Bovine oocyte maturation collected from abattoir and in vitro fertilization

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Abstract

This study was conducted at biotechnology center in Baghdad. Eighty (80) of bovine ovaries from Al-Shualah abattoir in Baghdad after slaughter have been collected. The total number of oocytes were obtained from ovaries by aspiration of follicles reached (71). The follicle selection method according to size from 3-7 mm. This oocytes classified into two groups according to the cumulus cells surrounded the oocyte, partially or completely. The first group included (37) oocytes completely surrounded by cumulus cells, and the second group (34) oocytes without cumulus cells. All oocytes subjected to incubation in TCM-199 medium at 38 C° in CO2 incubator about 24 hrs., the results indicates that (24) 64.88% of the oocytes in the first group and (16) 47.05% of the second group showed maturation. All matured oocyte were fertilized by frozen semen which had been brought from artificial insemination center (Abu Ghraib) as straws. The result appeared (13) 54.16% fertilized oocytes in first group while the number of fertilized oocyte in second group reached (6) 46.15%, when incubation had been continued for further development, the cumulus oocyte complex give (6) 37.50% embryos in two cells to four cells (Cleavage stages). But the second group give only (2) 33.33% embryos. No significant differences in maturation between oocyte with cumulus (WC) and without cumulus (WOC) among oocyte ($p>0.05$).

إنضاج بيوض الأبقار المأخوذة من المجازر وتخصيبها مختبرياً

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الخلاصة

تم جمع (80) مبيض للأبقار من مجزرة الشعلة بشكل عشوائي بعد ذبح الحيوان مباشرة وقد نقلت إلى مركز التقنيات الإحيائية في بغداد محفوظة بالمحلول الملحي المتعادل، استخدمت طريقة السحب بواسطة السرنجة لسحب (71) بويضة وصنفت اعتمادا على وجود الركمة المبيضية إلى (37) بويضة محاطة بشكل كامل بخلايا الركمة الاباضية و(34) خلية معراة من الركمة الاباضية وتم إهمال جميع البيوض التي ظهرت محاطة بشكل جزئي بالركمة الاباضية.

أخضعت جميع البيوض إلى الحضن في الوسط الزرعي (TCM-199) باستخدام الحاضنة المكيفة بغاز CO2 (ثاني اوكسيد الكربون) لمدة 24 ساعة وكانت النتائج من المجموعة الأولى المحاطة بخلايا الركمة المبيضية (24) بيضة أظهرت نضوجها من خلال ملاحظة تكوين الجسم القطبي الأول وبنسبة 64.86 ٪.

أما المجموعة الثانية فأعطت (16) بيضة ناضجة وبنسبة 47.05٪. تم استخدام السائل المنوي المجمد المزود من مركز التلقيح الاصطناعي في أبو غريب في تلقيح هذه البيوض وثم حضنها في الحاضنة المكيفة بغاز CO2 فأظهرت (13) بيضة مخصبة، حيث كان الإخصاب بنسبة 54.16٪ من البيوض الحاوية على الركمة المبيضية واستمر حضن البيوض إلى الانقسام الثاني فحصلنا على (6) أجنة بنسبة 46.15٪، بينما تم الحصول على (6) بيوض مخصبة (Zygot) وبنسبة 37.50٪ من الخلايا العارية من الركمة المبيضية علما إن نتيجة الحضن لهذه البيوض المخصبة أظهرت جنينين فقط بعد استمرار حضنها وكان بنسبة 33.33٪. لم يلاحظ وجود فرق معنوي على مستوى (P>0.05).

Introduction

Invitro fertilization which means (fertilization in glass) sperm and oocyte join in a laboratory dish (1). The first comprehensive account of IVF in the rabbit, including the birth of young (2). The mouse was the second mammalian species in which IVF was successfully accomplished, the event being reported by (3).

Invitro oocytes maturation studies are gaining momentum in developing countries, since it is the starting point of biotechnological processes like in vitro fertilization and cloning (4 – 5). In vitro maturation of oocytes helps in the generation of embryos from the ovaries of high producing slaughtered animals, which are otherwise wasted (6 – 7). So we can get high number and easy recovery of oocyte to obtained mature oocyte and fertilized it without disturbance of animals in a technique by surgical and non surgical**.** Therefore this study was designed to estimate recovery rate of bovine oocyte from collected ovaries.Oocytes maturation in vitro culture medium and In vitro fertilization of matured oocyte.

