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Effect of Sodium Dodecyl Sulphate (SDS) on the Viability and Fertility of Damascous Goat Spermatozoa

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Abstract: The objective of this study was to investigate the effect of sodium dodecyl sulphate (SDS) addition to Tris egg yolk glycerol extender on the viability and fertility of frozen-thawed goat spermatozoa. Two experiments were conducted to determine these effects. In the first experiment, semen was collected from five fertile Damascus bucks and frozen in Tris egg yolk glycerol extender alone or with the addition of 0.05, 0.1 or 0.2% SDS. The post-thawing sperm individual motility and acrosome integrity were assessed. Both acrosome integrity and individual sperm motility were significantly ($p<0.05$) increased by increasing the concentration of SDS with the highest values achieved by addition of 0.1% for individual motility (60.5±0.66) and 0.05% for acrosome integrity (72.0±1.02). However, the percentages of individual motility(48.5.±1.03) and acrosome integrity (55.00±1.2) were significantly ($p<0.05$) decreased when SDS was added by a concentration of 0.2% .In the second experiment, we investigate the effect of SDS on the fertility of frozen-thawed goat semen. Incorporation of SDS into the Tris egg yolk glycerol extender during freezing of Damascus buck semen significantly ($p<0.05$) improve the kidding rates than the control group with the highest rate (90%) was recorded for 0.05% SDS. In conclusion, the viability and fertility of frozen-thawed goat spermatozoa were significantly improved when sperm was frozen in Tris egg yolk glycerol extender containing SDS, especially at a concentration of 0.05%

Key words: Damascus goat · Sperm · Frezzability · SDS

INTRODUCTION

Membranes are thought to be a primary target of chilling or freezing damage in cells [1]. It is crucial to maintain the integrity of the membrane in order to produce spermatozoa that remain potentially functional after freezing. Egg yolk is beneficial for the preservation of sperm of domestic and exotic species [2]. Egg yolk is added to protect against cold shock [3, 4] and the low density lipoprotein fraction is now useful established as the active constituents [5, 7].

Dilution of goat semen with diluents containing egg yolk can have a detrimental effect on the quality of the spermatozoa during cooling and cryopreservation [8, 9]. The presence of egg yolk coagulating enzyme (phosopholipase A) in the seminal plasma has a lipase activity on egg yolk lecithin resulting in fatty acid and lysolecithin production [10]. In terms of actual freezing methods, egg yolk could not be readily solubilized in a solution of extender. Moreover, egg yolk is the major detrimental factor for the acrosome before and after freezing of goat spermatozoa [11, 12] . Egg yolk could be solubilized by the addition of sodium dodecyl sulphate (SDS) [7] . In that regard, SDS has been included in the freezing extenders of spermatozoa for many species [11]. Many diluents have been used for freezing goat spermatozoa, but there are few reports on the effects of the surfactant on the post-thaw viability and fertility of goat spermatozoa.

The present study was, therefore, initiated to investigate (i) the effect of SDS addition to Tris egg yolk glycerol extender on the viability of frozen thawed goat semen, (ii) to what extent SDS could affect the fertility of frozen-thawed goat spermatozoa.

MATERIALS AND METHODS

Media: The diluent, a Tris-Egg yolk glycerol solution defined by Salamon and Ritar [13], was composed of 375.0 mM (Hydroxymethyle amino methane) Tris

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(Sigma Chemical Co. St. Louis, Mo, USA), 124.0 mM citric acid monohydrate (Sigma, USA) and 41.0 mM glucose (Sigma, USA), fresh egg yolk (20 ml), glycerol (5ml), penicillin G sodium (500 i.u./ml)streptomycin sulphate (500μg/ml) and glass distilled water to 100 ml. The SDS (Sigma) was added to the freezing extender at final concentrations of 0.05, 0.1 and 0.2%.

