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Oocyte quality and *in vitro* embryo production of aged Nellore cows selected for fertility

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Decreased fertility with maternal aging has been well documented in cattle. The main causes of the low fertility in aged cows are attributed to the poor quality of the oocytes and to depletion of the number of oocytes, due to endocrine disturbances and exhaustion of the ovarian follicular reserve, respectively. However, in some circumstances, cows older than the age in which they are regularly discarded from the herd can still ovulate regularly a good-quality oocyte and give birth to healthy calves. The present study aimed to evaluate the oocyte number and quality of aged and young cows, submitted to ovum pickup (OPU). Lactating Nellore cows (*Bos indicus*) with a history of annual calving and ages of 17 to 20 years (n=12) and 8 to 10 years (n=15), were treated with a progesterone vaginal device (PD) containing 1 g of progesterone and injected, by IM route, with 2 mg estradiol benzoate and 0,5 µg of cloprostenol. Five days later, the PD was removed and the oocytes were recovered by OPU. A second OPU was done five days later in all cows. The number and quality of recovered cumulus-oocyte complexes (COCs) were registered. The quality of immature COCs was classified as good (viable) and poor (not viable), according the appearance of cumulus, compaction and granulation of ooplasm. The COCs were matured in TCM plus FSH and 10% estrous cow serum. After fertilization, presumptive zygotes were co-cultured with cumulus cells to assess developmental rates to blastocyst. Data were analyzed by Chi-square and Anova tests. In total, 826 oocytes were recovered, of which 510 (mean = 42.5 ± 7.4) were from aged cows and 316 (mean = 21.1 ± 6.7) from young cows, respectively ($P < 0.05$). Although the proportion of morphologically viable oocytes was not different among groups (59.8% and 55.7%, respectively ($P > 0.05$), more viable oocytes were recovered from aged than young cows (37.7 ± 6.8 vs 15.0 ± 6.1), respectively ($P < 0.05$). More total oocytes were recovered in the first OPU than in the second OPU in both aged (28.9 ± 5.4 and 12.6 ± 3.1) and young (15.6 ± 4.9 and 5.8 ± 2.4) cows ($P < 0.05$). Similarly, the number of viable oocytes was higher in the first than in the second OPU (older = 25.4 ± 4.8 and 12.3 ± 2.3; young = 11.7 ± 4.3 and 3.3 ± 2.1), respectively ($P < 0.05$). Three aged cows older than 20 years did not produce oocytes at the two OPUs. There were significant differences among groups ($P < 0.05$) in cleavage rate (aged = 75.7%, and young = 95.4%) and blastocyst formation (aged = 26.9% and young = 39.2%). The mean number of blastocysts per cow was 3.1 ± 0.3 and 2.9 ± 0.3 for aged and young cows, respectively ($P > 0.05$). In conclusion, although the oocyte quality appears to be lower in aged cows, the higher number of oocytes produced by aging cows, selected for fertility, makes embryo production no different from young cows. Furthermore, the age threshold at which cows stop producing oocytes seem to be around 20 years.

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Differences in transcriptomic data from preovulatory follicles of buffalo *(Bubalus bubalis)* and (*Bos indicus*) cattle: A meta-analysis

