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EFFECT OF ANTIBIOTIC TREATMENT ON BACTERIAL LOAD AND PRESERVABILITY OF CHILLED BOVINE SEMEN

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ABSTRACT

The semen samples which were collected from twenty four different bovine bulls were diluted and treated with Streptomycin + Benzyl Penicillin, Streptomycin + Benzyl Penicillin + Gentamicin and Streptomycin + Benzyl Penicillin + Ampicillin combination at different dose levels. The effect of different combinations of antibiotics on semen characteristics was non-significant but the effect on total bacterial count (TBC) was highly significant ($P < 0.01$). The period of preservation had a significant ($P < 0.01$) influence on individual motility of spermatozoa, eosinophilic count, sperm abnormalities, acrosomal integrity and TBC. The most efficient antibiotic combination was SPG followed by SPamp and SP to reduce the bacterial load in chilled semen without affecting the semen quality and preservability of semen.

Microbial contamination of bovine semen may infect the inseminated females and may affect spermatozoa directly or indirectly which will lead to low reproductive efficiency in a herd. The present investigation was, therefore, undertaken to study the effect of various combinations of antibiotics on bacterial load and preservability of bovine semen preserved at refrigerated temperature.

MATERIALS AND METHODS

Twenty four disease free bovine bull (3-5 years of age) belonging to Artificial Insemination Section, National Dairy Research Institute, Karnal were used as semen donor. The bulls were maintained under identical system of management and fed as per N.R.C. standards. Semen was collected by Artificial Vagina (AV) technique¹⁸ taking all aseptic precautions. Each ejaculate was extended by using egg yolk citrate (EYC) extender¹⁴ containing streptomycin and benzyl penicillin (Na salt) (SP), streptomy-

cin and benzyl penicillin and gentamicin sulphate (SPG), streptomycin and benzyl penicillin and ampicillin (SPamp) combinations by split sample technique. For this purpose each semen sample was split into three aliquots to mix EYC extender which were treated with different combinations of antibiotics at following dose level.

Combination of antibiotics	Dose
1. Streptomycin	@ 1000 ug/ml of extender
2. Benzyl penicillin (Na-salt)	@ 1000 IU/ml of extender
3. Gentamicin	@ 1000 ug/ml of extender
4. Ampicillin	@ 1000 ug/ml of extender

Part of the semen sub-samples was kept in ice bucket for microbiological investigation. SP combination was used as control group and neat semen (NS) was used as a benchmark of bacterial count of the samples. The individual motility

eosinophilic spermatozoal abnormalities⁸, acrosomal abnormalities, standard plate count (SPC) (just after dilution) (p_1), hours of preservation at 4°C, processes were carried out in a sterile free air zone by using a laminar flow cabinet which was sterilised by AV sterilisation for 40 minutes. Semen was sterilised in hot air oven at 121°C for 15 minutes. Surface sterilisation was done by exposure to UV rays. Latent infections were checked at weekly interval. The data were analysed¹⁶ for the inter-

Mean value of semen

	P_1	P_2
Individual motility		
SP	67.92 ±1.35	57.2 ±1.6
SPG	69.46 ±1.25	57.9 ±1.5
SPamp	68.00 ±1.36	57.7 ±1.4
Over all av.	68.46 ±1.12	57.6 ±0.8
Sperm abnormalities		
SP	17.33 ±1.06	21.0 ±0.9
SPG	17.29 ±1.05	21.2 ±0.9
SPamp	17.63 ±0.90	21.3 ±0.9
Over all	17.42 ±0.58	21.2 ±0.9

eosinophilic spermatozoa^{3,7}, morphological abnormalities⁸ acrosomal integrity⁷ and standard plate count (SPC)⁶ were studied at 0 (just after dilution) (p_1), 24 (p_2) and 48 (p_3) hours of preservation at 4 to 7°C. All these processes were carried out in contamination free air zone by using spirit lamp. AV was sterilised by AV steriliser (steam sterilisation) for 40 minutes. Glassware were sterilised in hot air oven at 110°C for overnight. Surface sterilisation was done by exposure to UV rays. Lab was fumigated at weekly interval. The data were statistically analysed¹⁶ for the interpretation of results.

RESULTS AND DISCUSSIONS

The data on semen characteristics of antibiotic treated and chilled samples at various intervals are given in Table 1. The effect of different combinations of antibiotic treatment on individual motility of spermatozoa, eosinophilic spermatozoa, abnormal spermatozoa and acrosomal integrity revealed that there was no significant difference between three different combinations of antibiotic treatment on semen characteristics for a particular period of preservation. The individual motility of

