EFFECT OF TWO DIFFERENT ANTIBIOTIC COMBINATIONS ON FERTILITY OF FROZEN BUFFALO AND SAHIWAL BULL SEMEN

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ABSTRACT

This study was carried out to identify the suitable antibiotic combinations in semen extender for improvement in fertility of frozen semen of buffalo and cow (Sahiwal) bulls to obtain better pregnency rate through artificial insemination (AI). For this study eight first ejaculates, four each from a buffalo and a cow (Sahiwal) bull were used. The ejaculates were split-sampled and diluted with Tris-citric acid extender (at 37°C; 50×10^6 spermatozoa/ml), containing either SP (streptomycin 1000 µg/ml and penicillin 1000 iu/ml) or GTLS (gentamycin 500 µg/ml, tylosin 100 µg/ml and linco-spectin 300/600 µg/ml). There was no difference in post-thaw motility for these samples. Fertility test based on 75-days first service pregnancy rate was determined under field conditions. A total of 400 inseminations were recorded, 200 for each buffalo and cow (Sahiwal) with 100 of each antibiotic combination, respectively. Fertility rates for SP-based frozen semen of buffalo bull were 41.66% and were 55.2% for GTLS-containing frozen semen, respectively. The results for GTLS were higher ($P<0.0001$) than SP. Similarly, fertility rates were higher ($P<0.0001$) for GTLS-based frozen semen of Sahiwal bull (78.78%) than SP-containing frozen semen (69.6%) of the same specie. Fertility rates also differed due to species of donor bulls. They were better ($P<0.0001$) for the frozen Sahiwal bull semen than that of the buffalo bull in both SP and GTLS-based frozen semen samples, respectively. In conclusion, seminal quality measured by field fertility trial indicated GTLS combination of antibiotics added to the semen extender was better for improvement in the fertility of frozen buffalo and Sahiwal bull semen, by yielding better pregnancy rates through AI.

Keywords: antibiotic combination, frozen semen, fertility, buffalo, Sahiwal

INTRODUCTION

The main purpose of preserving semen is to maintain the fertilising capacity of spermatozoa, while diluting the ejaculate to maximise the use of superior genetic bulls (Kommisrad *et al.*, 1996). Moreover, the risk of spreading pathogens through semen needs to be effectively reduced (Hasan *et al.*, 2001).

Use of antibiotics in semen extenders to check the growth of several organisms originating from bulls or from contamination during semen processing, provided a major contribution for the development of AI (Almquist, 1951). Traditionally, at different Semen Production Units (SPUs) of Pakistan, streptomycin and penicillin (SP) is the antibiotic combination that is being added to the semen extender. However, current international standards with regard to the antibiotic components of semen extenders have made it necessary to look for alternatives for the SP-containing extender (CSS®, 1993; Hasan *et al.*, 2001).

It has been demonstrated that the combination of gentamycin, tylosin and linco-spectin (GTLS) is more effective for controlling micro-organisms including Mycoplasmas, Ureaplasmas, *Campylobacter fetus*, *Haemophilus somnus*, and Pseudomonas in bovine semen than the standard combination of SP (Shin *et al.*, 1988; Hasan *et al.*, 2001). These micro-organisms can cause infection in the reproductive tract leading to abortion, lowered fertility or infertility (Friberg, 1980; Hasan *et al.*, 2001). Also systemic studies of this new antibiotic combination (GTLS) has revealed that it is not deterimental to semen quality (Lorton *et al.*, 1988a; Krause *et al.*, 1989; Hasan *et al.*, 2000) or viability (Ericsson *et al.*, 1990) in bovines.

The significance of buffalo and Sahiwal cow in the Livestock economy of Pakistan is unequivocal in terms of milk, beef and hide production (Economic Survey, 1998-99). Effects of non-conventional antibiotics in frozen semen on fertility has been sparsely assessed in cattle (Ahmad and Foote, 1985 and 1986; Lorton *et al.*, 1988b; Kupferschmied *et al.*, 1991; Kommisrud *et al.*, 1996), while no information is available in case of water buffalo. The present study was conducted to determine the suitable antibiotic combination in semen extender for improvement in fertility of frozen buffalo and Sahiwal cow bull semen to obtain better pregnancy rate through AI.

