Comparative study of DNA extraction from semen of cauda epididymis in Ram and Bull

دراسة مقارنة لاستخلاص ال DNA للسائل المنوي من ذيل البربخ للكباش والثيران

Saad Akram Hatif*
College of Vet Med Baghdad

Sadeq Jaafer Zalzala*
College of Vet Med Karbala

Abstract

This study included semen collection from 20 adult rams and 20 bulls, via aspiration from tail of epididymis. All semen samples subjected to sperm count after staining with eosin. The high number of sperm count in rams show 1180000 sperm/ml, while the lower sperm count reached 540000 sperm/ml, the mean of total samples 1139000 sperm/ml .The maximum sperm count of bulls samples gives 990000 sperm/ml while the minimum appeared 744000 sperm/ml and the mean 953000 sperm/ml. Lysis buffer used for DNA extraction from semen and spectrophotometer in order to assessment concentration of DNA samples, the concentration of DNA in rams and in bulls semen gives 3.5908g/l and 4.4319 g/l. We are concluded that the mean of sperm count in the tail of epididymis in ram more than in the bull ,and DNA concentration in ram samples less than in bull

المستخلص

تضمنت الدراسة الحاليه جمع 40 نموذجا للسائل المنوي منها 20 نموذج من الكباش و20 نموذج من الثيران البالغة , وذلك بسحب السائل المنوي من ذيل البربخ لها . وقد اخضعت جميع العينات لحساب عدد الحيامن بعد صبغها بالايوسين.

تم حساب عدد الحيامن في الكباش فكان اعلى عدد لها 1180000 حيمن/ مل ,اما اقل عدد فكان 540000 حيمن/مل , وبمعدل 1139000 حيمن/مل . فيما كان اعلى عدد لها في الثيران 990000 حيمن/مل , اما اقل عدد فكان 744000 حيمن/مل , اما معدلها فسجل 953000 حيمن /مل . تم استخلاص ال DNA الكلي من نماذج السائل المنوي باستخدام الدارئ الحال .وتم تعيين تركيز ال DNA باستخدام جهاز المطياف الضوئي, فكان معدل تركيزه في نماذج الكباش 3.5908g/l اما في نماذج الثيران فكان تركيزها 4.4319 g/l .نستخلص من ذلك ان معدل عدد الحيامن في ذيل البربخ للكباش اكبر منه في الثيران وان تركيزال DNA في عينات الكباش اقل من الثيران .

Introduction

The mammalian epididymis has two principal functions. First, it creates a unique microenvironment within the lumen of the duct that helps transform immotile, immature testicular spermatozoa into fully fertile competent cells, and second, it stores fertile spermatozoa in a viable state within the cauda epididymis until they are ejaculated (1). Another function of the epididymis of which influence on spermatozoal maturation has been gradually elucidated (2). Sperm in the epididymal fluid are highly concentrated (roughly 1 x 10^6 /ul) (3) due to the head is entirely filled with a nearly homogenous nuclear material (DNA) surrounded by the nuclear membranes and each spermatozoon contains about 2.5 billion bits of information (4).The molecular techniques require isolation of genomic DNA of suitable purity (5).

The aim of this study determined the sperm count of cauda epididymis in rams and bulls, and used these sperms for DNA extraction.

Materials and methods

Twenty samples of semen collected randomly from alive awasi rams,1-4years old and (20) samples from cross breed bulls,1.5-3years old (one weekly) by aspirated semen(used needle gauge 21) from cauda epididymis from Baghdad region,(from October 2009-April 2010). All samples subjected to sperm count and DNA extracted in the obstetric lab and DNA concentration and electrophoresis in lab of physiology of veterinary med and lab of genetic engineering and biotechnology in Baghdad.

