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Short Communication

RELATIVE EFFICIENCY OF STERILIZATION METHODS FOR THE TREATMENT OF GLASSWARE PART OF ARTIFICIAL VAGINA TO BE USED FOR BOVINE SEMEN COLLECTION

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ABSTRACT

Present investigation was conducted on Sahiwal, HF crosses and Murrah bulls maintained at A.B. Complex, NDRI, Karnal (India) to identify the relative efficiency of various sterilization methods for the treatment of artificial vagina (AV) to be used for bovine semen processing. The glassware part of AV was sterilized by hot air oven at 160^{0}C for 60 min. and microwave oven for 3 min. A total of 72 ejaculates were taken from 3 collections each from 4 bulls of each breed/species subjected to 2 types of treatments, then total plate count (TPC) of all these combinations was carried out using plate count agar media. Standardization of the best sterilizing method was done by selecting the method whose total bacterial count on the media was the least and the best semen quality (mass activity, initial motility, non-eosinophilic percent and abnormalities) was obtained. The microwave oven method of sterilization of glassware part of artificial vagina was found superior over hot air oven method.

Key words: Artificial vagina, bovine semen, sterilization methods

INTRODUCTION

The important factor in effective handling of semen, *viz.*, the proper cleaning and sterilization of wares, is the one aspect that has remained neglected in semen handling. The procedures for cleaning and sterilization have often been followed in a casual way with little emphasis being given to the critical control points. Even under ideal conditions, some microbial contaminations can occur. In general, accepted norms for microbial populations in semen are 500 non-pathogenic microorganisms per dose of semen (ISI, 1962). The minimum standard protocol (MSP) for semen production as uniform procedure in the semen production is not followed in the entire country. Also, there is a lack of legislative backup for the standardization and certification of semen

collection and processing stations. The quality of semen varies from state to state and there is need to follow minimum standard protocol in semen production. The present programme was envisaged to identify the various loopholes in the existing methodologies of semen production and processing and to develop a suitable package which could be recommended to a semen bank manager for routine use.

MATERIALS AND METHODS

Four healthy adult bulls, each from Sahiwal (SW), Karan-Fries (KF) and Murrah (MU) breed, maintained under identical nutrition and management conditions were selected randomly from the herd at NDRI, Karnal (Haryana). The bulls were washed 30 min. before taking them to the site of collection adjacent to semen processing laboratory in the morning hours at 7.30 AM. Semen was collected by using artificial vagina (AV) of 30 cm size "Danish model" with smooth lining, over a male dummy bull once a week. The temperature of AV was maintained at 42-45°C with sufficient pressure and proper lubrication with sterilized vaseline jelly. On each collection, two ejaculates were taken in succession, and each ejaculate was preceded by a period of sexual preparation consisting of at least two false mounts separated by 1 min. restraint. The second ejaculate was collected 15 min. after the first. Immediately after the collection, each ejaculate was placed in a water bath at 30°C and examined for various standard laboratory tests *viz.*, mass activity, individual motility, non-eosinophilic count, sperm abnormalities, etc.

Sterilization method for artificial vagina (AV)

The glassware part of AV was sterilized by two ways i) hot air oven at 160°C for 60 min., and ii) microwave oven (frequency 2450 MHz, model No. MC-805AA, LG Microwave Appliance, India) for 3 min. A total of 72 ejaculates were taken for study from 3 collections each from 4 bulls of each breed/species subjected to 2 different types of treatments. Then total plate count (TPC) of all these combinations were carried out by using plate count agar media. The standardization of the best sterilizing method was done by selecting the method whose total bacterial count on the media was least and the best semen quality *viz.*, mass activity, initial motility, non-eosinophilic percent and abnormalities was obtained. The number of bacteria (cfu mL^{-1} sample) was calculated as under:

$$\text{No.of bacteria per mL} = \frac{\text{No. of colonies}}{\text{Dilution factor} \times \text{Amount of specimen plated}}$$

The physical parameters of semen recorded were:

Volume: The semen was collected in 15 mL graduated metal free glass tube (0.1 mL accuracy).

Mass activity: The mass motility was assessed just after the semen collection. Gross swirl rating (GSR) of undiluted semen was performed within one min. of collection. Undiluted semen (10 µL) was placed on two places of warmed slide placed on stage warmer (37°C) and scored on a scale of 0-4 using 10X objective lens on the phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan). Motility was expressed qualitatively on a motility scale (0-4) as per the method of Matharoo *et al*. (1985).

Motility estimate (%): Manual progressive motility and percentage motile spermatozoa were determined by placing and mixing 100 µL of undiluted semen into pre-warmed tubes containing 900 µL tris buffer. Twenty microliters of diluted semen was placed on a warmed glass slide (37°C) and allowed to spread uniformly under the cover slip. The strength of motility rating was scored on a scale of 0 to 5 by using 200X magnification with phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) equipped with a 37ºC-heated stage.

Sperm concentration: Sperm concentration was estimated by haemocytometer (Neubauer's chamber) method.