Semen Collection, Evaluation and Dilution: Semen was collected twice a week by means of an artificial vagina from five fertile Damascus bucks (25-36 months old) belonging to Sakha Experimental Station, Kafr El Sheikh, Animal Production Research Institute, Egypt. An anoestrus doe was used as a mount animal for semen collection. Immediately after collection the ejaculates were transferred to the laboratory and kept in a water bath at 30°C for the initial evaluation. Fresh semen was evaluated for volume, individual sperm motility and sperm cell concentration. Only ejaculates of at least 70% sperm progressive motility and 2500 x 10^6 sperm cells per milliliter were used for freezing. The prepared semen extender was divided into 4 parts; part 1 without SDS (control), part 2 contains 0.05% SDS part 3 has 0.1% SDS and part4 with.0.2%. Dilution of semen was performed by one-step dilution (1:4) method (at 30°C). The diluted semen was cooled to 5°C within 3 hours. Sperm cell concentration was adjusted to 300 x 10^6 / ml. The diluted semen was then equilibrated at 5°C for 15 min before freezing.

Freezing and Thawing Procedure: Semen was frozen in a pellet form (0.3 ml) into a plate made up of polytetrafluoroethylene cooled to (-80 to -140°C) beforehand by immersion in liquid nitrogen for 15 min [14]. Thawing of frozen semen was performed using a thawing solution (TCG) without glycerol or egg yolk at 37°C. Three pellets were dropped into a pre-warmed glass test tube containing 3 ml of thawing solution. To ensure uniform thawing of the pellets, the test tube was shaken in the water bath until complete melting of the pellets.

Semen Evaluation: Sperm progressive motility(%) was subjectively assessed under a phase contrast microscope (x400) equipped with a thermal stage at 37°C. Sperm cell concentration was determined by using Neubauer heamocytometer chamber (light microscope x 400) after dilution of fresh(1:400) and cooled (1:20) semen with 513.35 mM sodium chloride solution containing 0.32 mM eosin B [15].

The percentage of intact acrosome was assessed [16]. Briefly, 250 μL semen was fixed with 25 μl of 1% solution of formal citrate (99 ml of 2.9% trisodium citrate dehydrate and 1 ml of 37% commercial formaldehyde). Two hundred sperms were counted for the presence of normal apical ridges under phase contract microscope X1000.

Synchronization and Artificial Insemination: Eighty does were synchronized with 45 mg of fluorogestone acetate by vaginal sponge. (Crono-gest, Intervet International B.V. Boxmeer-Holland. The sponge was inserted (Day 0) and remained intravaginal for 11 days. On day 9, each doe was treated with 300 I.U.i.m. PMSG (Folligen, Intervet, International B.V. Boxmeer-Holland) and 50 μg chlorprostenol (Estrumate, Coopers, Animal Health Ltd, Berkhamsted England). The sponges were removed on the day 11, starting from device withdrawal, estrus was detected four times daily (30 min each) by using an aproned intact fertile buck. All does showed estrous signs were artificially inseminated with frozen thawed semen at 48 and 56 hr of sponge removal according to the technique of Ritar *et al.* [17]. An inseminate volume of 1.1 ml (containing 250-300 x 10^6 PMS) was used for cervical insemination of does. For insemination, the hind quarters of females were raised over a rail and the semen was deposited as deeply as possible into the cervical canal using a simple plastic disposable inseminating pipette with fine blunt bent end and a vaginal speculum. After 60 days of insemination, the does were screened for pregnancy by Trans-abdominal ultrasonography. Fertility was defined as the number of pregnant over that of inseminated as well as the number of kidding does over the number of inseminated does.

Experimental Design

Experiment 1: This experiment was carried out to investigate the effects of the addition of different concentrations of SDS to the Tris egg yolk glycerol extender on the viability of frozen-thawed goat spermatozoa. Sperm were frozen in the Tris egg yolk glycerol extender containing different concentrations of (0.05, 0.1 and 0.2%) SDS. The viability of the frozen-thawed sperm was evaluated in terms of progressive motility and intact-acrosomal membrane percentages.

Experiment 2: A fertility test was undertaken to explore if the improvement in post-thaw sperm viability observed in results of the experiment 1 from treatment of semen with SDS would be resulted in an improvement in the pregnancy and kidding rates. A total number of 80 multifarious Damascus does (55.5 kg body weight) were used in this experiment, the does were synchronized and divided into four groups (each of 20 does)

group, 1 as control, inseminated with frozen –thawed semen without addition of SDS; group, 2 inseminated with frozen-thawed semen provided with 0.05% SDS; group, 3 inseminated with semen diluted with Tris egg yolk incorporated with 0.1% SDS and group, 4 inseminated with frozen semen treated with 0.2% SDS.