Sperm cells stained by eosin were observed under a light microscope X 1000 to account sperm (6)

Fresh semen samples were collected into labeled 1.5 ml microfuge tubes and then mixed with1.5 ml of extraction TE buffered, the cauda epididymal sperm suspension was prepared in normal saline (7) Sperm count , by Haemocytometer , add 0.1 ml raw semen with 20 ml DW to get 1:200 dilution with addition eosin 5% (8). Total DNA was isolated from cells by digestion with proteinase K in buffer TE (10 mM Tris, 1 mM EDTA, pH 7.5), containing 0.5% SDS(sodium dodecyl sulfate) (9) with phenol- chlorophorm for purification of DNA .

Generally, the variety of methods can be used for DNA isolation from different biological materials (10) We are used spectrophotometer in order to determining the DNA purification and concentration ,the A260:A280 ratio for the sample , using the spectrophotometer , was1.80,indicating highly purified DNA free from contaminating protein (11) DNA quality and concentrations were evaluated by spectrophotometer (12). , insure by electrophoresis **(Figure 1).**

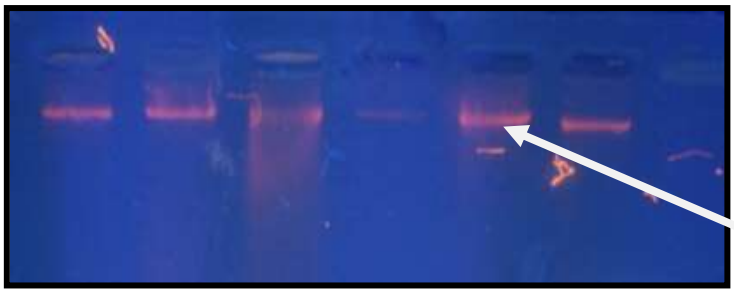

Figure 1- Bands of total DNA on 1% agarose gel , one hour electrophoresis .

Results

This study was concentrated on the sperm count and DNA extracted from sperms were aspirated from the tail of epididymis of 20 rams and 20 cross breed bulls, The mean of sperm count in rams 1139000 sperm / ml and the mean of sperms in bulls 953000 sperm / ml **(table 1)**. The maximum number of sperm reached1180000 sperm / ml in the ram and the minimum reached 540000 sperm per/ml, and the maximum number in bull 990000 sperm / ml and the minimum reached 744000 sperm / ml.

The total DNA extracted gives the concentration of DNA3.5908g /l in rams and the concentration in bulls gives 4.4319 g/l. by spectrophotometer. The findings of this study show the sperms in the epididymis of ram more concentrated than the sperms in the epididymis of bull, and the DNA extracted in bull more concentrated than ram. May be due to the set of chromosomes are more in bull 30 and in ram 27 (table1) .

Table1- show the maximum and minimum sperm number in tail epididymis in ram and bull

Animal	Maximum no sperm / ml	Minimum no sperm / ml	Mean
Ram	**1.180.000**	**540. 000**	**1.139.000**
Bull	**990.000**	**744.000**	**953.000**

Discussion

The cauda epididymis large and firm used for aspirated semen. A large, firm tail is indicative of good reserves whereas a small, soft tail would indicate the opposite (13), collected semen from cauda epididymis has been more practical for sperm count and concentration than collection by other methods, as artificial vagina or electroejaculater in ram and bull, because the male must be adaptation for mating, furthermore the semen not concentrated as in the tail of epididymis in consecutively. The semen less than 5% of a mixture is sperm in natural ejaculation (14). Therefore the epididymis absorbs testicular fluid and concentrates the spermatozoa into a tightly packed mass enabling large quantities of sperms to be stored in the smallest possible space (13).

The healthy, well-fed and sexually rested rams may hold up to 100.000 million sperm, of which about 75 per cent will be stored in the tail of the epididymis (1). Bull have about 70billion spermatozoa outside the testes, 37 billion 53% in the tail of epididymis (4).

