protocol from Fluidigm. Samples were run in duplicate on a single 48.48 microfluidic chip using intercalating dye qPCR technology. Each primer set was run in duplicate as well, resulting in 4 data points for each sample and primer set combination. Data were analysed using the relative standard curve method. We were able to produce preliminary data on how reproducible data generation is with this platform on single oocyte and blastocyst samples. Correlations between assay and sample replicates were high ($R^2 = 0.998$ and $R^2 = 0.967$, respectively). Among the oocyte samples, a fold change difference of 2 or more was found in 9 of the 24 primer sets. A similar analysis of the blastocyst samples yielded fold changes over 2 for 12 of the 24 primer sets. These preliminary data suggest that the use of nanofluidic qPCR technology may be useful for the study of gene expression variation among single oocyte and blastocyst samples.

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137 DIFFERENTIAL GENE EXPRESSION OF *IN VITRO*–MATURED BOVINE OOCYTES WITH OR WITHOUT A POLAR BODY

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Oocyte competence is associated with the amount of transcripts stored in the ooplasm and oocyte ability to extrude polar bodies (PB). To our knowledge, however, no data comparing mRNA levels between bovine oocytes matured *in vitro* with or without PB are available. The aim of the present study was to compare the relative abundance of transcripts of glucose transporter 1 (*GLUT1*), insulin-like growth factor 1 receptor (*IGF1R*), insulin-like growth factor 2 receptor (*IGF2R*), growth differentiation factor-9 (*GDF9*) and aquaporin 3 (*AQP3*) genes between oocytes with and without PB (PB and NPB groups, respectively) following *in vitro* maturation. Immature bovine oocytes were obtained by follicular aspiration and matured in TCM-199 (Gibco Life Technologies, New York, NY, USA) containing 10% of oestrus cow serum and 20 μg mL^{-1} of FSH (Pluset, Serono, Italy) for 24 h under 5% CO_2 in air at 38.5°C. Subsequently, oocytes were visually classified according to the presence or absence of PB and then denuded and rapidly frozen in liquid nitrogen. Three pools of 10 oocytes for each group were subjected to total RNA extraction using the RNeasy Micro Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions and treated with DNase. Reverse transcription and cDNA amplification were performed using the TransPlex Complete Whole Transcriptome Amplification Kit (WTA2, Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. Relative abundance of the target transcripts was performed by quantitative RT-PCR (Applied Biosystems Prism 7300 Sequence Detection Systems, Foster City, CA, USA) using a mixture of SYBR® Green PCR Master Mix (Applied Biosystems), 200 ng of cDNA, nuclease-free water and specific primers for each reaction. Expression of the β-actin gene was used as an endogenous reference. Relative gene expression analysis was performed using the software REST© 2005 using the Pair Wise Fixed Reallocation Randomization Test©. The relative expression values are presented as mean ± standard error. The relative abundance of *GLUT1* (0.81 ± 0.07), *IGF2R* (0.72 ± 0.07) and *GDF9* (0.82 ± 0.10) genes was lower ($P < 0.05$) for NPB oocytes. There was no difference ($P > 0.05$) in relative abundance between PB and NPB groups for the other genes. The results suggest that the amount of some transcripts stored in the matured ooplasm is associated with the presence of PB.

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138 THE EFFECT OF FOLLICLE SUPERSTIMULATION ON mRNA LEVELS IN BOVINE OOCYTES COLLECTED BY OVUM PICKUP

