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FERTILITY OF FROZEN BUFFALO SEMEN EXTENDED IN TWO COMMERCIAL DILUENTS

BY

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SUMMARY

An experiment was carried out to compare between the effect of Triladyl (Tris based patent diluent) and milk diluent used for freezing buffalo semen on the motility, percentage of spermatozoa with intact acrosomes during two hours of incubation period as well as the pregnancy rate. The results revealed that, the milk diluent was significantly better than Triladyl diluent with regarding to the percent motile sperm directly after thawing, motility percent 2 hrs after incubation at 37°C as well as the pregnancy rate. Meanwhile, the percent of intact acrosome was similar in both diluent

INTRODUCTION

The choice of an extender is an integral part of a successful cryopreservation process. Different extenders were used for bull semen with good results of freezing and fertility. Extenders of bull semen produce different results when used for the preservation of buffalo semen in A.I. centres. Tris based diluent was used with successful results for preservation of bull and buffalo bull semen (Becker, Senger, Aalseth and Marshall, 1977, Senger, Mitchell and Almquist 1983, Dudeja, 1990). Milk diluent was considered one of the best extenders for cryopreservation of bovine semen (Foote and Arriola, 1987, Dhami and Sahni, 1993, Kumar, Sahni and Mohan 1994 a).

An attempt has been made in the present work to test the fertility of frozen buffalo semen using two commercial diluents, Triladyl and milk as diluents for buffalo semen in A.I. centres in Egypt.

MATERIALS AND METHODS

This experiment was conducted in Abassia Centre of frozen buffalo semen, belonging to General Organization for Veterinary Services, Ministry of Agriculture.

Semen was collected by an artificial vagina from four adult (4-6 years old) buffalo bulls. After collection, semen was evaluated for individual motility and sperm concentrations. Good ejaculates with a motility more than 75 % and a sperm cell concentration more than 0.8 x 10^9 sperm cells / ml semen were splited into two portions. First portion was diluted in tris based commercial patent diluent-Triladyl (Mini-Tube Comp. Germany) prepared as one part Triladyl, one part egg yolk and three parts bidistillated water to give a final glycerol concentration of 7 % . Diluents were fortified by 500 I.U Pencillin G-sodium and 0.5 mg Streptomycin/ml diluent. Diluted semen was cooled gradually to + 5°C within 45 minutes. After cooling diluted semen was packaged in 0.25 ml French straws with sperm concentration of 30x10^6 sperm cells/straw and was left to equilibrate in a cold cabinet at 5°C for 2 hours. After equilibration, straws were frozen horizontally in liquid nitrogen vapour for 10 minutes and were rapidly plunged in liquid nitrogen for storage. Skin milk diluent was prepared by dissolving 11 gm skimmed milk powder and 1 gm fructore/100 ml, bidistillated water ,70ml of milk solution with fructose was added to 20ml egg yolk and antibiotices were added to diluent as mentioned with Triladyl diluent. The diluent was splited into two portions, portion A without glycerol (50ml) and 10ml glycerol was added to portion B which kept in cold cabinet at 5°C. The second portion of semen samples was primary diluted to the half rate of dilution with portion A at 35°C. Primary diluted semen was cooled gradually to 5°C within 45 minutes. After cooling, the prediluted precooled semen samples were finally diluted with portion B to give final glycerol concentration 10%. Finally diluted semen was left to equilbrate in cold cabinet at 5°C for 2 hours. After equilbration diluted semen was packaged in 0.25ml straws and frozen as discribed with Triladyl.

For invitro evaluation, two straws of each diluent were thawed in a water bath at 37°C/30 sec. Thawed semen was transfered into prewarmed, clean, narrow glass vials and incubated at 37°C. Semen was examined for individual motility and the percentage of spermatozoa with intact acrosomes was scored in wet unstained mounts using dark field microscopy immediately after thawing and after one and two hours of incubation period.

For deterimining the effect of the two diluents on the fertility of frozen buffalo semen, 308 buffaloes were inseminated with 0.25 ml straws (171 with milk diluent and 137 with Triladyl) thawed in water bath at 35°C at Aussim A.I. Centre at Giza. The inseminated buffaloes were rectally examined 60 days after insemination for pregnancy diagnosis. Statistical analysis (F. test, t. test and Chi-Square) was done according to Snedecor and Cochran (1980).

RESULTS

The percentage of motile spermatozoa and the percentage of spermatozoa with intact acrosomes (PIA), recorded during two hours of incubation period are depicated in table 1. Analysis of variance and Student's (t) test revealed that frozen semen diluted in milk diluent had a significantly higher post-thaw s perm motility immediately after thawing as well as during the first and second hour of incubation period than frozen semen

diluted with Triladyl. On the contrary, there was no significant difference in the PIA between semen samples diluted in milk or Triladyl diluent during the two hours of incubation period.

The effect of time on sperm motility in semen samples diluted in milk diluent significantly appeared during the first hour of incubation period only while the effect of time on the PIA in the same samples was significantly noticed after two hours of the incubation period. On the other hand, the effect of time on sperm motility in semen samples diluted in Triladyl significantly appeared after two hours of incubation period. Nevertheless, the PIA in Triladyl decreased significantly during the two hours of incubation.