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For years investigating the differences in reproduction between species has been restricted to describing the differences in clinical or biological parameters evaluated at different levels including gene expression using "omics," but very few publications have reported this information to understand the source of the differences or to analyze their influence on the reproductive performance of the species. Despite that buffalo and cattle belong to the bovine family, it has been reported that buffalo has a smaller ovary size with less number of primordial and antral follicles and higher incidence of atresia compared with cattle. This may provide a possible explanation for the reduced response in buffaloes to ovarian follicular hyperstimulation, low recovery of embryos and oocytes and low number of transferable embryos. The ovulatory process in mammals follows the same developmental pattern, the meiotically arrested oocyte must grow inside the follicle until ovulation, and if two closely related species have the same pattern in their estrus cycle, it can be deduced that the physiological and the molecular events are also similar. The aim of this paper was to compare the gene expression pattern of the granulosa cells of preovulatory follicles of buffalo and cattle, based on the available and comparable data reported in PubMed GSE39589 and GSE11312. In both experiments RNA was extracted, converted to cDNA, evaluated using Affymetrix Gene- Chip Bovine Genome Arrays, which contained 24,128 probe sets. Genes with =2 fold change as cut-off for identification where considered differentially expressed. The comparison of relative expression of the top 15 up- regulated genes from buffalo and cattle were very different, only 3 genes (20%) PLAT (plasminogen activator tissue), STAR (steroidogenic acute regulator, similar to Steroidogenic acute regulatory protein), F2RL- 1 (Coagulation factor II (thrombin) receptor-like 1) were expressed in the two species. It is very important to note the relative expression of the genes was very different, 9.7 vs 17.5, 8.7 vs 3.4, and 7.7 vs 5.3 fold for PLAT, STAR AND F2RL-1, respectively. All the shared genes are not related specifically with follicle development and are not considered as markers of ovulation. Although the two species are very close bovines and with the simplicity of the analysis, it seems that each one has its own different pathways leading to the same endpoint; i.e., ovulation. More sophisticated bioinformatic tools will be necessary to analyze the data to compare directly the data sets to construct a theory to explain the differences observed in the field. Asociación Colombiana de Criadores de Búfalos.

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Histone deacetylase inhibitor during pre-maturation (PIVM) and/or *in vitro* maturation (IVM) of bovine oocytes: effect on transcript levels of histone acetylation related genes

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Pre-maturation (PIVM), a period that precedes *in vitro* maturation (IVM), has been reported as a means to provide oocytes additional time to acquire competence and increase their developmental potential. The rationale behind PIVM approaches is to allow mRNA and protein accumulation within the ooplasm before meiotic resumption. However, for mRNA transcription to occur oocyte chromatin should be in A permissive or hyperacetylated status. The objective of the present study was to determine if the presence of a histone deacetylase inhibitor during PIVM and/or IVM could affect the expression profile of genes involved in the histone acetylation/deacetylation processes in bovine oocytes. Cumulus oocyte complexes (COCs) obtained from slaughterhouse ovaries were submitted to a PIVM using 100 nM of C-type natriuretic peptide (NPPC), in the presence or absence of a deacetylase inhibitor (500 nM of scriptaid). Grade 1 and 2 COCs were distributed into 5 groups: T1- IVM for 22 h; T2- PIVM for 6 h and IVM for 22 h; T3- PIVM with Scriptaid for 6 h and IVM for 22 h; T4- PIVM for 6h and IVM with Scriptaid for 22 h; and T5- PIVM with Scriptaid for 6h and IVM with Scriptaid for 22 h. For gene expression analysis, oocytes from all groups were collected at 0h of IVM and PIVM, 6 h of PIVM and at 22h of IVM. Levels of transcripts for genes coding for enzymes involved in acetylation (HAT1 and KAT2A) and deacetylation (HDAC1 and HDAC3) of histones were determined by qPCR, using the constitutive gene PPIA for normalization. Total RNA was extracted from 3 pools of 20 oocytes from each treatment. Data were analyzed by ANOVA, and the means compared by Tukey test ($P < 0.05$). Expression of all genes studied was similar among treatments at any of the different time points evaluated. However, when the profile of the genes during PIVM and IVM was analyzed it was observed that transcript levels for HAT1 in the control group decreased during maturation, being lower at 22 h compared to 0 h ($P < 0.05$). A different profile was noted when Scriptaid was present during either PIVM, IVM or both, since no decrease in transcript levels for the HAT1 gene was observed during maturation, but rather the levels were similar to those at the beginning of maturation ($P > 0.05$). For the other genes no alterations were observed during PIVM and/or IVM ($P > 0.05$). In conclusion, the presence of a histone deacetylase inhibitor during PIVM and/or IVM affected the transcript level of HAT1, preventing its decrease that occurs during *in vitro* maturation of bovine oocytes. Financial support: FAP-DF, CNPq, Embrapa.

