RECOVERY AND FERTLIZATION RATES OF GOAT SPERMATOZOA AS AFFECTED BY DIFFERENT LEVELS OF EGG YOLK, DILUTION RATES, FREEZING METHOD AND MONTHS OF THE YEAR

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ABSTRACT

Semen from10 fertile 1/2 Damascus-1/2 Baladi crossbred bucks (3-5 years old) was collected by artificial vagina twice weekly for 12 months. Ejaculates with mass motility ≥80% were pooled to be used in series of five experiments. In the 1st experiment, semen was diluted with Tris-citric acid-glucose-glycerol extender to select the best level of egg yolk among the levels 2.5, 5.0, 7.5, 10.0, 15.0 and 20% verifying the highest sperm post-thawing motility where it was found as 2.5%. Experiment II, was to select the best dilution rate among the rates 1:4, 1:5, 1:8, 1:10 and 1:16 which was found to be 1:5. Semen diluted with 2.5% egg yolk in Tris-based extender at a rate of 1:5 was used to compare freezing in straws or in pellets form (Exp. III). In experiment IV, post-thaw sperm motility and recovery rate were compared among months of the year. Fertility rate by using frozen semen in pellets form with different sperm cell doses (100, 200 and $300x10^6$/cervical inseminate) was assessed (Exp. V).

The results revealed that sperm motility percentage was the highest in post-thaw semen diluted with Tris extender containing 2.5% egg yolk at a rate of 1: 5 (54.5%, $P<0.05$) and the lowest (45.4%, $P<0.05$) at a rate of 1:16. Freezing goat semen showed higher post-thaw motility when was in pellets compared to straws (49.04% *vs.* 43.54%, $P<0.05$). Sperm motility in fresh, post-diluted and post-thaw semen showed the highest values during autumn months (Sept.-Nov.) and the lowest during summer months (June-Aug.). Fertility rate in does artificially inseminated with 100, 200 and 300 x 10^6 motile spermatozoa in pellets form was 31.57, 42.10 and 50.00%, respectively.

Keywords: Goat semen, freezing, egg yolk, dilution rate, pellets.

INTRODUCTION

The addition of the egg yolk to semen extenders plays a major role during the freezing steps of goat sperm cryopreservation (**Aboagla and Terada, 2004**). Egg yolk is common constituent in semen extenders, protecting spermatozoa against cold chock and has been thought to confer protection during freezing and thawing (**Simpson et al., 1986**).

On the other hand, egg yolk might show negative effect on frozen goat semen due to the presence of phospholipase, which is an enzyme in the seminal plasma catalyzes the hydrolyses of lecithin in egg yolk to fatty acids and lysolecithins, which are toxic to spermatozoa and cause coagulation of the storage medium (**Iritani and Nishikawa, 1972**). The toxicity of egg yolk coagulating enzyme was found to be influenced by season of the year (**Loubser *et al.*, 1983**) and varied among individuals (**Leboeuf *et al.*, 2000**). **Iritani *et al.* (1961)** observed that the toxic effect is substantially reduced after removal of the seminal plasma by washing the spermatozoa, or when semen is obtained from cowperectomized bucks. Removal of the seminal plasma (**Corteel, 1975**) or Cowper's glands (**Corteel, 1981**) is beneficial when the spermatozoa are frozen in a diluent containing egg yolk.

Semen is diluted to protect spermatozoa during preservation, but the rate of dilution is often changed for technical reasons (**Salamon and Maxwell, 1995**).

Some studies have indicated that semen production in bucks is influenced by seasonal changes (**Loubser *et al.*, 1983**). Goat spermatozoa are very sensitive to cold shock and can't be cooled to 5°C in the absence of egg yolk (**Salamon and Maxwell, 1995**).

Semen packaging (straw or pellets) during freezing had variable effects on post-thaw sperm motility. This was recognized in ram (**Awad, 1989**), bull (**Zeidan, 1994**) and rabbits (**Daader and Zeidan, 2008**).

