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Effect of diluents type and preservation periods on some sheep semen characteristics

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ABSTRACT

The present study aimed to evaluate three seminal diluents and three conservation periods (0, 48 and 96 hours) on quality of Barki rams. The three diluents tests were citrate-egg yolk , tris – egg yolk and skim milk – egg yolk. The dilution ratio was 1 diluent:10 semen. Semen was collected from barki rams using artificial vagina ram one time monthly in the morning during September, October and November. Semen samples after dilution with the tested diluents were conserved for 48 and 96 hours, then semen quality was evaluated. The results obtained are summarized as following :

1- tris –egg yolk diluent showed the best sperm movement and the livability of the sperm was better compared to the skim milk diluent.
2- tris + skim milk showed the lowest abnormality percentage compared to citrate.
3- The conservation periods had significant effects where the tris- diluent was the best in semen conservation using cooling compared to citrate.

Keywords: Barki rams , semen quality , diluents , preservation.

INTRODUCTION

Semen of rams can be diluted and stored to keep its quality in good level and this was achieved through conserving rams semen for some hours or days without any reverse effects on its quality or reducing its efficiency on fertilization (Lopez *et al.*[1]. Kulaksiz *et al.*[2] showed that the success of artificial insemination in sheep led to the importance of establishing new methods for ram sheep dilution and conserving through cooling or freezing to be used out of breeding season. In this respect Chemineau *et al.*[3] reported that semen diluted increased the possibility of conserving the ram sperms alive for long period, thus the diluents supply the sperms with energy and nutrients to remain alive. Similarly semen diluents contain compounds that product the sperms from the unfavorable impacts of cooling and freezing. The present study was conducted to evaluate the effects of some diluents on Barki rams during the conservation periods 48 or 96 hours and to test three semen characteristics of barki rams. The possibility of conserving and diluting sheep semen will make sheep keeping and intensive production more economically, it will reduce the number of rams in the farm and increased the capability to distribute the genetic makeup of the superior rams in wide areas of Libya.

MATERIALS AND METHODS

1. **Animal and management** :

Five Barki rams of about 50kg $\pm$ 1.00 5kg live body weight (LBW) were fed at rate of 2% of LBW for animals. The wheat straw was fed to animals' ad.li The experimental animals were kept under routine veterinary supervision of feed stuffs as illustrated in Table (1)

Table (1). The chemical composition of feed stuffs (on DM basis).

feed stuffs	DM%	OM%	CP%	CF%	EE%	ASH%	MFE%
C.F.M	100	93.50	14.00	12.90	2.45	6.50	64.15
W.S	100	94.70	4.11	36.00	1.51	5.30	53.08

C.F.M: concentrate feed mixture W.S: Wheat straw

2-**Analytical methods:**

The experimental rams were trained for semen collection for one month using the artificial vagina. Semen collection was performed using the artificial vagina. Semen was collected from the experimented rams once every month from each animal and the collected semen was transfers to the laboratory where samples were kept in a water bath at 37^{o}C , then the ejaculate was diluted into three equal parts where each part was diluted using the tested diluents as presented in Table (2). The tested diluents were prepared according to the method described by Verberckmoes *et al.* (2005)[(4)]. After diluting the semen samples, the diluted semen was conserved for 0, 48 and 96 hours in a Refrigerator at 5^{o}C. There after the diluted and conserved semen were evaluated for the percent of individual sperm movement, alive sperms and abnormal sperms and semen PH according to the method of Chemineau *et.al.* (1991)[(3)]. Live and dead sperms were detected using Wells-Awa acrosome stain[(5)] (Wells and Awa, 1970). Semen PH was determined using a digital PH – meter after semen collection directly.

Table (2) diluents used in semen treatment

Material	Citrate diluent	Tris diluent	Skim milk diluent
Tris (gm)	-	3.63	-
Glucose (gm)	0.50	-	1
Fructose (gm)	-	0.50	1
Sodium citrate (gm)	2.37	-	-
Citric acid (gm)	-	1.99	-
Skim milk (%)	-	-	80
Egg yolk (v/vml)	15	14	10
Pinclin(iu/ml)	1000	1000	1000
Streptomycin (gm)	100	100	100
Distilled water	100	100	100

3-**Statitical analysis**:

Data obtained was evaluated statistically according to **Snedecor and Cochran** (1980)**[(6)]** and the significance among means experimental groups were tested by Duncans multiple rang test (Duncans , 1995)**[(7)]**.

