate were obtained of Fair (Grade B) oocyte (53.4%) followed by Good (Grade A) oocyte (31.4%) and poor (Grade C) oocyte (15.14%). In a slaughter house ovaries. Similar observations have been made by Rahman et al (5) in goats and Wani et al (3) in sheep. There was a significant difference ($P<0.05$) in recovery rate of different Grade oocytes. Concerning the method of recovery there was no significant difference in (Grade A) between different method of recovery. While there was a significant difference between Grade B and Grade C in different methods of oocytes collection.

The observation of low quality or Grade oocyte recovered might be due to slaughters of low quality does.

The results showed in Table -2- that aspiration yielded a significantly higher maturation rates of oocytes ($P<0.05$) as compared with the slicing method. Similar observations have been made in goats (8, 10, 11) and in sheep (12). This might be attributed to more antral oocytes collected by aspiration as compared with more pre-antral oocytes collected by slicing (13).

It was concluded from this study that the method of collection of oocytes from ovaries taken from a slaughter house effect on recovery and maturation rate of oocytes in Black Iraqi Goat.

Table (1) Effect of two different collection techniques on oocyte recovery in Black Iraqi Goat

Method of Collection	Total No. of ovaries				Grade		
		No. follicles	oocytes	Oocytes/ ovary	Good (A)	Fair (B)	Poor (C)
Aspiration	614	1821	1401 (76.93%)	2.28 ± 0.23a	30.04%	62.52%	7.4%
Slicing	584	1264	884 (69.93%)	1.51 ± 0.28b	33.7%	38.9%	27.37%
Total	1198	3085	2285 (74.06%)	1.90 ± 0.25			

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

Table (2) Effect of collection technique on in vitro maturation in Black Iraqi Goat

Method of Collection	Total No. of oocytes	No. of matured oocytes	Maturation (%)
Aspiration	1297	527	40.63a
Slicing	642	198	30.84b
Total	1939	725	37.39

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

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