

In Vitro Production of Ovine Embryo

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Abstract

The study was conducted in order to know the effect of certain factors on in vitro maturation and fertilization of sheep oocytes in vitro during the period from November 2010 to March 2011, 195 oocytes were aspirated from 60 ovaries of adult an prepubertal lamb slaughtered in AL-Falluja slaughter house, and transported to reproductive lab of coll .vet .Med. / AL -Anbar university at Falluja. Aspirated oocytes were graded and incubated at 38.5 °C, 5% CO₂ with relative humidity 90% for 24 hrs. Capacitated sperm were prepared for IVF. Then incubation of matured oocyte with capacitated sperm (CO₂ 5%, 38.5°C, 90% relative humidity for 24 hrs).

The results showed that the right ovary give more oocyte ($P < 0.05$) the left one. The large follicles showed oocyte recovery and in the percent of maturation and IVF as compared ($P < 0.05$) with the smaller one. The results showed also that the TCM -199 is the best for IVM and IVF ($P < 0.05$) as compared with DPBS. It was concluded for the study the ability of producing ovine Embryo in vitro, However further study is required.

إنتاج أجنة الأغنام مختبرياً

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الخلاصة:

أجريت الدراسة لبيان تأثير بعض العوامل على إنضاج وإمكانية إخصاب بويضات النعاج مختبرياً للفترة من تشرين ثاني 2010 لغاية آذار 2011 حيث استخدمت في هذه الدراسة 195 بويضة سحبت من 60 مبيض لنعاج بالغة وفطائم ذبحت في مجزرة الفلوجة ثم نقلت إلى مختبر كلية الطب البيطري جامعة الانبار موقع الفلوجة . حضنت البويضات المستخرجة بظروف 38.5 م , 5% CO₂ ورطوبة تصل إلى 90% لمدة 24 ساعة. تم تحضير الحيامن للأخصاب الخارجي وحضن مع البويضات بنفس الظروف المذكورة ولمدة 24 ساعة. بينت النتائج تفوق المبيض الأيمن ($p < 0.05$) على المبيض الأيسر في نسبة استحصاء البيوض وفي نسبة الإنضاج كما أشارت النتائج تفوق الجر بيئات الكبيرة على الصغيرة ($p < 0.05$) في الإنضاج والإخصاب كما وضحت النتائج تفوق الوسط الزراعي TCM_199 على DPBS ($P < 0.05$) . وقد استنتج من الدراسة إمكانية إنتاج الأجنة مختبرياً بعد توفر الإمكانيات والموضوع يحتاج إلى دراسات أخرى.

Introduction

In bovine, the capacity to produce embryos by IVM/IVF has progressed very rapidly during the past few years (Freitas and Melo, 2010). However, in sheep only a limited number of offspring have been produced using this technique also in small ruminant a demand exists for basic research on zygote development and on the production of transgenic offspring (Wani *et al.*, 2000). The steps of IVF (in vitro fertilization) system involves three steps; (a) collection and recovery of oocytes; (b) the in vitro maturation of these oocytes (IVM) and (c) the in vitro fertilization of the matured oocytes (IVF) (Wani, 2002). The aim of this study is to detect the ability of producing an embryo from an oocytes collected from sheep ovaries at the slaughter house of AL-Fallouja city by the IVM and IVF the ova in two culture media.

Materials and Method

Collection of oocytes and in vitro maturation (IVM)

The ovaries (60 in number) from the local ewes, of different ages, were collected from AL-fallouja slaughter house and transported with cool box containing warmed normal saline (37°C) immediately within 1-2 hours to the reproductive laboratory, college of vet. Medicine, AL-Anbar University. Follicle (2-8mm) were obtained by aspiration of follicles from the ovary using a 18 gauge needle fitted with 3ml syringe containing 2ml from the media. Only cumulus oocyte complex (COC) or oocyte covered with one layer granulosa cells or the filled cytoplasm oocytes were used for the in vitro maturation IVM. The collected oocytes were in vitro matured in TCM-199 supplemented with (fetal

calf serum (10% fcs), eCG hormone (10 µg/ml, LH (10 µg/ml), estradiol

10 µg/ml, antibiotics 50 µg/ml and streptomycin 50 µg/ml) 10% heat inactivated ovine serum and in Dulbecco's phosphate buffer media supplemented with: D-glucose, Na pyruvate bovine serum Albumin and Gentamycin sulphate at pH 7.0.

