STUDY THE EFFECT OF DIFFERENT CULTURE MEDIA ON SOME FROZEN SEMEN CHARACTERISTICS OF IRAQI BLACK LOCAL GOAT SPERMATOZOA

Khairi G. Al-Rikabi ,Abdulrazak N. Khudair ,Taher A.Fahad

Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah , Iraq

(Received 2 October 2017 ,Accepted 13 december 2017)

Key words: Bucks, DMEM, Sperm motility.

Corresponding author E.mail: abdulrassaq.khudair@uobasrah.edu.iq

ABSTRACT

The semen was collected from five adult Iraqi black local bucks by the artificial vagina and using estrous induced doe the semen was diluted with egg yolk 10% extender and frozen by liquid nitrogen for three months, macroscopic and microscopic evaluation were done after collection, dilution and thawing, three media(TCM-199, DMEM and SOF)were used to see the their effect on activation of spermatozoa. The results showed that TCM-199 media presented higher significance in sperm motility, progressive motility compared with DMEM and SOF media. There was a great decrease in the concentration of spermatozoa after activation using these media which didn't differ significantly among them and they were higher significantly compared with control in normal morphology.

INTRODUCTION

Goat production has importance in agriculture economy worldwide. However, selection programs specific for goat production have been developed relatively recently, compared to those developed for dairy cattle breeding (1). When the purpose is the genetic progress of a particular population, it is compulsory to improve a selection program and in this context the use of artificial insemination is essential for the connection of the herds to diffuse high quality genetics of sires between organizations and breeders (1).The rate of diluted semen cooling from temperature

just above 0C° can drastically affect the survival of spermatozoa after freezing and thawing (2). In addition mammalian spermatozoa do not have the ability to fertilize immediately after ejaculation ; however, they gain this ability after passing the capacitation and hyper-activation process(3). **S**perm preparation techniques, culture media and the properties of semen sample itself can determine the outcomes of assisted reproductive technology(ART)(4),so the aim of the study is to evaluate the effect of culture media on improving sperm characteristics of Iraqi black local bucks.

MATERIALS AND METHODS

1- **Extenders preparation:** Egg yolk extender (EY10%) consist of 10 ml egg yolk , 0.5 gm glucose ,20 mg gentamycin sulfate , 1000 mg streptomycin sulfate and 7 ml glycerol, volume completed with distal water up to 100 ml(5)**.**

2- **Semen collection and evaluation.**

Semen was collected from five trained, healthy and sexually mature local goat bucks making pool,using artificial vagina, bucks aging approximately three years and average weight of 55 kg . Goats were raised on extensive management without restrictions of feed and water. The experiment was conducted from February 2016 to February 2017 in the animal field of veterinary college of Basrah university.

after collection, the semen evaluation was performed within nearly 15 min, and only those semen samples showing at least 80% individual progressive motility (6), volume of 1.5 ml and mass activity higher than 3 (0 to 5 score) were chosen, pooled and treated further.(7).

3-Semen dilution, freezing, and thawing

Semen samples washed with phosphate buffer saline(PBS) (1:9) at 1200 × g for 10 min at room temperature and seminal plasma was removed. After that, washing samples were diluted with (EY10%).diluted semen evaluated as for fresh semen. At the end of this time, the diluted semen was loaded into 1 mL plastic eppendr of and frozen over nitrogen vapors for 10 min, 4 cm above the nitrogen level, plunged and stored in liquid nitrogen for three months. Sperm samples were frozen to a final concentration of 140–200 × 10^6 spermatozoa/mL. (8).

4-Sperm preparation for in vitro activation

The samples were thawed at room temperature for 5 min and transported to a 37ºC incubator for 20 min and a small aliquot (0.1 mL) was removed from each sample for evaluation of spermatozoa, three media were used DMEM(Dulbecco's Modified Eagle Medium-Gibco) ,TCM-199 (tissue culture media-Gibco) and SOF (synthetic oviduct fluid media-Iraq)) in addition to control(frozen thawed semen before activation) to see the effect of media on capacitation and activation of spermatozoa.The spermatozoa were separated by layering 50 µl of fresh or frozen–thawed semen under 2 ml medium(simple layer technique),and allowing the spermatozoa to swim-up during incubation for 1 h at 39 C°(9). After incubation, the top 1 ml from each tube was removed and pooled in a sterile 15 ml centrifuge tube and centrifuged (300 _ g for 10 min). (10) . The resulting sperm pellet was over layered with 1 ml of medium +3% fetal bovine serum (FBS).