MATERIALS AND METHODS

Semen collection and evaluation

In this study eight first ejaculates, four each from a buffalo bull (P4) and a Sahiwal bull (S2) maintained at Livestock Research Station, National Agricultural

Research Centre, Islamabad, Pakistan were used. Semen was collected in artificial vagina (42°C) at weekly intervals during the months of May and June 2000. The frequency of collection from each bull was two consecutive ejaculates per week. Each ejaculate was taken to the laboratory within a minute and visual motility was assessed by using phase contrast microscope (400X; Olympus BX 40) attached with a closed circuit television. Sperm density was assessed by digital spectrophotometer (Dr. Lange LP 300 SDM, Germany). Semen samples possessing more than 60% motility were used. The semen was given a holding time of 15 minutes at 37°C in water bath before dilution.

Dilution with Tris-citric acid extender

Tris-citric acid (TCA) was used as the buffer for the experimental extenders. It consisted of 1.56 gm citric acid (Fluka, Switzerland) and 3.0 gm tris–(hydroxymethyl)-aminomethane (Sigma, St. Louis, MO) in 74 ml distilled water. The pH of buffer was 6.8 and the osmotic pressure was 320 mOsmol/Kg. Egg yolk (20%; vol/vol), fructose (0.2%; wt/vol; Merck, F.R Germany) and glycerol (6%; vol/vol; Merck, F.R Germany) were added to each of the two experimental extenders.

First extender (SP) contained streptomycin (Sigma, St. Louis, MO) available as streptomycin sulphate 761 iu/mg added at the rate of 1000 µg/ml and penicillin (Antibiotics, Mianwali, Pakistan) available as benzyl penicillin 500,000 iu added at the rate of 1000 iu/ml. Second extender was a combination of four antibiotics (GTLS). It comprised of gentamycin available as gentamycin sulphate (Gibco, Madison, WI) 561 µg/mg, which was added at rate of 500 µg/ml, tylosin tartrate (Elanco, Indianapolis, Indiana) was added at the rate of 100 µg/ml and linco-spectin commercially available as lincomycin hydrochloride (Upjohn Co, Kalamazoo, MI) 50 mg/ml, and spectinomycin sulphate (Upjohn Co, Kalamazoo, MI) 100mg/ml, added at the rate of 300/600 µg/ml (Shin *et al.*, 1988).

Semen processing

The ejaculates were split and diluted at 37°C in a single step with one of the two experimental extenders in order to contain approximately 50×10^6 spermatozoa/ml. After dilution, the semen was cooled to 4°C in 2 hours and equilibrated for 4 hours at 4°C. Semen was then filled in 0.5 ml straws with suction pump at 4°C in the cold cabinet unit (Minitub, Germany) and frozen in programmable cell freezer (KRYO 10 series III, UK) from 4°C to -15°C at the rate of 3°C/minute and from -15°C to -80°C at the rate of 10°C/minute. Straws were then plunged into liquid nitrogen (-196°C) and stored.

Semen quality control and AI

After 24 hours of storage in liquid nitrogen (-196° C), post-thaw seminal quality (motility) was assessed by routine procedure as previously described (Hasan et al., 2001). The percentage of progressively motile spermatozoa in both buffalo and Sahiwal bull were similar (55%) in either of the two extenders (SP and GTLS).

A total of 400 inseminations with frozen semen were recorded, 200 for buffalo bull in Tehsil Hazro, District Attock and 200 for Sahiwal bull in Tehsil Karore Pacca, District Lodhran with 100 of each antibiotic combination, respectively. The artificially bred animals were examined for pregnancy through rectal palpation 75 days post-insemination. The inseminations were performed over three months.

Statistical analysis

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

RESULTS AND DISCUSSION

The data on effect of two different antibiotic combinations added to extender on fertility of frozen buffalo and Sahiwal bull semen is presented in Table 1.

Table 1: Effect of two different antibiotic combinations in extender on fertility rate (%) of frozen buffalo and Sahiwal bull semen.