The fresh semen samples were used for the evaluation of semen. Various parameters recorded were i) non-eosinphilic and ii) morphological abnormality. The data was analyzed using the analysis of variance technique (Snedecor and Cochran, 1994). Prior to the analysis, proportionality data (in case of sperm concentration, non-eosinophilic count and motility) was transformed using the arcsine transformation and abnormality data transformed using the square root transformation with adjustment to allow for zero values. Comparison between different treatment groups was done by Fisher's least significant difference (LSD) test. The differences at $p \leq 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

A comparative study of sterilization of glassware part of artificial vagina using hot air oven (HAO) and microwave oven (MO) was conducted. In between the two sterilization methods, overall there was no significant ($p>0.05$) difference in various semen characteristics and microbial load (log cfu mL^{-1}) in bovine bull semen (Table 1). Among the various abnormalities, no significant difference ($p>0.05$) was observed, except the tail abnormality which was slightly ($p< 0.01$) higher in collection tube (CT) sterilized by MO in KF bull semen. There was significantly ($p<0.05$) more total abnormality in the CT treated with MO in KF bull semen as the tail abnormality was the major

Table 1: Semen characteristics (%) and microbial load (log cfu mL^{-1}) of bovine semen (N = 12) obtained after different methods of collection tube sterilization

Parameters (%)		Breed/ species						Overall mean	
		Sahiwal		Karan Fries		Murrah			
		HAO*	MO*	HAO	MO	HAO	MO	HAO	MO
Mass activity	Mean	2.25	2.25	2.54	2.54	2.67	2.67	2.49	2.49
	SE	0.16	0.16	0.11	0.11	0.19	0.19	0.15	0.15
Sperm motility	Mean	53.33	52.92	57.92	56.25	64.17	64.58	58.47	57.92
	SE	4.94	4.75	3.51	3.54	5.29	5.52	4.58	4.60
Non-osinophilic Count	Mean	57.75	57.00	63.42	62.00	68.58	69.08	63.25	62.69
	SE	4.89	4.77	2.99	3.37	5.31	5.16	4.40	4.43
Abnormalities: Head	Mean	2.33	2.17	2.67	3.25	2.33	1.92	2.44	2.45
	SE	0.38	0.24	0.22	0.33	0.43	0.43	0.34	0.33
Mid Piece	Mean	1.92	1.33	1.67	2.17	1.75	1.67	1.78	1.72
	SE	0.26	0.31	0.22	0.41	0.46	0.14	0.31	0.29
Tail	Mean	12.25	13.42	10.08***	12.25***	9.75	10.33	10.69	12.00
	SE	1.40	1.43	0.83	1.00	1.22	1.33	1.15	1.25
Total	Mean	16.50	16.92	14.42**	17.67**	13.83	13.92	14.92	16.17
	SE	1.82	1.37	1.03	1.29	1.84	1.81	1.56	1.49
Microbial load€	Mean	3.13	3.11	3.02	2.96	3.47	3.48	3.21	3.18
	SE	0.09	0.10	0.12	0.13	0.13	0.15	0.11	0.13

*HAO = Hot air oven sterilization method, MO = Microwave oven sterilization method;
€Microbial load expressed in log cfu mL^{-1}.
** = significant at 5% level within the same row; *** = significant at 1% level within the same row

contributor to the total abnormality. The microbial load was on higher side in HAO treated CTs, but they were not significantly ($p>0.05$) different from the MO treated CT.

Recently, the use of microwave irradiation is being commonly used in the food industry as a tool to inactivate microorganisms (Rosenberg and Bogl, 1987; Kakita *et al*., 1995). Therefore, the studies were taken up to study the effectiveness of microwave oven over conventional hot air oven. The effectiveness of sterilization can be attributed to the energy transfer which utilizes microwaves and are quite different from these conventional heating processes. These conventional heat processes transfer the energy to the material to be heated-up by means of conduction, convection and irradiation while microwaves interact directly with the material, causing temperature increment due to the interaction of molecular electric fields with the electromagnetic waves at their different phases and producing ionic conduction and dipolar rotation (Kingston and Jassie, 1988). Also, short time and lower temperature combination for pasteurization and sterilization of materials by using microwaves is the most important aspect involved when compared to conventional processes which is based on long time and high temperature. After 3 minutes in microwave oven glassware become free of microbes (Border and Rice-spearman, 1999).

Method of microwave sterilization is more easy-to-perform, economical and reproducible than the conventional autoclaving method (Xix *et al*., 2002). In the present study, except increase in the total abnormality, no other factor was affected and thus taking into account the time saving, readiness, working efficiency and effectiveness of the method, MO method of sterilization can be recommended as even the abnormalities obtained after this treatment are also within the permissible limit. Significant difference in tail ($p<0.01$) and total ($p<0.05$) abnormality for KF bull semen may be due to less number of observations as there was no significant ($p>0.05$) difference in these attributes when overall observations were taken. Also the sterilization efficiency of both the methods was equal as evidenced from microbial load.

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