Statistical analysis

Statistical analysis is done by using SPSS software version 19, one-way ANOVA is used to assess statistical significance.

RESULTS

Sperm quality

The characteristics of the fresh ejaculates used in this experiment are presented in Table 1.

Table 1 Mean values, standard deviation, maximum and minimum values observed for motility (%), volume (mL) , concentration ($x10^9$/mL), pH, in fresh ejaculates used in Experiment.

	N	Mean	Std. Dev.	Max.	Min
Mass motility (%)	5	90	3.31	92	85
individual motility(%)	5	85	3.00	90	80
Volume (ml)	5	1.5	0.15	1.6	1.2
Concentration($x10^9$/ml)	5	2.9	0.28	3.4	2.7
Live spermatozoa	5	90	3.16	94	86
Normal spermatozoa	5	95	1.87	97	92
PH	5	6.9	0.15	7.1	6.7

Table-2 Effect of media on some Spermatological characteristics of frozen-thawed semen prepared from EY10%

Treatment	Sperm conc.milion/ml	Sperm motility%	Prog. Motility%	Normal morph.
control	200±0.00 a	45±1.34 c	32±1.78 c	85±1.84 b
DMEM	32.5±0.50 b	60±2.23 b	40±1.70 b	75±3.53 a
TCM-199	33.0±0.70 b	70±2.98 a	46±2.12 a	78±4.47 a
SOF	32±0.65 b	58±2.54 b	38±1.78 b	75±3.68 a
LSD	0.00	10.00	6.00	7.00

The sperm concentration in control was(200±0.00)which was significantly reduced after activation(32.5±0.50 , 33.0±0.70 and 32±0.65) for DMEM, TCM-199 and SOF respectively which didn't differ significantly among them.

TCM-199 media presented higher significance in sperm motility compared with control, DMEM and SOF media (70.0±2.98 *vs*45±1.34,60±2.23 and 58±2.54 respectively), and Prog. Motility% (46±2.12 *vs*.32±1.78,40±1.70 and 38±1.78 respectively) Non-significant difference among three median normal morphology(78±4.47, 75±3.53 and 75±3.68) respectively while they were significantly higher than control(85±1.84).

DISCUSSION

Direct swim-up technique showed enhancement in the sperm quality of selected population allowing for a good recovery of sperm. These results agreed also with (11) in the efficiency of this method for preparing normo zoo spermia samples. However, this technique was an simpler way and this approved with (12). Generally, It was noticed in the current study that the use of in vitro culture media causes a significant increases in the sperm motility and the reason may be that, the seminal fluid with high viscosity impedes sperm progressive motility so that the uses of in

vitro media with aqueous nature lead to reduction seminal fluid viscosity and as a result sperms move more freely because of their aqueous nature with lower viscosity

than of seminal plasma resulted in making spermatozoa move more easily ,also the presence of calcium in the incubation medium is very important because extracellular

calcium acts as unlimited reservoir of the ion [13].Three media(TCM-199, SOF and DMEM) were used for incubation of spermatozoa to see the effect of each media on an activation of caprine spermatozoa which are prepared fromEY10% extenders. The result showed there was a significant reduction in the concentration of spermatozoa following in vitro activation in all used media . This is due to the inability of the dead and abnormal sperms with poor motility spermatozoa to swim up and migrate from sperm pellet to the upper layer of culture medium, In addition to the contents of culture media that used for activation select only intact sperm to swim up to the upper surface of the medium (14). These results were in agreement with other studies using culture for the separation and activation of sperm in vitro (15).

