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Application of the microflow technique for the selection of stallion semen

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Routine semen preparation techniques are not always satisfactory for all stallions. Some stallions, despite poor semen parameters, are valuable for breeders who want to produce equally worthwhile offspring. In recent years, advanced sperm selection methods such as the microflow technique have been developed and successfully applied to humans. The aim of this study was to adapt the microflow technique for selection of stallion semen, focusing on their predicted benefits for sperm quality. The experiment was performed with semen of 6 stallions, which after combining in equal volumes created pooled research sample. Semen was left for 24 h in order to reduce the viability and motility of spermatozoa and divided into 3 parts, the control and the two research groups separated using ZyMōt Devices with two different incubation times (30 and 45 minutes). For each group, the basic sperm characteristics were assessed by SCA/CASA system, and fragmentation was evaluated using the TUNEL technique. The Z-score test was used for statistical analysis. In the sample incubated for 30 min in ZyMōt Devices, sperm motility increased from 28% to 52.15% ($p=0.001$), including sperm with progressive motility which increased from 8.47% to 41.91% ($p<0.00001$). After extending the incubation time for 45 minutes, these parameters were even higher: motility 62.43% ($p<0.00001$), progressive movement 55.33% ($p<0.00001$). It should be noted, however, that in the case of both research samples, the concentration of sperm decreased from 804.68 M/ml in the control to 32.35 M/ml ($p<0.00001$) after 30 min incubation and 40.37 M/ml after 45 min incubation, respectively ($p<0.0001$). Sperm selection did not affect vitality. The TUNEL technique showed a very low percentage of spermatozoa with apoptosis in the semen fraction after selection at both incubation times. In summary, the microflow technique is an effective method for selection of stallion sperm with high motility, progressive movement and low DNA sperm fragmentation, with unchanged vitality parameters. Comparing the two incubation times, it was found that extending the incubation time to 45 minutes improved sperm concentration and spermatozoa with progressive motility. Nevertheless, prolonged incubation caused an increase in apoptotic spermatozoa that penetrate through a membrane with microchannels. It should be considered that sperm selection by microflow technique drastically reduces sperm concentration, which in practice translates into a significant reduction in the number of insemination doses which will increase costs significantly. However, this method can be successfully used for sperm selection for the ICSI procedure.