In our study the mean of sperm count reached 1.180.000 sperm/ ml in ram. We are recorded the mean of sperm count reached 953.000 sperm/ml in bulls , The sperm cells concentration in the tail of the epididymis in bulls is 4.000.000 or more per cmm (4) Semen aspirated from tail of epididymis for DNA extraction more concentrated than normal ejaculation . The semen less than 5% of a mixture is sperm in natural ejaculation (4) furthermore the sperms more delicate than other tissues, therefore the sperm cells direct contact with lysis buffer in the test tube, that's mean all cells involved by DNA extraction otherwise, in the other body tissues not all cells contact with lysis buffer during DNA extraction like liver or skin cells and contain many components like fibrous or protein. The variability in DNA quality and purity can be explained by tissue specific structural complexity, liver, kidney, and brain tissues are composed of delicate membrane cells with few fibrous cells on the other hands, the skin consist of stratified tissue with keratin and other fibrous cells and the muscle tissue is constituted by many proteins within the cell (15), therefore the DNA extracted from total sperms were found in the lysis buffer. The total DNA were extracted from semen in rams reached (3.5908 g/l) and (4.4319 g/l) in bulls 300billion spermatozoa make, this variant may be the different between the set of chromosomes number in ram 54 and 60 chromosomes in bull. (4) Mention, 300billion spermatozoa make 1gram of DNA.

References

1- Wilson, K.(2010). Sheep breeding sperm production in rams. Agency for Food and Fiber Sciences. e-mail callweb@dpi.qld.gov.au.

2- Herao,K.and Iizuka.(1986).Electrophoretic characteristics of boar epididymal fluid proteins .J. Coll.Dairying, 11:303-3

3- Center for Male Reproductive Medicine and Microsurgery. 1998. webmaster@aswas.com**.**

4- Roberts, S.J. (1971). Veterinary obstetrics and genital diseases. Theriogenology. Second edition: 619.

5-Ogunkanmi,A.L.;Oboh,B.Onifade,B.;Ogunjobi,A.A.;Taiwo,I.A.and Ogundipe,O.T.(2008).An improved method of extracting genomic DNA from preserved tissues of Capsicum Annuum for PCR application. EurAsianJournal of Biosciences .2 ,115-119.

6- Tajik **,** P.; Mirshokraee, P. and Khosravi A. (2007)**.** Effects of Different Concentrations of Aflatoxin B on Ram Epididymal and Ejaculatory Sperm Viability and Motility in vitro . Biology of Reproduction .10. 3923 .4500.4504.

7-Chenoy,N.J.and Sharma , A .(1998). Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice .Digital Archive of Fluoride Journal .vol 31 .no 4 .203-216.

8-Al-Rekabi ,Z.G.(2005). Comparative study of season and pollution on reproductive traits and cytogenetics on sheep grazed on pasture area and Al- Twuaitha region – 42 Thesis. Baghdad university.

9- Bayona-Bafaluy,M.P. ;Acín-Pérez,R.; Mullikin,J.C[1.] ;Park,J.S.; Moreno-Loshuertos,R. ;Hu,P.; Pérez-Martos,A ;Fernández-Silva,P.; Bai,Y and Enríquez ,J.A.(2003).Revisting the mouse mitochondrial DNA sequence. Nucleic Acids Res. 15. 31, 18: 5349–5355.

10- Hosek , J.;Savastova ,P.;Moravkova , M . ;Pavlik,I .and Bartos , M.(2006).Methods of mitochondrial DNA isolation from different biological material. Veterinarni Medicina,51(5):180-192.

11- Hengen,P.N.(1994).Methods and reagent :Determination DNA concentration and rescuing PCR primers. pnh@ncifcrf.gov. Flp. ncifcrf.gov.

12-Biase,F.H.;Franco,M.M.;Gaulart,L.R.andAntunes,R.C.(2002). Protocol for extraction of genomic DNA from swine solid tissues. Genetic and Molecular Biology 25, (3):313-315.

13- Roy Jones. (2004). Sperm Survival Versus Degradation in the Mammalian Epididymis: A Hypothesis[1] Biology of Reproduction 71(5):1405-1411.

14- Ferriere, R. (2005). Reproductive behavior .Bio 182, lecture 9. University of Arizona.

15– Junqueira, L. C. and Carneiro, J. (1995). Histologia Basica. Rio de Janeiro,text book: 512.