Incubation of EY10% diluted semen with the TCM-199 medium showed a greater TM and PM than for DMEM and SOF but the sperm concentration and normal morphology were similar, these result was in agreement with those obtained by (16) which cryo preserved human sperm in media mixed with EY 30% then incubated in ferticult media. Improvement of sperm quality may be considered as natural response for sperm physiology after the exclusion of seminal plasma, pus cells and agglutinated spermatozoa using sperm preparation techniques. Furthermore, it was reported that only the active motile sperms will swim-up to the upper layer of culture medium in vitro human sperm activation (17).The beneficial of using egg yolk in frozen semen increase sperm resistance against cold shock and enhance sperm survival (18).

Presence of HEPES in TCM-199 formula may be the main cause of an improvement of total and progressive motility of spermatozoa, HEPES(4-(2-hydroxyethyl)-1-piperazineethanesulfonicacid)is a zwitter ionic organic chemical buffering agent. HEPES is commonly used in cell culture, mostly because it is better at preserving physiological pH in spite of changes in carbon dioxide concentration (produced by cellular respiration) when compared to bicarbonate buffers, which are usually used in cell culture(19). The dissociation of water declines with dropping temperature, but the dissociation constants (pK) of many other buffers do not change much with temperature. HEPES is like water in that its

dissociation decreases as the temperature falls. This makes HEPES a more effective buffering agent for preserving enzyme structure and function at low temperatures

(19). In addition TCM-199 contain less amount of glucose (1000 mg/L) than DMEM (4500/L) and absence of glucose from SOF. Glucose is source of energy but excess quantities may be has inhibitory effect on sperm motility which was observed by several researchers on bull semen, these observations suggested that the inhibitory effect of glucose could operate by two possible routs : improvement of the activity of the enzyme phosphor diesterase to lower the intracellular content of cyclic adenosine monophosphate(cAMP), or inhibition of the rise in intracellular pH(20).

In all treatment there was no significant difference in normal morphology post - activation using centrifugation swim up technique, this is because the sperm preparation techniques for ART were developed to separate the motile morphologically normal spermatozoa and let off leucocytes, bacteria, and dead spermatozoa produce oxygen radicals that negatively affected the ability to fertilize the egg (11). In conclusion TCM-199 is best culture media for in vitro activation of frozen thawed Iraqi local bucks spermatozoa.

صفات السائل المنوي المجمد لذكور المعز دراسة تأثير استخدام أوساط زرعيه مختلفة في المحلي الأسود

خيري غركان الركابي عبد الرزاق نعيم خضير طاهر عبد الحسين فهد
فرع الفسلجة،كلية الطب البيطري،جامعة البصرة،البصرة،العراق

الخلاصة

جمع السائل المنوي من خمسة ذكور معز محلي أسود بالغة بواسطة المهبل الاصطناعي وباستخدام انثى مستحدثة الشبق وخفف مع مخفف صفار البيض ١٠٪،تم تجميد السائل المنوي بالنيتروجين السائل لمدة ثلاثة أشهر،وتم إجراءا لفحص ألمجهري والعياني بعد الجمع والتخفيف والإذابة بعد التجميد،استخدمت ثلاثة اوساط زرعيه(TCM-199و DMEMوSOF) لحضن الحيامن ومعرفة تأثير الأوساط المختلفة على تنشيط الحيوانات المنوية. أظهرت النتائج أن الوسط الزرعيTCM-199 اعطى نسبة أعلى في الحركة الكلية والتقدمية للحيوانات المنوية مقارنة مع وسطي DMEMوSOF.هناك انخفاض واضح في تركيز الحيامن بعد التنشيط باستخدام الاوساط الثلاثة بينما لم تختلف تلك الاوساط معنويا فيما بينها كذلك هناك انخفاض معنوي في نسبة التشوهات بعد التنشيط والتي لم تختلف معنويا فيما بينها ايضا.

REFERENCES

1- **Dubeuf, J.P., Boyazoglu, J.,** 2009. An international panorama of goat selection and breeds.Livestock Science 120, 225–231.

2- **Salamon S. and Maxwell. W. M. C**. 2000. Storage of ram semen. Anim. Reprod. Sci. (62).77-111.

