Effect of cryopreservation on some semen parameters of Awassi rams.

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Abstract :

This experiment was conducted at the station of sheep and goat research's / the state board of agricultural research ,the artificial Insemination Department belongs to the Ministry of Agriculture and in the High Institute of Infertility Diagnosis and Assisted Reproductive Technology at Al-Nahrain university, Baghdad, Iraq to investigate the effects of cryopreservation on some ram semen characteristics. Six Turkish Awassi rams were used for semen collection and evaluation. several samples of ram semen were collected and evaluated for Volume, pH,color,Sperm concentration ,Mass activity and individual motility using computer assisted semen analysis (CASA) Dead and abnormal sperm and DNA fragmentation index (DFI). Then the semen was diluted 1:19(semen: diluents v/ v) using a Tris diluents and subjected into slow cryopreservation program .The semen samples were assessed after freezing-thawing. Cryopreservation causes a clear decadency in some parameters of ram semen .Result shows that there was a significantly ($p< 0.01$) affect of cryopreservation on the dead sperms percentage (31.6 ± 1.92), individual motility(IM)(31.33± 1.20%) and DNA fragmentation index (DFI) (29.83± 4.45%).

Introduction :

The sheep industry has not been able to utilize many of the assisted reproductive technologies (ART)in general and in Artificial Insemination(AI) in particular or multiple ovulation and embryo transfer (MOET), that widely adopted into normal breeding programs in dairy cattle (Maxwell and Evans,2006). Semen cryopreservation is an important aspect of Artificial Insemination (AI) in sheep while it was long term conservation of ram semen is essential when *in vitro* fertilization (IVF) or AI is performed at a future date (Merlo et al., 2008). It has been established that ram spermatozoa are sensitive to the conditions of low-temperature and freezing (Nauk

,1991).So that this study was conducted to investigation the effect of cryopreservation on some ram semen traits .

Materials and methods :

Six Turkish Awassi rams of 2.5-3 years old and average body weight was75-85 kg, maintained under preventive and clinical care, were used as semen donors for this experiment . This study was done from October /2011to April 2012.The experiment was carried out at ruminants research's station, Ministry of Agriculture, Baghdad. Iraq(20 km south west of Baghdad).

Semen collection and evaluation

Ejaculates were collected from each ram by artificial vagina (at 40-42ºC). Semen quality was directly evaluated. Volume, pH (using pH meter. hanna ,Romania),color, ,sperm concentration according to Smith and Mayer(1955) (using Neubaure hemocytometer chamber, Germany), mass activity,individual motility (Evans and Maxweel 1990) using computer assisted semen analysis (CASA), dead sperm according to Swanson and Beardom(1951) and abnormal sperm according to Hancock (1951) .Then, the semen sample were diluted 1:19(semen: diluents , v/v) using a Tris diluents containing 3.63g (Tris, hydroxymethylamin methan) 20% egg yolk ;1.99g Citric acid; 0.5g Fructose; 100000IU penicillin; 100mg streptomycin; 6% glycerol(BDH, England) and completion the volume to 100 ml by distilled water according to ,Moce *et al*(2010) .

Semen cryopreservation

After initial evaluation and dilution, the semen was loaded in 15ml test tube, then transferred to the Artificial Insemination Department/ Abu. Graib. Ministry of Agriculture, using insulant container (Thermos).Then the samples were getting into the refrigerator and gradually cooled to 5ºC for 120 minutes using ice molds alternately .At 5ºC ,the diluted semen was loaded into 250µL straws(IMV ,l'Aigle, France).The free end of straws was sealed and placed horizontally on special rack to 2-2.5 hours equilibrium period. Thereafter the straws were exposed to vapor of liquid nitrogen (at -75 ºC)for 10min and then plunged into liquid nitrogen(at -196ºC)until the later time of evaluation.

Results :

Some individual characteristics of rams semen pre- cryopreservation compared with post-cryopreservation showed a significantly increase ($p<0.05$) in the percentage of abnormal sperms(6.29 vs.9.91%), and higher($p<0.01$) significantly increase in the percentage of dead sperms(9.94 vs. 31.6%) and the individual motility was decreased after cryopreservation (73.3vs.31.3%)(Table.1).Cryopreservation of rams semen yielded a highly significant increase ($p<0.01$) in the percentage of DNA fragmentation(DFI) (29.83±4.45%) as compared with fresh semen (4.62±0.56%) (Figure.1 and plate 1,2).

Table 1.Some semen characteristics of Awassi rams during pre and post cryopreservation technique.

