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EFFECT OF THIOUREA ON MOTILITY AND VIABILITY OF CHILLED-STORED AND FROZEN-THAWED GOAT SPERMATOZOA

M.M.WAHEED , T.A.A. KHALIFA and K.H. EL-SHAHAT

Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt.

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SUMMARY

The aim of the present study was to elucidate the effect of thiourea on motility and viability of chilled-stored and frozen-thawed goat spermatozoa. Five male Baladi goats were used in three experiments during this work. Three different concentrations (10,50 and 100 mg%) of thiourea were added to semen extenders before processing. Tris-based egg yolk and Tris-egg yolk free extenders were used for chilled-storage of spermatozoa in the first and second experiments, respectively. In the third experiment, motility and viability of frozen-thawed goat semen were evaluated after inclusion of 50 mg% thiourea in the extender. In vitro augmentation of semen extender with 50 mg % thiourea exerted a pronounced improvement in incubated and post-thawed sperm motility percentages and viability indices of goat spermatozoa either in presence or absence of egg yolk. It is concluded that Tris-egg yolk free extenders could be used in presence of 50 mg % thiourea for both incubation and preservation of goat semen.

INTRODUCTION

Artificial insemination has an important role in goat breeding, specially in intensive systems of production, to control reproduction, progeny testing and to improve production of milk, hair and meat (Leboeuf et al.,2000).

Urea and uric acid are normal constituents of seminal plasma not only in man where their occurrence is not limited to semen , but in other mammals, including bull, ram and boar, in which the metabolism of purines in the body does not normally terminate in uric acid (Buonaguidi et al.,1991). It has been recognized that in addition to the cryoprotective effect of urea on living cells (Salamon and Maxwell,1995) , urates and thiols represent the major chain-breaking antioxidants in mammalian seminal plasma (Gavella et al.,1997;

Hughes et al.,1998).

Recent biotechnological advances in artificial insemination have emphasized on the adverse effect of goat bulbourethral gland secretions for the survival of goat sperm chilled or frozen in media containing egg yolk (Amoah and Gelaye,1997). Subsequently, it has been suggested that the adverse effect of bulbouretheral gland secretions could be minimized by simple dilution of goat semen with Tris-based extenders containing low concentration (2.5%) of egg yolk (Roca et al.,1997). Since egg yolk was found to protect sperm viability from the deleterious effects of lipid peroxidation (Jones and Mann,1977), the reduction in egg yolk concentration may render semen extenders less effective in moderating the oxidative stress that generated in consequence of short term (Cerolini et al.,2000) and long term (Bilodeau et al.,2000) preservation of semen.

Consequently, the present study was designed to investigate whether inclusion of thiourea (2.5 %) in egg yolk based and egg yolk free extenders had an ameliorative effect on motility and viability of chilled-stored and frozen-thawed goat spermatozoa.

MATERIALS AND METHODS

Chemical reagents

Unless otherwise stated, thiourea (thiocarbamide, CH_4N_2S) and other chemical reagents used were of the highest available grade and were purchased from Sigma- Aldrich Co., Deisenhofen, Germany.

Animals

Five male Baladi goats aged two years and weighed 45 to 50 kg were used in the current study. This study commenced in August,2001 and lasted for four months in indoor pens belonging to the Department of Theriogenology , Faculty of Veterinary Medicine , Cairo University. Throughout the experimental period, each buck was daily offered 5 kg barseem hay and one kg of a balanced concentrate.

Semen collection and evaluation

Semen was collected twice a week by means of an artificial vagina using an anestrous doe as a mount animal. Within 2 to 3 minutes following collection, the ejaculates were transferred to the laboratory and kept in a water bath at 30°C for evaluation by means of conventional methods.

Semen extenders

Two types of diluents were used for short-term (5°C) and long-term (-196°C) preservation of semen :

1- Egg yolk-based diluent (Evans and Maxwell,1987) which was composed of Tris (hydroxymethyl) amino-methane (3.786g) , glucose (0.625g) , citric acid mono-hydrate (2.172g), fresh chicken egg yolk (2.5 ml), glycerol (5ml), penicillin (100,000 IU), streptomycin (100mg) and glass-distilled water to 100 ml.