Statistical Analysis: Data were analyzed according to the general linear models procedures of the statistical analysis systems (SAS, [18]). Data were expressed as mean ± SEM. ANOVA was used to examine the effect of treatments on the viability indices of goat's spermatozoa. In the fertility test the differences in pregnancy and kidding rates among control and treated groups (n=4) was analyzed by Chi-square test. A probability (P) value of ≤0.05 was selected as a criterion for a statistically significant differences.

RESULTS

Effect of Freezing on the Viability of Goat Spermatozoa: Ejaculates used in this study have the following characteristics; total volume of 1.1 + 0.03 ml, sperm cell concentration of 2718 + 32.60 x 10^6 sperm /ml) and 79.58 + 0.64% individual motility. In addition to 91.23 + 0.84% intact acrosome (Table 1).

Table 1: Semen characteristics on Damascus goat (Before treatment with SDS).

Sperm parameter	Mean ± SEM
Semen volume (ml)	1.1 ± 0.03
Individual motility (%)	79.58 ± 0.64
Sperm cell concentration (x 10^6/ml)	2718 ± 32.60
Intact acrosome (%)	91.23 ± 0.84

This experiment was carried out for 12 weeks to study and stabilize the normal semen characteristics before addition of SDS. The cryopreservation process greatly affected the sperm viability parameters (progressive motility and intact acrosome %). The studied sperm parameters showed significant (P<0.001) differences after freezing-thawing process (Figure 1).

Effect of Sds on the Viability of Goat Spermatozoa (Experiment, 1): Table 2 shows the effects of the addition of different concentrations of SDS to Tris egg yolk glycerol on the percentages of individual motility and intact acrosome of goat spermatozoa.

Fertility after Cervical Insemination Using Frozen Thawed Buck Semen (Experiment, 2): The outcomes of cervical insemination with frozen thawed semen after addition of SDS are delineated in Table, 3

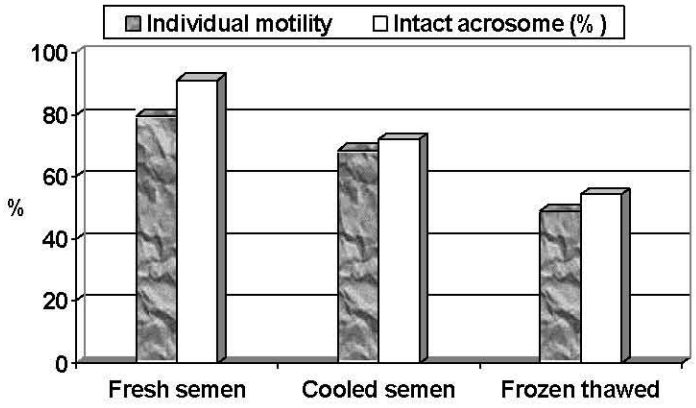

Fig. 1: Individual motility and intact acrosome percentage of fresh and frozen thawed spermatozoa from Damascus bucks

Table 2: Individual motility and intact acrosome (%) of goat spermatozoa during freezing in Tris egg yolk glycerol extender containing different concentrations of SDS

	Individual motility %			Intact acrosome %		
SDS%	After dilution	After cooling	After thawing	After dilution	After cooling	After thawing
0.05%	78.75± 0.59 a	70.50± 0.69 b	54.00± 0.45 b	90.50± 1.02	86.60± 1.50 a	72.00± 1.02 a
0.1%	77.50± 0.45 a	74.25± 1.00 a	60.50± 0.66 a	89.00± 0.85	80.00± 1.20 b	63.50± 0.95 b
0.2%	79.00± 0.40 a	71.25± 1.00 b	48.50± 1.03 c	90.30± 1.03	75.50± 0.95 c	55.00± 1.20 c
Control	77.0±0.61 a	65.75± 0.59 c	39.00± 0.71 d	91.00± 0.92	71.80± 0.81 c	54.25± 0.82 c

Values are means ± SEM (n= 60)

In the same column, means followed by different letters are significantly different at P≤0.05.

Table 3: Effect of SDS on the fertility of frozen thawed goat spermatozoa

SDS%	No. of pregnant does/No. of inseminated	No. of kidding does /No. of inseminated	Kidding rates(%)
0.05	18/20	18/20	90 a
0.1	15/20	15/20	75 a
0.2	16/20	16/20	80 a
Control	12/20	12/20	60 b
Overall	61/80	61/80	76.25

Values followed by different letters are significantly different at P<0.05.