The present study aimed to assess optimal egg yolk level, dilution rate, month of the year and type of freezing that allows efficient processing and use of Damascus x Baladi crossbred buck semen.

MATERIALS & METHODS

The present study was carried out at Sakha Research Station, Animal Production Research Institute, Agricultural Research Center, during the period from January to December, 2006.

Experimental design

A series of five experiments was carried out to study the effect of some protocols for freezing semen on sperm motility as well as live sperm concentration, yet

fertilization rate. Factors studied were egg yolk level, dilution rate, type of freezing and month of collection.

Experiment I: was carried out to investigate the effect of different levels of egg yolk (2.5, 5, 7.5, 10, 15 and 20%) on post-thawing motility and recovery rate of spermatozoa in relation to sperm motility before freezing (fresh semen).

***Experiment II*:** aimed to define the effect of different semen dilution rates (1:4, 1:5, 1:8 1:10 and 1:16) with Tris-extender containing 2.5% egg yolk (according to the best results obtained in experiment I) on post-thawing motility and recovery rate of spermatozoa in relation to sperm motility before freezing (fresh semen).

Experiment III: 1:5 diluted semen with 2.5% egg yolk in Tris-based extender (according to the best results of experiments I and II) was frozen in straws or in pellets form to investigate the best packaging method (straws or pellets) on motility and recovery rate of spermatozoa in relation to sperm motility before freezing (fresh semen).

Experiment IV: Values of pre- and post-freezing sperm motility percentage, as well as, recovery rate in relation to fresh semen were recorded monthly from January to December. Semen (either fresh or frozen) was evaluated for sperm motility and sperm concentration using Haemocytometer. According to the results of experiments I, II and III, 2.5% egg yolk level, dilution rate of 1:5 and freezing in pellets form as optimum were only included in comparison between months of the year. The comparison among months was implemented on different samples collected and frozen every month.

Experiment V: In this experiment, naturally in estrous does were intracervically inseminated during September breeding season with frozen semen diluted in Tris-based extender containing 2.5% egg yolk at a dilution rate of 1:5 in pellets form. Insemination was carried out using 30 does divided into three groups (10 does each), each was inseminated with three different counts of motile sperm cells (100, 200 and 300 x 10^6/ml). Each doe was inseminated twice; at the onset of heat and 12 hr later. Fertility rate was calculated as the percentage of does kidded in each group.

Semen collection

Semen samples were collected from each of 10 fertile 1/2 Damascus-1/2 Baladi crossbred bucks (3-5 years old) with an artificial vagina twice weekly between 0.8 to 0.9 a.m. throughout the experimental period. At each ejaculation time, ejaculate of each buck was taken immediately to the laboratory, then evaluated and held in a water-bath at 35-37°C. Ejaculates of all bucks in each collection showing mass motility ≥80% were pooled before use in the study.

Semen dilution

The extender used in this study was Tris (hydroxymethyl-aminoethane)-citric

acid consisting of (375.0 mM) Tris (Sigma Chemical Co., St. Louis, MO, USA), (124.0 mM) citric acid (Sigma), (41.0 mM) glucose (Sigma Aldrich), (5%) glycerol and egg yolk. Egg yolk was separated from the albumin and added to the extender at different levels in experiment I then 2.5% level only was used. . Semen was diluted to different rates in experiment II then 1:5 rate only was used.

Freezing and thawing

Semen was frozen as pellets (0.25 ml) on dry ice or in straws (0.25 ml) in nitrogen vapor and then plunged in liquid nitrogen. Glycerol was added to the cooled semen and was left at 5°C for 2 hours as equilibration period before freezing process. Frozen semen was thawed using thawing-solution 1: 3 as thawing medium (2.9 sodium citrate/100 ml water) for 120 sec. at 37°C for pellets or directly in water bath at 37°C for straws.

