SELECTION OF BOVINE EPIDIDYMAL SPERM WITH THE USE OF A MICROFLUIDIC DEVICE

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INTRODUCTION

Sperm selection is a fundamental step for in vitro embryo production and the use of microfluidic devices can provide more appropriate methods to isolate viable sperm, with greater motility and with less cellular damage. Thus, this study aimed to perform the selection of epididymal sperm of cattle, testing different chemoattractive solutions, from the use of microfluidics.

MATERIAL AND METHODS

Samples obtained from 10 pairs of bovine epididymis were recovered and placed in the microfluidic device designed with an inlet reservoir and four outlet reservoirs. Each output reservoir was filled with a different medium to evaluate the best chemoattractive for sperm. Four experimental groups were formed: CG = Control Group (saline solution 0.9%); GIVM= medium only with in vitro maturation medium; GIVM+O= IVM medium + matured oocytes; GIVM+MPA = IVM medium + medroxyprogesterone acetate at 0.05%. In addition, Percoll gradient was performed as control of the selection method. The medium was placed separately in the outlet reservoirs and each sample of epididymal sperm was placed in the inlet reservoir. After 30 minutes, at a temperature of 37.5ºC, the samples were collected from the outlet reservoirs for assessment. The kinetic parameters of sperm were evaluated using the computerized analysis system (CASA), morphology was performed with Bengal Rose, and plasma membrane integrity and mitochondrial sperm activity were evaluated using fluorescent microscopy probes. The data were submitted to descriptive statistics, followed by Mann-Whitey test, with a significance level of 5%.

RESULTS

The percentage values of total motility were higher ($p < 0.05$) in the samples collected from the control group (82 ± 22.1) and those that passed through the Percoll gradient (78.3 ± 15.7), and were still similar ($p > 0.05$) to GIVM (69.2 ± 23.9) and GIVM+O (66.7 ± 27.2). However, the GIVM+MPA presented the lowest values ($p < 0.05$) compared to the other groups. When analyzing progressive motility, it was observed that all means presented similar values ($p > 0,05$), ranging from 24.1 ± 14.7 to 47.6 ± 16.1. Regarding morphology, it was observed that the spermatozoa selected by Percoll presented a lower percentage ($p < 0,05$) of normal cells (49.2 ± 9.5) when compared to CG (58.1 ± 11.4), GIVM (62.7 ± 10.4), GIVM+O (69.2 ± 11.9), and GIVM+MPA (70.6 ± 8.6). Plasma membrane viability and mitochondrial activity showed that the samples from the Percoll, CG, and GIVM groups were similar ($p > 0,05$) and presented higher values than the other groups ($p < 0,05$). In conclusion, it was confirmed that the microfluidic device was able to select epididymal sperm with adequate total and progressive motility, being ideal to be used for in vitro fertilization. The highest percentage of morphological abnormalities of epididymal sperm selected by Percoll gradient probably occurred due to the centrifugation stage, necessary to perform the gradient. Since the plasma membrane viability of these cells was viable, there was no influence on the use or not of centrifugation.

CONCLUSION

The microfluidic device showed promising results for the selection of epididymal sperm of cattle and the medium containing only in vitro maturation medium (GIVM) and IVM + oocytes medium (GIVM+O) showed greater attraction to spermatozoa.