Materials and Methods

Eighty bovine ovaries were collected from Al-shualah abattoir, transported to the laboratory of Biotechnology Research Center in Baghdad. In container of normal saline within 3 hrs. Oocytes were collected from follicles (non atretic) by aspiratation method with follicular fluid. The diameter of these follicles ranged from 3 to 7 mm. After oocytes and follicular fluid aspiration, they transfered to apettri dish containing some of TCM-199 medium for oocyte examination under dissecting microscope, the oocytes with cumulus cells (WC) were isolated in apettri dish from oocytes without cumulus cells by using a micropipette(8). Oocyte surrounded with multilayer compacted cumulus cells (CoCs) washed three times in maturation medium (RPMI-1640 medium) in order to remove cumulus cells and obtained oocyte without cumulus cells (WOC) denuded oocyte(9). Oocytes were transmitted to a pettridish containing culture media (TCM-199) covered with paraffin mineral oil were incubated in 5% CO_2 incubator, at 37 C˚, with humidity 90% for at least 24 hrs (10). Oocytes were deposit in 1×10^6 sperm/ml in pettri dish containing TCM-199 medium, and then incubated for 24 hrs. at 37 C˚ and 5% CO_2. Thin layer of paraffin oil was used to cover the surface of media (10).

Results

The total number of ovaries gives 71 oocytes (Table 1), 37 (with cumulus cells) and 34 (without cumulus cells) (Fig. 1) from follicles, collected oocytes and subjected to maturation, show first poler body (Fig. 2) and in vitro fertilization. Only 24 oocytes (WC) maturated while matured oocytes (WOC) reached 16.

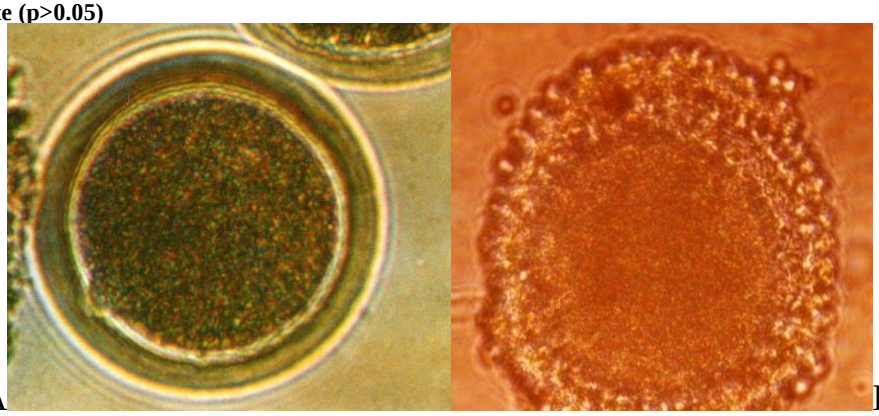
When oocytes developed to fertilized ova, gives 13 zygotes and 6 zygotes in the first class (WC) and the second class (WOC) respectively.

Eventually, all number of (WC) gives 6 embryos in the first stages and second (Fig. 3) of cleavages and the number of embryos from (WOC) two only.

Table (1) Shows the number of matured oocyte, fertilized oocyte and cleavage embryos

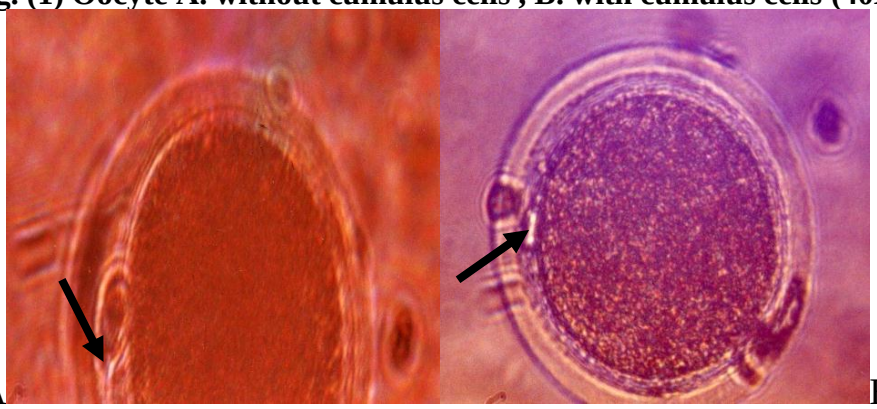
Oocyte situation	With Cumulus (WC)		Without Cumulus (WOC)		Total
	No.	%	No.	%	
Incubated oocyte	37	52.11	34	47.88	71
Matured oocyte	24	64.86	16	47.05	40
Fertilized oocyte	13	54.16	6	37.50	19
Cleavage embryo	6	46.15	2	33.33	8

***No significant differences in maturation between oocyte with cumulus (WC) and without cumulus (WOC) among oocyte (p>0.05)**



