In vitro capacitation of buck spermatozoa

A. A. Omar
Collage of Veterinary Medicine\ University of Fallujah

Abstract

Testes of adult Buck (n=15) were taken from Al-Fallouja slaughter house and transferred with cool box containing normal saline to the lab of Vet. Med. College, Al-Anbar University during the period from October 2013 to December 2013 within 30 minutes. Semen were collected from the tail of epididymis by aspiration with 3_{ml} (18-guage) disposable syringe containing $1\text{-}2_{ml}$ of MEM media. It's also the tail of epididymis subjected to several incisions with a sharp surgical blade and applying a pressure at the base of epididymis to collect the remnant of spermatozoa. The collected materials were put in a sterile petridish and incubated at 35°c. samples were examined to evaluate the degree of sperm's maturation. The semen then incubated in 5% co_2, 35°c with 90% relative humidity for 4-6 hours. After maturation of spermatozoa the suspension was diluted 1:1 with heparin ($100_{\mu g/ml}$ heparin salt) and incubated for 15 minutes at 5% Co_2, 35°c with 90% relative humidity. Head to Head agglutination of spermatozoa was considered to be a criteria of sperm capacitation. The result showed the possibility of sperm maturation obtained from cauda epididymis of Buck in MEM media in vitro. The presence of distal protoplasmic droplet was the criteria of spermatozoa maturation. It was concluded from this study that it's possible of sperm maturation and capacitation taken from tail of epididymis of buck from slaughter house source in vitro.

E-mail: alhadithyali@gmail.com

Keyword: buck, spermatozoa, *in vitro* capacitation

تكييف حيامن ذكر الماعز مختبرياً

علي عبد الفتاح عمر
كلية الطب البيطري/ جامعة الفلوجة

الخلاصة

أجريت الدراسة على (15) خصية أخذت من مجزرة الفلوجة وتم نقلها بحاوية مبردة تحتوي على محلول ملحي خلال 30 دقيقة إلى مختبر التناسل التابع لكلية الطب البيطري – فلوجة/ جامعة الأنبار للفترة من تشرين الأول إلى كانون الثاني 2013. تم جمع السائل المنوي من ذيل البربخ بواسطة السحب بمحقنة سعة 3 مل حاوية 1-2 مل من وسط (MEM). وتم أيضاً تقطيع ذيل البربخ باستخدام مشرط مع الضغط على ذيل البربخ لاستخراج بقايا الحيامن. وضعت النماذج في حاضنة وبدرجة حرارة 35°م. فحصت النماذج تحت المجهر لتقييم درجة نضوج الحيامن. بعد ذلك وضعت النماذج في حاضنة 5% CO_2 وبدرجة حرارة 35°م 90% رطوبة نسبية لمدة 4-6 ساعات. بعد انضاج الحيامن خفف السائل المنوي بنسبة 1:1 مع الهيبارين (100 مايكرو غرام لكل مل من وسط MEM) وتم الحضن في 5% CO_2 ، 35°م و90% رطوبة نسبية لمدة 15 دقيقة لغرض الحصول على تكييف الحيمن. تلازن رؤوس الحيامن تعتبر الطريقة المقبولة لمعرفة تكييف الحيامن. أظهرت النتائج إمكانية انضاج حيامن التيس (ذكر الماعز) المأخوذ من ذيل البربخ باستخدام وسط MEM. ان وجود القطرة الهيولة بموقع سفلي يعطي دلالة على نضوج الحيامن. وقد استنتج من الدراسة إمكانية إحداث نضوج وتكييف الحيامن ذكور الماعز المأخوذة من ذيل البربخ المأخوذة من المجزرة في المختبر.

الكلمات المفتاحية: ذكور الماعز، الحيامن، تكييف.