2- Egg yolk free diluent which was composed of the same ingredients and concentrations of the above mentioned diluent without egg yolk.

Semen processing and experimental procedure

Only ejaculates of at least 70% initial motility and 2000 x 10^6 sperm cells/ml were used in three in-vitro experiments. For each experiment, 10 trials were carried out.

In each trial, three consecutive ejaculates from one buck were pooled to yield one semen sample with a total volume of 2 to 3 ml.

Experiment 1 :-

This experiment was conducted to study the effect of thiourea on the viability of chilled-stored semen in egg yolk-based diluents . Semen samples were split and diluted (1:4) at 30°C with semen extenders supplemented with or without 10 , 50 and 100 mg % thiourea . Immediately after dilution, the extended semen was incubated at 5°C for 168 hours. Sperm progressive motility was assessed after dilution as well as after 6 , 24 , 48 , 72 , 96 , 120 , 144 and 168 hours of incubation period. The viability index of incubated semen was calculated according to Milovanov et al. (1964).

Experiment 2 :-

This experiment was designed to find out the impact of thiourea on the viability of chilled-stored spermatozoa in egg yolk free diluents. Semen samples were split, processed and assessed as previously described in Exp.1.

Experiment 3 :-

This experiment was set out to study the influence of thiourea on freezability of goat semen in 0.50 ml straws. Semen samples were split and diluted (1:4) at 30°C with egg yolk free extenders supplemented with or without 50 mg% thiourea (the concentration gave the best results in the previous two experiments) and egg yolk-based extenders supplemented with or without 50 mg% thiourea. The diluted semen was then cooled to 5°C and loaded into 0.50 ml straws over a period of 4 hours. For freezing, the straws were placed horizontally on cold (5°C) freezing racks and lowered into liquid nitrogen vapour inside a foam box (71 x 37 x 29 cm, containing 10 liters of liquid nitrogen) at a height of 2 cm above the level of liquid nitrogen, for 15 minutes . The straws were then immersed in liquid nitrogen and transferred into liquid nitrogen storage container. After three weeks of storage, frozen semen was thawed in a water bath at 40°C for 30 seconds and incubated at 30°C for 3 hours. Sperm motility was assessed immediately after dilution and thawing as well as after 1 , 2 and 3 hours of thawing. The post-thaw viability index was calculated according to Milovanov et al. (1964).

Statistical analyses:

All data were subjected to analysis of variance (ANOVA) By using the general models procedures of the Statistical Analysis Systems (1990).

RESULTS

The mean percentages of progressive motile sperm, live spermatozoa and total sperm abnormalities in freshly collected semen samples were 72.50% , 79.80% and 13.45% , respectively.

Experiment 1 :

Table 1 declares the effect of thiourea on the motility and viability of preserved (at 5°C for 168 hours) goat semen in Tris-based egg yolk (2.5%) extenders.

In-vitro provision of semen extenders with two concentrations (10 and 50 mg%) of thiourea significantly (P<0.05) exerted a stepwise augmentation in the motility percentages and viability indices of preserved spermatozoa. The maximum percentages of sperm motility after dilution, 6, 72 and 168 hours of incubation period as well as the highest value of viability index were recorded after treatment of goat spermatozoa with 50 mg% thiourea. On the contrary, inclusion of 100 mg% thiourea in semen extenders significantly (P<0.05) minimized the motility percentages and viability indices of stored spermatozoa.

Experiment 2 :

Table 2 depicts the influence of thiourea on the motility and viability of stored (at 5°C for 168 hours) goat spermatozoa in Tris egg yolk free extenders.

Table 1: Effect of thiourea on sperm motility (%) and viability during incubation of goat semen at 5°C in a Tris-based egg yolk (2.5%) extender (Mean±SE).

