31 DEVELOPMENT AND APOPTOSIS IN BOVINE CLONED EMBRYOS RECONSTRUCTED WITH OOCYTES COLLECTED BY REPEATED OVUM PICKUP SESSIONS OR FROM SLAUGHTERED COW OVARIES

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The oocyte has important components for nuclear reprogramming and its cytoplasmic background may influence the somatic cell nuclear transfer success. The current study attempted to evaluate the competence of cytoplasm from oocytes recovered by repeated ovum pickup (OPU) in living cows (OPU group) or obtained from ovaries collected at slaughterhouse from unknown source crossbred cows (SH group) to produce nuclear-transferred bovine embryos. For the OPU group, oocytes were recovered from 4 *Bos indicus* × *Bos taurus* crossbred cows in 4 repeated OPU sessions. Oocytes of OPU and SH groups were matured *in vitro* for 17 to 18 h, denuded and exposed to Hoechst 33342 (Sigma, St. Louis, MO, USA) and cytochalasin (Sigma) before enucleation. Embryos of OPU ($n = 100$) and SH ($n = 105$) groups were reconstructed with somatic cells from adult Gyr (*Bos indicus*) cow, fused with double electric pulse of 2.4 kV cm^{-1} for 30 µs and activated with ionomycin (Sigma) and 6-DMAP (Sigma). Embryos were cultured in CR2aa medium supplemented with 2.5% fetal calf serum (Nutricell, Campinas, Brazil) under 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. Cleavage and blastocyst rates were evaluated at 72 h and 168 h post-activation, respectively. Blastocysts at 168 h post-activation were fixed and permeabilized for TUNEL assay (DeadEnd™ Fluorimetric TUNEL System, Promega, Madison, WI, USA), according to the manufacturer instructions. IVF bovine blastocysts (IVF group; $n = 245$) obtained with oocytes of slaughtered cows were used as control group. Fusion, cleavage, and blastocyst rates were analysed by chi-square test and total cell number, apoptotic cell number, and apoptotic cell index (calculated by dividing the apoptotic cell number by total cell number) were analysed by ANOVA. There were no differences ($P > 0.05$) in fusion (71.0% and 61.0%), cleavage (74.6% and 78.1%) or blastocyst (32.3% and 31.2%) rates between OPU and SH groups, respectively, but both groups presented greater ($P < 0.05$) blastocyst rates than the IVF group (15.1%). Total cell number (80.66 ± 5.36 and 82.10 ± 4.79), apoptotic cell number (12.66 ± 3.20 and 15.60 ± 3.04), and apoptotic cell index (0.15 ± 0.03 and 0.20 ± 0.04) were also similar ($P > 0.05$) between OPU and SH groups, respectively. However, apoptotic cell number (7.40 ± 0.93) and apoptotic cell index (0.07 ± 0.01) were lower ($P < 0.05$) in the IVF group than the SH group and similar ($P > 0.05$) to the OPU group. In conclusion, oocytes cytoplasm from both groups (OPU and SH) have the same potential to produce nuclear-transferred bovine embryos but only blastocysts from the OPU group present apoptosis levels similar to its *in vitro*-fertilized counterpart.

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32 A CLONED FOAL PRODUCED USING OOCYTES RECOVERED BY TRANSVAGINAL ASPIRATION OF IMMATURE FOLLICLES

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Closure of all the horse slaughterhouses in the US has reduced the availability of equine oocytes in this country. We investigated the use of oocytes collected from immature follicles of live mares for cloning research. Because blastocyst development of equine cloned embryos is typically low (< 10%), we also investigated the effect of Scriptaid, a histone deacetylase inhibitor that increases blastocyst development, live birth rate, and neonatal health in cloned mice and pigs. Immature oocytes were transvaginally aspirated from all follicles ≥8 mm diameter in a herd of 11 mares. The oocytes were cultured in modified M199 for 24 to 26 h. Donor fibroblasts from a 27-year old stallion were treated with roscovitine for 24 h, then were directly injected into enucleated oocytes using the Piezo drill. Reconstructed oocytes were activated with ionomycin followed by injection of sperm extract and culture with 6- dimethylaminopurine (6-DMAP) for 4 h. Recombined oocytes in the Scriptaid treatment were cultured in the presence of Scriptaid, 250 nM, starting at the onset of 6-DMAP treatment and continuing for a total of 18 to 20 h. After embryo culture, blastocysts were shipped for transfer to recipient mares. Overall, each oocyte donor mare underwent aspiration up to 10 times; 653 follicles were aspirated and 271 oocytes were recovered. The *in vitro* maturation rate was 65% (172/263). After nuclear transfer procedures, 147 oocytes survived; 130 were used for the study. The blastocyst development rate was 2/47 (4%) in the control treatment and 1/83 (1%) in the Scriptaid treatment. All 3 blastocysts yielded pregnancies after transfer. Both control pregnancies were lost, 1 at 30 days and other at 9 months. The mare pregnant with the embryo from the Scriptaid treatment foaled at 326 days of gestation. The foal had medical issues at birth similar to those seen in some cloned foals previously, including maladjustment, patent urachus, and poor oxygenation. These issues were resolved with medical care; the foal is 3 months of age and healthy at the time of writing. These results indicate that immature oocytes obtained from a limited number of mares can be used successfully for nuclear transfer, providing the opportunity to control the mitochondrial identity of the host cytoplast. Scriptaid treatment did not improve the rate of blastocyst development or prevent health problems at birth; however, transfer of 1 embryo in this treatment produced a viable foal. More work is needed to determine the effect of histone deacetylase treatment on efficiency of cloning in the horse.