One hundred and fifteen (115) non return buffaloes out of one hundred seventy one (171) oestrous buffalo cows inseminated with thawed semen diluted in milk diluent, were diagnosed to be pregnant by rectal palpation after 60 days post-insemination while 56 animals returned to estrus after 20 days post insemination.

On the other hand, out of 137 oestrous buffaloes inseminated with thawed semen diluted in Triladyl, 74 non oestrous females were diagnosed as pregnant while 63 animals showed symptomes of heat after 20 days post-insemination. The pregnancy rate of buffaloes inseminated with thawed semen diluted with milk (67.25 %) was significantly higher [(x2 = 5.244) at $P < 0.05$, 0.025 than that (54.02 %) of animals inseminated with semen diluted in Triladyl.

Table 1: Effect of milk and Triladyle diluent on different semen parameters during two hours of incubation (Means ± S.E)

	Incubation period	Motility %	Intact acrosome %
Milk	0 hr.	64 ± 1.2a *	69.2 ± 1.1 a *
Triladyl		54 ± 1.2 b A	69.2 ± 0.8 aA
Milk	1 hr.	59.5 ± 1.2 a **	63.6 ± 1.1 a **
Triladyl		50 ± 1.5 b AB	62.3 ± 1.4 a BC
Milk	2 hr.	56.5 ± 1.5 a **	59.1 ± 1.1 a ***
Triladyl		47 ± 0.8 bB	60.1 ± 1.5 a C
Milk		67.25	Pregnancy rate (%)
Triladyl		54.02	

Means with different alphapetical superscripts a, b for effect of diluent or A, B,... and stars for the effect of time on semen diluted in Triladyl and milk are significnatly different at least at $P < 0.05$.

DISCUSSION

Dilution and freezing of buffalo semen in skim milk diluent resulted in a significantly high post-thaw sperm motility immediately after thawing as well as during two hours of incubation. Compared with other studies, the loss of motility and live spermatozoa was lower in milk and tris diluent than in citrate one (Kumar Sahni and Mohan, 1994 b). The highest post-thawing motility was recorded in semen diluted with milk diluent with fructose than in tris and citrate diluent.

In another experiment, Kumar, Sahni, Mohan and Bisht (1994) recorded that the highest post-thawing percentage of motile and live spermatozoa in milk diluent containing 5 % egg yolk, 6 % glycerol and 1 % fructose.

Both milk and Triladyl diluents maintained a good post-thaw PIA in frozen buffalo semen without significant differences between them immediately after thawing or during two hours of incubation.

For the bull and buffalo bull semen, the highest values of keeping quality, freezability, post-thawing thermoresistance and fertility and the lowest enzyme leakage were observed in Tris diluent (Dhami and Sahni, 1994). They claimed that tris fructose egg yolk glycerol is a better diluent than citrate egg yolk or lactose egg yolk glycerol and that inclusion of 0.1 % cysteine or EDTA has a beneficial effect on fertility of frozen and liquid semen of bull and buffalo bull semen. Semen diluted in tris diluent with 14 % glycerol and a slightly nonsignificant lower decrease in motility and percentage of live spermatozoa. A higher acrosome maintenance Tris diluent contains 11 % glycerol was reported by Bhosrekar, Mokashi and Purohit, Gokhale and Mangurkar, (1993). A successful trial for freezing buffalo semen in milk diluent without yolk but in the presence of glycerol and sugars was undertaken by Kumar, et al. (1994 a). They claimed that the milk diluent with 3 % glycerol produced the best motility for semen stored in 1.0 or 1.5 % sucrose. Concerning tris diluent, Kumar, Sahni , and Mohan (1994 c) recorded the highest post-thawing motility in frozen buffalo semen diluted in tris diluent with 7 % glycerol, 5 % yolk and 1 % raffinose. Contradicting to our results, Bhosrekar and Ganguli, (1978) reported that egg yolk citrate extender supported greater progressive spermatozoal motility of buffalo semen than skim milk egg yolk diluent and sodabicarbid glucose-fructose egg yolk diluent. Bovine spermatozoa extended and frozen in egg yolk tris diluent had a greater post-thaw viability than those extended in skim milk or egg yolk citrate (Senger, et al. 1983). Dudeje (1990), registered that the maximum motility and live sperm in buffalo semen diluted in tris egg yolk diluent followed by egg yolk citrate and citric acid whey respectively.

Our present fertility trials, the buffalo cows inseminated with semen extended in milk diluent resulted in a higher pregnancy rate than those inseminated with semen extended in triladyl, In

agreement, the fertility trials of Foote and Arriola (1987) resulted in a significantly higher percent of nonreturns cows inseminated with whole milk diluted semen (73.4 %) than those inseminated with egg yolk tris diluted semen (69.5 %)

Moreover, Dhami and Sahni (1993) concluded that both tris and milk diluents were equally efficacious for cryopreservation of bovine semen. They added that the whole milk diluent can be an efficient, inexpensive and readily available substitute for tris diluent for deep freezing of bovine semen.

It can be concluded that milk diluent was efficacious in cryopreservation of buffalo semen with an optimum freezability and a higher fertility rate.

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