Thiourea concentrations	Incubation periods (hours)				Overall means	Viability indices
	0	6	72	168		
Control	72.00 ±2.00	69.00^{a} ±1.87	50.00^{a} ±5.70	12.00^{a} ±1.22	51.50^{a} ±5.83	78.45^{a} ±6.12
10.00 mg%	77.00^{b} ±1.22	73.00^{b} ±1.22	59.00^{b} ±5.10	18.00^{b} ±1.22	56.75^{b} ±5.50	93.39^{ab} ±4.93
50.00 mg%	79.00^{c} ±1.00	75.00^{c} ±0.00	63.00^{c} ±5.39	30.00^{c} ±3.16	61.75^{c} ±4.65	104.19^{b} ±4.94
100.00 mg%	76.00^{ab} ±1.87	68.00^{a} ±3.39	40.00^{d} ±5.48	7.00^{d} ±1.22	47.75^{d} ±6.40	61.02^{c} ±6.28

Means with different superscripts in the same column are significantly different (P<0.05).

In-vitro replenishing of semen extenders with different concentrations (10,50 and 100 mg%) of thiourea significantly (P<0.05) procured a pronounced improvement in motility percentages and viability indices of stored spermatozoa.The maximum percentages of sperm motility after 6, 72 and 168 hours of incubation period as well as the superior value of viability index were detected after supplementation of goat semen with 50 mg% thiourea.

Experiment 3 :

Table 3 outlines the influence of thiourea on the motility and viability of processed (freeze-thaw) goat spermatozoa in Tris-egg yolk free and Tris-based egg yolk extenders.

In-vitro augmentation of semen extenders with (50 mg %) thiourea significantly (p<0.01) exerted a pronounced improvement in post-thaw sperm motility percentages and viability indices of processed goat spermatozoa either in the presence or absence of egg yolk in the extender. Furthermore, Tris-based thiourea free extenders significantly (p<0.01) minimized the motility percentages and viability indices of preserved spermatozoa even in inclusion of egg yolk in the extender (Table 3).

Table 2 : Effect of thiourea on sperm motility (%) and viability during incubation of goat semen at 5°C in a Tris-egg yolk free extender (Means ± SE).

Thiourea concentrations	Incubation periods (hours)				Overall means	Viability indices
	0	6	72	168		
Control	72.00 ±2.00	68.00 ±2.55	49.00 ±4.00	11.00 ±1.00	50.00^{a} ±5.67	72.90^{a} ±3.60
10.00 mg%	76.00 ±1.87	74.00 ±1.00	58.00 ±7.18	19.00 ±3.32	56.75^{b} ±5.58	86.52^{ab} ±7.89
50.00 mg%	77.00 ±2.55	76.00 ±2.92	64.00 ±2.92	30.00 ±4.74	61.75^{c} ±4.64	102.12^{b} ±5.21
100.00 mg%	78.00 ±2.00	75.00 ±1.58	59.00 ±4.58	21.00 ±5.10	58.25^{d} ±5.47	92.28^{bc} ±6.58

Means with different superscripts in the same column are significantly different (P<0.05).

Table 3 : Effect of thiourea on sperm motility (%) and viability during various stages of freeze-thaw processing of goat semen (Mean ± SE).

Semen treatments	Sperm motility (%)			Overall means	Post-thaw viability indices
	After dilution	0 hour after thawing	3 hours after thawing		
* TGCG extender	81.00 ±1.00	35.00[a] ±2.24	12.00[a] ±2.55	42.67[a] ±7.74	68.50[a] ±6.10
TGCG extender + 50.00 mg% thiourea	83.00 ±2.00	50.00[b] ±3.16	20.00[b] ±4.74	51.00[b] ±7.12	113.00[b] ±9.30
TGCG extender + egg yolk	81.00 ±1.00	41.00[a] ±2.92	12.00[a] ±2.55	44.67[a] ±7.66	87.50[a] ±6.07
TGCG extender + egg yolk + 50.00 mg% thiourea	81.00 ±1.00	48.00[b] ±2.00	19.00[b] ±2.92	49.33[b] ±6.86	115.00[b] ±5.00