Oocyte were incubated in 24 cell culture wells and kept at incubator (38.5°C, 5% CO₂ in air) for 24hr.

The In Vitro Fertilization (IVF)

Fresh semen were collected for the IVF, by electroejaculator, from Awassi ram with proven fertility and prepared according to Salvik *et al.*, (1992) method. Diluted 0.5ml of semen in 5ml tyrode—albumin lactate pyruvate media, centrifuge and washed two times. Heparin 10 µg/ml (20 i.u.) were added to the capacitation media and kept for 1hr at 39 °C incubator. The capacitated sperms were added to oocytes with 1×10^6 sperm/ml in 2ml petridish wells (containing TCM-199 media or Dulbecco's phosphate buffer media) and kept at incubator for IVF (38.5°C, 5% CO₂, 90% relative humidity for 24hrs). T-test and Chi-square test used for analysis of the data.

Result and Discussion

From 30 ewes 60 ovaries were collected 195 oocytes were aspirated from 2-8mm size follicles. The oocytes were classified according to their morphological appearance into four graded groups according to Aloti and AL-himaidi (2004); 1- Excellent oocytes when more than one of granulosa cell layer around (20 oocytes, 10% of collected oocytes). 2-good oocytes: an oocytes with very good cytoplasm appearance with very few intact granulosa cell (96 oocytes 50%). 3-poor

oocytes :an oocytes with shrink cytoplasm and good zona pellucida membrane(60 oocytes 30%) 4- bad oocytes :with fragmented cytoplasm and damaged zona pellucida membrane(19 oocytes,-10%). From this data , only low fertile female is allowed to be slaughtered similar observation have been made by Alofi and AL Himaidi(2004). The low grade of oocyte recovered might be due to collection in non –breeding season (Farag *et al.*,2010,. Rao *et al.*,2002). The effects of the types of the ovary on the percentage of follicles recovery rate and maturation were shown in **table-I** .the results indicates that the right ovary more active than the left one in the recovery rate of oocytes.

There was a significant difference ($P < 0.05$) between the right and left ovaries in the percent of recovery and maturation . similar observations have been made by several workers (wani ,2002;Freitas and melo, 2010 ;IM *et al.*,1995). While Al-Jumaily(2003) showed no significant difference between the right and the left ovaries in recovery and maturation. This might be due to breed difference and seasonality (Rao *et al.*, 2002; wani,2002). The effects of follicle size an recovery rate and maturation of oocytes percent have been shown in **table –II**. Results showed that large follicles given high recovery rates and maturation of oocytes percent. There was a

significant difference ($P < 0.05$) in recovery rate and maturation percent between the large and small follicles. Similar observation have been made by wani.(2008) , AL-Jumaily(2003), Majeed *et al* .(2008) and forag *et al.*(2010). This might be due to large follicles contained well developed oocytes as compared .with smaller one (Hafez and hafez, 2000; wani 2002, Gordon,2007;).