Introduction

It has been reported that the most important aspect of the sperm preparation are capacitation of spermatozoa, culture conditions, temperature and PH of culture medium used (1, 2). Mammalian spermatozoa undergo series of biochemical and biophysical change prior to fertilization, called capacitation (3). Capacitation involves the removal of sperm coating material acquired during epididymal transit or during exposure to seminal plasma and cholesterol depletion resulting in increased membrane permeability to calcium (4, 5). Capacitation involves the modification at the plasma membrane level, such as protein and phospholipid transfer and antigen redistribution and changes in intracellular calcium and other ion concentration (4). Capacitation also involves enzymatic removal of a substrate or coating, probably a polysaccharide on the sperm cell head that is acquired in the seminal plasma (2). Head to head agglutination of spermatozoa is considered to be a criteria of sperm capacitation (3). Heparin is used for in vitro capacitation spermatozoa. It modulates gamete interaction in cattle and sheep IVF system (6). The aim of this study was designed to show the capacitation of Buck spermatozoa taken from the tail of epididymis.

Materials and Methods

Testes of adult Buck (n=15) were taken from Al-Fallouja slaughter house and transferred with cool box containing normal saline to the lab of Vet. Med. College, Al-Anbar University during the period from October 2013 to December 2013 within 30 minutes. Semen were collected from the tail of epididymis by aspiration with 3_{ml} (18-guage) disposable syringe containing $1\text{-}2_{ml}$ of MEM media according to the size of the tail of epididymis as reported by (7). It's also the tail of epididymis subjected to several incisions with a sharp surgical blade and applying a pressure at the base of epididymis to collect the remnant of spermatozoa. The collected media together with the sperm were put in a sterile petridish and then transported to be incubated at 35°c. Samples were taken from media to observe the degree of maturation and evaluate sperm according to the presence of protoplasmic droplet and their position on the sperm. Evaluation of semen included individual motility and sperm abnormalities. The sperm then were incubated in 5% Co_2, 35°c with 90% relative humidity for 4-6 hours in sterile test tubes with 45 angle without cover. Minimum Essential medium (MEM) with L-glutamine: 11mg of media was dissolved in approximately 600ml of Bi-distilled water and then the following components were added: Sodium bicarbonate powder (23_{gm})., HEPES buffer [(Gib.co., USA), (JM) 10_{ml}], Benzyl penicillin 100 $_{I.u/ml.}$, Streptomycin $10_{mg/ml}$., Fetal calf serum (F.B.S.) 100_{ml} 10%. The final volume of the media was completed to one liter with Bi-distilled water and finally the medium was sterilized by using Nalgen filter 0.22 $_{\mu,m}$ filter unit. After maturation of spermatozoa the suspension was diluted 1:1 with heparin containing media ($100_{mg/ml}$ heparin salt) and incubated for 15 minutes in co_2 incubator at 35°c according to the procedure described by (3). Head to Head agglutination of spermatozoa was considered to be a criteria of sperm capacitation (3).

Result and Discussion

The result of this study showed the possibility of sperm maturation obtained from cauda epididymis of Buck in MEM media in vitro. The presence of distal protoplasmic droplet was the criteria of spermatozoa maturation. Similar results have been observed by (3). Several investigators indicated that the cauda epididymal sperm could be successfully used from slaughtered bucks, several hours after their death, for IVF of goat oocytes (8). MEM cultured medium for in vitro maturation of cauda epididymal spermatozoa were easily prepared medium in the lab. (9). This might be attributed to their biochemical content materials. The result also showed the phenomena of head to head agglutination, which improves sperm capacitation. It has been claimed that heparin

stimulates the fertilization rate through improvement of sperm capacitation (10). Heparin plays a role in spermatozoa uptake of calcium through its binding to the sperm (11). Up till now, some workers not applied heparin in the capacitation media and have still obtained a cleavage rate of about 70% (12). Capacitation involves the alternation of the plasma membrane such as removal of capacitation factors, removal of cholesterol, influx of calcium, an increase in intracellular pH, and increase in protein tyrosine phosphorylation. The majority of changes that occur during capacitation involve sperm head; however no morphological changes occur. It has been believed that capacitation allow sperm to undergo the acrosome reaction. Although the viability (life span) of capacitated sperm is limited; as a result, precise timing between capacitation and acrosome reaction is necessary for proper fertilization to occur (6). It was concluded from this study that it's possible of sperm maturation and capacitation taken from tail of epididymis of buck from slaughter house source in vitro.

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