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Heritability and genetic correlations for scrotal circumference at different ages in Brahman bulls raised under tropical conditions

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Beef herds in the tropics are based on the utilization of *Bos indicus* and its crosses bred naturally. Genetic progress has been traditionally restrained by many factors including social, climatic, nutritional and management, among others. Besides, there is little selection of sires through variables correlated to their profitability, such as scrotal circumference (SC). This fact justifies attention to and research on those valuable characteristics as selection criterion to benefit genetic improvement in those systems. This paper aimed to determine the heritability index and genetic correlations for SC at different ages in Brahman bulls, to establish its use as a robust selection criterion at early ages in sires raised under tropical conditions. Heritability index (h^2) and genetic correlations (r^g) for SC at different ages were determined in 485 full blood Brahman bulls distributed in 8 herds from the North dry Pacific region of Costa Rica. Monthly SC measurements were performed by the same operator from 7 to 24 months of age. The genetic estimates were determined with a bivariate random regression animal model (SAS, ver 9.3, 2010). The model considered the fixed effects of herd, year and season of birth, nutritional plane, body weight, age at weaning and calving number of the mother. In addition, the random permanent effect of environment and animal additive genetic effect were considered. The genealogy data base included 3000 animals distributed in 7 ancestors´ generations. The average h^2 index for SC was 0.58 (range 0.48-0.72), being highest at 20 months of age. In addition, SC h^2 was higher in the period comprising 14 to 20 months of age compared to younger ages. The h^2 index found in this study in Brahman bulls is higher than previous reports in crossbred zebu sires in Australia (Brahman and Sahiwal x Shorthorn; h^2=0.40 and 0.45 at 18 and 24 months respectively; Fordyce et al., 1996. Aust J Exp Agric, 36:9-17). In contrast, they are lower than those published for most *Bos taurus* breeds raised under sub-tropical climate. Coulter for instance (Coulter *et al*., 1976. J Anim Sci, 43:9-12), reported average h^2=0.68 in Holstein bulls under conditions of an experimental station. The SC r^g coefficients obtained in this study in Brahmans aged 7 to 24 months ranged from 0.43 to 1. Furthermore, r^g among yearling and bulls 18, 20, 22 and 24 months of age were higher (0.95, 0.92, 0.89 and 0.84 respectively) than those obtained among 7 months-old bulls and the same age range (0.43, 0.43, 0.44 and 0.45). These findings indicate that selection of Brahman sires by their SC can be performed as early as 12 months of age with high confidence (>80%) of their SC when adults (24 months). In addition, the lower h^2 and r^g for SC obtained among bulls <11 months and older ages (20-24 months), suggest that the initial post weaning is a stressful and adaptation period in Brahman steers. This fact should be considered by breeders and practitioners when choosing the right age for selection of prospective Brahman sires. This study was funded by the Andrology Section-UNA.

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The embryo regulation of the immune system in day 14 endometrium is affected by the level of nutrition