The effects of culture media on percentage of oocytes maturation and in vitro fertilization are shown in **table –III**. The result showed that the TCM-199 given the best result in maturation and fertilization percent as compared with DPBS media. There was a significant differences ($P < 0.05$) between TCM_199 and DPBS in maturation and IVF percent. Similar observation have been made by wani(2002),farag(2009),farag *et al.*(2010). This might be due to the TCM-199 maintained requirements for both ova and sperm IVF. Techniques (Motlagh *et al.*,2008; Rao *et al.*,2002). The beneficial effect of TCM-199 media on IVM and IVF of animal oocytes may be related to some factors in its composition such as essential amino acids and glutamine that stimulate DNA and RNA synthesis and enhance cell division (pawshe *et al.*, 1996; Gordon, 2003). It was concluded from this study the possibility of production of sheep embryo in vitro, however; further studies is required.

Table-I: effects of the type of the ovary on the % of follicles, recovery rate and maturation

Type of ovary	% of follicles	%Oocyte recovery	%matured oocyte
R. ovary	3.4 ± 0.8a	74.0 ± 1.4a	40.0 ± 1.8a
L. ovary	2.8 ± 0.6b	68.0 ± 2.0b	38.0 ± 1.3b

a,b, there was a significant difference between different litters($P < 0.05$)

Table-II: effects of follicle size on the recovery rate and matured oocytes:

Size of follicles	%Oocyte recovery rate	% of matured oocytes
Small follicles(2-4mm)	62 ± 1.5a	39.3 ± 1.4a
Large follicles(5-8mm)	68 ± 1.8b	48.20 ± 1.6b

a,b, there was a significant difference between different letters($P < 0.05$)

Table-III: effects of culture media on percentage of oocyte maturation an IVF.

Culture media	% of maturation	% fertilization
TCM-199	46 ± 1.6a	31 ± 1.2a
DPBS	32 ± 1.4b	22 ± 2.4b

a,b, there was a significant difference between different letters ($P < 0.05$).

