COMPARATIVE EFFECT OF DIFFERENT LEVELS OF GENTAMICIN IN VIABLE BACTERIAL COUNT OF COW BULL SEMEN

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SUMMARY : Semen of two cross bred (Sahiwal and Fresian) and one Sahiwal cow bull was used in this study. Bull wise and ejaculate wise bacterial count was recorded after diluting the semen samples in Lactose-fructose, egg yolk extender. Gentamicin at a dose rate of 0.00, 50.00, 250.00 and 500 µg/ml was added to four fractious of each semen sample. The significant difference was observed in bacterial count between bulls and ejaculates. It was concluded that gentamicin 500 µg/ml is very effective in controlling the bacterial microflora of cow bull semen.

Key Words : Gentamicin, bacterial count, semen.

INTRODUCTION

Improvement of livestock breeds for increased milk and meat production is being carried out in under developed countries and artificial insemination (A. I.) is extensively used for this purpose. Numerous accidents have happened where semen born infectious diseases have been transmitted by A. I. Semen from the perfectly healthy bulls collected under hygienic conditions is supposed to be bacteria free, but it is contaminated by micro organisms derived from the testes, epididymis, vas deferens, accessory sex glands, urethra, preputial cavity and artificial vagina. Contamination may also occur from the atmosphere, teaser animals, un-sterilized equipments and semen extenders (3).

For the success of A. I. technique, it is necessary that semen to be used for breeding is properly evaluated and is free of microbial abnormalities. So antibiotics are added to semen by commercial bovine artificial insemination organizations as a routine practice. It has been proved that gentamicin is more effective antibiotic against the contaminants isolated from the semen samples, but the level of gentamicin at which optimum be obtained is yet to determined. Keeping there facts in mind, a project was planned to study the comparative effect of different levels of gentamicin in the viable bacterial count. It is hoped that results thus obtained will go a long way in improving the quality and fertility of diluted semen.

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MATERIALS AND METHODS

A. Collection of semen

For this study the semen of two cross bred (Sahiwal and Friesian) named S x F_{1Z} and S x F_{1R} and one Sahiwal cow bull

Table 1: Composition of the experimental diluters.

Ingredients	Experimental diluters			
	A	B	C	D
Lactose (gms)	6.05	6.05	6.05	6.05
Fructose (gms)	1.08	1.08	1.08	1.08
Egg Yolk (ml)	20.00	20.00	20.00	20.00
Gentamicin sulphate (µg/ml)				

named S9 kept at semen production unit, Department of Animal Reproduction, University of Agriculture, Faisalabad was used. A total of 12 semen samples, 4 from each bull comprising two first and two second consecutive ejaculates were used.

B. Extension of semen

After collection, evaluation each selected ejaculate was divided into five parts. One part was kept undiluted. While remaining four parts were diluted 1 : 10 with one of the four experimental diluters. The composition of which has been given in Table 1.

After dilution, each sample was further devided into two parts, one was proceeded immediately at 0 hour, while others were kept in refrigerator at 4°C to process after 24 hours.

C. Bacteriological count

For the determination of viable bacterial count the medium was prepared by dissolving 23 gms of dehydrated nutrient agar (Gilco Lab. USA) in 1000 ml of distilled water and heating it to boiling point. The pH was adjusted at 7. The medium was autoclaved at 121°C under 15 lb/inch pressure for 20 minutes. By cooling down to 50-55°C 10% fresh de-fibrinated blood was added and subsequently poured in sterilized petri dishes. In order to check the sterility of media, petri dishes were kept in incubator at 37°C for 24 hours. The viable bacterial count in all semen samples was determined by using spead plate method (4). The bacterial count for undiluted portion of samples was performed once at 0 hour, while for diluted parts performed twice at 0 and 24 hours of storage at 4°C. As a procedure 0.5 ml of semen was added into a test tube having 4.5 ml of Phosphate Buffer solution step wise 1:10, 1:1000 and 1:10.000 dilutions were obtained. By using double set of petri dishes for each dilution 0.5 ml from each diluted samples was spread on nutrient agar plates. The petri dishes were than incubated at 37°C for a period of 24 hours and the number of colonies that arose were counted by colony counter. The number of bacteria present in each petri dish was calculated by multiplying the number of colonies with the dilution rate at which the colonies developed.

RESULTS AND DISCUSSION

The bull wise and ejaculate wise viable bacterial count recorded for undiluted semen at 0 hour and diluted with four experimental diluters at 0 and 24 hours after storage are seen in Tables 2 and 3.

In the present study significant difference was observed in viable bacterial count between bulls. The values for bulls being 31.24, 26.64 and 41.99 thousand/ml of semen respectively. Almquist (1) also observed marked differences in bacterial count between samples from different bulls. Similarly, Bush *et al.* (2) reported that there was a considerable difference between bulls with respect to the range in the number of bacterial/ml of diluted semen. Some bulls consistently had low bacterial count, while others varied considerably from one ejaculate to another in this respect. These views support the findings of present study in which the viable bacterial count for first and second ejaculate averaged 38.27 and 34.95 thousand/ml. The difference being significant. The viable bacterial count,

Table 2: Bull wise viable bacterial count (thousand/ml) recorded at 0 and 24 hours after storage at 4°C of cow bulls semen diluted with four experimental diluters.

