6 individual embryos in similar groups showed relatively low variability compared to embryos at similar stage but produced in different conditions. Interestingly, the parthenogenetic embryos showed a level of gene expression comparable to that of the *in vivo* ones, notwithstanding their culture *in vitro*. In conclusion, morphological observations and similarity in developmental stage alone cannot guarantee the uniformity of embryo samples, and a minimum of 4–6 replicates per treatment is needed. Moreover, we showed that culture condition itself has an effect on housekeeping gene expression, which, if neglected, might result in misinterpretation of data.

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261 PHENOTYPIC CHARACTERISTICS AND TISSUE-SPECIFIC *IGF2R/IGF2* EXPRESSION PARTITION BOVINE FETAL OVERGROWTH ASSOCIATED WITH *IN VITRO* FERTILIZATION AND SOMATIC CELL NUCLEAR TRANSFER CLONING

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Large offspring syndrome (LOS) in ruminants refers to various poorly defined organ pathologies that are associated with fetal overgrowth and are encountered after a range of embryo manipulations (Rhind *et al.* 2003 Nat. Rev. Genet. 4, 855–864). We have explored the effects of somatic cell nuclear transfer (NT) and *in vitro* fertilization (IVF) on phenotype and relative expression levels of 2 imprinted genes important for fetal growth, insulin-like growth factor 2 (IGF2) and its receptor (IGF2R). Viable bovine fetuses were recovered near the end of the first trimester of pregnancy, and skeletal muscle, liver, and lung were sampled for real-time RT-PCR analyses. We compared NT-fetuses ($n = 23$), IVF-fetuses ($n = 24$), and fetuses generated by artificial insemination (controls, $n = 24$) in order to separate abnormalities specific to cloning from effects of *in vitro* gamete and embryo manipulation. Nuclear transfer and IVF-fetuses, both derived from embryos cultured with 10% estrous cow serum, demonstrated significant fetal overgrowth. The increase in body weight relative to controls was similar for both groups (+22%, $P < 0.001$, and +19%, $P < 0.001$, respectively), but further analyses clearly separated the NT phenotype from the IVF phenotype. The NT-fetuses were characterized by a shorter crown-rump length but larger thorax circumference, which consequently produced a significantly reduced fetus length-to-thorax circumference ratio in comparison with IVF-fetuses and controls (−9% each, $P < 0.0001$). Absolute liver weight was significantly increased in NT- and IVF-fetuses (+62%, $P < 0.0001$, and +20%, $P < 0.0001$, respectively), but relative liver weight was increased only in NT-fetuses (+30%, $P < 0.0001$). Heart ($P < 0.0001$) and kidney ($P = 0.0003$) mass also showed disproportionate organomegaly in NT-fetuses only, but relative lung weight (NT, $P = 0.263$; IVF, $P = 0.317$) was not affected by either embryo technique. Transcript abundance for *IGF2* and *IGF2R* genes were strongly correlated in muscle ($r = 0.835$, $P < 0.0001$), liver ($r = 0.922$, $P < 0.0001$), and lung ($r = 0.772$, $P < 0.0001$). The *IGF2* and *IGF2R* transcript levels in muscle tissue from NT-fetuses were markedly reduced in comparison with both IVF-fetuses (−31%, $P < 0.0001$, and −41%, $P < 0.0001$, respectively) and controls (−31%, $P < 0.0001$, and −41%, $P < 0.0001$, respectively). In liver tissue, however, transcript levels for NT-fetuses were similar to those of controls, and IVF-fetuses showed markedly elevated, albeit non-significant, *IGF2* (+86%, $P = 0.0591$) and *IGF2R* (+54%, $P = 0.1305$) mRNA levels relative to controls. Our data demonstrate that seemingly similar syndromes caused by NT or IVF procedures can be clearly partitioned with respect to phenotype and *IGF2/IGF2R* expression.

262 ASSESSMENT OF HSP70-1 TRANSCRIPTION LEVELS IN IMMATURE OOCYTES FROM *BOS TAURUS* AND *BOS INDICUS* COWS RAISED IN A TROPICAL CLIMATE

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Heat stress is one of the main causes of low conception rate in *Bos taurus* cows in a tropical climate. On the other hand, in this environment, oocytes from *Bos indicus* show greater developmental capacity after *in vitro* fertilization than those from *Bos taurus*, suggesting an adaptation to the hot climate. Heat shock proteins (HSP) are chaperones that promote protection against heat damage, and their transcription is associated to stress. The aim of this study was to evaluate the expression of HSP70-1 gene (Genbank NM174550), a member of HSP family, in oocytes from *Bos taurus* (Holstein) and *Bos indicus* (Gyr) cows raised in the tropical climate located at 21°35′′S latitude, 43°51′′W longitude, and 435 m altitude. Cumulus–oocyte complexes were recovered by oocyte pickup from mature non-lactating Holstein ($n = 4$) and Gyr ($n = 4$) donor cows during the hot season. Cumulus cells of viable oocytes were removed by vortexing in TALP-HEPES plus BSA, and pools (3 for each breed) with 12 immature oocytes were rapidly frozen in liquid nitrogen and subsequently thawed for RNA extraction. Total RNA extraction was performed using Rneasy® Micro kit (Qiagen, Valencia, CA, USA), and first strands were synthesized using Superscript™ III First Strand Synthesis kit (Invitrogen, Chicago, IL, USA). Relative quantification was performed in duplicate using real-time PCR (ABI Prism® 7000; Applied Biosystems, Foster City, CA, USA);

