

Effect of Addition of Antibiotics on Liveability, Liveability Index and Viable Bacterial Count of Lohi Ram Semen

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ABSTRACT

Semen of six Lohi rams of two age groups (12-18 and 19-36 months) was used. The effect of addition of different antibiotics in extender on sperm motility percentage, liveability of spermatozoa, absolute index of liveability and bacterial count was recorded. The sperm motility percentage did not differ significantly ($P < 0.05$). Liveability of spermatozoa and absolute index of liveability was significantly lower in control samples ($P < 0.05$). The viable bacterial count was higher in the semen of older rams. The addition of gentamycin sulphate and kanamycin sulphate significantly decreased the viable bacterial count ($P < 0.05$). It was concluded that gentamycin sulphate and kanamycin sulphate are very effective in controlling the bacterial microflora of Lohi ram semen.

Key Words: Lohi ram semen; Antibiotics; Bacterial count

INTRODUCTION

The sheep population in Pakistan has been estimated to be 23.5 million heads (Anonymous, 1998). Sheep and goats contribute 4 per-cent of the total milk produced in the country, bulk of which is produced in the hilly areas, where they constitute the major livestock population. In spite of its vital role in the livestock economy lesser efforts have been made to explore its productive and reproductive potentials.

Because of its polytocous nature and early maturity, sheep population can be increased tremendously using the artificial insemination. The knowledge and expertise in allied techniques especially that of semen preservation bear significance if artificial insemination has to be used with its fullest potential. Semen from apparently healthy male contains many types of microorganisms (Rehman *et al.*, 1983). Additionally the environmental bacteria may also gain access and further increase the number of microflora in the semen. Addition of antibiotics in the extender controls the growth of microorganisms (Kohler &

Samuilidis, 1984). Much work has been done on the preservation of cattle and buffalo semen using different antibiotics in semen extender but little attempts have been made so far to test the efficacy of adding different antibiotics to the ram semen extender.

The project was therefore designed to study the effect of different antibiotics i.e. combiotic, ampicillin, gentamycin sulphate and kanamycin sulphate on the semen characters and viable bacterial count in liquid semen of Lohi rams.

MATERIALS AND METHODS

The study was conducted at Livestock Production Research Institute Bahadurnagar, District Okara. Two groups of Lohi rams each comprising of three rams were used in this study. Rams of group I ranged from 12-18 months of age (younger rams) while those in group II were 19-36 months of age (older rams). Rams were kept under similar managemental conditions. Semen was collected hygienically using artificial vagina. Two semen collections each comprising of two

Table I. Composition of experimental extenders

Ingredients	Experimental diluters				
	A	B	C	D	E
Tris (gm)	3.63	3.63	3.63	3.63	3.63
Citric acid (gm)	1.99	1.99	1.99	1.99	1.99
Fructose (gm)	0.4945	0.4945	0.4945	0.4945	0.4945
Egg yolk (ml)	10.00	10.00	10.00	10.00	10.00
Glycerol (ml)	5.00	5.00	5.00	5.00	5.00
Combiotic ($\mu\text{g/ml}$)	-	1000	-	-	-
Ampicillin ($\mu\text{g/ml}$)	-	-	250	-	-
Gentamycin sulphate ($\mu\text{g/ml}$)	-	-	-	500	-
Kanamycin sulphate ($\mu\text{g/ml}$)	-	-	-	-	500
Redistilled	Water upto 100ml				

ejaculates were made at 6 hours interval twice a week for four weeks. At each collection the semen from two ejaculates was pooled and subjected to macro and microscopic examinations. Semen showing at least 60% sperm motility was further processed. Each semen sample was divided into five parts, four of which contained different antibiotics and one served as control. The composition of extenders with and without the addition of antibiotics is given in Table I. The motility percentage, liveability and liveability index of spermatozoa in pooled semen samples was determined after dilution. The viable bacterial count was made by spread plate method (Harry & Paul, 1981). The data was subjected to Complete Randomized Design with factorial experiment (Steel & Torrie, 1980). Means were compared by Duncans Multiple Range test (Duncans, 1955).

RESULTS AND DISCUSSION

There was no difference in the sperm motility percentage immediately after dilution in all the semen sample. Sperm motility percentage (Mean \pm S.D) of 75 ± 5.77 and 78.75 ± 2.50 was recorded at 0 hours collection for younger and older rams respectively, while 61.25 ± 2.50 and 70.00 ± 4.08 was recorded at 6 hours collection in both the age groups, respectively.