Evaluation of sperm motility

Percentage of sperm motility was evaluated in fresh, pre-freezing (post-dilution) and post-thawing semen. Sperm motility was compared after and before cryopreservation to evaluate the effect of cryoprotectant on the cryosurvival (recovery rate). Recovery rate (%) was calculated according the following formula:

Recovery rate= Motility after freezing/ Initial motility × 100

Statistical analysis

Data were analyzed by one-way analysis of variance according to **Snedecor and Cochran (1982)**. The statistical model was: $Y_{ij} = \mu + A_i + e_{ij}$, where: Y_{ij} = Observed values, μ = Overall mean, A_i = Level of egg yolk / dilution rate / type of freezing or month of the year and e_{ij} = Random error.

The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages. Duncan new multiple range test **(Duncan, 1955)** was used to test the differences among means. Significance level was tested at a probability of $P<0.05$. Fertility rate results were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Effect of egg yolk level

Results in Table (1) show that post-thawing sperm motility was negatively affected by egg yolk level. Sperm motility decreased but insignificantly from 54.8% with the egg yolk at a level of 2.5% to 50.4% with the egg yolk at a level of 10% and

Table (1): Effect of egg yolk level on the percentage of post-thawing motility and recovery rate in goat semen.

Egg yolk level (%)	post-thawing sperm motility (%)	Recovery rate (%)
2.5	54.8±1.67[a]	65.9±0.25[a]
5	51.3±1.93[a]	61.7±0.12[a]
7.5	50.8±1.65[a]	61.1±0.23[a]
10	50.4±1.68[a]	60.6±0.20[a]
15	45.4±1.51[b]	54.6±0.15[b]
20	39.0±1.90[c]	46.9±0.16[c]

a, b and c: Means having different superscripts within the same column differ significantly (P<0.05).

significantly then after reaching 39.0% with 20% egg yolk. Changes in recovery rate followed the same trend.

Similar trend of changes in sperm motility have been reported by **Ritar and Salamon (1982).** These findings may indicate that when egg yolk level increased in the diluted semen, hydrolysis of lecithin led to further deterioration of sperm motility. Frozen goat semen motility after thawing was 35.50, 36.00, 26.80, 19.80 and 15.30% at 0, 1.5, 3.0, 6.0 and 12.0% egg yolk concentrations, respectively. This may be attributable to the activity of the egg yolk coagulating enzyme (phospholipase) in the buck seminal plasma. As the egg yolk increase in the buck diluted semen, more hydrolysis of lecithin occurs which leads to further deterioration of sperm.

The present results came in line with those obtained by **El-Maghraby (2007)** who found an optimal level of 2.5% egg yolk with Tris-based extender for dilution of goat semen.

On the other hand, many extenders containing 20% egg yolk were used for washed semen with accepted post-thaw semen quality (**Memon *et al.*, 1985, Deka and Rao, 1986 and Tuli *et al.*, 1991**). At studying the suitable level of egg yolk with Tris-egg yolk citric acid-fructose glycerol extender for freezing washed buck semen, **Deka and Rao (1986)** found that 20% was better than 10 or 7% egg yolk for post-thaw motility (68.35, 67.35 and 58.40%, respectively) and percentage of damaged acrosomes was 11.70, 16.80 and 24.10%, respectively.

Effect of dilution rate

Results presented in Table (2) show that post-thawing motility of spermatozoa was significantly (P<0.05) affected with dilution rate. Dilution rate of 1: 5 showed

significantly (P<0.05) the highest post-thawing sperm motility (54.5%) and recovery rate (65.7%), but did not differ significantly than those diluted at rates 1:4 or 1:8. However, increasing dilution rate to more than 1:8 decreased significantly (P<0.05) sperm motility and recovery rate.

Table (2): Effect of dilution rate with Tris-based extender containing 2.5% egg yolk on the percentage of post-thawed sperm motility and recovery rate in goat semen.

