ACTIVATION OF EPIDIDYMALS SPERM ,OOCYTES MATURATION AND EMBRYOS PRODUCTION IN VITRO IN ARABI SHEEP OF DIFFERENT AGE

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ABSTRACT

The study was conducted at the Laboratory of Molecular Genetics / College of Agriculture / University of Basra , from 1/5/2013 to 18/12/2013. The aim was to study the possibility of fertilization of Oocytes in vitro by sperm withdrawn from the epididymis . Reproductive tracts were brought from Basra slaughter house with a total of 410 samples. Withdrawing the sperm from the epididymis and oocytes from small follicles (less than 4 mm) and from large (more than or equal to 4 mm) .Rams aged (1.2 -2) years showed significant superiority (P <0.01) in sperm individual motility and live sperms in comparison with older ones . In vitro fertilization by using semen of (1.2 – 2) years rams and an oocyte of greater than or equal to 4 mm was superiorly significant (P <0.01) and showed a division into two or more cells.

INTRODUCTION

Agricultural production played an important role in the national economy , Livestock production represents the biggest part of this production , and breeding sheep represent important sector in terms of numerical or productivity . Reproductive efficiency is one of the main basis pillars of sheep production and breeding , as the economical success of any animal project depends on fertility (1), There are many ways to improve reproduction in sheep , which aims to increase their fertility and fecundity and thereby increase their birth rate (2).

The epididymis carry sperm from the testicle to the vas deferens and sperm formulation continue to maturity within the epididymis , which is a place to store sperm , so the sperm is unable to move after configured directly while in the testis acquires the

ability to move while passing and capacitation in the epididymis (3) . Note that the sperm remain in the body and head of the epididymis (2-5) days and in the tail of the epididymis (6-14) days (4) . (5) showed that the number of sperm stored in the tail of the epididymis have a positive relationship with the rate of sperm production in the testes Sperm and ova age within female reproductive system within the limits of a short time (20-48 hours) in most mammals . animals ovaries Contain large number of those as early as birth , however small number of these are used during production life . Therefore , slaughtered animals can be cheaper source of mature oocyte that can be fertilized in vitro (6).

To get successful oocyte and sperm capacitation in vitro. The study aims at the possibility of using epididymall sperms in fertilizing Oocytes in vitro.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Molecular Genetics / College of Agriculture / University of Basra from 1/5/2013 to 18/12/2013 . A total of 410 samples Males and Females reproductive system from Basra main slaughter house . Rams and ewes aged from (1.2 -4) years old . Reproductive system were completely isolated and put in a nylon bag containing normal saline (0.9Nacl) and then transferred to the laboratory ,Follicles classified by diameters to follicles equal to or greater than 4 mm , and the second is less than 4 mm . Incubated oocytes were withdrawn from the follicles in Co2 incubator for 27 hours at the end of incubation period , mature oocytes with 1^{st} polar body were isolated to be ready for fertilization . Fresh semen from the tail of epididymais was collected and a comprehensive evaluation was practiced as discussed by (6) . An equipped sterile Petri dishes contain 2ml of TCM-199 Hub was used for in vitro fertilization of sperms (0.2ml) and 5-10 oocyte added to the Petri dishes. They were incubated with Co_2 (5%) temperature (38.5 C^o) and 90% humidity for 24 hours . oocyte were examined for fertilization occurrence and the appearance of 2^{nd} polar body. Oocyte that showed signs of fertilization were transferred to dishes containing the culture media TCM-199 Hup axis and incubated for 24 hours , to examine cell division (7) .

Statistical analysis was performed using Data was viewed as mean ± standard deviation SPSS, (8)

RESULTS AND DISCUSSION

Results in table (1) indicated the existence of significant (p <0.01) differences in rams mass and individual Motilities of ram of different ages . Rams aged (1.2-2) exceeded the other age group in mass and individual motilities , live sperm % (P <0.01) and dead sperm % (P<0.05) .

Age has great impact on reproduction system function , as age progress testes are unable to produce normal sperm and testosterone levels (9) .

Table (1) : Mass and individual Motilities live sperm and dead sperm percentages ± standard deviation .

