The transcripts of KPNA5 were detectable only in GV and MII oocytes. No significant changes in the amount of KPNA6 transcripts were detectable at the stages analyzed. Transcript levels of KPNA7 were reduced in BL embryos as compared to the GV oocytes (1165-fold, BL *v.* GV). Throughout all these stages examined, KPNA5 had the lowest transcript abundance and was not detectable in 4C and BL stages. Transcripts levels of KPNA7 were in higher abundance than KPNA1-6 in GV and MII oocytes. Results suggest that KPNA7 is a new member of the KPNA family. Our results also suggest that porcine oocytes and embryos, at discrete stages of development, have differing requirements for individual KPNA molecules.

199 EFFECT OF PRODUCTION SYSTEM AND BREED ON RELATIVE GENE EXPRESSION IN BOVINE EMBRYOS

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Embryos produced *in vivo* and *in vitro* show morphological and developmental differences, which can be related to culture environment. Nevertheless, there are a few studies showing the effect of *in vitro* environment on embryos from different bovine subspecies, such as Gyr (*Bos indicus*) and Holstein (*Bos taurus*). The aim of this study was to evaluate the relative abundance of aquaporin 3 (AQP3) and ATPase-α1 (Na/K-ATPase alpha 1) transcripts in blastocysts produced *in vivo* or *in vitro* from Gyr and Holstein cattle. The production system effect (*in vivo* × *in vitro*) for Gyr cattle and the breed effect (Holstein × Gyr) for *in vitro*-produced embryos were evaluated. For each group, blastocysts ($n = 15$) distributed in 3 pools were used for RNA extraction (RNeasy MicroKit, Qiagen, Valencia, CA), followed by RNA amplification (Messageamp II amplification kit, Ambion-Applied Biosystems, Foster City, CA) and reverse transcription (SuperScript III First-Stand Synthesis Supermix, Invitrogen, Carlsbad, CA). The cDNA obtained were submitted to real-time PCR, using the H2a gene as endogenous control, and analyzed with *REST software*© using the pair wise fixed reallocation randomization Test. There was no difference ($P > 0.05$) in gene expression for AQP3 and ATPase-α1 between *in vivo*- and *in vitro*-produced Gyr embryos, although the results suggest that the APQ3 gene was down-regulated (0.81 ± 0.31) and the ATPase-α1 gene was up-regulated (1.20 ± 0.65) in embryos produced *in vitro*. For breed effect within *in vitro* production system, ATPase-α1 gene was down-regulated in Holstein (0.56 ± 0.30) when compared with Gyr embryos ($P < 0.05$). The same trend was observed for AQP3 (0.58 ± 0.25), but with no difference ($P > 0.05$). In conclusion, the data suggest that embryo production system does not interfere with the transcript amount of the genes studied for Gyr cattle; however, the *in vitro* production system may have different effects on gene expression according to embryo breed. Other genes should be evaluated for a better understanding of these differences.

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200 REPROGRAMMING OF Oct-4 FOLLOWING EQUINE SOMATIC CELL NUCLEAR TRANSFER

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Oct-4, a POU domain-containing transcription factor encoded by Pou5f1, is selectively expressed in pre-implantation embryos and pluripotent stem cells, but not in somatic cells. Because of such a unique expression feature, Oct-4 can serve as a useful reprogramming indicator in somatic cell nuclear transfer (SCNT). Compared with data of Oct-4 expression in mouse and bovine cloned embryos, little is known about this gene in equine nuclear transfer. In the present study, we investigated Oct-4 expression in donor cells, oocytes, and SCNT embryos to evaluate reprogramming of equine somatic cells following nuclear transfer. Horse ovaries were obtained from a local slaughterhouse and the oocytes collected from the ovaries were matured *in vitro* in an M199-based medium (Galli *et al.* 2003 Nature **424**, 635) for 24 h. Donor cells were derived from biopsy tissue samples of adult horses and cultured for 1 to 5 passages. Standard nuclear transfer procedures (Zhou *et al.* 2008 Mol. Reprod. Dev. **75**, 744–758) were performed to produce cloned embryos derived from equine adult somatic cells. Cloned blastocysts were obtained after 7 days of *in vitro* culture of reconstructed embryos. Total RNA were extracted using Absolutely RNA Miniprep/Nanoprep kits (Stratagen, La Jolla, CA) from oocytes ($n = 200$), donor cells, and embryos ($n = 5$). DNase I treatment was included in the procedure to prevent DNA contamination. Semiquantitative RT-PCR was performed with optimized cycling parameters to analyze Oct-4, GDF9, and β-actin in equine donor cells, oocytes, and cloned blastocysts. The RT-PCR products were sequenced to verify identity of the genes tested. The relative expression abundance was calculated by normalizing the band intensity of Oct-4 to that of β-actin in each analysis. No transcript of Oct-4 was detected in equine somatic cells used as donor nuclei, consistent with its expression patterns in other animal species, whereas Oct-4 was abundantly expressed in equine SCNT blastocysts derived from the same donor cell line. Oct-4 transcripts were also detected in equine oocytes and whether any maternally inherited Oct-4 mRNA persisted up to the blastocyst stage was unclear in this study. We selected GDF9 to address this question; GDF9 was abundantly detected in equine oocytes, consistent with its expression pattern in mouse and bovine, but not detected in donor cells and cloned blastocysts, suggesting that the GDF9 mRNA from the oocyte was degraded at least by the blastocyst stage. The results from this study imply occurrence of Oct-4 reprogramming in equine SCNT blastocysts, and future analysis for more developmentally important genes is needed to better understand reprogramming at molecular levels in this species.