Dilution rate	Post-thawing sperm motility (%)	Recovery rate (%)
1:4	53.7±1.67[a]	64.8±0.24[a]
1:5	54.5±1.74[a]	65.7±0.16[a]
1:8	52.0±1.70[ab]	62.7±0.17[a]
1:10	47.9±1.64[b]	57.8±0.23[b]
1:16	45.4±1.83[c]	54.8±0.15[b]

a, b and c: Means having different superscripts within the same column, differ significantly (P<0.05).

In accordance with the present results, **Ritar and Salamon (1982)** indicated that best sperm characteristics obtained with media dilution from 1:4 to 1:8. **Singh *et al.* (1995)** also found the highest post-thaw motility (47.8%) and live sperm percentage (67.1%) found with goat semen diluted at rate 1: 5. In goat semen stored at refrigerator, **Salamon and Maxwell (1995)** observed the highest survival rate (64.3%) at dilution rate 1: 7, followed by 1: 5 (63.1%) compared with 1: 9 (54.2%).

The success of deep freezing depends, to a notable degree, on the rate of dilution of semen. Semen is diluted to protect spermatozoa during freezing and thawing, but the rate of dilution was often change for technical reasons (**Salamon and Maxwell, 1995**). Greater dilution rate had a severe effect on the cation concentration of spermatozoa (**Robertson and Watson, 1987**).

Effect of type of freezing

Freezing of goat semen diluted with Tris-based extender containing 2.5% egg yolk at a dilution rate 1:5 showed significantly (P<0.05) higher post-thaw motility as pellets than in straws (49.04 *vs.* 43.54%), as shown in Fig. (1).

Freezing of goat semen in pellets form or in straws decreased significantly (P<0.05) the percentage of post-thawing motile spermatozoa. The difference from fresh was more in semen frozen in straws than in pellets. The merit of pellets has been reported by **Awad (1989)** in ram, **Zeidan (1994)** in bull and **Daader and Zeidan (2008)** in rabbit semen. This could be related to the less surface area in relation to

volume of pellets compared with straws which allows extenuation of freezing and thawing shocks.

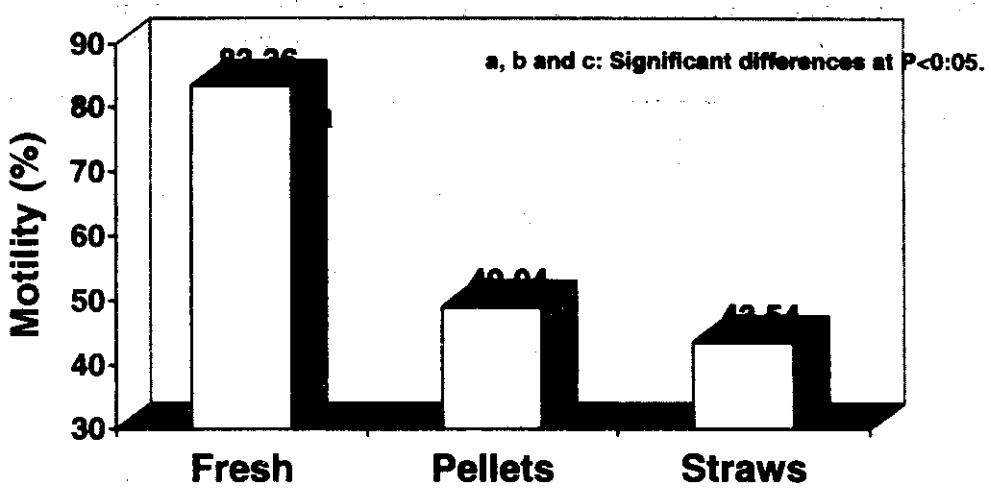

Fig. (1): Effect of freezing method on post-thaw sperm motility in buck semen diluted with Tris extender containing 2.5% egg yolk at a dilution rate of 1:5.