reactions consisted of a mixture of iTaq™ SYBR® Green Supermix with ROX (Bio-Rad, Waltham, MA, USA) and cDNA equivalent to 1.2 oocytes and gene specific primers. Expression of the GAPDH gene was used as endogenous reference. Calculations of relative quantification were performed by the comparative Ct method, using the lowest value found in *Bos indicus* oocytes as calibrator; values (mean ± SE) are shown as *n*-fold difference relative to the calibrator. Statistical comparison between breeds was performed by analysis of variance. Oocytes from Holstein cows showed a higher level ($P < 0.05$) of HSP70-1 expression (1.82 ± 0.22) than oocytes recovered from Gyr cows (1.12 ± 0.11). Previous study reported that oocytes from Gyr cows in a tropical climate showed a higher blastocyst rate after *in vitro* fertilization than Holstein oocytes (Camargo *et al.* 2006 Reprod. Fertil. Dev. 18, 243 abst). The lower level of HSP70-1 in Gyr oocytes suggests that they were less subject to stress than the Holstein ones, which may reflect their capacity to develop after fertilization. This effect may be, at least in part, due to the ability of *Bos indicus* cows to regulate body temperature in a hot environment, causing less stress on oocytes.

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263 GENE EXPRESSION PROFILING OF IMMATURE AND *IN VITRO*-MATURED BOVINE OOCYTES USING AFFYMETRIX GENECHIP TECHNOLOGY

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Previous studies by our group have demonstrated that oocyte maturation is a crucial event in the determination of subsequent developmental competence. The objective of the current study was to characterize changes in gene expression profiles of bovine oocytes during meiotic maturation. To this end, 5 replicate pools of 200 bovine cumulus–oocyte complexes (COCs) were collected from the ovaries of slaughtered heifers. Upon recovery, 100 COCs from each replicate were immediately denuded, and the oocytes were snap frozen in liquid nitrogen. The remaining 100 COCs were matured *in vitro* in TCM-199 supplemented with 10% (v/v) fetal calf serum and 10 ng mL^{-1} epidermal growth factor for 24 h at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity. Following maturation, the remaining COCs were denuded and snap frozen. Total RNA was isolated (mean total RNA content 106.08 ± 38.87 ng per 100 oocytes) and subjected to 2 rounds of amplification incorporating biotin-labeled nucleotides during the second *in vitro* transcription reaction (mean total RNA content 155.15 ± 51.14 μg per 100 oocytes post-amplification). The resulting labeled antisense RNA was hybridized to a GeneChip Bovine Genome Arrays (Affymetrix, Inc., Santa Clara, CA, USA) (10 chips, 5 replicates each of immature and mature oocytes, $n = 100$ oocytes/chip). Expression data were analysed using Genespring software (Agilent Technologies, Palo Alto, CA, USA), and data were normalized to the median. Overall, 54.9 ± 1.3% and 53.3 ± 3.3% of the 24 178 probe sets representing 23 000 transcripts spotted on the arrays were expressed in immature and *in vitro*-matured oocytes, respectively. Across the 5 array comparisons, 52 genes were consistently exclusively present in immature oocytes, whereas 16 genes were exclusively present in mature oocytes. A further 821 genes were found to be differentially expressed (≥2-fold) between the 2 groups ($P < 0.05$), of which 209 were up-regulated and 612 were down-regulated in the *in vitro*-matured oocytes compared with their immature counterparts. The differentially expressed transcripts were classified according to their gene ontology (http://benzer.ubic.ca/ermineJ). The existing Affymetrix annotation was updated by blasting the sequences against bovine, human, and murine databases (≥90% homology; increasing molecular function annotation from 14% to 42%). In terms of molecular function, the majority of these genes were associated with protein or nucleic acid binding (>42%), catalytic activity (24%), signal transduction (7%), transporter activity (5%), and structural molecule activity (5%). In conclusion, we have established the molecular transcriptome blueprint of immature and *in vitro*-matured bovine oocytes. Through comparisons with *in vivo*-matured oocytes, this resource will be invaluable in determining genes that are involved in controlling the developmental competence of oocytes.

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264 ANALYSIS OF DNA METHYLATION PATTERN IN PRE-IMPLANTATION-STAGE EMBRYOS DERIVED FROM NUCLEAR TRANSFER USING PORCINE EMBRYONIC GERM CELLS

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Somatic cell nuclear transfer (SCNT) has been successfully used to produce live cloned offspring in various mammals. However, some studies had reported that cloned embryos by SCNT had many problems in reprogramming or epigenetic modification, such as DNA methylation. DNA methylation is an essential process in epigenetic modification for development, and aberrant methylation in cloned embryos gives rise to abortion, high birth weight, and perinatal death. In this study, embryonic germ (EG) cells were used as donor cells for nuclear transfer. EG cells may have less reprogramming or demethylation than SCNT because these are already in erased status. However, little is known about methylation state or developmental capacity of the EG cell as a donor. The objective of this study was to analyze the methylation pattern of pre-implantation embryos cloned from porcine EG cells. Two regions, PRE-1 and microsatellite (MS), were analyzed for methylation patterns of cloned embryos from porcine EG cells and compared with the pattern of mature oocytes and *in vitro*-fertilized (IVF) embryos as a control. Cumulus–oocyte complexes were collected from prepubertal gilt ovaries and matured *in vitro* for 44 h, followed by use for IVF and NT with porcine EG cells. The porcine EG cells were prepared from 28-day-old fetuses after mating; genital ridges were isolated from fetuses, and then transferred into a culture medium on a feeder layer. The number of embryos for analysis was 300 for matured oocytes, 50–80 for 4–8 cell embryos, 30–40 for morulae, and 20–30 for

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