# FERTILITY OF FROZEN-THAWED SEMEN COLLECTED FROM YOUNG, ADULT AND OLD BUFFALO BULLS DURING LOW AND PEAK BREEDING SEASONS

M. Younis, H.A. Samad, N. Ahmad and I. Ahmad
*Department of Animal Reproduction, University of Agriculture, Faisalabad-38040, Pakistan*

## ABSTRACT

Ejaculates were collected from 18 Nili-Ravi buffalo bulls belonging to young (3-4 year), adult (6-8 year) and old (12-15 year) age groups during the low and the peak breeding seasons. These ejaculates were diluted with lactose-fructose-egg yolk glycerol extender, frozen and stored at -196°C at least for 24 hrs. Frozen-thawed ejaculates were used to inseminate 971 buffaloes with clinically normal reproductive tract. These buffaloes were examined for pregnancy through rectal palpation two months after insemination. The results showed that semen from young (42.81 ± 1.48%) and adult (45.14 ± 1.37%) bulls had higher ($P<0.05$) fertility rate than old (37.46 ± 1.75%) bulls. Similarly, semen samples collected during the peak breeding season showed higher ($P<0.05$) fertility (44.87 ± 1.01%) than those collected during the low breeding season (38.73 ± 1.72%). Fertility of frozen-thawed semen was correlated positively with post-thaw motility ($r = 0.40$, $P<0.05$) and liveability ($r = 0.44$, $P<0.01$) of spermatozoa. Abnormal sperm heads ($r = -0.52$, $P<0.01$) and abnormal acrosomes ($r = -0.41$, $P<0.05$), and GOT ($r = -0.45$, $P<0.01$) and GPT ($r = -0.49$, $P<0.01$) activities in frozen-thawed seminal plasma showed negative correlations with fertility.

## INTRODUCTION

In the Nili-Ravi buffaloes of Pakistan, most of the calvings take place in July and August and the maximum breeding takes place during September-November, which is usually considered the peak breeding season for animals of this species. On the other hand, the breeding activity in the buffalo is minimal during May to July i.e. the low breeding season (Jainudeen *et al.*, 1982; Heuer *et al.*, 1987; Pedersen, 1989).

Achievement of high fertility rates is the aim of every farmer and the seasonal changes may also influence the fertilizability of spermatozoa. Semen from cow and buffalo bulls is preserved in frozen form to ensure its long term storage without damaging its fertilizing capability. However, the fertilizing ability of frozen-thawed spermatozoa can be affected by the season when the semen is collected.

The present project was, therefore, carried out to study the conception rates in buffaloes inseminated with frozen-thawed semen collected from young, adult and old bulls during the low and the peak breeding seasons. Attempts were also made to correlate the conception rates with some characteristics of the frozen-thawed semen.

## MATERIALS AND METHODS

### Experimental animals

Eighteen Nili-Ravi buffalo bulls with clinically normal reproductive tract and aged 3 to 15 years were used in this study. Depending on their age, these bulls were divided into 3 groups viz. young (3-4 years), adult (6-8 years) and old (12-15 years), with six bulls in each group (Table 1).

### Feeding and management

The experimental animals were kept at the Semen Production Unit, Qadirabad, District Sahiwal. The bulls were housed in individual pens having sufficient cross ventilation and protection against heat during summer, and an open space for sun-bath in winter. They were fed good quality seasonal green fodder at the rate of 35 to 65 kg per bull. In addition, two to three kg of concentrate (cottonseed cakes and wheat bran) was offered per bull per day. Clean water was provided *ad libitum*.

### Collection and processing of semen:

Semen from experimental bulls was collected once a week with two ejaculates per collection, using an artificial vagina. Immediately after collection, the first and the second ejaculates were pooled and evaluated for the sperm concentration and motility (Nazir, 1988). Semen samples with at least 60% motility were selected for processing. The semen was diluted at 25-30°C by slow and one step dilution method, using lactose-fructose-egg-yolk-glycerol extender (Nazir, 1988) at such a rate to achieve a final concentration of 30 million

spermatozoa per insemination dose of 0.5 ml and filled in 0.5 ml french straws. After an equilibration period of 6 hrs at 5°C, the straws were frozen in liquid nitrogen by rapid method of freezing (Ahmed *et al.*, 1980) and stored in a liquid nitrogen container for at least 24 hrs before thawing at 37°C for 15-20 seconds in a water bath. Thawed semen samples were evaluated for post-thaw motility and liveability of spermatozoa at 37°C. The activities of GOT and GPT enzymes in the seminal plasma were determined by photometric method recommended by the International Federation of Clinical Chemistry (Anonymous, 1980), using Randox GOT and GPT kits.

**Insemination of buffaloes**

A total of 971 buffaloes with clinically normal reproductive tract were used for insemination during the peak breeding season. In these buffaloes, heat was detected through rectal palpation and animals showing signs of true heat were inseminated with frozen thawed semen. These buffaloes were examined for pregnancy through rectal palpation at least two months after insemination.