Cryopreservation period / Semen characteristics	Ram 1		Ram 2		Ram 3		Ram 4		Ram 5		Ram 6		Means	
	Pre	Post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post	Pre	post
Sperm abnormality %	5.20	9.32	5.82	11.24	7.13	10.11	6.40	10.82	7.62	8.55	5.55	9.43	6.29	9.91*
Sperm dead %	8.00	33.3	13.1	36.43	12.33	37.00	12.17	27.20	9.81	29.77	4.24	25.9	9.94	31.6 **
Individual Motility %	75.0	30.0	75.0	28.00	70.00	30.00	70.00	30.00	70.0	35.00	80.0	35.0	73.3	31.3**

* (P<0.05), ** (P<0.01)

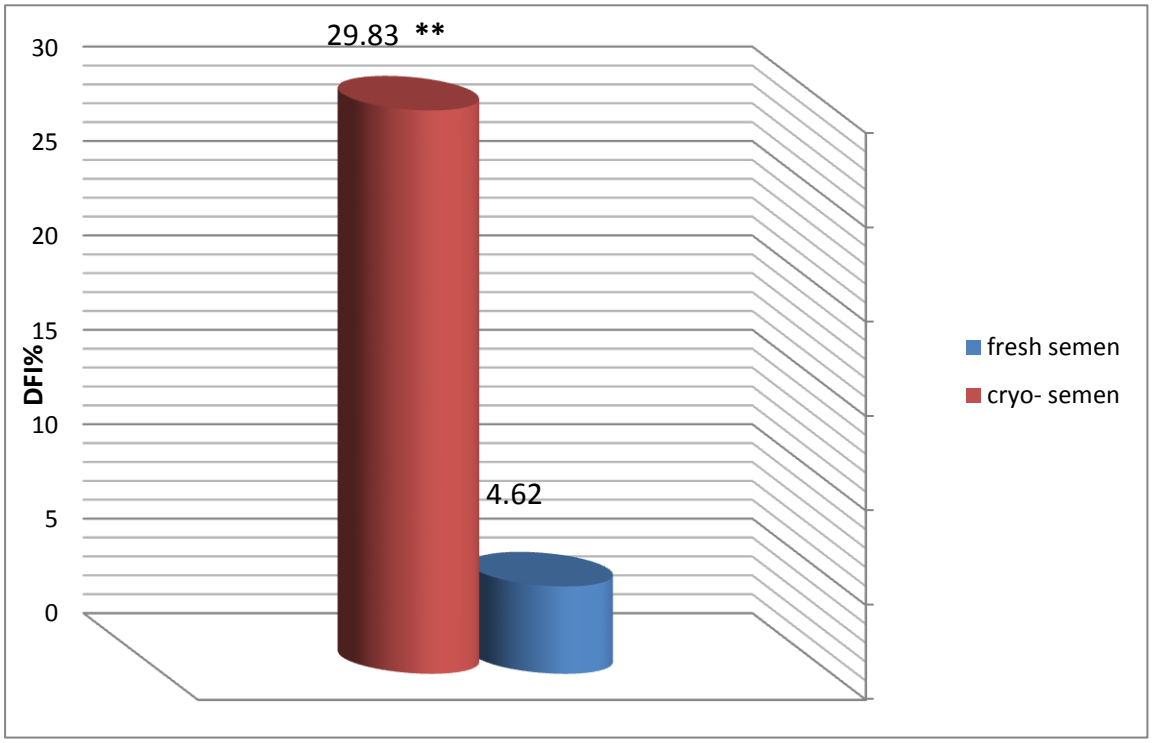

Figure 1: Effect of cryopreservation on the DFI percentage of rams semen.

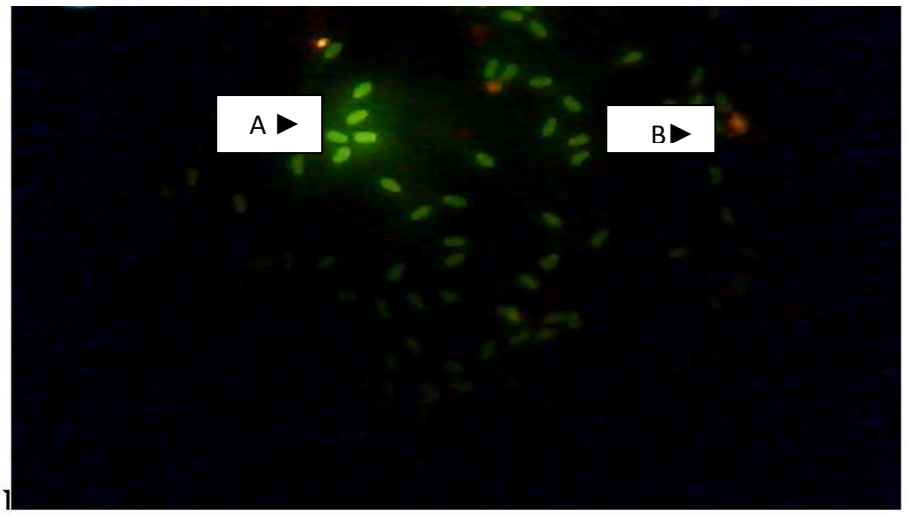

Plate1: DNA fragmentation in the fresh semen: Intact sperms (A),fragmented sperm (B).