Means with different superscripts in the same column are significantly different (P<0.01).
* TGCG = Tris Glucose Citric acid Glycerol

DISCUSSION

It is well known that storage of goat semen, particularly in frozen state, causes ultrastructural , biochemical and functional damage to the spermatozoa resulting in a reduction of motility, viability and impaired fertility (Leboeuf et al.,2000). This damage of stored spermatozoa, specially in egg yolk-based extenders, resulted from the inevitable oxidative stress that accelerated by the inability of stored spermatozoa to scavange superoxide anions (0_2) and hydrogen peroxides (Bilodeau et al.,2000) . In turn, hydrogen peroxide either initiates sperm capacitation and acrosome reaction (O'Flaherty et al.,1997) or interacts with superoxide anions to give rise to the formation of the hydroxyl radicles which are powerful initiators of lipid peroxidation cascade in spermatozoa (Calamera and Quiros,1996).

The current study indicated that prestorage incorporation of thiourea (50 mg%) in goat semen extenders (with or without egg yolk) was able to maintain motility and viability of spermatozoa during in-vitro incubation at 5°C for more than 168 hours. This improvement in the quality of stored spermatozoa might be due to thiourea at this concentration could scavange preferentially the stronger oxidants such as hydroxyl radical (Halliwell and Gutteridge,1990). An alternative mode of the antioxidative action of thiourea is its protective effect for ascorbate through chelation of trace amounts of catalytically active iron (Gavella et al.,1997) present in goat seminal plasma (El

Anwar and Badr,1996). Also , thiourea in the beneficial dose (50 mg %) binds to iron leading to inhibition of lipid peroxidation (Gutteridge et al.,1979) and protection of thiol group from oxidation (Ochsendorf et al.,1998). Nevertheless , in comparison to the beneficial dose (50 mg %) of thiourea, low values of sperm motility and viability was recorded in the present study through incubation (at 5°C) of goat semen with the low dose (10 mg %). The most plausible explanation for this reduction is a disturbance in the structure of proteins and enzymes such as lactate dehydrogenase as its concentration increased beyond the protective concentration of alanin and taurine in goat seminal plasma (Jones and Mann,1977). Concomitantly, in-vitro storage (at 5°C) of goat semen with high dose (100 mg %) of thiourea resulted in dramatically reduction in sperm motility and viability. This reduction might be due to increased avilability of asarbic acid lipid peroxidation (Gavella et al.,1997).

On the same bases, the mammalian spermatozoa were very susceptible to oxidative stresses during the freeze/thaw process (Erokhin and Deryazhentsev,1991). The previous investigators found that the freeze/thaw process could reduce glutathione (GSH) level and superoxide dismutase (SOD) activity in spermatozoa with a subsequent elevation of reactive oxygen species (mainly superoxide anions, hydroxyl radicals and hydrogen peroxide) in thawed semen. As expected, it was observed from the present study that in-vitro augmentation of semen extenders with (50 mg %) thiourea exerted a pronounced improvement in post-thaw sperm motility and viability of processed goat spermatozoa either in the presence or absence of egg yolk in the extender. This cryoprotective effect of thiourea is evident by its support to glycerol through chelation of metal ions such as iron (Karow,1969). Consequently, thiouea increases glycerol avilability to prevent denaturation of proteins and enzymes like superoxide dismutase and glutathione peroxidase.

In accordance with Roca et al.,1997, egg yolk has a detrimental effect on goat spermatozoa. The presence of phaspholipase A (egg yolk coagulating enzyme) in goat seminal plasma (Roy,1957) was shown to hydrolyze egg yolk phospholipids into lysophospholipids such as lysolecithins, which are toxic to spermatozoa (Aamdal et al.,1965) . It is tempting to speculate from the current study that Tris egg yolk free extenders could be used for both incubation (at 5°C) and preservation (at -196°C) of goat semen in presence of 50 mg % thiourea in the extender.

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