

# **Selection of X chromosome of buffaloes sperm with Percoll gradients**

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**ABSTRACT** - The aim of the present study was to evaluate the selection of X chromosome of buffaloes sperm with Percoll gradients. The stock solution of Percoll was prepared in the proportion of 1:11 (1 part of Percoll:11 parts of a solution containing KCl 1M, NaH<sub>2</sub>PO<sub>4</sub> 0.1M, NaCl 1.5M and sodium HEPES 23.8 g/ml). In order to prepare 9 different gradients were added to the stocked Percoll the A solution (glycine-yolk extender) in the following proportions: 90, 80, 72, 65, 57, 49, 34 and 25%. A sample of 0.7 ml of the fresh semen was deposited at 2 ml of Percoll 80% for the sperm wash. The precipitate was put in tube with 0.7 ml of each gradient. Then, the precipitated was washed in TES solution by centrifugation (500xg for 10 minutes), and collected again and diluted in TES solution to be freeze. The presence of the F body in the spermatozoa was observed in 58.7 ± 5.4% of the control group and in 41.2 ± 5.4% of the treated group ( $p < 0.01$ ). This result showed an increment of 17.55 of male sperm in the Percoll's group. The reduction of the centrifugation force did not improve the percentage of X sperm.

**Key words:** Buffaloes, X chromosome, Sperm, Percoll gradients.

**INTRODUCTION** - The techniques of artificial insemination and embryo transfer, when used in genetic improvement programs, as well as a demand of more efficient production systems led to the development of protocols for sex selection of spermatozoa and pre-implanted embryos in all species with commercial interest. The sex selection will lead to a more efficient and fast genetic gain, because of the improvement of the annual gain (Van Vleck *et al.*, 1987).

Kaneko *et al.* (1983) working with human sperm using 6 and 8 different density gradients had 73.1± 3.3 and 27.4±3.4% of Y spermatozoa in the fraction with more and less selection, respectively. Further, Schwiderski *et al.* (1991) had 75% of males and 92% of female embryos produced by *in vitro* fertilization (IVF) with sperm obtained from the upper and lower fractions of different density gradients. Hossepiian (1998) working on the separation of the sperm in those who detain the X or the Y chromosome had shown in 9 and 12 different gradients of Percoll, 74.45 and 74.55%, respectively with X chromosome, evaluating these

results using the fluorescent probe cinacrine mustard, coloring with fluorescence the F body in the Y chromosome.

Ramalho *et al.* (2000) with the proposal to test at field conditions the possibility of the sperm sedimentation using 9 different Percoll gradients, inseminating Holstein cows with the precipitated sperm, obtained 52% of pregnant rate with 85.71% of female fetuses confirmed by ultrasonography sexing.

**MATERIAL AND METHODS** - The stock solution of Percoll was prepared in the proportion of 1:11 with 1 part of Percoll (Amersham Biosciences 17-0891-01) in eleven parts of a solution containing KCl 1M (Ecibra - 1233), NaH<sub>2</sub>PO<sub>4</sub> 0.1M (Sigma - 229903-10G), NaCl 1.5M (Merck 21635) and sodium HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid – buffering agent) 23.8 g/ml (Sigma - H7006/100). In order to prepare the nine different gradients were added to the stocked Percoll the A solution of the glycine-yolk extender (Board 2) in the following proportions: 90, 80, 72, 65, 57, 49, 34 and 25% with a density of 1.06, 1.05, 1.04, 1.03, 1.02, 1.01, 1.00 and 0.9 g/ml, respectively.

Soon after the semen collection of the Murrah breed buffaloes, was retired a sample of 0.7 ml of the fresh semen which were deposited at 2 ml of Percoll 80% inside in a conic tube, for the sperm wash, according the method of Hossepi (1998) modified in the centrifugation force, 250xg for 30 minutes in the original, to 200xg for 10 minutes. The precipitate was collected with a Pasteur pipette and put, in the conic tube with 0.7 ml of each gradient deposited from the higher to the lower in density, above the nine gradients. Then, the precipitated was washed in TES solution (diluent that contains TES – methyl-2-aminoäthan-sulfonsäure/Merk 8320, TRIS – Sigma T6066, D (-) fructose, streptomycin and penicillin) by centrifugation of 500xg for 10 minutes, and collected again and diluted in TES solution to be freeze.

To confirm the results of sex selection were performed the fluorescent probe probe cinacrine mustard (Ogawa *et al.*, 1988) modified by Mota (2005).

**RESULTS AND CONCLUSIONS** - The presence of the F body in the spermatozoa was observed in  $58.7 \pm 5.4$  percent of the control group (semen diluted in TES) and in  $41.2 \pm 5.4$  percent of the treated group (semen treated with centrifugation in Percoll gradients), with a statistical difference ( $p < 0.01$ , Student's t test – Microsoft Excel). This result showed an increment of 17.55 of male sperm in the Percoll's group, as seen in Hossepi (1998), with sperm F body account, and worse than the results obtained by Ramalho *et al.* (2000), with the sexing by ultrasonography of the fetuses of Holstein cows inseminated with the selected semen, both of them working with nine gradients of Percoll. The reduction of the centrifugation force did not improve the percentage of X sperm in the precipitate, and, led to a reduced number of sperm recovering.

This study showed an increment of 17.55 of male sperm in the Percoll's group and that the reduction of the centrifugation force did not improve the percentage of X sperm in the precipitate, and, led to a reduced number of sperm recovering.

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