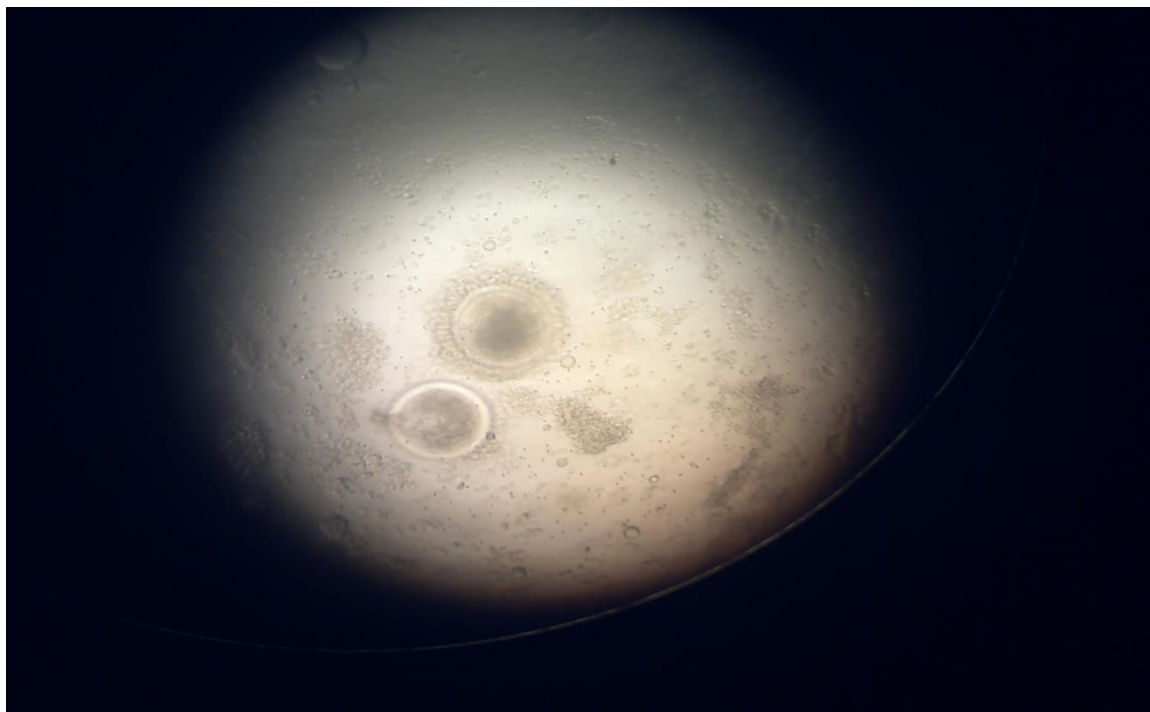
Collection of the ovary



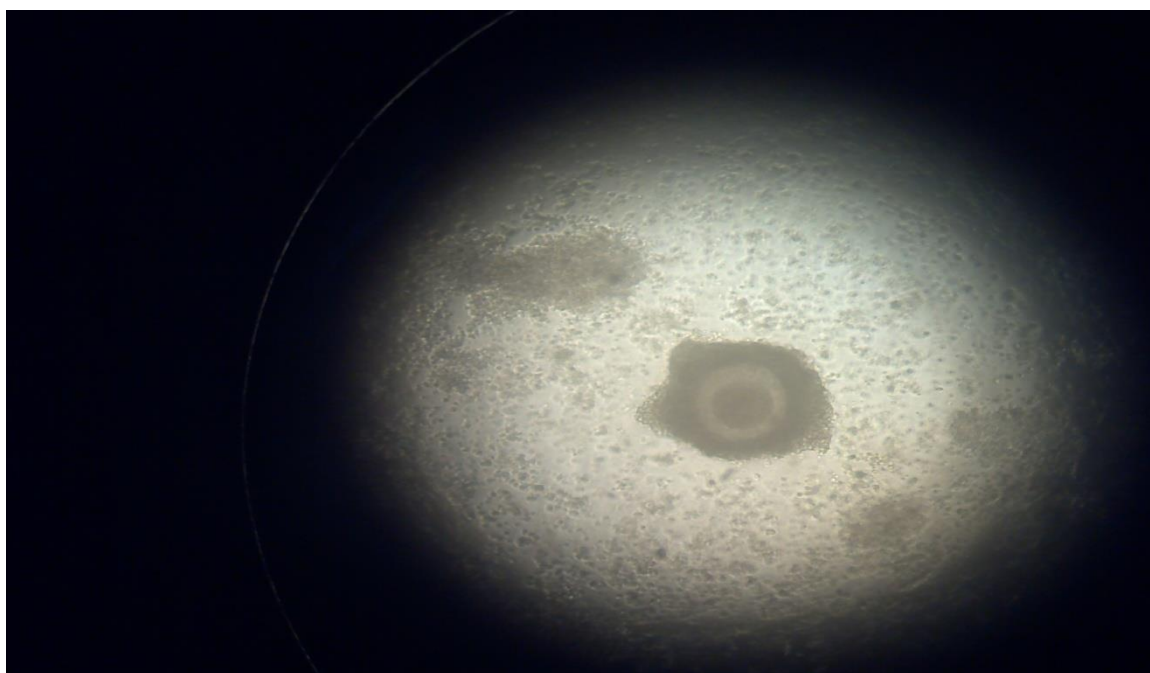
Method of aspiration of Oocyte from the ovary



