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# Microbial Assay of Nystatin in Buffalo Bull Semen

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**ABSTRACT**

Semen samples from five healthy breeding buffalo bulls were collected at regular intervals. Nystatin at concentration of 5, 20, 30 & 40 µg/ml was added to the semen, extended in Tris and frozen. After thawing, the samples were centrifuged (3000 rpm/10 mts) and the drug was estimated in the seminal plasma. The estimation of Nystatin was done by using *Saccharomyces cervisiae* as test organism. The zone of inhibition in the seed layer was taken for calculating the amount of nystatin recovered from the seminal plasma in comparison to reference concentration after incubating at 30°C for 48 hrs. The assay was done to assess the suitable dose of nystatin to be added for effective control of fungi without significantly affecting the quality of semen. It was found that 30 µg/ml of Nystatin is sufficient to prevent the growth of fungi in preserved semen.

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Nystatin, a antifungal, antibiotic drug is widely used in veterinary clinical practice against mycotic organisms. It has also been found effective when added to liquid semen (Natalia, 1988; Mohanty, 1989). Fungal infections may develop during processing of semen for preservation (Natalia, 1986; Bindra, 1991). In addition, fungal infections of prostate and other accessory glands may result in presence of fungal organisms in the semen which deteriorate the quality of semen (Ristuccia, 1984). However, no report is available on estimating nystatin in semen so as to find out the appropriate dose to be added in semen for effectively controlling the fungi in frozen semen and also to observe the adverse effects on semen quality. Accordingly, recovery of nystatin in seminal plasma was estimated to obtain a suitable dose to be added in buffalo semen.

## MATERIALS AND METHODS

**Animals and collection of samples:** The experiment was conducted on five healthy buffalo bulls (4-10 years of age) maintained at dairy farm at Punjab Agricultural University, Ludhiana, India. Semen was collected from each bull by using a sterilized artificial vagina (temperature 42-45°C). After collection, ejaculate was kept in water bath at 37°C. Two ejaculates from same bull were pooled.

**Addition of drug in semen:**–Nystatin (Hi media Laboratories Pvt. Ltd., Bombay, India) 10 mg was dissolved in 10 ml of dimethylformamide. Further dilutions were made in phosphate buffer (pH 6.0±0.05) and finally 20, 30 and 40 µg/ml of nystatin was added in extended semen. The extender used was modified tris yolk glycerol, with and without chloroquine diphosphate, a membrane stablizer. Further processing of semen was done by standard techniques.

**Estimation of Nystatin:** The concentration of nystatin in post freeze thaw seminal plasma samples were determined by employing standard cylinder plate bio-assay technique using **Saccharomyces cerevisae** as the test organism (Arret *et al.,* (1971).

## RESULTS AND DISCUSSION

The results revealed that the average recovery of nystatin in seminal plasma was highest in samples with 20 μg/ml nystatin followed by samples containing 30 μg/ml and it was lowest when the samples contained 40 μg/ml nystatin.

A concentration i.e. 20 μg/ml in seminal plasma was similar to minimum therapeutic plasma concentration. In clinical practice, keeping all safety points in view, atleast two-fold higher concentration is desired (Power and Power, 1985). At higher concentration i.e. 40 μg/ml the recovered drug concentration in seminal plasma was approximately same as was obtained after adding 30 μg/ml. After addition of nystatin in different concentrations in semen extender, the sperm morphology was studied. It was found that the head abnormalities with 20, 30 and 40 μg/ml of nystatin in post freezed thaw semen were 6.59±0.30, 5.06±0.35 and 7.12±0.19% respectively, as compared to 2.64±0.26 in fresh semen. Similarly, tail abnormalities (%) were 6.72±0.29, 5.39±0.28, 6.59±0.78 and 2.78±0.13, with 20, 30, 40 μg/ml of nystatin and fresh semen, respectively. On the basis of results obtained, it is concluded that addition of higher concentration may be mere wastage of drug. Moreover, the excess drug concentration in semen may produce injurious effect to sperms. The therapeutic index of nystatin is low (Hubee, 1984). In addition, it was also observed that when high concentration of nystatin was added in semen, the semen quality deteriorated with higher values of sperm head and tail abnormalities.

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