RESULTS AND DISCUSSION

It was obvious from data in Table (3) and Figures (1 & 2) that there were highly significant differences (P≤ 0.01) for diluents on individual sperm mortality (%) and a live sperms (%) for tris and skim milk diluents. These results agreed with the findings of many workers**[(8,9)]**, also the preservation period had a significant effect on diluted and cooling individual motility % and live sperm %, however the tris- diluents was superior dilution for preserving Barki semen at 5^{o}C for 96hours. This results were in accordance with those obtained by Blackshow[(10)], Deka and Rao[(11,12)] and Yaniz *et al.*[(13)].

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Table (3). Effect of diluents types and preservation periods on individual motility and live sperms.

Preservation Period (hr)	Individual sperm motility %			Live sperm %		
	Citrate diluent	Tri diluent	Skim milk diluent	Citrate diluent	Tri diluent	Skim milk diluent
0	88.56 ±1.753 Ca	90.17 ± 2.075 Ba	91.50 ±4.187 Aa	86.30 ±3.221 Ba	87.65 ±2.600 Aa	88.10 ±3.339 Aa
48	69.25 ±1.319 Cb	74.50 ±3.690 Ab	73.60 ±4.464 Bb	70.40 ±2.389 Bb	74.50 ±2.941 Ab	74.61 ±3.935 Ab
96	44.82 ±5.920 Cc	52.21 ±3.515 Ac	46.50 ±2.691 Be	50.00 ±3.279 Bc	55.50 ±4.176 Ac	50.27 ±2.871 Bc

(ABC) capital letters compare between columns while (abc) small letters compare between rows.