Age Rams (year)	Mass Motility **%**	Individual Motility **%**	Live Sperm **%**	Dead Sperm **%** *
1.2 -2	88.33^{a}±1.67	86.67^{a}±2.88	83.67^{a}±1.45	11^{a}±1.15
>2 -4	73.33^{b}±1.67	71.67^{b}±2.88	74.0^{b}±1.73	14.67^{b}±1.20

Different letters indicate significant differences at (P <0.01) or * (P <0.05)

. The results indicated the possibility of fertilization of oocyte of sheep in the culture media TCM-199 hup by activating sperm retrieved from the tail of the epididymis after the slaughter of the animal. Table (2) showed a significant effect between studied treatments in maturation of oocyte *in vitro* (P <0.01) through the formulation of 1^{st} polar body and reaching the highest percentage of maturation in the two treatments of follicles greater than or equal to 4 mm and was % (77.537 , 77.226). The lowest percentage of mature oocytes percentage were 70.836 and 70.947 for treatments of follicles less than 4 mm. While the results of growth of these Oocytes and division of treatment used rams aged 1.2 to 2 years and follicles size greater than or equal to 4 mm in vitro after 24 hours showed higher (p<0.01) fertilization and division (30.743 , 53.58% respectively). However the lowest (P<0.01) ratios (13.71 , 40.87 % respectively)were exhibited by the treatment used rams aged more than two years and follicles sizes equal or less than 4 mm. Hence the present study was identical with the results of (10), whenever the larger size of the follicles gave rise to a high percentage in the maturation of oocyte compared to oocytes originated from the small follicles and produce embryos with high vitality

due to the lack of maturation of the cytoplasm which affect the development of the embryo. As there is a close relationship between the size of the follicles and integration development oocytes up to the stage of the embryo because of the size of the follicles has a correlation with the diameter of the oocyte and the quality of cumulus cells surrounding the oocyte, leading to the division and completion development of the oocyte. As well as the age of the rams have a significant impact on the rate of production of embryos (11).

Table (2): The effect of age of rams and sizes of follicles on the proportion of ripening and fertilization of oocyte in vitro ± standard deviation

Age Rams (year)	Size of follicle	Maturation %	Fertilization %	Division %
1.2 -2	Less than 4 mm	70.836^{b}±0.74	47.693^{b}±2.0	24.366^{b}±1.097
	Greater than or equal to 4 mm	77.537^{a}±2.313	53.58^{a}±0.614	30.743^{a}±1.166
	The average age of the ram	74.182±3.973	50.636^{a}±3.485	27.555^{a}±3.636
<2 - 4	Less than 4 mm	70.947^{b}±1.933	40.87^{b}±2.161	13.71^{b}±4.052
	Greater than or equal to 4 mm	77.226^{a}±1.22	45.187^{a}±1.74	27.687^{a}±5.206
	The average age of the ram	74.087±3.731	43.028^{b}±2.944	20.698^{b}±8.718
Average size of the follicle	Greater than or equal to 4 mm	70.892^{b}±1.31	$44..282^{b}$±4.175	19.038^{b}±6.412
	The average age of the ram	77.377^{a}±1.661	49.383^{a}±4.743	29.215^{a}±3.766

Different letters indicate significant differences at (P <0.01)

تنشيط النطف البربخية وأنضاج البويضات لانتاج أجنة خارج الجسم من ألاغنام العرابية المختلفة الاعمار

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الخلاصة

أجريت الدراسة في مختبر الوراثة الجزيئية / كلية الزراعة / جامعة البصرة من 2013/5/1 ولغاية 2013/12/18 بهدف دراسة أمكانية أخصاب البويضات خارج الجسم بالنطف المسحوبة من البربخ بعد ذبح الحيوان (الكباش ، النعاج) ، أذ جمعت الأعضاء التناسلية من مجزرة محافظة البصرة و بلغ المجموع الكلي للعينات 410 عينة . سحب السائل المنوي من البربخ والبويضات من الجريبات الصغيرة (اقل من4 ملم) والكبيرة (تساوي أو أكبر من 4 ملم) وكانت نتائج الدراسة على النحو الآتي :

أظهرت الكباش التي عمرها (1.2-2) سنة تفوقاً معنوياً عند مستو ى ($P<0.01$) في حركة النطف الجماعية والفردية والنطف الحية مقارنة بالكباش التي عمرها أكبر من سنتين ، بيّنت النتائج انخفاضا معنوياً للنطف الميتة عند مستوى ($P<0.05$) للكباش التي عمرها (1.2-2) سنة مقارنة بالكباش التي عمرها أكبر من ذلك . أثبتت النتائج تفوق معنوي عند مستوى ($P<0.01$) للكباش التي عمرها (1.2-2) سنة عند ألاخصاب الخارجي للبويضات التي حجم جريباتها أكبر أو تساوي 4 ملم وكذلك انقسامها إلى خليتين أو أكثر .

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