Effect of month of the year

The results shown in Table (3) reveal that the percentage of post-thawing sperm motility as well as recovery rate was significantly ($P<0.05$) high during autumn months (Sept, Oct.. and Nov.), compared with other months of the year. The lowest values have been reached in summer in July and August. **El-Maghraby (2007)** found the highest percentage of sperm motility, in fresh, pre-freezing and post-thawing semen for different goat breeds, during autumn.

Insemination

Results of insemination with pelleted frozen semen extended at a rate 1:5 with Tris-based extender containing 2.5% egg yolk (Fig. 2) reveal differences among inseminate sperm cell doses. Fertility rate was 50% due to large inseminate (300×10^6 motile sperm cells), 42.10% for the medium (200×10^6 motile sperm cells) and 31.57% for the small one (100×10^6 motile sperm cells). Difference was only significant ($P<0.05$) between the large and small inseminates.

Results are seemingly reasonable as cervical insemination in sheep resulted in lambing rates of 40% (**Halbert et al., 1990**). **Sallam (1999)** concluded fertility rate in sheep around this figure resulted from research studies for many investigators.

Table (3): Sperm motility in post-thawed semen pellets and recovery rate of spermatozoa diluted with 2.5% egg yolk Tris-based extender at a rate 1:5.

Month of the year		Post-thawing	Recovery rate
Winter	Jan.	46.42 ± 2.82^{bcd}	58 ± 0.03^{f}
	Feb.	44.28 ± 2.02^{cde}	56 ± 0.03^{g}
Spring	March	46.90 ± 1.14^{de}	56 ± 0.01^{e}
	April	49.28 ± 2.97^{bcde}	60 ± 0.03^{c}
	May	41.42 ± 0.92^{ef}	56 ± 0.01^{d}
Summer	June	41.48 ± 0.80^{ef}	56 ± 0.01^{d}
	July	32.85 ± 3.59^{g}	43 ± 0.05^{i}
	Aug.	35.71 ± 2.64^{fg}	51 ± 0.04^{h}
Autumn	Sept.	57.14 ± 2.64^{ab}	67 ± 0.03^{b}
	Oct.	52.85 ± 4.20^{bc}	62 ± 0.05^{e}
	Nov.	62.85 ± 1.84^{a}	78 ± 0.02^{a}
winter	Dec.	47.85 ± 2.14^{cde}	59 ± 0.02^{h}

a, b... i: Means having different superscripts within the column differ significantly ($P<0.05$).

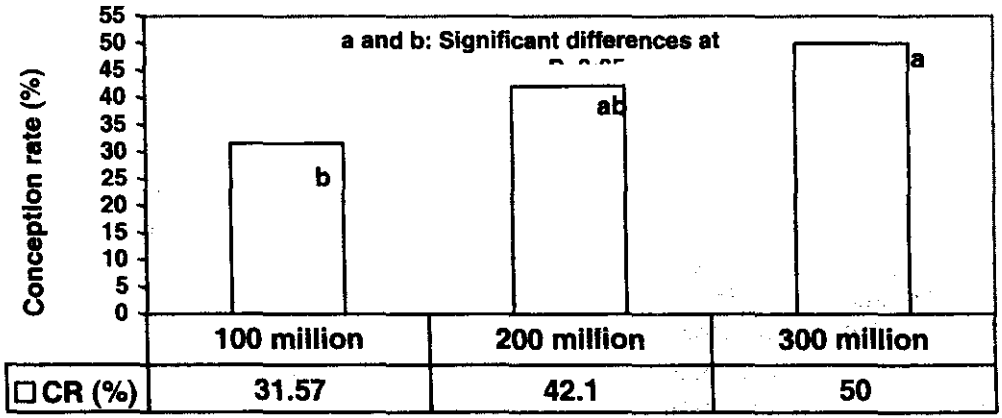

Fig. (2): The effects of the inseminated pelleted sperm cell number on fertility rate.

