

## EFFECT OF ANTIBIOTICS IN EXTENDER ON FERTILITY OF LIQUID BUFFALO BULL SEMEN

S. AKHTER, M. SAJJAD, S. M. H. ANDRABI<sup>1</sup>, N. ULLAH<sup>b</sup>, M. QAYYUM<sup>a</sup>

<sup>a</sup>Department of Zoology, University of Arid Agriculture, Rawalpindi 46300, Pakistan; <sup>b</sup>Animal Reproduction Laboratory, Animal Sciences Institute, National Agricultural Research Centre, Islamabad 45500, Pakistan

### ABSTRACT

This study was carried out to determine if a new antibiotic combination comprising of gentamycin, tylosin and linco-spectin (GTLS) in extender is suitable for improvement in fertility of liquid buffalo bull semen through artificial insemination (AI). Two consecutive ejaculates per week (4 weeks) were collected from three Nili-Ravi buffalo bulls of known fertility by using artificial vagina (42°C). The pooled ejaculates were split-sampled and diluted with skimmed milk extender (37°C; 10x10<sup>6</sup> motile spermatozoa/ml) containing either SP (streptomycin 1000 µg/ml and penicillin 1000 iu/ml) or GTLS (gentamycin 500 µg/ml, tylosin 100 µg/ml, lincomycin 300 µg/ml, and spectinomycin 600 µg/ml). Liquid semen was stored at 5°C for seven days. Fertility test based on 90-days first service pregnancy rate was determined under field conditions. The fertility rates for SP-based vs. GTLS-containing liquid semen of buffalo bull were 58.55 and 60%, respectively, the difference was non significant. The fertility rates also did not differ (P>0.05) due to antibiotics at different days of storage of liquid semen at 5°C. In conclusion, GTLS, in skimmed milk extender compared to SP, did not significantly improve the fertility of chilled buffalo bull semen.

**Key words:** Antibiotics, fertility, liquid semen, buffalo bull.

### INTRODUCTION

Addition of antibiotics to semen extender was one of the first major advances to significantly improve the fertility potential of artificial insemination (AI) in bovine (de Jarnette et al., 2004). Traditionally, streptomycin and penicillin (SP) is the antibiotic combination that has been added to the diluents for buffalo bull semen (Hussain et al., 1990; Ali et al., 1994; Sansone et al., 2000; Andrabi et al., 2001). However, current international standards (Certified Semen Services (CSS), 2002) with regard to the antibiotic components of semen extenders have made it necessary to look for alternatives for the SP-containing extender for buffalo bull semen preservation.

It has been demonstrated that the combination of gentamycin, tylosin and linco-spectin (GTLS) is more effective for controlling various micro-organisms including mycoplasmas, ureaplasmas, *Campylobacter fetus*, *Haemophilus somnus*, and *Pseudomonas* in bovine semen than other antibiotics or combination of antibiotics added to extenders (Shin et al., 1988; Guerin and Thibier, 1993). Also systematic studies of the relatively new antibiotic combination (GTLS) have revealed that it is not detrimental to post-thaw semen quality or fertility in bovine (Gerard et al., 1995; Bousseau et al., 1998, Hasan et al., 2001; Andrabi et al., 2001).

Effect of the relatively new antibiotic combination (GTLS) on fertility of frozen-thawed semen has been assessed in cattle (Kommisrud et al., 1996; Bousseau et al., 1998), and sparsely reported in buffalo (Andrabi et al., 2001). While, there is no information available on effect of GTLS on fertility of liquid buffalo semen. Therefore, the present study was conducted to determine the suitability of GTLS in extender for improvement in fertility of chilled buffalo bull semen through AI under field conditions.

### MATERIALS AND METHODS

#### Extender preparation

Skimmed milk (10%; w/v) based extenders were prepared for the study. The first extender (SP) comprised of streptomycin sulphate (Hebei, China) added at the rate of 1000 µg/ml and benzyl penicillin (Hebei, China) added at the rate of 1000 iu/ml. Second extender (GTLS) contained gentamycin sulphate (500 µg/ml; Reckitt Benckiser, Pakistan), tylosin tartrate (100 µg/ml; VMD, Belgium), lincomycin hydrochloride (300 µg/ml; Pharmacia & Upjohn, Belgium) and spectinomycin hydrochloride (600 µg/ml; Pharmacia & Upjohn, Belgium) as described previously (Andrabi et al., 2001).

### Semen collection and evaluation

Two consecutive ejaculates per week (4 weeks) were collected from three adult Nili-Ravi buffalo bulls of known fertility by using artificial vagina (42°C). The bulls were maintained at Semen Production Unit, Qadirabad, District Sahiwal, Pakistan.

After collection, semen was immediately transferred to laboratory. Visual motility was assessed microscopically (x400; Olympus) with closed circuit television. Sperm concentration was assessed by using Neubauer haemocytometer (Germany). The neat semen samples with more than 60% motile spermatozoa were used for further dilution. Semen of the three buffalo bulls was pooled before dilution to eliminate bull effect. The semen was given a holding time of 10 to 15 minutes at 37°C in water bath before extension.

### Semen processing

The pooled ejaculates were split-sampled and diluted at 37°C in a single step with one of the two experimental extenders in order to contain approximately  $10 \times 10^6$  motile spermatozoa/ml. After dilution, the semen was cooled to 5°C in 2 hours and finally stored at 5°C for seven days. Inseminations were performed with experimental semen starting after 24 hours of storage.

