

annexin microbeads, and subjected to column separation as described above. MACS resulted in a significant increase in live annexin-negative sperm in the negative fraction ($64 \pm 6.5\%$) when compared to the unsorted sample ($34 \pm 2.0\%$).

In conclusion, magnetic activated cell sorting of equine sperm resulted in an enriched population of live, membrane-intact sperm. However, there are a number of obstacles with the methodology that remain to be overcome, including the binding buffer which negatively affects sperm motility, the cost of the reagents that potentially limits routine clinical application and adaptation of the protocol to handle higher volumes and cell concentrations.

Keywords: Equine sperm; Cell separation; Annexin; MACS

DOI: 10.1016/j.theriogenology.2007.05.021

EFFECT OF ARTIFICIAL INSEMINATION PROTOCOL AND SPERM DOSE ON PREGNANCY RESULTS IN MARES

J. Govaere¹, M. Hoogewijs¹, C. De Schauwer¹, S. De Vlieghe¹, L. Duchateau², A. Van Soom¹, A. de Kruif¹

¹Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Science, Ghent University, Belgium

²Department of Physiology and Biometry, Faculty of Veterinary Science, Ghent University, Belgium

Objectives: The objectives of this study were to evaluate whether: (1) deep horn insemination with a low sperm dose is feasible with commercially available semen doses; (2) those low doses require deep horn insemination instead of conventional insemination into the corpus uteri; (3) the device used for deep horn insemination [Ghent device (Beldico, Belgium) versus Minitüb pipette (Minitüb, Germany)] affects pregnancy rates.

Materials and methods: Twenty-five cyclic, non-lactating French trotter mares with an average age of 5 years (range, 3–7) were housed in pens with access to pasture. Twenty-one were maiden, three had foaled once and one had foaled twice. The mares had no history of reproductive failure and were clinically normal. During spontaneous estrous cycles, the dominant follicle was measured by ultrasonography every 6 h; mares were inseminated with frozen/thawed semen [(high dose: 138×10^6 pms in five straws (2.5 mL) or low dose: 27×10^6 pms in one straw (0.5 mL)] within 6 h post

ovulation. Only mares that had no intrauterine fluid at that time were included. For each ovulation, mares were assigned randomly to one of four artificial insemination (AI) protocols (cross-over design): (1) HUB = high dose AI into the uterine body; (2) LUB = low dose AI into the uterine body; (3) LDHgd = low dose AI deep into the horn with the Ghent Device; (4) LDHmp = low dose AI deep into the horn with the Minitüb pipette. Semen of two stallions with a known fertility status (first: ‘good fertilizer’ and second: ‘bad fertilizer’) was used. Fourteen days after insemination, pregnancy diagnosis was made by ultrasonography (5 MHz, Pie Medical, Falco). Data were analysed using logistic regression with pregnancy result as outcome variable, AI protocol as predictor variable of main interest, and mare as random effect to account for repeated measurements.

Results and conclusions: In total, 143 inseminations were performed, resulting in 40 pregnancies (27.9%). In the HUB and LUB groups, 14 out of 35 and 5 out of 35 inseminations respectively, were successful. The average difference in pregnancy result between HUB and LUB was 26.4% in favour of HUB (95% CI: 0.05–46.8, $P = 0.01$).

LDHgd insemination resulted in 12 pregnancies out of 48 inseminations and LDHmp in 10 pregnancies out of 25 inseminations. The average difference in pregnancy results between LDHmp and LUB was 30.6% (95% CI: 7–54, $P = 0.01$).

Pregnancy results were not different between the HUB and LDHmp protocols (4.2% difference in favour of LDHmp, 95% CI: –30.7 to 0.22, $P = 0.74$) and between the HUB and the LDHgd group (12.4% difference in favour of HUB, 95% CI: –8 to 33.8, $P = 0.24$).

The conclusion is that deep horn insemination with a reduced sperm dose using the Minitüb pipette resulted in the same pregnancy rate as uterine body insemination with a full sperm dose.

Keywords: Deep horn insemination; Low sperm dose; Frozen-thawed semen

DOI: 10.1016/j.theriogenology.2007.05.022