Microfluidic Sperm Selection Improves Number and Quality of In Vitro Produced Bovine Embryos

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For successful in vitro embryo production, male factors such as sperm motility and DNA integrity play an important role. Sperm selection by density-gradient centrifugation (DGC) before in vitro fertilization (IVF) is widely used to select progressive motile sperm but potentially damages sperm DNA. Therefore, a microfluidic sperm selection (MFSS) method has been developed to reduce DNA damage by avoiding centrifugation. While MFSS is expected to improve the production efficiency of IVF embryos, the impact of sperm DNA integrity before selection on the efficiency of MFSS remains unknown. Thus, the present study aimed to determine the impact of MFSS on in vitro production of bovine embryos using sperm with high or low DNA integrity.

Cryopreserved semen samples from 11 Simmental bulls were selected depending on their DNA fragmentation index (%DFI), where six bulls showed low %DFI values (%DFI<3.5%) and 5 bulls had high %DFI values (%DFI>7.0%). Progressive motility, viability, and %DFI of sperm were analyzed before (Pre-selection (PS)-sperm) and after selection with DGC using Bovipure^{TM} (DGC sperm) or microfluidic chips (Fertile Plus, KOEK Biotechnology: MFSS sperm). Abattoir-derived cumulus-oocyte complexes of dairy cows were matured in vitro and fertilized with DGC- or MFSS-IVF sperm. At 18 h post-insemination, the number of pronuclei was determined to evaluate the fertilization. Another cohort of zygotes was examined by time-lapse microscopy for 48 hours to determine the timing of the first cleavage and its pattern, then cultured for seven days. At day 7, the bioenergetic profile of the embryos was analyzed by measuring the oxygen consumption rate (OCR) of pools of five expanded blastocysts using an extracellular FLUX-analyzer (Seahorse XFp, Agilent).

Progressive motility and viability increased, and %DFI decreased in MFSS-sperm compared to PS-sperm and DGC-sperm in all bulls, regardless of pre-selection %DFI values ($P<0.01$). In bulls with high %DFI pre-selection, %DFI increased in DGC-sperm ($P<0.0001$) and decreased in MFSS sperm ($P<0.01$). Compared to the DGC-IVF zygotes, MFSS-IVF showed a lower proportion of abnormal fertilization (more than two pronuclei, 34.5% vs. 22.7% in low DFI bulls; 36.1% vs. 7.3% in high DFI bulls, $P<0.05$). The proportion of embryos showing direct cleavage, the cleavage from one cell to three or more blastomeres, was lower in MFSS-IVF (12.5% and 2.5% in low and high DFI bulls, respectively, $P<0.05$) than DGC-IVF (32.4% and 22.4% in low and high DFI bulls, respectively). The developmental rate to the blastocyst was higher in MFSS-IVF (35.0% and 29.5% in low and high %DFI bulls,

respectively, $P<0.05$) compared to DGC-IVF (28.5% and 21.8% in low and high %DFI bulls, respectively). In high %DFI bulls, the OCR of blastocysts was higher in MFSS-IVF embryos than in the DGC-IVF embryos ($P<0.05$), whereas DGC-IVF and MFSS-IVF embryos showed comparable OCR values in low %DFI bulls ($P>0.05$).

In summary, MFSS substantially improved bovine sperm quality and embryonic development compared to DGC. These improvements occurred both in ejaculates with low and high DNA damage before the selection. The blastocysts produced with MFSS-sperm also showed a better bioenergetic profile, but only in ejaculates with high DNA damage. These results indicate an increase in the number of bovine embryos after IVF with sperm with low and high DNA damage and an improvement of embryonic quality after IVF with sperm with high DNA damage by using microfluidics compared to DGC for sperm selection.