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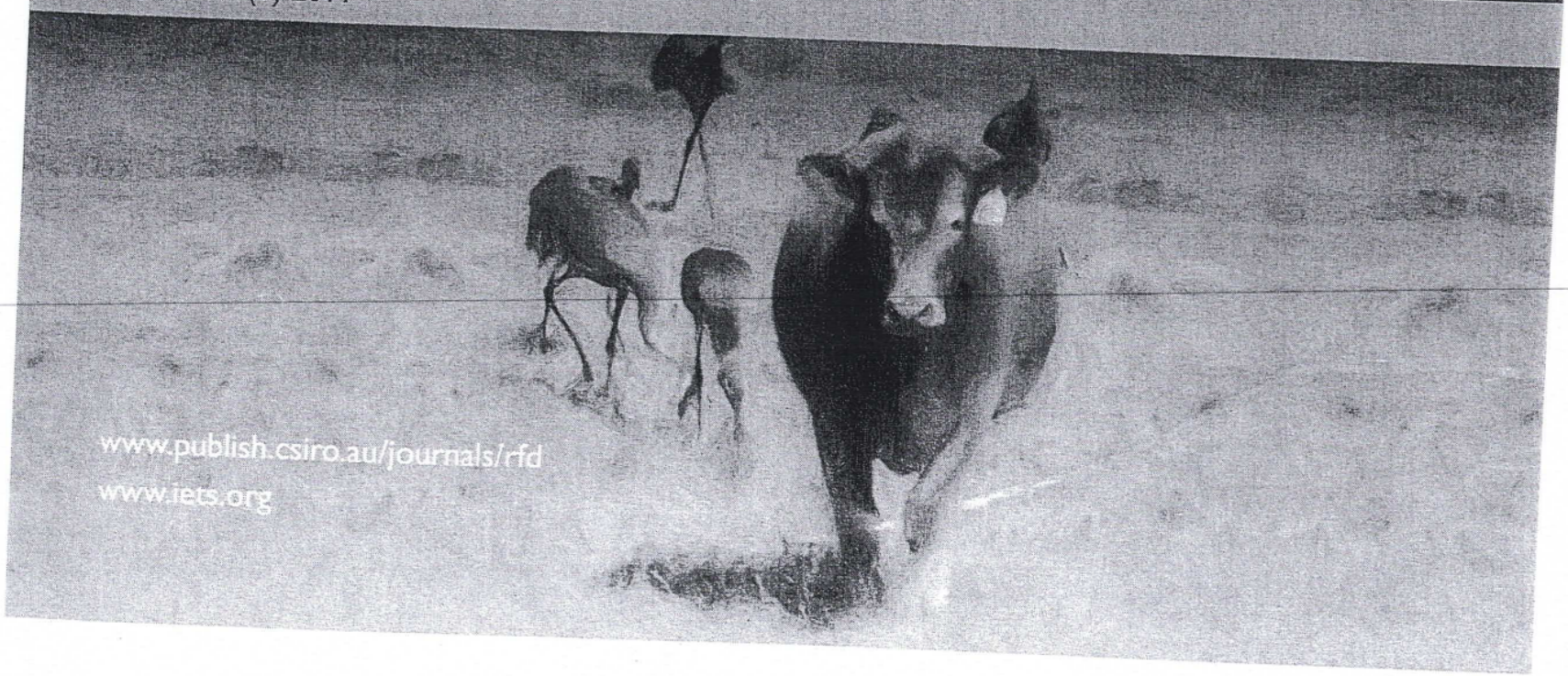

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