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Corrigenda

198 AGE EFFECT ON RELATIVE GENE EXPRESSION OF BOVINE SPERMATOGONIA

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Because of mistakes in the information supplied, the original list of authors was incorrect. The correct author list is:

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the Student–Newman–Keuls test (SNK; $P < 0.05$). The FUGW plasmid promoted a higher rate of transfection and fluorescence intensity than pEGFPN2 in all cell types evaluated. When the FUGW plasmid was used, higher transfection rates were obtained with fetal fibroblasts (FF: 17.8 ± 2.82; AF: 10.66 ± 0.65, CC: 3.9 ± 1.97), while higher fluorescence intensity was observed in adult fibroblasts (FF: 4542 ± 497.09; AF: 9367.5 ± 3490.9, CC: 3496 ± 2638.92). The $pEGFPN_2$ plasmid showed percentage of transfected cells and fluorescence intensity significantly higher than the control only in cumulus cells (pEGFPN2 – FF: 4.9 ± 0.14 and 206.47 ± 755; AF: 760 and $2.4 \pm 0.70 \pm 330.92$; CC: 3.9 ± 1.97, and 1418 ± 36.06, respectively; control – FF: 0.15 ± 0.07 and $249 \pm 6 : 36$; AF: 0.15 ± 0.07 588 ± 213.54, and CC 0.05 ± 0 214 ± 0.07, respectively). We conclude that the plasmid construction may influence the overall efficiency in transfected cells; however, the transfection percentage and fluorescence intensity is greatly influenced by the cell type. We suggest that transgenesis of a specific cell type may be enhanced by the proper choice of the expression vector.

198 AGE EFFECT ON RELATIVE GENE EXPRESSION OF BOVINE SPERMATOGONIA

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Spermatogonial stem cells (SSCs) have important applications in treatments for infertility and animal transgenesis. However, the isolation of bovine SSCs is less efficient than for other species and it is lower for adult bulls. The goal of this study was to verify whether the relative gene expression of spermatogonia is affected by age after the laminin differential plating. Ten grams of parenchyma of testicles from pre-pubertal animals (5 months of age, $n = 5$) and bulls (3–4 years of age, $n = 5$) were minced and digested: colagenase (1 mg mL^{-1}) for 30 min at 37°C and trypsin (2.5 mg mL^{-1}) for 5 min at 37°C. Cells were plated (3×10^6 viable cells) in 60-mm culture dish covered with laminin (20 ng mL^{-1}) and they were cultured for 15 min in high-humidity atmosphere with 5% of CO_2 at 37°C. The adherent cells were recovered by enzymatic digestion with 0.1% trypsin for 1 min at 37°C. Viable cells were selected by the Trypan exclusion method, RNA was extracted from ~200.000 viable cells (Ilustra RNAspin Mini RNA, GE Healthcare, Waukesha, WI, USA) and cDNA synthesis was performed (SuperScript® VILO™, Invitrogen, Carlsbad, CA, USA). *ITGA6* (*integrin*, α 6), *SELP* (Selectin P) and *ICAM* (Intercellular Adhesion Molecule 1) relative gene expression were determined by real-time RT-PCR (Mastercycler ep Realplex, Eppendorf International, Hamburg, Germany); *GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase) and *ACTB* (actin β) were used as housekeeping genes. The statistical analysis was performed by QPCR_MIXED (SAS®, SAS Institute Inc., Cary, NC, USA). An α level at 0.05 was always adopted. *ITGA6* and *ICAM1* relative expression weren't effected by age; respectively, $P = 0.2367$ and $P = 0.3583$. However, *SELP* was highly expressed in adult bulls ($P = 0.0022$). Previously, *ICAM1* and *SELP* have also been shown to mark aging haematopoietic stem cells and were more highly expressed in spermatogonia from adult mice than younger. However, SELP is expressed in more differentiated spermatogenic cells such as human sperm. The expression levels of *ITGA6* did not seem to be significantly altered by age, indicating that age affects only certain SSC properties.

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199 *IN VITRO*-DEVELOPED BOVINE BLASTOCYSTS ARE MARKED WITH ABERRANT HYPER- AND HYPO-METHYLATED GENOMIC REGIONS

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Most often, *in vitro* produced embryos display poor quality and altered gene expression patterns compared to their *in vivo* counterparts. Aberrant DNA methylation occurring during *in vitro* embryo development is believed to be one of the multifaceted factors which may cause altered gene expression and poor embryo quality. Here, we investigated the genome-wide DNA methylation patterns of *in vitro* derived embryos using the recently developed Bovine EmbryoGENE Methylation Platform (BEGMP) array (Shojaei Saadi *et al.* BMC Genomics 2014 **15**, 451. doi: 10.1186/1471-2164-15-451) to unravel the aberrantly methylated genomic region in *in vitro* developed embryos. For this, *in vitro* and *in vivo* produced blastocysts were produced and used for genome-wide DNA methylation analysis. *In vitro* blastocysts were produced from oocytes retrieved from ovaries collected from the local abattoir and matured, fertilized, and cultured *in vitro* using SOF media. The *in vivo* blastocysts were produced by superovulation and AI of Simmental heifers followed by uterine flushing. Genomic DNA (gDNA) was then isolated from four replicates (each 10 blastocysts) of *in vivo* and *in vitro* derived blastocysts using Allprep DNA/RNA micro kit (Qiagen, Valencia, CA, USA) and the gDNA was then fragmented using the MseI enzyme. Following this, MseLig21 and MseLig were ligated to the MseI-digested genomic fragments in the presence of Ligase enzyme. Methyl-sensitive enzymes, *Hpa*II, AciI, and Hinp1I, were used to cleave unmethlayted genomic regions within the MseI-MseI region of the fragmented DNA. The gDNA was subjected to two rounds of ligation-mediated polymerase chain reaction (LM-PCR) amplification. After removal of the adapters, the amplified gDNA samples from *in vivo* or *in vitro* groups were labelled either Cy-3 or Cy-5 dyes in dye-swap design using ULS Fluorescent gDNA labelling kit (Kreatech Biotechnology BV, Amsterdam, The Netherlands). Hybridization was performed for 40 h at 65°C. Slides were scanned using Agilent's High-Resolution C Scanner (Agilent Technologies Inc., Santa Clara, CA, USA) and features were extracted with Agilent's Feature Extraction software (Agilent Technologies Inc.). The results have shown that from a total of 414 566 probes harboured by the BEGMP array, 248 453 and 253 147 probes were detected in *in vitro* and *in vivo* derived blastocysts, respectively. Data analysis using the linear