TABLE 1
Mean value of semen characteristics of chilled samples of various interval

	p_1	p_2	p_3	Overall average	p_1	p_2	p_3	Overall average
Individual motility (%)					Non-eosinophilic spermatozoa (%)			
SP	67.92 ±1.35	57.21 ±1.69	45.83 ±1.99	56.99 ±1.43	76.92 ±1.12	71.81 ±1.41	64.75 ±1.29	71.13 ±0.94
SPG	69.46 ±1.25	57.92 ±1.56	45.79 ±1.92	57.72 ±1.47	76.88 ±1.16	71.13 ±1.48	64.92 ±1.38	70.97 ±0.97
SPamp	68.00 ±1.36	57.79 ±1.46	46.84 ±2.01	57.28 ±1.42	76.49 ±1.20	70.83 ±1.49	64.46 ±1.38	70.58 ±0.98
Over all av.	68.46 ^a ±1.12	57.64 ^b ±0.89	46.15 ^c ±1.15		76.75 ^a ±0.68	71.22 ^b ±0.85	64.71 ^c ±0.79	
Sperm abnormality (%)					Acrosomal integrity (%)			
SP	17.33 ±1.06	21.08 ±0.93	25.08 ±0.93	21.17 ±0.68	96.17 ±0.34	93.38 ±0.38	91.25 ±0.47	93.60 ±0.33
SPG	17.29 ±1.05	21.29 ±0.91	25.13 ±0.86	21.24 ±0.66	96.04 ±0.28	93.46 ±0.33	91.21 ±0.37	93.57 ±0.30
SPamp	17.63 ±0.90	21.33 ±0.93	25.79 ±0.87	21.58 ±0.65	95.88 ±0.28	93.08 ±0.37	91.25 ±0.51	93.40 ±0.32
Over all	17.42 ^a ±0.58	21.24 ^b ±0.53	25.33 ^c ±0.57		96.03 ^a ±0.25	93.31 ^b ±0.21	91.24 ^c ±0.26	

spermatozoa significantly ($P < 0.01$) dropped as the preservation time passed. It is consistent with the observation of various workers⁹. The non-significant effect of antibiotic treatment on individual motility was agreed by others^{2,1} but according to Panda¹¹ motility was higher in gentamicin treatment as compared to ampicillin or penicillin + streptomycin treatment at 0, 24 and 48 h of preservation. There was significant ($p < 0.01$) influence of period of preservation on live spermatozoa. The eosinophilic count gradually increased after 24 h and 48 h interval but the figures are appreciably lower than those reported in previous studies¹⁵. The harmless effect of antibiotic treatment on sperm survival also get substance from earlier report¹⁷. Panda¹¹ have reported beneficial effect of certain antibiotics on sperm survival. On the other hand, some lethal effect of different antibiotic treatment on spermatozoa has been reported by Codazza et al. (1971). Abnormal spermatozoa count significantly ($p < 0.01$) increased as preservation time passed. Although sperm abnormalities rose with the passage of time but the increment was identical for all antibiotic combinations used in study. The harmless effect of different combinations of antibiotic on sperm morphology is in agreement with the observation of Sykes et al. (1991). There was significant ($p < 0.01$) effect on period of preservation on intact acrosome. It is in conformity with

Chinnaiya (1978) who also reported that the increased acrosomal damage with the process of semen preservation at $4-7^{\circ}\text{C}$ temperature but there is no report available on the study of the effect of different antibiotic combinations on acrosomal integrity. Table 2 reveal the data of mean values of TBC on chilled semen sample at 0, 24 and 48 h of preservation. Different combinations of antibiotic treatment were highly significant ($p < 0.01$) on bacterial count. The reduction in TBC in semen samples after antibiotic treatment recorded during this investigation is consistent with the observations of Nimai Singh et al. (1990). The significant ($p < 0.01$) difference of TBC was also observed due to preservation time. The maximum reduction of TBC was observed by SPG treatment followed by SPamp and SP combination. Same declining trend was seen in each time of preservation. As a whole, maximum reduction was observed at 48 h followed by 24 h as compared with 0 h. It could be attributed to injury of some bacterial cells (mesophilic, thermophilic) sensitive to refrigerated temperature (5°C) used for storage of semen samples. The high efficacy of gentamicin followed by SPamp and SP combination is in agreement with Saikia¹³. Gentamicin controlled the bacterial growth completely without affecting semen quality at different hour of preservation which is also supported by other worker¹.

TABLE 2
Mean Value of SPC (cfu/ml) of chilled semen of various intervals

	P ₁	P ₂	P ₃	Overall average
NS	32727.08	19289.58	14833.33	22283.33
SP	1074.58	780.83	432.08	762.50
SPG	523.33	413.33	229.67	388.78
SPamp	798.75	472.50	239.17	388.78
Overall av.	8780.94	5239.06	3933.56	

The result of SPG treatment is more effective in reducing the bacterial load to bovine semen preserved at $4-7^{\circ}\text{C}$ temperature without affecting motility and preservability. SPamp and SP treatment also showed

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The result of study suggests that the SPG treatment is most efficient to reduce the bacterial load to a great extent in bovine semen preserved at refrigerated temperature without affecting semen quality and preservability. It is followed by SPamp and SP treatment.

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Results

Overall average

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MATERIALS AND

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