**Statistical analysis**

Conception rates for semen samples collected from bulls of three age groups during the low and the peak breeding seasons were worked out. In order to see the magnitude of variation in conception rates among bulls of three age groups and the two seasons, the data were subjected to analysis of variance, using factorial experiment under completely randomized design (Snedecor and Cochran, 1989). Duncan's Multiple Range test (Duncan, 1955) was applied for multiple mean comparisons, where necessary. Correlation coefficients between conception rates and some parameters of frozen-thawed semen were also worked out.

Table 1: Identification (brand) numbers and years of birth of experimental bulls of the three groups

| Group 1 | | Group 2 | | Group 3 | |
|---|---|---|---|---|---|
| Brand No. | Year of birth | Brand No. | Year of birth | Brand No. | Year of birth |
| B-421 | 1991 | B-300 | 1987 | B-64 | 1980 |
| B-422 | 1991 | B-305 | 1987 | B-125 | 1983 |
| B-427 | 1992 | B-308 | 1987 | B-226 | 1985 |
| B-432 | 1992 | B-339 | 1988 | B-294 | 1983 |
| B-438 | 1992 | B-345 | 1989 | B-237 | 1983 |
| B-447 | 1992 | B-263 | 1989 | KD-5 | 1982 |

## RESULTS AND DISCUSSION

**Fertility of frozen-thawed semen**

Using frozen-thawed semen, an overall mean conception rate of 41.80 ± 1.02% was observed in the present study (Table 2).It compares favourably with that reported by Chaudhry (1971), Bhavsar *et al.* (1990) and Dhami and Kodagali (1991) who used the frozen-thawed semen collected from buffalo bulls for insemination and achieved 37.90 to 40.1% pregnancy rates. Conception rates of frozen-thawed semen of young (42.81 ± 1.48%) and adult (45.14 ± 1.37%) bulls were higher ($P<0.05$) than old bulls (37.46 ± 1.75%) the difference between the former two groups was non significant (Table 2). Gupta *et al.* (1978) stated that the age of bulls had significant effect on the semen quality, freezability and fertility in buffalo bulls. A significantly higher conception rate ($P<0.05$) was obtained with semen collected in the peak breeding season (44.87 ± 1.01%) than that collected during the low breeding season (38.73 ± 1.72%). These findings are comparable to those reported by Heuert *et al.* (1987) who observed lower conception rates for semen collected in June (34%) than that in November (41%). Similarly, conception rate of 34.61 and 30.64% in winter and summer seasons, respectively, have been reported in Indian buffaloes (Singh *et al.*, 1992), although the values are lower than those observed in the present investigation. Besides semen quality, the conception rates are affected by a number of other factors including female reproductive status and genetic, managemental and nutritional factors.

Table 2: Mean values (± SEM) of conception rates (%) in buffaloes inseminated with frozen-thawed semen collected from bulls of three age groups during the low and the peak breeding seasons.

| Age Groups | Low breeding season | Peak breeding season | Overall mean |
|---|---|---|---|
| Young | 40.02±2.29a | 45.59±1.14a | 42.81±1.48a |
| Adult | 41.84±1.53a | 48.81±0.76a | 45.14±1.37a |
| Old | 34.70±1.02b | 40.22±2.50b | 37.46±1.75b |
| Mean | 38.73±1.72A | 44.87±1.01B | 41.80±1.02 |

Values with different lower case letters in a column and upper case letters in a row differ significantly ($p<0.05$).

**Correlation of semen attributes with fertility**

The results of the present study showed that there was a significant positive correlation between post-thaw motility ($r = 0.40$, $P<0.05$) and liveability of

spermatozoa ($r = 0.44$, $P<0.01$) and fertility. However, the fertility was negatively correlated with abnormal sperm heads ($r = -0.52$, $P<0.01$) and abnormal acrosomes ($r = -0.41$, $P<0.05$). According to Saacke and White (1972), correlation between post-thawed sperm motility and fertility for frozen semen of buffalo bulls varied from 0.21 to 0.79.

The GOT and GPT activities in frozen-thawed seminal plasma had significant negative correlation ($r = -0.45$ and $-0.49$, respectively, $P<0.01$) with fertility. It supports the idea that the conception rate was greatly influenced not only by post-thaw motility but also by leakage of transaminase enzymes during freezing of buffalo semen (Nath *et al.*, 1991).

Higher conception rates for ejaculates collected from young and adult than the old bulls might be due to higher post thaw motility and liveability of spermatozoa from young and adult bulls (Younis, 1996). Same may be true for higher conception rates during the peak than the low breeding season. This idea is supported by the significant positive correlations recorded between the post thaw motility and liveability of spermatozoa and their fertility.

The results of this study suggest that for achieving higher conception rates with frozen semen, young and adult bulls with outstanding genetic potential may be preferred over old bulls. Furthermore, attempts should be made to collect maximum possible ejaculates during the peak breeding season. These ejaculates may be frozen for use as and when required.

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