The overall kidding rate was 76.25%. In spite of the kidding rate achieved with 0.05% SDS (90%) was superior to that achieved with 0.1 (75%) and 0.2 (80%) SDS. This superiority did not reach to the level of statistical significance (x^2 = 2.76). There were highly significant (x^2 = 6.65) differences between the control (without SDS) and each of the SDS concentrations with the higher values for different SDS concentrations.

DISCUSSION

The results of the present study revealed that the percentages of progressive sperm motility and acrosomal membrane integrity decrease following cryo preservation. However, the obtained average values may be considered acceptable semen quality for use of frozen-thawed goat spermatozoa [11, 19].

The freezing-thawing process reduces the individual motility to lesser extent than acrosomal integrity. This means that the acrosomal membrane is more vulnerable than the parts of the spermatozoa involved in locomotion [20]. Cryopreservation leads to detrimental effects in sperm cells [21] , resulting in a reduction of motility and fertilizing ability [22]. Under the best experimental conditions, about half the population of motile spermatozoa survives the freeze-thaw process [23].

The results of the present study revealed that increasing the concentration of SDS in the diluents significantly increased both the progressive motility of the cooled and frozen-thawed goat spermatozoa, with the highest values reached by the addition of 0.1% SDS. However, the intact acrosome percent was significantly higher due to addition of 0.05% SDS in frozen thawed and cooled semen. Moreover, the percentages of individual motility and intact acrosome were significantly lowered when SDS was added at a concentration of 0.2%. The present results proved the protective role of SDS against freezing-induced damage. Our results are in agreement with the results of Aboagla and Terada [11] who found that incorporation of SDS in semen diluents containing egg yolk protect spermatozoa against freezing induced damage as indicated by the significantly higher progressive motility as well as intact acrosome in the treated semen samples.

Previous studies have found the same effects of SDS when added to the egg yolk containing diluents in many species. Martin *et al.* [24] found that addition of SDS to a lactose-EDTA extender increased the past-thaw motility of stallion spermatozoa. Addition of 0.035% SDS to a medium containing 20% egg yolk preserves the motility and fertilization capacity of frozen mouse spermatozoa [7]. Pursel *et al.* [25] reported that the addition of sodium lauryl sulphate to the extender improved motility, acrosome integrity and fertilizing capacity of boar spermatozoa. In addition, freezing of ram spermatozoa in extender containing sodium lauryl sulphate achieved the best viability [26].

The protective effect of SDS may be due to that the surfactant acts to solubilize and increase the dispersion of egg yolk globules within the diluents enhancing the contact between the egg yolk and sperm cell membrane [27]. Thereby increasing the cryoprotective effects of egg yolk. The results of present study, reported that high concentration of SDS significantly decreased the individual motility and intact acrosome percentages. There is no explanation for this effect, but Dewit *et al.* [7] found that the integrity of mouse spermatozoa was significantly reduced when the SDS was added by more than 0.05% to the extender. However, when SDS is used at a high concentration in the diluents, free SDS molecules increase and may bind directly to the sperm membrane, with devastating results [11].

Fertility rate of 60% obtained herein by cervical insemination in control group (without SDS) was higher than fertility results obtained in other breeds with frozen semen 47.62% in Florida bucks [28], 39.1% in the Cashmere goat [19], 50.53% in the Beetal and Bengal goats [29] and nearly similar to that recorded (52.17%) in Damascus bucks [15] , Alpine (57.7%) and Saanen (55.4%), buck [30].

Incorporation of SDS into the Tris egg yolk glycerol extender in Damascus buck semen significantly improve the kidding rates than the control group with the highest value (90%) recorded for 0.05% SDS. Doubtless,

the favorable effects of SDS on post-thaw sperm motility and acrosomal integrity were a mean able for the improvement in the fertility of frozen-thawed goat semen. Several authors revealed that the addition of SDS to semen extender significantly increase the fertilization capacity of semen. In goat [11], in boar [25], in ram [26] and in mouse [7].

In conclusion, the viability of goat spermatozoa was significantly improved due to incorporation of SDS to the Tris egg yolk glycerol extender, especially at a level of 0.05%. In addition to improvement of kidding rates (fertility rates) in frozen-thawed goat semen provided with SDS.

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