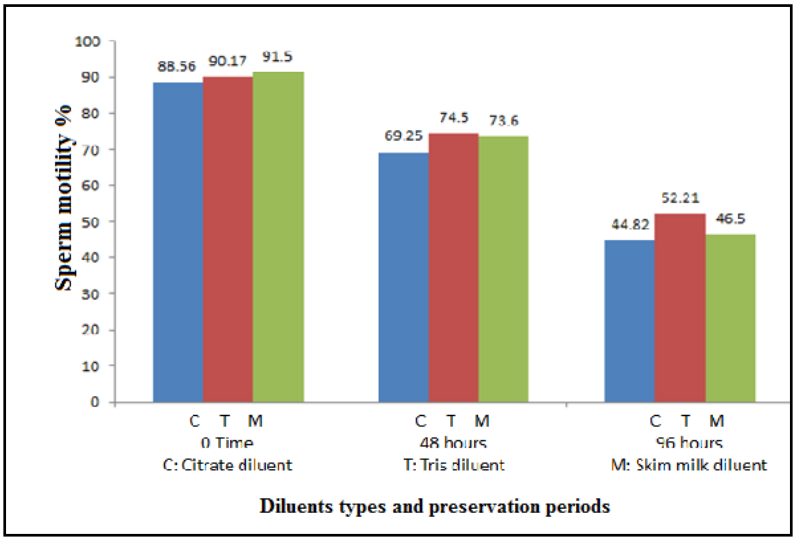


Fig. (1). Effect of diluents types and preservation periods on sperm motility %

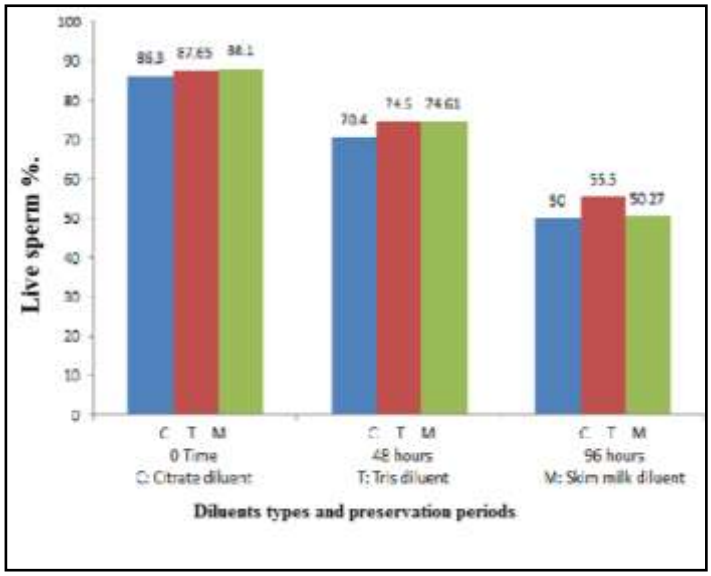


Fig. (2). Effect of diluents types on live sperm %.

Results of Table (4) and Figures (3 & 4) indicated highly significant differences ($p<0.01$) among diluents for abnormal sperm (%) and pH of semen. Tris diluents were less significant ($p<0.01$) with respect to sperms abnormality in diluted semen than citrate and skim milk dilutions. The abnormal sperms percent increases while pH decreases with tris diluents when preserving the sperms compared to the other two investigated diluents at different preserving periods. These results are in agreement with those reported by Azawi *et al.*[14], Shamsudden and Chanda[15], and Gundigan[8].

Table (4). Effect of diluents type and preservation periods on Abnormal sperm (%) and pH of semen.

Preservation Period (hr)	Abnormal sperm (%)			PH of semen (%)		
	Citrate diluent	Tri diluent	Skim milk diluent	Citrate diluent	Tri diluent	Skim milk diluent
0	Ac 11.00 ±0.707	Bc 10.60 ± 1.025	Cc 9.40 ±0.899	Ba 6.70 ±0.324	Aa 6.75 ±0.315	B a 6.70 ±0.342
48	Ab 12.00 ±1.340	Bb 11.20 ±1.069	Cb 10.02 ±2.561	Ab 6.50 ±0.300	Ab 6.56 ±0.317	Ab 6.47 ±0.349
96	Aa 15.03 ±2.588	Ca 13.00 ±1.719	Bc 13.68 ±2.325	Bc 6.25 ±0.361	Ac 6.33 ±0.249	Bc 6.28 ±0.286

)AB.C) Capital letters compare between columns while (a.b.c) small letters compare between Rows.