In the present work, the large inseminate (300 x 10^6 motile sperm cell) resulted in the highest fertility, could be recommended only in case of availability of goats semen. If semen is not available enough, the optimal dose could be the medium one (200 x 10^6 motile sperm cell) as the difference was not significant from the large.

CONCLUSION

The current study indicate that addition of egg yolk to Tris-based extender at a level of 2.5% and dilution of goats semen at a rate 1:5 frozen in pellets form will be more efficient in maintaining sperm post-thawing . Best season to collect and freeze goat semen is autumn. Sperm-cell dose of 300 x 10^6/inseminate is recommended for A.I. of does to obtain satisfactory fertility rates in goats. In case that availability of semen is not enough, the optimal dose could be the medium one (200 x 10^6 motile sperm cell). The low cost and simple preparation technique of pellets could preferably recommend the use of pellets rather than straws especially in the developing countries like Egypt,.

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الحيوية قبل وبعد التجميد للسائل المنوى للماعز تحت تأثير مستويات مختلفة من صفار البيض ، معدل التخفيف ، نوع التجميد والشهر من السنة

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ملخص

تم جمع السائل المنوي من 10 تيوس خليط 2/1 دمشقى × 2/1 بلدى عمر (3-5 سنوات) باستخدام المهبل الاصطناعي مرتين أسبوعيا على مدى 12 شهر . فى كل مرة يتم خلط القذفات المحتوية على حيوية اكبر من 80% فقط لاستخدامها فى 5 تجارب. فى التجربة الاولى : تم تخفيف السائل المنوى بمخفف ترس -حامض الستريك - الجلوكوز - جليسرول لتقييم استخدام مستويات مختلفة من صفار البيض (2.5 و 5 و 7.5 و 10 و 15 و 20 %) على حيوية الحيوانات المنوية بعد الاسالة. في التجربة الثانية : تم تخفيف السائل المنوي بمستوى صفار البيض 2.5٪ فى مخفف صفار البيض بمعدل تخفيف (1: 4 ،1: 5 ، 1: 8 ، 1: 10 و 1: 16). بناء على نتائج الإختبارين تم فقط إستخدام السائل المنوى المحتوى على صفار البيض 2.5٪ والمخفف بالترس بمعدل 1: 5 . تم تجميده فى قصيبات أو ككريات لتقييم تأثير نوع التجميد (التجربة الثالثة). فى التجربة الرابعة ، تم عمل اسالة للسائل المنوى المجمد وحساب معدل الحيوية خلال شهور السنة. أما التجربة الخامسة فكانت عن معدل الخصوبة باستخدام السائل المنوي المجمد في شكل كريات مع اختلاف تركيز وعدد الحيوانات المنوية الملقح بها (100 و 200 و 300 × 10 ^{6} / تلقيحة داخل عنق الرحم).

أوضحت النتائج أن أعلى نسبة حيوية للحيوانات المنوية بعد الاسالة (54.5%) كانت للسائل المخفف بالترس المحتوى على 2.5% صفار بيض وبمعدل تخفيف 1: 5 بينما كانت أقلها حيوية (45.4%) لمعدل التخفيف 1: 16. حيوية السائل المنوى المجمد بعد الاسالة والمخفف بالترس والمحتوى على 2.5% صفار بيض وبمعدل تخفيف 1: 5 كانت اعلى عند تجميده فى صورة كريات عنه فى صورة قصيبات (49.04٪ مقارنة بـ 43.54% على الترتيب). كان هناك اختلافات خلال شهور السنة فى حيوية السائل المنوى طازجا أو بعد التخفيف أو بعد الاسالة حيث كان اعلاها خلال شهور الخريف (سبتمبر- نوفمبر) وأدناها خلال شهور الصيف (يونيو- اغسطس). معدل الخصوبة فى الماعز الملقحة إصطناعيا بتركيز 100، 200، 300 مليون حيوان منوى حى مجمد فى صورة حبيبات كان 31.57 ، 42.10 ، 50% بالترتيب .