COMPREHENSIVE STUDY OF *IN VITRO* FERTILIZATION OF LOCAL GOAT

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ABSTRACT

Goat ovaries brought from local abattoir within two hour of slaughter at 30° C , oocytes were asprated and then washed in Phosphate Buffer Solution(PBS)for cumulus - oocyte complexes (COCs) removed and these oocytes were divided in to two groups (with and with out cumulus) . Granulated cytoplasm were sellected and divided randomly to35 – 40 oocytes in 4-well dishes and incubated for 27 hours at 38.5° C with 5% CO_2 atmosphere in air with 95% humidity . *In vitro* fertilization were performed after maturation oocytes by capacitated fresh sperms taken by artificial vagina from proven fertile bucks , by addition of a microdrops($1x10^6$sperm) and culturing with matured oocytes for 24 hours at the same environement mentioned above . The large gravian folliculs (g.fs.) had more identified oocytes than the small ones .

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المستخلص

تم جلب المبايض من المجزرة القريبة للمختبر خلال ساعتين بعد ذبح الحيوانات عند درجة حرارة 30 م . وتم سحب البيوض و غسلها بمحلول داري، الفوسفات المتعادل (PBS) وتم ازالة الخلايا الركامية الزائدة وذلك بغسلها بذلك المحلول وتم اختيار البيوض ذات السايتوبلازم الحبيبي المتجانس وقسمت عشوائيا كل 40-35 بيضة في جفنة وحضنت بدرجة حرارة38.5 م مع غاز CO_2% بنسبة 5 % بهواء رطب بدرجة رطوبه 95% لمدة 27 ساعة لغرض تنضيج البيوض . وتم جمع السائل المنوي من ذكور ماعز ذات قدرة وخصوبة تلقائية جيدة بواسطة المهبل الاصطناعي وتم تكييف السائل المنوي وبعدها تم اضافة قطرات منه(تحوي5، املیون حيمن) الى الوسط الزرعي الذي يحتوي البيوض المنضجة و غطيت بالزيت وحضنت لمدة 24 ساعة بنفس الحاضنة اعلاه. اعطت الجريبات الكبيرة اعداداً اكبر للبيوض المنتخبة عن تلك الجريبات الصغيرة وكانت الفروقات معنوية ($P < 0.05$) لكافة النتائج المطلوبة في عدد البيوض ، وجود الخلايا الركامية ، انضاج البيوض، اخصاب البيوض ، الانقسام الخلوي للبيوض المخصبة. اضافة الى ان البيوض ذات الخلايا الركامية كانت اعلى معنويا($P<0.05$) بقدرتها على الانضاج والتخصيب والانقسام.

Introduction

Goat oocytes maturation show oftenlly during the *in vitro* maturation and fertilization a low incidence of male pronuclear formation , first cleavage division , and low developmental competence to advanced zygote development (14,19). Relatively few reports concerning the IVF in goats despite its usefullness for both basic research and in commercial applications (4) . The time required for maturation of oocytes *in vitro* is slightly longer (27 h) in goats than in sheep and cattle (4,7,8,15,17) . There is a decondensation and transformation into a male pronucleus , and the mechanisms by which these events take place in the egg cytoplasm are largely unknown . The transformation of the sperm nucleus during IVF has been shown to be related to the

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levels of intracelluler glutathione participates in sperm decondensation and in the transformation of the fertilizing sperm head into the male pronucleus (3,22,27,28) , which leads to the impairement in the decondensation of the sperm nucleus (11). Therefore the ability of oocytes to induce the nuclear decoudensation of spermatozoa seems to be directly related to high levels of intracellular glutathione (non – protein sulphydryl) compound in mammalian cells that protects cells from oxidation and has important role in cellular metabolism (18) , and including an effect on amino acid transport , DNA and protein synthesis , and a reduction of disulphides (16).

In a contrast to the numerous comparisons of sperm treatments for IVF in cattle and human , few direct comparisons have been reported for the goat (20). The present study were to declear the major limitation and the slow growth rate and low percentage of developing embryos reaching the morula and blastocyst stage in comparison to the normal embryos *in vivo* .