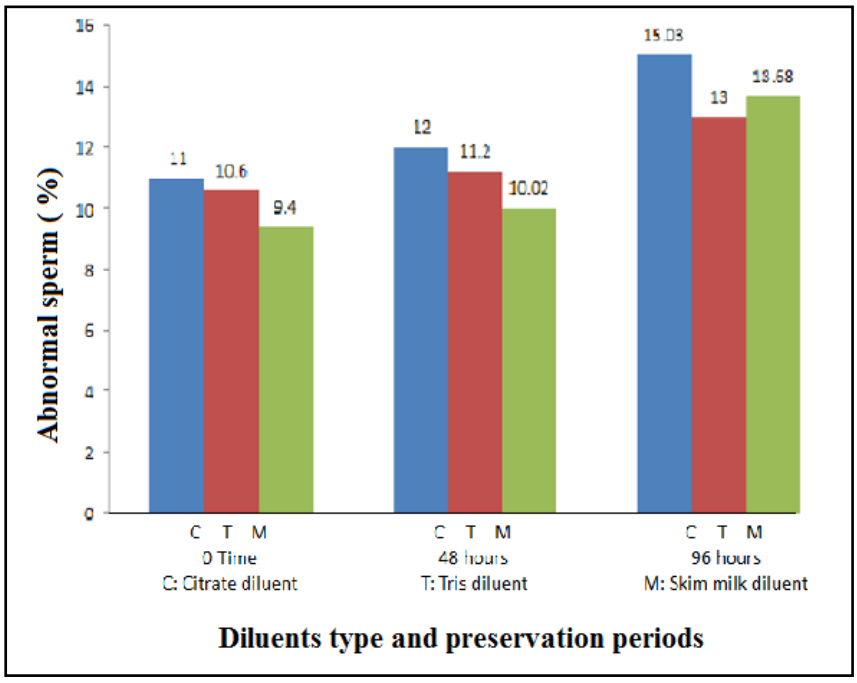
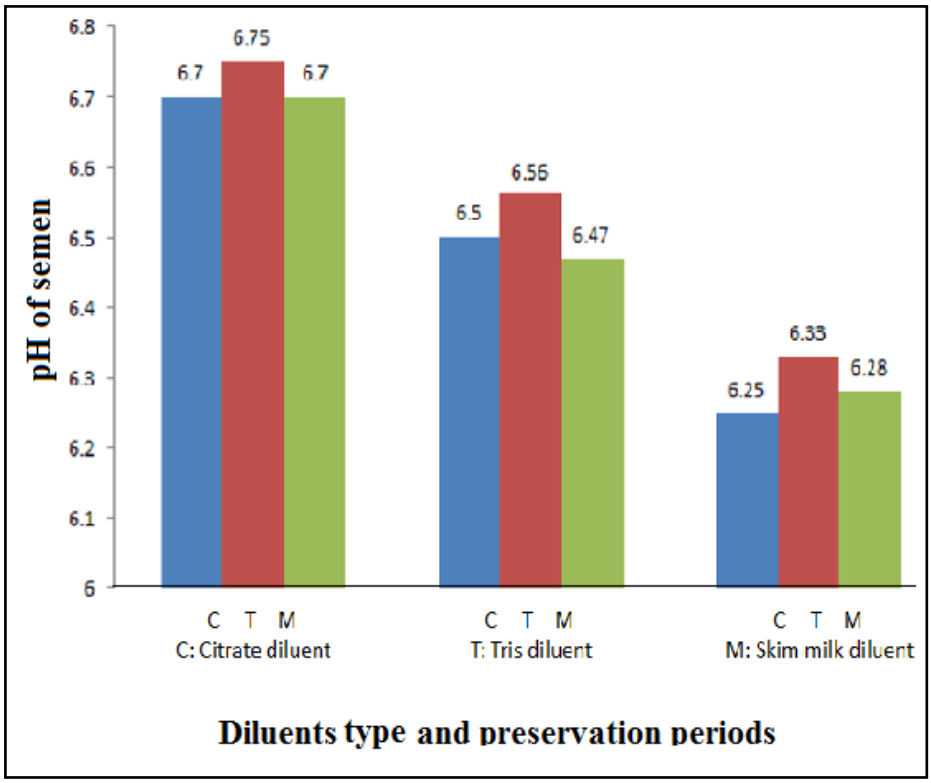


Fig. (3). Effect of diluents type and preservation periods on Abnormal sperm (%).



Fig, (4). Effect of diluents type and preservation periods on pH of semen.

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تأثير نوع المخففات وفترات الحفظ على بعض خصائص السائل المنوي للأغنام

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المستخلص

هدفت الدراسة الحالية إلى تقييم ثلاث مخففات منوية وثلاث فترات حفظ (0 ، 48 ، 96 ساعة) على جودة الكباش البركى. وكانت الاختبارات الثلاثة للمخففات هي: صفار - سترات ، تريس - صفار بيض وحليب خالي الدسم - صفار بيض. كانت نسبة التخفيف 1 مخفف: 10 منوي. تم جمع السائل المنوي من كباش البرقي باستخدام كبش مهبل صناعي مرة واحدة شهرياً في الصباح خلال شهري سبتمبر وأكتوبر ونوفمبر. تم حفظ عينات السائل المنوي بعد التخفيف بالمواد المخففة لمدة 0، 48 ، 96 ساعة ثم تم تقييم جودة السائل المنوي. يتم تلخيص النتائج التي تم الحصول عليها على النحو التالي:

1- مخفف تريس + صفار البيض أظهر أفضل حركة للحيوانات المنوية وحيوية الحيوانات المنوية أفضل مقارنة بمخفف تريس + اللبن الخالي من الدسم.
2- أظهر تريس + حليب اللبن خالي الدسم أقل نسبة شذوذ مقارنة بالسترات.
3- كان لفترات الحفظ آثار معنوية حيث كان المخفف تريس هو الأفضل في حفظ السائل المنوي باستخدام التبريد مقارنة بالسترات.