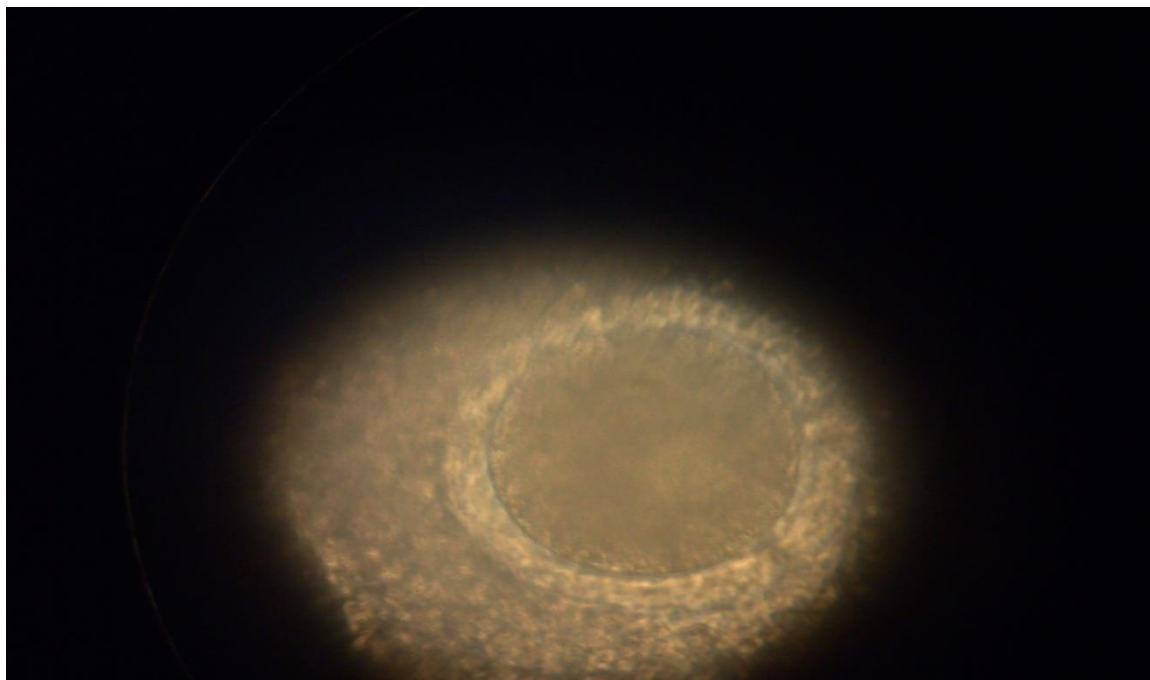
Two Immature Oocyte, One with Layer of Granulosa Cells & Other with Compact Cytoplasm