### Artificial insemination and pregnancy diagnosis

A total of 206 inseminations with liquid buffalo semen were recorded, in Tehsil Chichawatani, District Sahiwal and Tehsil Kahore Pacca, District Lodhran, Pakistan. The dose of AI was 1 ml and inseminations were performed over three months during the peak breeding season (October to December, 2005). 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 90 days post-insemination under field conditions.

### Statistical analysis

The data on fertility rate were compared by using chi-square statistics (MINITAB® Release 12.22, 1998).

## RESULTS

The data on effect of two combinations of antibiotics i.e. SP or GTLS, added to extender on fertility of chilled buffalo bull semen is presented in Table 1. The fertility rates for SP and GTLS-containing liquid semen of buffalo bulls were 58.55 and 60%, respectively, the difference was non significant. The

fertility rates also did not differ significantly due to antibiotics at different days of storage of liquid semen at 5°C (Table 2).

## DISCUSSION

The findings of present experiment are in line with previous studies which have shown that GTLS as a component of various semen extenders had no negative influence on pregnancy or non-return (NR) rates in bovine when compared with other antibiotics used alone or in combinations. Sullivan et al. (1988) reported no significant effect on seminal quality as measured by field fertility using GTLS or dihydrostreptomycin, penicillin and polymyxin B sulphate with or without linco-spectin in heated whole-milk or egg yolk-sodium citrate extenders. Bousseau et al. (1998) reported a similar trend for *in vitro* and *in vivo* fertility tests conducted with GTLS-based Biociphos plus® extender and Laiciphos® extender containing SP plus linco-spectin. Kupferschmied et al. (1991a) found no difference in fertility rate in cows on using SP or GTL with or without spectinomycin in Tris-diluents prior to deep freezing. Kupferschmied et al. (1991b) also reported no significant difference in NR rates in cows inseminated with frozen-thawed semen containing either SP or GTLS.

However, Andrabi et al. (2001) reported a significantly higher pregnancy rate in buffaloes with GTLS as compared to SP in cryodiluents. Better efficacy of GTLS could be due to difference in method of addition of antibiotics in semen diluent i.e., one step vs. two step. Also the lowered fertility rates in buffaloes reported by Andrabi and colleagues (2001) with SP as compared to GTLS-based AI doses could be due to occurrence of pathogenic strains of bacteria particularly pseudomonas in buffalo bull semen resistant to SP (Aleem et al., 1990; Hasan et al., 2001). Results of Shin et al. (1988) have indicated that GTLS has a broader spectrum of microbial control in frozen bovine semen than SP with or without polymyxin B. Thus, presence of effective antibiotics in semen extender significantly reduces the concentration of bacterial metabolites and increases the available energy for spermatozoa (Din et al., 1990; Tanyildizi and Bozkurt, 2003), resulting in better seminal quality/fertility (Lorton et al., 1988).

The presence of micro-organisms, especially the bacteria in the ejaculates can affect fertilization directly, by adhering to spermatozoa (Diemer et al., 1996), impairing their motility (Kaur et al., 1986) and inducing acrosome reaction (El-Mulla et al., 1996). Microbes can also have an indirect effect by producing toxins (Morrell, 2006). Besides semen quality, the fertility

**Table 1: Effect of antibiotics in extender on fertility of liquid buffalo bull semen**

Antibiotics	Inseminations Recorded	Pregnancies achieved	Fertility rate (%)	Chi-square	P- value
SP	111	65	58.55	0.04	0.8338
GTLS	95	57	60.00		

SP=streptomycin sulphate and benzyl penicillin.

GTLS=gentamycin sulphate, tylosin tartrate, lincomycin hydrochloride and spectinomycin hydrochloride.

**Table 2: Effect of antibiotics in extender on fertility of liquid buffalo bull semen at different days of storage**

Antibiotics	Storage time (Day)	Inseminations recorded	Pregnancies achieved	Fertility rate (%)	Chi-square	P- value
SP	2	18	11	61.11	0.55	0.4583
GTLS		15	11	73.33		
SP	3	37	23	62.16	0.13	0.7204
GTLS		24	16	66.66		
SP	4	25	13	52.00	0.18	0.6678
GTLS		10	6	60.00		
SP	5	10	6	60.00	0.23	0.6279
GTLS		14	7	50.00		
SP	6	8	5	62.50	0.18	0.6728
GTLS		15	8	53.33		
SP	7	13	7	53.84	0.00	0.9607
GTLS		17	9	52.94		

SP=streptomycin sulphate and benzyl penicillin.

GTLS=gentamycin sulphate, tylosin tartrate, lincomycin hydrochloride and spectinomycin hydrochloride.

rates can be affected by a number of other factors including female reproductive status and genetic, management and nutrition (Younis et al., 1999; Graham and Moce, 2005). Also this variation might be due to technical know how and geo-climatic reason (Rodriguez-Martinez, 2003). It should also be noted that large numbers of animals are needed to average out the extraneous variation associated with insemination of each female (Amann, 2005).

In summary, the new antibiotic combination, GTLS, in milk based extender compared to the conventional antibiotic combination, SP, did not significantly improve the fertility of chilled buffalo bull semen. However, fertility trials based on higher number of AI are suggested for future studies on antibiotics in extender.

#### Acknowledgements

The authors wish to thank Mr. Iftikhar Mehdi for assistance in artificial insemination and pregnancy diagnosis. Thanks also to Dr. Abdul Sattar for providing the facilities to prepare the experimental buffalo bull semen doses at Semen Production Unit, Qadirabad, District Sahiwal, Pakistan.

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