

SHORT COMMUNICATION

ASSESSMENT OF BACTERIAL LOAD IN ACCESSORIES
USED IN ARTIFICIAL INSEMINATION

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ABSTRACT

The bacterial load of Artificial Insemination (A.I.) equipments and semen dilutors have a role in contributing the bacterial contamination of semen. The Artificial Vagina (AV), glasswares, Egg Yolk Citrate (EYC) extender were subjected to assessment of bacterial load. The sterilization of AV was carried out by steam, alcohol and soap water. Twenty-five ml of sterilized Phosphate Buffer Saline (PBS) was used to wash the A.V. Glasswares were sterilized in hot air oven. Total bacterial count (TBC) was taken in sterilized as well as in unsterilized state. One ml of egg yolk citrate dilutor was used for Standard Plate Count (SPC). The TBC data obtained from this study, revealed that steam sterilization of AV was the most efficient in reducing bacterial contamination in semen. It was followed by alcohol flush and washing with soap water. The glasswares sterilized by hot air oven resulted in reduced contamination of semen. Bacterial contamination through extender was minimum during extension of semen.

The different equipment used in artificial insemination, the various semen extenders and processing procedure used for cryopreservation of semen can very well preserve the microorganisms present in the extended semen. Even under careful conditions, semen may get contaminated at the time of collection or subsequent handling. Artificial vagina, glasswares, semen extender coming in contact with semen during processing are contributing to the bacterial load of the semen. Keeping above in view, A.V., glasswares, extender used, were subjected to assessment of bacterial load.

Artificial vagina was subjected to assess the bacterial load in sterilized and unsterilized conditions. A.V. was sterilized by different methods, viz. steam sterilization for 40 minutes, with alcohol flushing, washing with soap water. Sterilized A.V. when taken just after semen collection was called unsterilized. In sterilized and unsterilized A.V., 25 ml of sterilized

phosphate buffer saline (PBS) was poured and A.V. was shaken vigorously for 3 to 5 minutes after closing the open ends by sterilized lid of petridish and washing was collected in sterilized vial. Glasswares (semen collection tube, cylinder, flask, pippet, glass rod) were sterilized by hot air oven in 110°C for overnight. Glasswares, when taken just after using in semen processing was called unsterilized. Sterilized and unsterilized glasswares were washed with 10 ml of sterilized PBS and washing was collected in sterilized vial. Egg yolk citrate⁴ was used as a dilutor of semen. Sodium citrate buffer was properly autoclaved at 121°C, 15 lb pressure for 20 minutes before it's use. All washings and 1ml of EYC dilutor were brought to Microbiology Laboratory on ice for Standard Plate Count¹. Trypton Dextrose Agar (TDA) media² was used as the plating medium for determining the Total Bacterial count. All these processes were carried out in contamination free air zone by using spirit

lamps. Surface sterilization was done by exposure to ultraviolet rays. Laboratory was fumigated at weekly interval. The analysis was carried out by using standard statistical methods⁵.

The average values of TBC (cfu/ml) for A.V. sterilized by steam, flush with alcohol, washing with soap water and sterilized and just after semen collection were 616.67, 1312.5, 96166.67 and 195000 respectively. Glasswares which were subjected to contact with semen during processing and handling showed the average value of TBC (cfu/ml) in sterilized and unsterilized condition were 130 and 940000, respectively. The average bacterial load of EYC dilutor was 390 cfu/ml of dilutor.

The TBC data generated from this study clearly indicated that sterilization of AV by steam sterilization was most efficient followed by alcohol flush and washing with soap water. The AV just after its proper use should not be used repeatedly, because it contributed maximum contamination to semen samples. 'Steam sterilization' procedure should be followed to

sterilize the AV before its use, because it minimized the contamination. Glasswares sterilized by hot air oven at 110°C for overnight, contributed a minimum contamination compared to that of unsterilized glasswares. Hence, glasswares should be sterilized by hot air oven before its actual use for semen processing. EYC dilutor contributed a minimum contamination to semen during its extension. The TBC values obtained in sterilized and unsterilized glassware and in EYC dilutor were less than other studies³. Low bacterial count in A.V., glasswares and EYC dilutor due to the procedures used for sterilization were rigorously followed at the semen production centres as confirmed by the results obtained in the present investigation.

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REFERENCES

1. Cruick Shank, R.; Duguid, J.P.; Marmolin, B.P. and Swain, R.H.A. (1975). Medical Microbiology: The Practice of Medical Microbiology 12th edn., Vol.2, Churchill, Livingstone, Edinburgh.
2. Indian Standards Institution (1962). Methods of test for dairy industry. Part III; and Bacteriological Analysis of milk IS: 1479, Part III. Indian Standards Institutions, New Delhi.
3. Kher, H.N. and Dholakia, P.M. (1987). Sources of contamination in bovine semen. *Indian J. Anim. Sci.* 57(5) : 436
4. Salisbury, G.W.; Fuller, M.K. and Willet, E.L. (1941). Preservation of bovine spermatozoa in yolk citrate diluent and field results from its use. *J. Dairy Sci.*, 24 : 905
5. Snedecor, W. George and Cochran, G. William (1981). Statistical Methods. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.

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