The liveability of liquid semen for different diluters both in younger and older rams at 0 and 6 hours of collection has been shown in Table II and III. The liveability of spermatozoa in diluter A which did not contain any antibiotic was significantly lower ($P < 0.05$) than in all other diluters at both collections in both groups of rams. A longer liveability of spermatozoa was recorded when gentamycin (Diluter D) or kanamycin sulphate (Diluter C) was added to the diluters. The

findings of this study are in agreement with those of Sevine *et al.* (1984) and Shahid (1986) who reported that the addition of antibiotics to semen diluter significantly improved sperm motility and liveability. Troster (1979), Kuradshhki (1980) and Saikia *et al.* (1987) also found that the addition of gentamycin sulphate in semen improved the motility percentage and liveability of spermatozoa. However, the results of this study differ from those of Hakim (1991) who found greater sperm motility percentage and liveability hours when ampicillin was added to the semen. Similarly, the absolute index of liveability of diluter A was significantly lower ($P < 0.05$) than rest of the diluters at both collection hours in both the groups of rams (Table II and III). The addition of antibiotic to diluters significantly improved the index of liveability which is in line with the findings of Sevine *et al.* (1984), Shahid (1986) and Hakim (1991), who reported greater sperm motility percentage, liveability and absolute index of liveability by the addition of antibiotics to the diluters. However, greater liveability and liveability index was reported when the ampicillin was added to the diluter. This may be due to the presence of different population of microorganism in the ram semen.

Highest bacterial count was observed in diluters containing no antibiotics in both the age groups at 0 and 6 hours collection. The addition of gentamycin sulphate (Diluter D) resulted in a significant reduction in the bacterial count in both age groups. The increased bacterial count in older group (Table III) may be because of the development of crypts on penile mucosa which harbor the bacteria (Roberts, 1971). Higher number of bacteria were reported in the initial collection which decreased in the successive collection in both age groups. Gunsalus *et al.* (1941), Almquist *et al.* (1949), Meredith (1970) and Pospelov *et al.* (1973) reported

Table II. Liveability, liveability index and viable bacterial count following the addition of antibiotics in the semen of younger rams

Diluters	0 Hours			6 Hours		
	Liveability (Means \pm SD)	Liveability Index (Means \pm SD)	Viable Bacterial Count 10^{-3} (Means \pm SD)	Liveability (Means \pm SD)	Liveability Index (Means \pm SD)	Viable Bacterial Count 10^{-3} (Means \pm SD)
A	^c 12.5 \pm 0.577	^c 430.15 \pm 2.887	^a 127.75 \pm 9.359	^b 10.50 \pm 1.732	^a 268.63 \pm 34.37	^a 121.25 \pm 7.500
B	^b 18.0 \pm 1.414	^b 627.78 \pm 102.054	^a 26.25 \pm 3.755	^a 15.0 \pm 0.957	^d 413.13 \pm 46.295	^b 24.25 \pm 2.986
C	^{ab} 19.25 \pm 1.500	^b 644.88 \pm 105.408	^a 23.50 \pm 5.447	^a 16.25 \pm 1.258	^{cd} 459.88 \pm 45.544	^b 20.75 \pm 4.787
D	^a 20.50 \pm 1.732	^a 761.25 \pm 109.116	^d 11.75 \pm 3.867	^a 17.25 \pm 1.258	^{bc} 537.75 \pm 43.260	^c 9.50 \pm 4.203
E	^a 20.25 \pm 1.50	^a 755.95 \pm 112.141	^d 16.25 \pm 5.439	^a 16.00 \pm 1.258	^{cd} 484.38 \pm 78.458	^c 14.75 \pm 6.131

The values with same superscripts in the same column do not differ ($P > 0.05$)

Table III. Liveability, liveability index and viable bacterial count following the addition of antibiotics in the semen of older rams

Diluters	0 Hours			6 Hours		
	Liveability (Means±SD)	Liveability Index (Means±SD)	Viable Bacterial Count 10 ³ (Means±SD)	Liveability (Means±SD)	Liveability Index (Means±SD)	Viable Bacterial Count 10 ³ (Means±SD)
A	^a 13.25±0.500	^a 447.5±35.709	^a 145.75±5.82	^a 12.00±0.816	^a 375.6±49.091	^a 139.00±4.546
B	^c 17.75±1.258	^c 664.0±76.67	^b 31.00±2.160	^b 16.75±1.258	^b 546.0±58.271	^b 28.50±2.646
C	^b 19.25±41.500	^b 723.3±85.172	^c 25.25±1.50	^c 17.75±1.258	^c 611.1±67.043	^c 24.25±2.500
D	^a 21.00±1.414	^a 816.88±71.776	^d 15.50±2.646	^d 19.00±1.414	^d 672.1±74.260	^d 10.00±3.107
E	^a 20.25±1.500	^a 783.75±83.702	^d 18.00±2.160	^d 18.50±1.291	^d 653.63±74.450	^d 13.54±3.167

The values with same superscripts in the same column do not differ (P>0.05)

variable number of bacteria in semen. The bacterial count was lowest in diluter containing gentamycin sulphate followed by kanamycin sulphate, combiotic and ampicillin. However, the bacterial count did not differ significantly when either gentamycin sulphate or kanamycin sulphate was added to the semen. These results are partially in agreement with the findings of Troster (1979) who recommended that addition of gentamycin sulphate to semen diluent can be used as prophylactic measure to check the bacterial growth. Rehman *et al.* (1983) and Wayda (1991) also reported gentamycin sulphate to be more effective when compared with other antibiotics.

CONCLUSIONS

The result of this study revealed that addition of gentamycin sulphate or kanamycin sulphate at the dose rate of 500 µg/ml was most effective amongst the antibiotics used in checking the bacterial growth and improving the liveability and liveability index of Lohi ram semen.

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