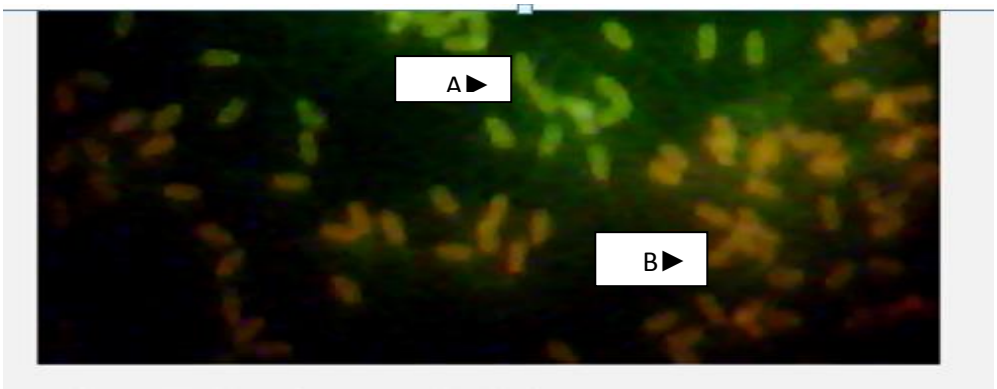

Plate2: DNA fragmentation in the cryo- semen: Intact sperms (A),fragmented sperm (B).

Discussion

In this study, several samples of ram semen were assessed after freezing-thawing. Cryopreservation causes a clear decadency in some parameters of ram semen such as sperm individual motility (%), sperm abnormal morphology(%) and dead sperms(%).

It is well known that freezing and thawing of ram sperm lead to severe structural changes in membrane integrity lead to reduced motility and fertilizing ability. The structural changes produced in the freeze-thawed sperm cells membrane ware primarily linked to altered abilities for energy sourcing. This would later influence both cellular metabolism and other sperm functions such as motility and abnormality (Cerolini *et al*, 2001; Gillan *et al*, 2004 and Dziekonska *et al*,2009).

Spermatozoa requires many of nutrients to gain the strength needed for the long journey from the epididymis to the ovum in the female reproductive tract. These nutrients are metabolized intracellularly, resulting in the release of useable energy available for cellular processes primarily in the form of ATP.The ATP for spermatozoa is mainly derived either by glycolysis in the cytoplasm or through oxidative phosphorylation in the mitochondria (Dziekonska *et al*, 2009; Januskauskas and Zillinskas., 2002).

Among the different alterations of activity of the intracellular enzymes, glucose-6-phosphate-dehydrogenase is the first enzyme which leaves the cell when the cellular membrane is damaged during cold shock. Generally, the intracellular concentration of ATP is decreased or lost and the AMP/ADP-rate is increased by the cryopreservation (Lemma, 2011).

In this study, the percentage of DNA damage tends a negative path to other sperm parameters, when the sperm motility and viability decreased, the sperm DNA

damage increased. These results are coincidence with the previous studies in bulls (Januskauskas *et al.*,2002) , bucks (Peris *et al.*, 2004) and boars (Chanapiwat *et al*.,2010).They reported that the DNA fragmentation might disrupt the vitality of spermatozoa during freezing thawing process.

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تأثير التجميد في بعض صفات السائل المنوي للكباش العواسي

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المستخلص

أجريت هذه الدراسة في كل من محطة بحوث الأغنام والماعز في عكركوف التابعة للهيأة العامة للبحوث الزراعية / وزارة الزراعة ، ومركز التلقيح الاصطناعي التابع إلى الشركة العامة لخدمات الثروة الحيوانية في أبي غريب والمعهد العالي لتشخيص العقم والتقنيات المساعدة على الإنجاب التابع إلى جامعة النهرين لمعرفة تأثير التجميد في بعض صفات السائل المنوي للكباش العواسي . استخدمت فيها ستة كباش من سلالة العواسي التركي لغرض جمع وتقييم السائل المنوي وكانت بأعمار تتراوح بين 2.5- 3 سنوات وأوزان تتراوح بين 75- 80 كغم ، تم جمع السائل المنوي منها باستعمال المهبل الاصطناعي الخاص بالأغنام بواقع قذفه لكل كبش اسبوعيا ، اجري تقييم أولي لصفات الحجم ، الأس الهيدروجيني ، اللون ، تركيز النطف ، الحركة الجماعية ، الحركة الفردية ، النسبة المئوية للنطف الميتة والمشوهة والنسبة المئوية لضرر الـ DNA للسائل المنوي قبل التخفيف وتم تخفيف السائل المنوي بمخفف الترس بنسبة 1:19. وتم بعد ذلك تجميد السائل المنوي المخفف بالنيتروجين السائل(– 196 مْ).

أظهرت نتائج الدراسة وجود تأثير عالي المعنوية ($p<0.01$) للتجميد في النسبة المئوية للنطف الميتة (31.6 ± 1.92)، الحركة الفردية للنطف (31.33± 1.20%) والنسبة المئوية لضرر الـ DNA (29.83± 4.45%).