Species	Antibiotics	No. of insemination s	Fertility rate (%)	Chi-square
Buffalo	SP[1]	100	41.66	43.43 (P<0.0001)
	GTLS[2]	100	55.20	
Sahiwal	SP	100	69.60	13.74 (P<0.0001)
	GTLS	100	78.78	

[1] SP=streptomycin, 1000 µg/ml and penicillin, 1000 iu/ml.
[2] GTLS=gentamycin, 500 µg/ml, tylosin, 100 µg/ml plus linco-spectin, 300/600 µg/ml.

The fertility rates for SP-based frozen semen of buffalo bull were 41.66% and were 55.2% for GTLS-containing frozen semen, respectively. The results for GTLS were higher (P<0.0001) than SP. Similarly, fertility rates were significantly higher (P<0.0001) for GTLS-based frozen semen of Sahiwal bull (78.78%) than SP-containing frozen semen (69.6%) of the same specie. These findings are in line with previous studies which have shown that GTLS as a component of various semen extenders had no negative influence on

non-return rates in cattle compared with SPS (streptomycin, penicillin and polymyxin B sulphate) (Lorton *et al.*, 1988b; Kupferschmied *et al.*, 1991). Results of Shin *et al.* (1988) and Hasan *et al.* (2001) have indicated that these antibiotics (GTLS) have a broader spectrum of microbial control in frozen bovine semen than SP. Thus, presence of effective antibiotics in semen extender significantly reduces the concentration of bacterial metabolites and increases the available energy for spermatozoa (Ala-ud-Din *et al.*, 1990), resulting in better seminal quality/fertility (Lorton *et al.*, 1988b). Influence of GTLS has also been compared after *in vitro* fertilisation and culture of bovine embryos, with higher cleavage rate for GTLS-containing Biladyl® (TCA) extender (Lonergan *et al.*, 1994). In contrast to our findings, Komnisred *et al.* (1996) reported a little higher non-return rate with SP-containing semen extender as compared to GTLS-based extender in cattle. This variation may be attributed to the difference in semen dilution system. According to Younis *et al.* (1999) other than semen quality, the fertility rates are affected by a number of other factors including female reproductive status and genetic, management and nutrition.

The effect of two different antibiotic combinations added to extender on fertility of frozen buffalo and Sahiwal bull semen in relation to their specie is presented in Table 2. The fertility rates differed due to specie of donor bulls. They were better (P<0.0001) for the frozen Sahiwal bull semen than that of the buffalo bull in both SP and GTLS-containing frozen semen samples (41.66 vs 69.6% and 55.2 vs 78.78%; Average = 48.43 vs 74.34%), respectively. These results are similar to those of Ala-ud-Din *et al.* (1990), who observed lower conception rate with frozen buffalo semen compared with that of cow bull.

Table 2: Effect of two different antibiotic combinations in extender on fertility rate (%) of frozen semen of buffalo and Sahiwal bull in relation to their specie (donor bull).

Antibiotics	Species	No. of inseminations	Fertility rate (%)	Chi-square
SP[1]	Buffalo	100	41.66	43.43 (P<0.0001)
	Sahiwal	100	69.60	
GTLS[2]	Buffalo	100	55.20	13.74 (P<0.0001)
	Sahiwal	100	78.78	

[1] SP=streptomycin, 1000 µg/ml and penicillin, 1000 iu/ml.
[2] GTLS=gentamycin, 500 µg/ml, tylosin, 100 µg/ml plus linco-spectin, 300/600 µg/ml.

The occurrence of Pseudomonas and *E.coli* in buffalo bull semen (Aleem *et al.*, 1990) abundantly, as compared with that of Sahiwal bull semen (Hasan *et al.*, 2001), could also be linked to lowered fertility rates observed in buffaloes. However, results of Ali *et al.* (1985) and Farooq, (1998) regarding conception rate in buffalo and cattle varied from that of present study. They reported slightly higher conception rate in buffalo than cattle through AI. This difference may possibly be attributed to the improper handling of frozen semen in case of cattle. Also this variation might be due to technical know how and geo-climatic reason.

In summary, the new antibiotic combination, GTLS, in semen extender compared to the conventional antibiotic combination, SP, resulted in significant improvement in the fertility of frozen buffalo and Sahiwal bull semen, by obtaining better pregnancy rate through AI. In conclusion, seminal quality measured by field fertility trial indicates that GTLS combination of antibiotics is more suitable in semen extender for improvement in the fertility of frozen buffalo and Sahiwal cow bull semen.

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