Materials and Methods

This experiment was performed within the year 2002 in Biotechnological Researches Center/Al-Nahrain University . Goat ovaries were collected from a local Al -Shulla slaughter house near Baghdad (30 km) and transported to the laboratory in Dulbeccos phosphate buffer solution (PBS) containing gentamicin 50 μg / ml at 30° C within two hour of slaughtering . The ovaries were washed three times in PBS containing gentamicin . Cumulus - oocyte complexes (COCs) were removed by washing for the group with cumulus cells and those oocytes without cumulus were chosen for there evenly granulated cytoplasim for both groups . Oocytes were washed three times in TCM-199 solution and randomly distributed at the hood temperature (30° C).

In vitro maturation oocytes in groups of 35-40 cumulus enclosed were placed in 500 ul of maturation medium in 4-well dishes and incubated for 27 hours at 38.5° C in an atmosphere of 5% CO_2 in air with a maxmium humidity (95%) . The maturation medium was TCM-199 (Sigma) supplimented with 10 (v/v) fetal bovine serum ,10 μg / ml LH , 10 μg / ml FSH , 1 μg / ml 17B – estradiol and 50 μg / ml gentamicin and then conered by mineral oil (sigma).

The matured oocytes were characterised by the appearance of first polar body and expansion of cumulus cells. At the end of oocytes maturation period , they were inseminated with capacitaed sperms. The capacitation of sperms were done *in vitro* from bucks of proven fertility collected by artificial vagina and transported within 10 minutes to the laboratory at 37° C . Motility of sperm cells was assessed under light microscope(40x) and the motile sperm fraction was separated by swim-up , 70 μ l of semen (1x10^6 sperm)was placed in each of several conical tubes (2.29)under 2ml of HEPES-TALPand incubated for 45-60 minutes in a humidified atmosphere of 5% CO2 in air at 38.5° C . After incubation , 600 μ l from the top of each tube was removed and pooled in a sterile 15 ml centrifuge tube and centrifuged at 200 g for 10 minutes , then discarding the supernatants , the resulting sperm pellet was resuspended 1 : 1 with TCM-199 medium containing heparin (100 mg / ml heparin – sodium salt (sigma)). Finally it was incubated for 45-60 min in a humidified atmosphere of 5% CO_2 in air at 38 5° C (Final suspension 84 x 10^5 sperm / ml , approximately).

In vitro fertilization were performed after oocytes maturation , groups of 25-30 oocytes transferred into 100 μ l fertilization medium (TCM-199) , (21) and covered with 5 μ l mineral oil . An aliquot (5 μ l) of the sperm suspension was added to the fertilization microdrops and the culture was performed within 24 h under humidified atmosphere of 5% CO_2 in air at 38.5 C.

Statistical analysis

A one-way analysis of variance was performed to test whether group variance was significant or not. The differences between group means were tested through Duncan's multiple range test and the CRD for maturation, fertilization and cleavage means of with and without cumulus oocytes,the comparison between groups were used Duncan for analysis(1).

Results and Discussion

The graffian follicles (g.fs). brought from the abattoir (6044) were divided into small (3823) and large (2221) g.fs. The small g.fs. contains 2085 oocytes (54.53%) and the large ones contain 1548 oocytes (69.7%) and the differences between the small and large g.fs. found on the goat ovaries were non significant (P>0.05) as showen in Table 1 .

Table 1. The relationship between the number of ovarian oocytes and graffian follicles in does

g.fs state	Number of g.fs	Identified oocytes	
		Number	Percentage
Small	3823	2085	54.53 a
Large	2221	1548	69.7 a
Total	6044	3633	60.1

No significant differences (p>0.05) to compression rows

The differences in oocyte liberation between the small and large graffian follicles , may a cause of the follicle physiological state (preantral) in which it stays in small dimention with oocyte , shortage (10) and the large ones passing the dominance state in which there is a continuous follicular growth with increase in estrogen and androgens in follicular fluid which insist the follicular development (12).