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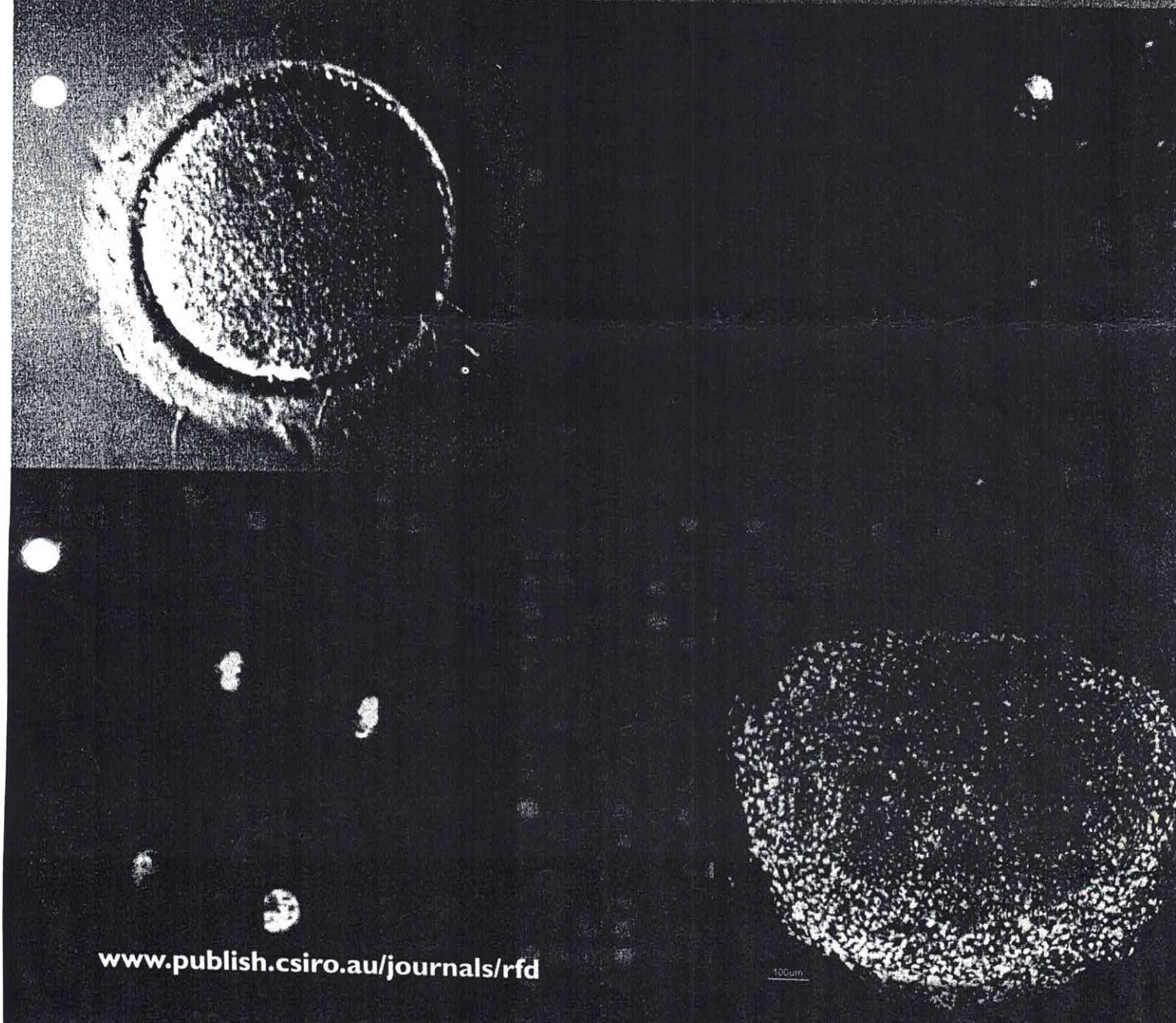

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