DILUTERS									
	A		B		C		D		Ave
	0	24	0	24	0	24	0	24	
S x F_{1Z}	165.50	81.56	1.48	1.08	0.28	0.00	0.00	0.00	41.82* 31.24^{c} 20.65**
Sx F_{1R}	178.25	110.25	2.43	1.53	0.55	0.13	0.00	0.00	45.31* 36.64^{bc} 27.98**
Sg	193.75	136.25	3.11	2.00	0.48	0.15	0.00	0.00	49.34* 41.91^{ab} 34.60**
AVE.	179.17	109.33	2.34	1.54	0.44	0.09	0.00	0.00	45.49* 27.24**
	144.25 ^{a}		1.94^{b}		0.27^{b}		0^{b}		

Table 3: Ejaculated wise viable bacterial count (thousand/ml) recorded at 0 and 24 hours after storage at 4°C of cow bulls semen diluted with four experimental diluters.

DILUTERS									
	A		B		C		D		Ave
	0	24	0	24	0	24	0	24	
E1	186.00	115.00	2.62	1.82	0.57	0.15	0	0	47.30* 38.27^{bc} 29.24**
E2	172.33	103.63	2.05	1.25	0.30	0.03	0	0	43.67* 34.95^{c} 26.49**
Ave.	179.10	109.33	2.34	1.54	0.44	0.09	0	0	45.49* 27.21**
	144.25^{a}		1.94^{b}		0.27^{b}		0^{b}		

Values with similar superscript in same row or column differ non significantly.

* observation at 0 hour.

** observation at 24 hours storage

less in ejaculate second than first is due to that bacteria residing in prepuce and duct system are washed away with first ejaculate. An average bacterial count of 45.49 and 27.74 thousand/ml of semen was observed in samples stored at 0 and 24 hours respectively. The difference being significant. It indicates that storage of semen after 24 hours at 4°C affected the viable bacterial count. These results are in line with the findings of Hendrikse (5) that the bacterial flora in 86 samples of bulls semen diluted with skim milk egg yolk diluent (without Antibacterial Agent) decreased during storage for 3 days. The average bacterial count being 198,000, 12,000 and 109,000 in fresh 24

Table 4: Analysis of variance showing the effects of diluters, bulls and storage periods on viable bacterial count.

Source	D.F.	S.S.	M.S.	F. Value
Ejaculate (E)	2	1858.699	929.350	8.2721**
Time (T)	1	7533.127	7533.127	67.0519**
ExT	2	171.759	85.879	0.7644 N.S
Diluter (D)	3	371000.063	123666.688	1100.7489**
ExD	6	5078.635	846.439	7.5341
TxD	3	21661.690	7220.563	64.2697**
ExTxD	6	553.547	92.258	0.8212 N.S.
Error	72	8089.040	112.348	
Total	95	415946.560		

** = Significant at 0.05 level of probability.

N.S. = Non significant.

Table 5: Analysis of variance showing the effects of diluters, bulls and storage periods on viable bacterial count.

Source	D.F.	S.S.	M.S.	F. Value
Ejaculate (E)	1	267.000	267.000	1.4440 NS
Time (T)	1	7538.443	7538.443	40.7696**
ExT	1	2.013	2.013	0.0109*NS
Diluter (D)	3	371021.876	123673.959	668.8573**
ExD	3	685.189	228.396	1.2352. N.S
TxD	3	21656.473	7218.824	39.0411**
ExTxD	3	5.063	1.688	0.0091 N.S.
Error	80	14792.268	184.903	
Total	95	415968.324		

** = Significant at 0.05 level of probability.

N.S. = Non significant.

and 48 hours old semen respectively. These results are however, not in line with the findings of Shahid (1980) when the semen was diluted with diluents containing antibiotics streptopenicillin, streptopenicillin + kanamycin and ampicillin are equally affective in controlling the bacterial contamination of semen.

Keeping in view the findings of the present study, it can be concluded that gentamicin 500 μg/ml is very effective in controlling the bacterial microflora of cow bull semen. When the effects of the levels of gentamicin sulphate on motility and survival of spermatozoa are considered, the level of gentamicin 500 μg/ml provide the better results than other two levels 250 μg/ml and 50 μg/ml of gentamicin sulphate.

Analysis of variance (Tables 4 and 5) showed that the effect is significant ($p<0.05$). Further analysis by Duncan's multiple A differed from the rest of three diluters B, C and D, significantly.

REFERENCES

1. Almquist JO, Glants RL and Shaffer HE : The effect of combination of penicillin and streptomycin upon the livability bacterial content of bovine semen. J Dairy Sci, 32:183-190, 1949.

2. Bush LJ, Ludwich TM, Ferguson LC and Fordyceely : The effect of bacteria on the fertility of bovine semen. J Dairy Sci, 33:633-638, 1950.

3. Carballo JRM : Materials and containers for processing and shipment of bovine semen. In : FAO Animal Health and Production Paper, No 23, "Diseases control in embryos and semen". Rome, FAO, pp 97-101, 1981.

4. Harry WSJ and Paul JV : Microbes in action. Laboratory manual of microbiology, 3rd ed, pp 37-40. Freeman and Com San Francisco and London, 1981.

5. Hendrikse J : Bacterial content of diluted and stored semen. Tijdscher, Diergeneesk, 88:506-509. (Vet Bull, 33:3362, 1963).

6. Rehman H, Dutta J C, Baro BR and Rajkonwar CK : Studies on bacterial flora of bull semen and their antibiotic spectra. Indian J Comp Microb Immun Infect Dis, 4:110-112, 1983.

7. Shahid H : Comparative effects of different antibiotics on bacterial count, motility and livability of spermatozoa in semen of cow and buffalo bulls. M Sc thesis, Dept Animal Reproduction, Univ Agri, Faisalabad, 1986.

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