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A previous study revealed that follicle superstimulation significantly improved the developmental competence of immature bovine oocytes collected by ovum pickup (OPU; Imai *et al.* 2008 *Reprod. Fertil. Dev.* **20**, 182). The aim of the present study was to investigate the effect of follicle superstimulation on the expression of developmentally important genes in bovine oocytes collected by OPU. Follicular oocytes were collected by OPU without (OPU group) or after follicle superstimulation by FSH (FSH/OPU group) by using a 7.5-MHz linear transducer with needle connected to an ultrasound scanner according to Imai *et al.* (2008). In the FSH/OPU group, after dominant follicle removal from Holstein dry cows by OPU, a CIDR was inserted on Day 5 (dominant follicle removal = Day 0). Cows then received 30 mg of FSH twice a day from Days 7 to 10 in decreasing doses (6, 6, 4, 4, 3, 3, 2, 2 mg) by IM injection. Cloprostenol (PGF; Clopromate C; Sumitomo Pharmaceuticals Co., Tokyo, Japan; 0.75 mg) was administered in the morning of Day 9 (third day of superstimulation). Oocyte collection by OPU was performed 48 h after PGF administration (Day 11) by the aspiration of follicles larger than 5 mm in diameter. In the OPU group, 3-to-6-mm follicles were aspirated without any previous hormone treatment. *In vitro* oocyte maturation (IVM) was performed according to Imai *et al.* (2006 *J. Reprod. Dev.* **52**(Suppl), 19–29). Gene expression was assessed before (0 h IVM) and after IVM (22 h IVM) by RT quantitative PCR. The following genes were investigated: *GAPDH, G6PDH, ACTB, H2A, CCNB1, MnSOD, OCT4, SOX2, CX43, HSP70, GLUT8, PAP, GDF9, COX1, ATP1A1, CDH1, CTNNB1, AQP3, DYNLL1, DYNC1I1* and *PMSB1*. In brief, mRNA was extracted from 20 oocytes per sample using Qiagen RNeasy Micro kit (Qiagen, Valencia, CA, USA). Gene expression was analysed by a Roche Light Cycler 480 device. The relative expression of each gene was normalized to *ACTB*. Three replications

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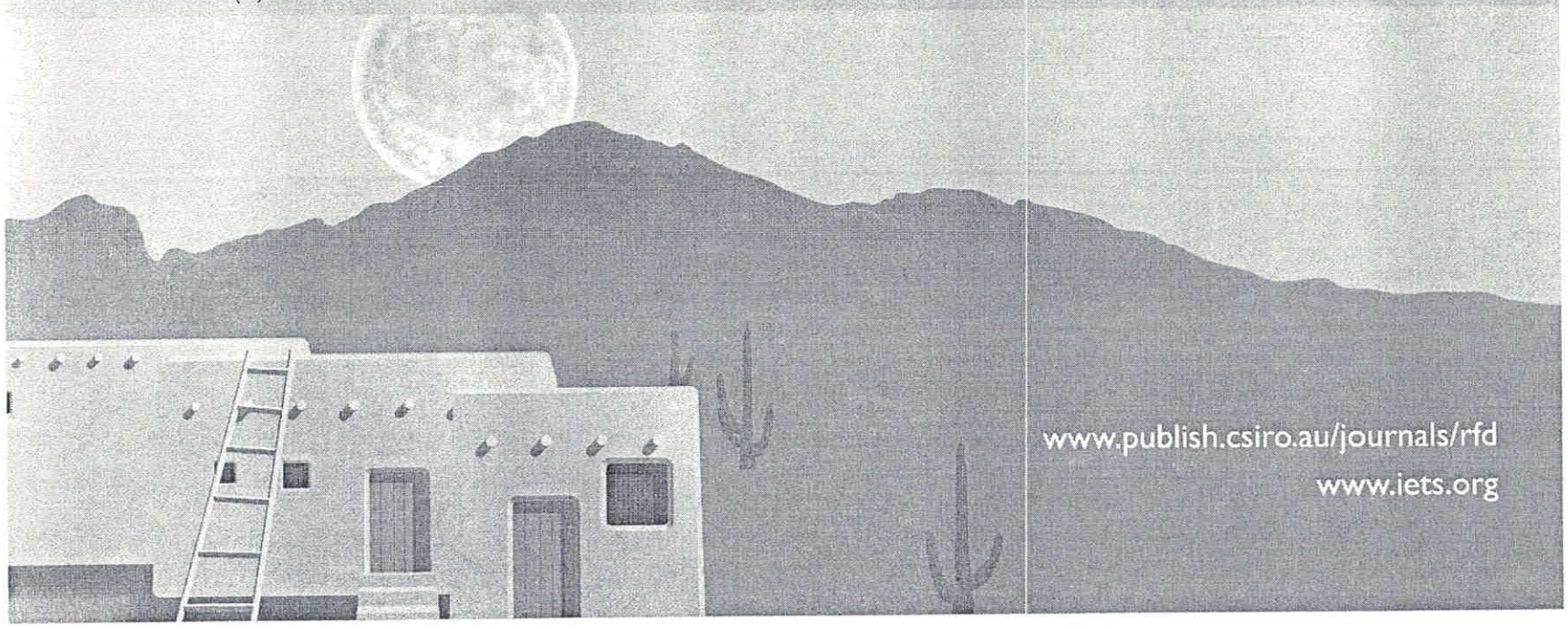

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