SHORT COMMUNICATION

Comparison of Photometer with Improved Neubauer Hemocytometer and Makler Counting Chamber for Sperm Concentration Measurement in Cattle

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ABSTRACT

The present study was designed to compare the photometer (Lp 300 SDM Minitüb GmHb) with improved Neubauer hemocytometer and Makler counting chamber for sperm concentration measurement in cow bulls. Data were based on 35 cow bull semen samples. The average sperm concentrations (10^9/ml) determined by photometer, hemocytometer and Makler chamber were 1.35±0.72, 1.17±0.53 and 1.49±0.60, respectively. Analysis of variance revealed that there was no difference among the three techniques of sperm concentration measurement of same semen samples in cow bulls. It was concluded that the use of photometer in semen evaluation for sperm concentration reduced chances of human error and time consumption effectively.

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INTRODUCTION

Artificial insemination (AI) technique has been used to raise the genetic potential of cattle by using genetically superior bulls (Wishwanath, 2003). Besides increasing the genetic potential of the livestock, AI offers other benefits like protection from venereal diseases, long term storage and transportation of the semen (Hafez, 1993b).

The success of an AI programme depends to a large degree on the accurate determination of sperm concentration. Photometric devices are most common methods of estimating sperm concentration. In these devices, a beam of light is passed through the sample and the amount of light transmitted is measured by phototube, which is then inversely correlated with sperm concentration in the sample. In case of Spermacue and other photometers, the instrument itself converts it to sperm/ml. Among other methods of sperm concentration measurement, improved Neubauer hemocytometer is the standard for sperm counting (Mahmoud *et al*., 1997). The Makler counting chamber allows the rapid and direct sperm count of an undiluted preheated sample (Makler, 1978). Photometer (LP 300 SDM Minitüb GmHb) is used in the Livestock Research Station, National Agricultural Research Center (NARC), Islamabad, Pakistan for the evaluation of bovine spermatozoa concentration. This study was designed to validate the photometer measure-

ments of spermatozoa concentration with the help of manual methods namely, improved Neubauer hemocytometer and Makler counting chamber. By comparing the measurements of three instruments with same semen samples the reliability of photometer measurements was assessed.

MATERIALS AND METHODS

The study was conducted on 35 semen samples collected from four cow bulls maintained as regular AI sires at the Livestock Research Station, NARC Islamabad, Pakistan. Semen was collected once a week using an artificial vagina warmed at 42°C. After recording the physical characteristics viz. appearance, volume and motility, the semen was evaluated for spermatozoa concentration. Semen samples with watery appearance were discarded because photometer was programmed to read sperm concentration of more than 40×10^6 sperms/ml.

Three different sets of procedures i.e. methods using photometer (LP 300 SDM Minitüb Gm Hb), improved Neubauer hemocytometer and the Makler counting chamber (Sefi Medical Industries, Haifa, Israel) were followed for spermatozoa concentration measurement. All the samples were analyzed by one person to exclude person to person variation.

Photometerically, spermatozoa concentration was determined at 546 nm wave length with the help of a prewarmed and calibrated photometer. Sperm concentration was also determined using improved Neubar hemocytometer as described by Hafez (2003a). A drop of thoroughly mixed 200 fold diluted (with 0.9% NaCl) semen was placed on the Makler chamber. The grid was located with 200X magnification under a phase contrast microscope. Number of spermatozoa was counted in 100 squares with the help of manual counter. Concentration of spermatozoa was calculated as described by Christensen *et al*. (2005). Data were statistically analysed using single factor analysis of variance.

RESULTS AND DISCUSSION

Non significant difference in the sperm concentration determined by the three methods tested was detected (Table 1). These results are in agreement with those reported by Brillard and McDaniel (1985) and Donoghue *et al*. (1996). Improved Neubauer hemocytometer is considered standard for the evaluation of sperm concentration (Mahmoud *et al*., 1997), but it is time consuming and cannot be used in routine evaluation of semen samples in an AI laboratory. Brillard and McDaniel (1985) determined average time to prepare and evaluate six replications of semen samples and found that the evaluation done by hemocytometer took longer (9min) as compared to the optical density method (2.3min). Makler counting chamber also takes much time for each semen sample. Makler counting chamber and improved Neubauer hemocytometer require a skillful and experienced laboratory technician because human error cannot be excluded from these methods. Jequier and Ukombe (1983) found that results differed when same semen sample was evaluated by different laboratory technicians.

It can be concluded from the present study that the use of photometer in semen evaluation for sperm concentration reduces chances of human error and time consumption effectively.

Table 1: Average sperm concentration (10^9/ml) in bulls measured by three methods

Method	Mean ± SD
Photometer	1.35 ± 0.72
Hemocytometer	1.17 ± 0.53
Makler chamber	1.49 ± 0.60

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