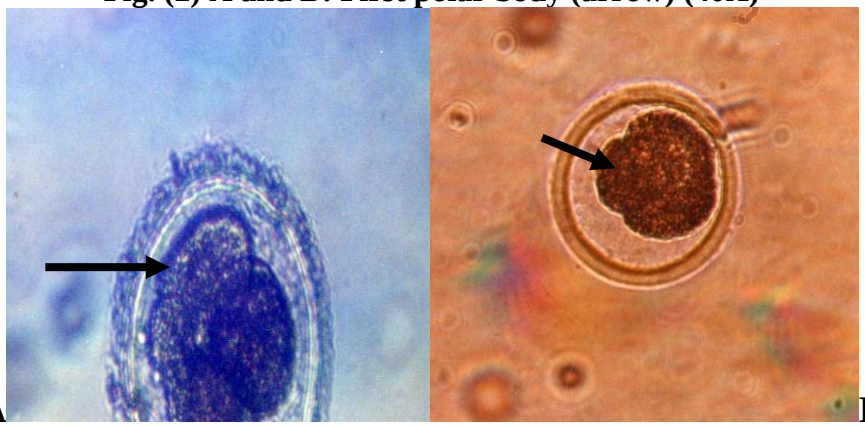
A B

Fig. (1) Oocyte A. without cumulus cells , B. with cumulus cells (40X)



A B

Fig. (2) A and B: First polar body (arrow) (40X)



A B

Fig. (3) A and B: second stage of Cell division (arrow) (20X)

Discussion

In comparison we obtained 71 oocytes (Table.1) from 80 ovaries while (12) obtained 549 oocytes from 115 ovaries. (13) recorded 70 oocytes which is recovered from 34 ovaries included small and large size of follicles. While (14) recorded 160 oocyte which is recovered from 50 ovaries by aspiration method and 287 oocytes from 50 ovaries by scoring method. In our study Oocyte were aspirated from follicles by syringe 18 gauge needle, while (15) mention two methods for the recovery of oocyte from the ovary, one approach involved the use of 6 ml disposable plastic syringe fitted with an 18 gauge needle for follicular fluid aspiration and the other utilized, the oocyte recovery for the human ovary method modified to suit the bovine ovary. All oocytes, (with cumulus cells and without cumulus cells) were obtained from bovine ovaries were matured in TCM-199 medium, as well as with the (16) who used the same media for maturing oocytes. Another studies showed that the TCM-199 medium, is the best of oocyte maturation (14,15,16). RPIMI- 1640 medium used in washing oocyte before preparing to maturation, but Spiropoulos and Long, (20) used TCM-199 medium for washing oocyte Kitiyanant et al., (17) oocytes recovered and washed three times in HEPES buffered Tyrodes media and oocytes were categorized non the basis of investment. We used TCM-199 medium, for washing oocyte, which does not required 5% CO2 atmosphere (18).

While Chung and Choi (19) in the study of oocyte maturation they used the tissue culture media (SECM) which mainly composed of salts, pyruvate and lactate, the oocyte were cultured in this media without addition of follicular fluid. Spiropoulos and Long (20) used TCM-199 culture medium with 202 g\1 sodium bicarbonate plus 50 mg/ml gentamicin supplemented with either 20% FCS or 20% estrus cow serum (ECS).

In our study, the total number of matured oocyte are 40 (56.33)%, while (21) recorded 73% to group I and 78.9% to group II, Longeran et al., (22) recorded 93.2% and (14) recorded 73.33% (19) 54.16% zygote we obtained from fertilization in this study. (21) recorded the fertilization percent 41.1% in the oocyte recovered from follicles with diameters 2-7 mm (Group I) while (Group II) with7-10 mm recorded 49.3% fertilized oocyte.

The differences between the studies might be due to different factors that effects the maturation and fertilization. The success of invitro culture is influenced by several factors, which includes media composition, culture atmosphere and temperature, Oxygen tension, osmotic pressure, Composition of nutrition, free radical scavengers, volume of culture drops and oocyte manipulation.

We get only 8 embryos from the total number 71 oocyte in percent of 11.26%, while Mustafa and Sukru (21) got percent of embryos from total incubated oocyte of group I reached to 41.1% and group II reached to 49.3%. This might be due to variables affecting development competence of oocytes, In vitro some of the variables affecting development competence of oocytes are: 1- The age of the females supplying the oocytes, 2- Their health and environmental stress, such as heat stress, 3– The size and maturity of follicles, 4– The size of the oocyte, 5– The presence and interaction of cumulus cells with the oocyte, 6- The conditions of oocyte maturation, such as temperature, pH and gas environment, 7– The number of cohort oocytes and, 8- The presence of cumulus cells and cell growth factors in the culture media (23).

It was concluded from this study, the time manipulation of oocyte through transmitted, aspiration, and invitro fertilization, plays a role in viable oocyte.

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