The results noticed in Table (2) showed the status of the identified oocytes in the small and large g.fs. , the number of oocytes with cumulus cells in 1247 oocytes examined in the large g.fs. were 701 (56.21) compared with 1671 oocytes of the small g.fs. which contains 737 (44.1%) oocytes with cumulas cells. These findings indicate that the small g.fs. had more oocytes percentage with COCs were statistically significant ($P < 0.05$) . The COCs has a big role on the nutritive maintainence and metabolic activities of goat oocytes along with the protein synthysis (25) a long with its role in the preparation of oocytes for maturation and fertilization (6) . The maturation of identified oocytes in the small g.fs.with cumulus were 254 (34.46%) inferior as compared with the highly significant differences ($P < 0.05$) noticed in the large g.fs. 333 (47.50) and on the contrary with the maturation observed in the large oocytes with out cumulus were 94 (17.22) inferior than those in the small g.fs 150 (16.05%) . The increased COCs increases the time needed for maturation of the oocytes (26) , so that we found the linear correlation between the presence of COCs around oocytes and the maturation process were noticeable.

Table 2. Relationship between the g.fs. size and the COCs occumulation and the oocytes maturation *in vitro*.

g.f.s.size	Oocytes number	Asparated oocytes				Maturation			
		w.c.		w.o.c.		w.c.		w.o.c.	
		No.	%	No.	%	No.	%	No.	%
Large	1247	701	a 56.21	546	a 43.8	333	a 47.5	94	a 17.22
Small	1671	737	b 44.1	934	b 55.9	254	b 34.4	150	a 16.05

Differences a,b are significant (p<0.05) to compression rows
W.C: with cumulus
W.O.C: with out cumulus

In other part of this study were performed on 842 small and 478 large g.f.s . In these follicles 493 (58.55%) and 389 (81.38%) identified oocytes in the small and large follicles respectively , off these oocytes their were 163 (33.06%) and 229 (41.13%) oocytes with cumules and 330 (66.93%) and 160 (41.13%) oocytes without cumlus for the small and large g.fs respectively . And the matured oocytes found in these four groups were 54 (33.12%) and 111 (48.47%) for the oocytes with cumulus where as 45 (13.63%) and 35(21.87%) for the oocytes without cumulus . The fertilized oocytes found in the maturated ova studied with cumulus were 9 (16.67%), and 26 (23.42%) and the oocytes with out cumulus where 2(4.44%) and 9 (25.71%) respectively. The cleavage rate of the four groups were 4 (44.4%), 15(57.69%), zero and 1 (11.11%) for the oocytes with cumuls and with out cumulus

oocytes in the small and large g.fs, respectively.(Table 3)

From the above mentioned results , we found that there were a significant ($P < 0.05$) difference in maturation rate between the oocytes groups with (superior) and without (inferior) cumulus in both of small and large g. fs. And the same finding were noticed for the fertilized and cleavage rates in the oocytes groups studied in these experiments and as shown in Table 3 .In addition to the highly significant($P<0.05$) difference of the large g.fs. than in the small g.fs. in the maturation , fertilization and cleavage rates.

Table 3. Relationships between graffian follicles and IVM , IVF and cleavage of goat oocytes, in large and small, with and with out cumulus.

Size and number of g.fs	Identified oocytes		Oocytes state		Oocytes maturaed		Oocytes fertilized		Xygote cleavage	
	No.	%	No	%	No.	%	No.	%	No.	%
Small 842	493	58.55	WC 163	a 33.06	54	a 33.12	9	a 16.67	4	a 44.44
			WOC 330	66.93	45	13.63	2	4.44	0	0
Large 478	389	81.38	WC 229	b 58.86	111	b 48.47	26	b 23.42	15	b 57.69
			WOC 160	41.13	35	21.87	9	25.71	1	11.11

Differences a,b are significant ($p<0.05$) to compression rows

In vitro maturation , fertilization and cleavage of caprine oocytes often showed deficient knowledge as indicated by a low incidence of male pronuclear formation , first cleavage division , and low developmental competence to blastocyst (14,19) and the major limitation in these studies has been the slower growth rate and low percentage of developing embryos reaching the morula and blastocyst stage in comparison to the normal embryo *in vivo* (15) . One of the main obstacles remaining to the production of caprine embryos *in vitro* is that of immediate availability of spermatozoa (8).

These findings in our study had a greed the findings of (7) that there is a relationship between the size of follicles and oocytes maturation and fertilization *in vitro*. On the other hand , the additions of co-cultures as the cAMP or granulosa and cumulus cells may lead to the maturation and developments (13,23,24).

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