3- **Suarez, S. S. and -C.Ho, H.** 2003. "Hyperactivatedmotility in sperm," *Reproduction in Domestic Animals*, vol. 38, no. 2, pp. 119–124.

4- **VandeVoort, C. A**. (2004). High quality sperm for non-human primate ART: Production and assessment. Reprod. Biol. Endocr., 2:33.

5- **Ferreira1,V.S., Mello2,M.R.B., Fonseca3,C.E.M., Dias4,A.C.F., Cardoso4,J.M., Silva4,R.B., Júnior,W.P.M.** 2014. Effect of seminal plasma and egg yolk concentration on freezability of goat semen. R. Bras. Zootec., 43(10):513-518.

6- **Bezerra,F.S., Castelo,T.D., Santos,E.A., Dantas,T.D., Simão,B.R., Silva.A.R.** 2012. Assessment of the interaction between straw size and thawing rate and its impact on in vitro quality of post-thaw goat semen. R. Bras. Zootec., v.41, n.3, p.592-597.

7- **Farshad, A. Akhondzadeh, S.** 2008. Effects of sucrose and glycerol during the freezing step of cryopreservation on the viability of goat spermatozoa.AsianAust J AnimSci, 21 (12) pp. 1721–1727.

8- **Jiménez-Rabadána, P. Ramóna, M. García-Álvarezb, O**. 2012. Effect of semen collection method (artificial vagina vs. electroejaculation), extender and centrifugation on post-thaw sperm quality of Blanca-Celtibérica buck ejaculates.AnimReprod Sci. 132(1-2):88-95.

9- **Parrish JJ, Susko-Parrish JL, Winer MA and First NL**(1988) Capacitation of bovine sperm by heparin *Biology of Reproduction* 38 1171-1180.

10- **Romagueraa, R., Mollb, X., Moratób, R.,** . 2011. Prepubertal goat oocytes from large follicles result in similar blastocyst production and embryo ploidy than those from adult goats. Theriogenology 76 : 1–11.

11- **Boomsma, C.M., Heineman, M.J., Cohlen, B.J.and Farquhar, C**. (2007). Semen preparation techniques for intrauterine insemination. Cochrane Database of Systematic Reviews, 4:1-16.

12- **Siam, E. M.** (2012). Pregnancy outcome after IUI for male and idiopathic infertility using a new simplified method for sperm preparation .Middle East Fertility Society Journal, 17: 30–36.

13- **Beduaddo, K. ;Barrall, C. L.; Kirkamn-Brown, J. C. and Publicover, S. J.** 2007.Pallerns of {Ca2+} mobilization and cell response in human spermatozoa exposed to progesterone. Dev. Biol. 302:324-332.

14- **Al-Dujaily, S.S ., Al-Zubaidi, S.FSouhayla O. Hussain.** 2012. Effect of in vitro activation by glycyrrhizaglabra extract on cryostorage. Euphrates Journal of Agriculture Science-4 (3): 15-25.

15- **Al-Delphi,M.S.** 2012. Effect of Pentoxifylline and Glycyrrhizaglabra activation on cryopreserved epididymal sperms. M.Sc. Thesis. Council of High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-NahrainUniversity, Iraq.

16- **Al –Azzawi, K. S**. 2013. The Benefit of Semen Cryostorage on Certain Sperm Function Parameters Following in Vitro Activation. Diyala Journal of Medicine. Vol. 4, Issue 1, April 2013.

17- **Henkel RR, Schill WB**. 2003. Sperm preparation for ART. Reprod. Biol. Endocrinol. 1:108-130.

18- **Gholami M, Faraji Z, and ZamiriMJ,** 2012: Effect of egg yolk of four avian species on the cryopreserved ram spermatozoa. *Iranian Journal of VeterinaryResearch,* 13(38), 23-27.

19- **Baicu SC, Taylor MJ** (2002). "Acid-base buffering in organ preservation solutions as a function of temperature: new parameters for comparing buffer capacity and efficiency . *Cryobiology*. 45 (1): 33–48.

20- **Storey, B.T.**2008.Mammalian sperm metabolism: oxygen and sugar, friend and foe. Int. J. Dev. Biol. 52: 427-437.