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EFFECT OF REDUCING SPERM NUMBERS PER INSEMINATION DOSE ON FERTILITY OF CRYOPRESERVED BUFFALO BULL SEMEN

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ABSTRACT

The objective of this study was to evaluate the effect of reducing sperm numbers per insemination dose on fertility of cryopreserved buffalo bull semen. For this purpose, semen was collected at weekly intervals from a Nili-Ravi buffalo bull (*Bubalus bubalis*) using an artificial vagina in two batches. The ejaculates were split-sampled and diluted at 37°C with tris-citric acid extender having $15x10^6$ or $30x10^6$ motile spermatozoa/0.5 ml. After dilution, the semen was cooled to 4°C, equilibrated for 4 hours, packaged in 0.5 ml straws and frozen in programmable cell freezer. Fertility test based on 75-days first service pregnancy rate was determined under field conditions. A total of 500 buffaloes were inseminated with frozen semen and out of these 431 could be followed, 209 for semen straws packaged with $15x10^6$ spermatozoa/straw and 222 for doses filled with $30x10^6$ spermatozoa/straw. The inseminations were performed in two batches and each batch was spread over a period of three months. The fertility rate for sperm concentration of $15x10^6$ spermatozoa/0.5 ml vs. $30x10^6$ spermatozoa/0.5 ml (49.28 vs. 56.75%) was similar (P>0.05). The fertility rates were also similar (P>0.05) in the first and second batch of inseminations performed with $15x10^6$ or $30x10^6$ spermatozoa/0.5 ml straw of cryopreserved semen. In conclusion, reduction of sperm number from $30x10^6$ to $15x10^6$ spermatozoa/0.5 ml dose of insemination did not affect fertility of cryopreserved buffalo bull semen.

Key words: Sperm number, frozen semen, fertility, AI, buffalo.

INTRODUCTION

Numerous efforts have been made to find the optimal sperm concentration per insemination dose without compromising the bull fertility (Abbas *et al*., 2001). The influence of number of spermatozoa per insemination dose of cryopreserved cow bull semen revealed that 5 to $15x10^6$ progressively motile spermatozoa are necessary to achieve an acceptable level of fertility (Sullivan, 1970; Hafs, 1971). Usually the sperm number varies from 10 to $30x10^6$/deep-frozen dose with varying results and in some studies even 1 or 5 to $20x10^6$ spermatozoa/cryopreseved dose has also been analysed in cattle (Foulkes *et al*., 1977; Schenk *et al*., 1987; Foote and Kaproth, 1997; Den-Daas *et al*., 1998; Nehring and Rothe, 2003). In buffaloes, different insemination doses of <20, 20-30, 30-40, 40-50, 50-60 and >60 million frozen thawed spermatozoa resulted in similar fertility rate (Tahir *et al*., 1981). Allen and Seidel (1996) suggested that with un-frozen cow bull semen, even $1x10^6$ or $2.5x10^6$ sperm per insemination dose produced reasonable pregnancy rates. Kommisrud *et al*. (1996) compared fertility rates following insemination with 12, 15 and 18 million spermatozoa per insemination dose of frozen Norwegian cattle bull semen, and found that there was no difference between 12 or 18 million sperm for high-fertility bulls. More recently, Anderson *et al*. (2004) found that an insemination dose of 2 million as compared with 15 million spermatozoa was too low for most bulls to achieve acceptable pregnancy rates in dairy cows. This study describes the effect of reducing spermatozoa per 0.5 ml dose of insemination on fertility of cryopreserved buffalo bull semen.

MATERIALS AND METHODS

Semen collection

Semen was collected by using an artificial vagina (42°C) at weekly intervals in two batches from an adult Nili-Ravi buffalo bull (*Bubalus bubalis*) maintained at the Livestock Research Station, National Agricultural Research Centre, Islamabad, Pakistan. The ejaculates for first and second batch were collected during the months of August/September 2001 and July/August 2002, respectively. Each batch of collection was spread over a period of five weeks. Immediately after collection, visual motility of each ejaculate was assessed by using phase contrast microscope (400X). Sperm concentration was assessed by digital spectrophotometer at 560 nm wave length. Semen samples possessing more than 60% motility and $500x10^6$ spermatozoa/ml were used. A total of six ejaculates were used in the first batch, whereas eight ejaculates were used in the second batch for further processing.