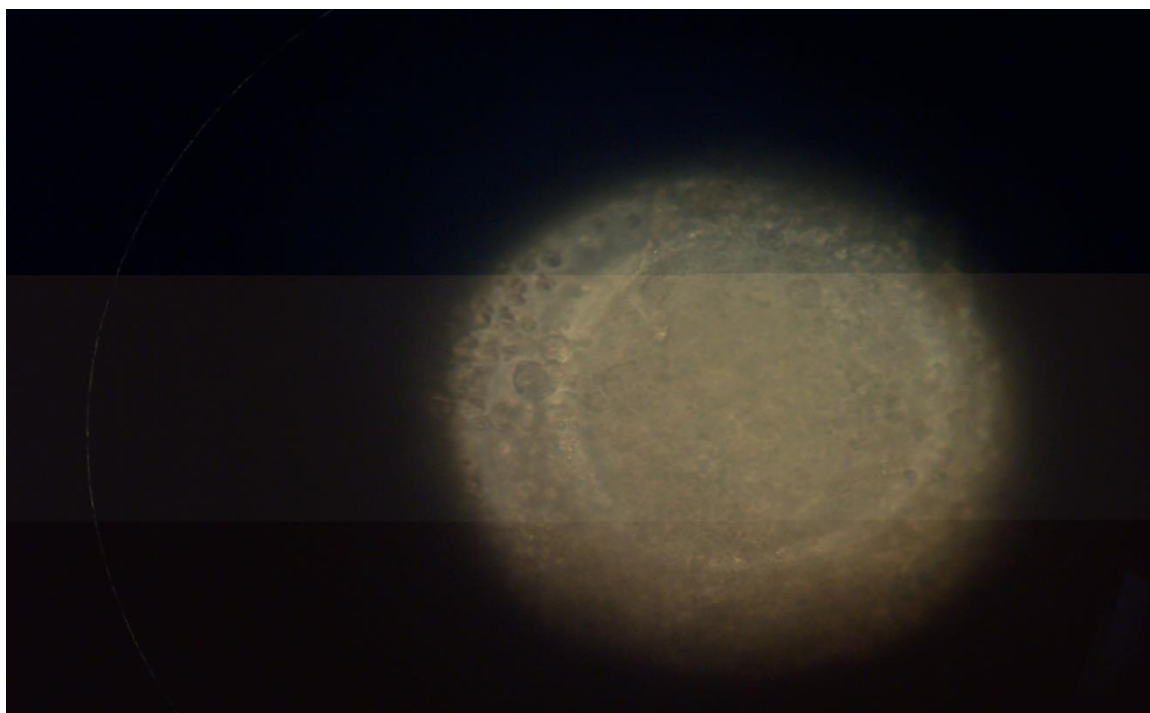
OOCYTE with cumulus oocyte complex



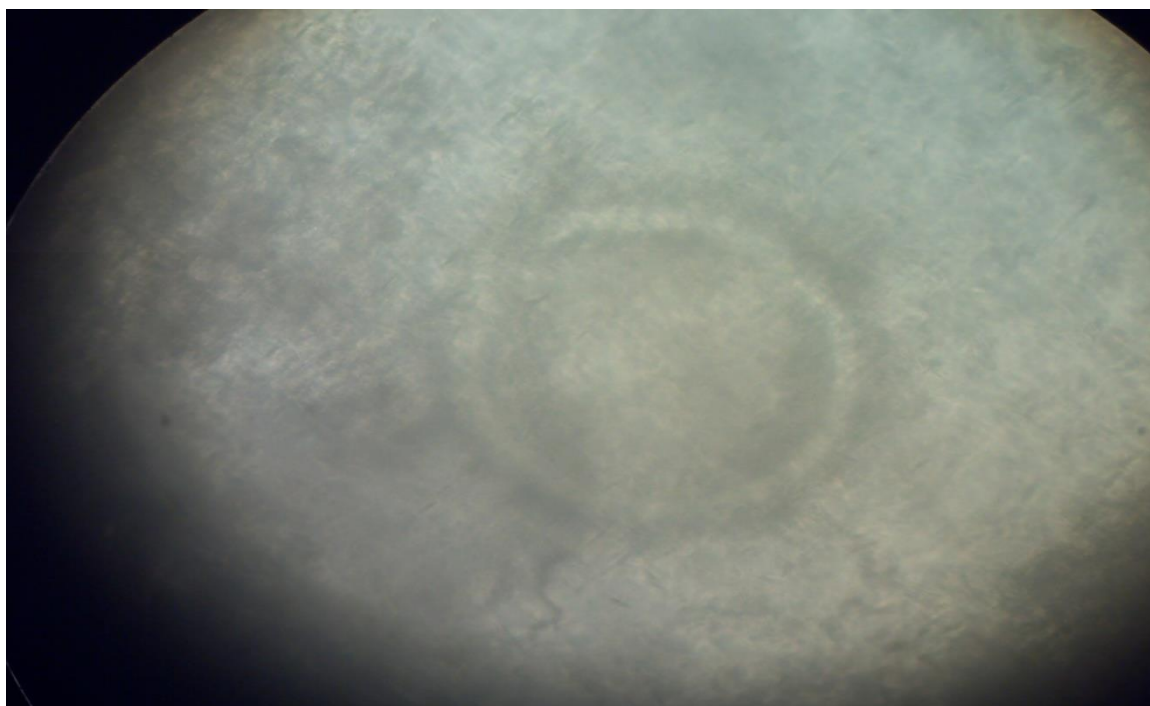
Mature oocyte with first polar body



Fertilized oocyte with second polar body



Oocyte with inner cell mass & blastocel



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