Semen dilution

Tris-citric acid (TCA) was used as a buffer for the experiment, which consisted of 1.56 g citric acid

(Fluka, Switzerland) and 3.0g Tris-(hydroxymethyl)-aminomethane (Sigma, USA) dissolved in 74 ml distilled water (pH 6.9 and osmotic pressure 320 mOsmol/kg). Egg yolk (20%), fructose (0.2%), glycerol (6%), benzyl penicillin (1000 IU/ml) and streptomycin sulphate (1000 µg/ml) were added to the experimental extender.

Semen processing

After a holding time of 10 minutes at 37°C, semen was diluted with TCA extender to achieve final concentration of $15x10^6$ or $30x10^6$ motile spermatozoa/0.5 ml. After dilution, the semen was cooled to 4°C in 2 hours @ 0.275°C/minute and equilibrated for 4 hours at 4°C. Semen was then filled in 0.5 ml straws and frozen in programmable cell freezer (Andrabi *et al*., 2001).

Artificial insemination and pregnancy diagnosis

A total of 500 buffaloes with clinically normal reproductive tract and showing signs of true oestrus were inseminated. Out of these, 431 animals could be followed, including 209 for AI straws packed with $15x10^6$ spermatozoa/straw and 222 for doses filled with $30x10^6$ spermatozoa/straw. All the experimental inseminations were performed approximately 24 hours after onset of heat. The artificially bred animals were examined for pregnancy through rectal palpation at least 75 days post-insemination. The inseminations were performed in two batches and each batch spread over a period of three months.

Statistical analysis

The data on fertility were compared by using chi-square statistics (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The data on effect of reducing sperm concentration per 0.5 ml insemination dose on fertility of cryopreserved buffalo bull semen are presented in Table 1. The fertility rate for sperm concentration of $15x10^6$ vs. $30x10^6$ spermatozoa/0.5 ml (49.28% vs. 56.75%) was similar (P>0.05). The fertility rates were similar (P>0.05) in the first and second batch of inseminations performed with $15x10^6$ or $30x10^6$ spermatozoa/0.5 ml straw of cryopreserved buffalo bull semen (Table 2).

These results are similar to those of Tahir *et al.* (1981), who observed similar conception rate with different spermatozoa concentrations per insemination dose of frozen buffalo semen ranging from <20 to >60 $x10^6$/0.5 ml dose. Similarly in cattle, Kommisrud *et al.* (1996) recorded no difference in fertility of frozen cow bull semen packed in 0.25 ml straws having concentration of 12 or 18 ($x10^6$) sperm. Our findings are also in concomitant with those of Foote and Kaproth (1997), who found that under better field conditions, total sperm concentration per 0.5 ml of AI straw can be reduced to $10x10^6$ sperm without compromising the non-return rates in dairy cattle. Recently, Nehring and Rothe (2003) found a sperm concentration of $15x10^6$ sperm/fine straw (0.25 ml) optimum for fertility of frozen dairy bull semen. Anderson *et al.* (2004) found an insemination dose of $15x10^6$ spermatozoa packaged in fine straw to be significantly better as compared with an AI dose of $2x10^6$ sperm.

The overall conception rate of the present study through AI in buffaloes with varying sperm concentration falls within range reported earlier (Tahir *et al*., 1981; Andrabi *et al*., 2001). However, Anzar *et al*. (2003) found a lower conception rate in buffaloes as compared to the present studies. This variation might be

Table 1: Effect of reducing sperm numbers per insemination dose on fertility of cryopreserved buffalo bull semen

Sperm concentration ($x10^6$/0.5 ml)	No. of inseminations performed	No. of pregnancy tests performed	Pregnancies achieved	Chi-square
15	250	209	103 (49.28%)	2.415^{NS}
30	250	222	126 (56.75%)	

NS=Non-significant.

Table 2: Effect of reducing sperm numbers per dose of insemination on fertility of cryopreserved buffalo bull semen in relation to batch of inseminations performed

Batch No.	No. of inseminations performed	Sperm concentration ($x10^6$/0.5 ml)	No. of pregnancy test performed	Pregnancies achieved	Chi-square
1	125	15	89	42 (47.19%)	1.750^{NS}
	125	30	104	59 (56.73%)	
2	125	15	120	61 (50.83%)	0.846^{NS}
	125	30	118	67 (56.78%)	

NS=Non-significant.

due to technical know how and geo-climatic reason (Andrabi *et al*., 2001). According to Younis *et al.* (1999), besides semen quality, fertility rates are also affected by a number of other factors including female reproductive status and genetic, management and nutrition.

In summary, reduction of sperm number from $30x10^6$ to $15x10^6$ spermatozoa/0.5 ml per insemination dose did not affect fertility of cryopreserved buffalo bull semen investigated through pregnancy rate under field conditions.

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