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The effects of the presence of conceptus and undernutrition on the endometrium transcriptome at Day 14 of the oestrous cycle or pregnancy were investigated. Adult Rasa Aragonesa ewes were allocated to one of two nutritional treatment groups: control, fed to maintenance requirements and undernourished, fed at 0.5-fold of daily requirements for maintenance. Sheep uterus transcriptome profiles were evaluated using an ovine oligonucleotide microarray in control and undernourished cyclic and pregnant ewes (n=4 each). Microarray data were analyzed with a mixed model using Proc MIXED (SAS Inst.). Functional bioinformatics analyses were performed using the Dynamic Impact Approach and Ingenuity Pathway Analysis. The upregulation of genes belonging to immune system pathways were consistent with the presence of an embryo regardless the nutritional treatment, underscoring their importance for pregnancy maintenance. Cytosolic DNA-sensing, RIG-I-like receptor signaling, and Toll-like receptor signaling pathways were upregulated in pregnant ewes. The presence of the embryo stimulated gene expression of the Interferon-induced helicase C domain 1 by 3.4-fold (control ewes) or 2-fold (undernourished ewes), which modulates local immune cells in the endometrium during pregnancy. Similarly, citokine *CXCL10* was upregulated by pregnancy with 6- (control) or 3- (undernourished) fold change, and is one classical interferon stimulated gene with biological effects on trophectoderm growth and adhesion in ruminants. While cytokine *CXCL12* (essential role in communication between trophoblast cells and the maternal endometrium) and Toll like receptor 7 (by acting in the trophectoderm influences conceptus development and IFNT production) were upregulated in control pregnant ewes, they remained unchanged in undernourished pregnant ewes. Activation of nuclear factor kappa B (3-fold change in undernourished pregnant vs. cyclic ewes) enhances uterine receptivity and development of conceptus during establishment of pregnancy. It is noteworthy that despite the similar flux among these pathways between control and undernourished ewes, the impact was lower in undernourished pregnant vs. cyclic animals, probably associated with a lower fold change in immune related genes, which could be associated with embryo losses after maternal recognition of pregnancy. Few genes of the immune system were affected according to nutritional treatment in pregnant ewes. In undernourished pregnant ewes, immune-related genes with known increased expression during pregnancy (CD180 molecule, Complement 5 and MYD88: myeloid differentiation primary response 88) were downregulated in undernourished vs. control pregnant ewes. CD180 is a TLR4 accessory protein that potentiates its action, and TLR4 through MYD88 induce activation of cytokines production involved in modulate the embryo-maternal tolerance. Indeed, C5 compensates for the decreased adaptive immunity observed in normal pregnancy, and is aimed to protect the mother and fetus from antigens. Clearly, downregulation of these important genes in undernourished ewes could be detrimental for successful uterine development, a scenario that agrees with the embryo mortality occurring after the period of maternal recognition of pregnancy.

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Dynamic changes in bovine endometrial stem cells throughout estrus cycle and postparturition

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We isolated and characterized bovine endometrial mesenchymal stem cells (beMSCs) during follicular (FP), early luteal (ELP) and late luteal (LLP) phases of the estrous cycle and after parturition in healthy cows (PPHE) and in cows with subclinical and clinical endometritis (PPSE and PPCE). Estrous cycle stages were confirmed by plasma P4 and 17ß estradiol measured by RIA, and health status by cytological and microbiological diagnosis. Characterization of putative beMSCs was based on fibroblast-like morphology, adherence to plastic and proliferation and colony formation at low density (cloning efficiency; CE) and also by expression of embryonic and MSC markers at mRNA (OCT4, NANOG, SOX2, and CD44, CD117 via RT-qPCR) and protein (Oct4, Sox2, and Cd44) levels. For the latter we used immunohistochemistry in fixed tissues and/or Western blot (WB) for both tissue and cells. We also evaluated the ability of isolated cells to differentiate into chondrogenic(C), adipogenic(A) and osteogenic(O) lineages after 7 and 14 days of induction, as measured by lineage-specific staining (Alcian blue, Oil Red and Alizarin Red, respectively) and by specific gene (AGGRECAN and SOX9 for C, PAPF1 for A, and SPARC and RUNX2 for O) and protein expression (Aggrecan for C, Ppar? for A, and Sparc for O) expression via RT-qPCR and WB. Cell lines were derived from endometrial biopsies by enzymatic digestion and cultured in supplemented DMEM-F12 (10% FCS, 1X AAM solution, 1mM sodium pyruvate, 2mM L-glutamine) at 5% CO_2, 39°C and full humidity. Results are shown separately for estrous cycle (group1) and post- partum (group2) or mixed. Statistical analysis of RT-PCR and CE was conducted using Kruskal–Wallis nonparametric test. All statistical analyses were tested for a=0.05. Putative beMSCs isolated from all groups showed fibroblast-like morphology and adherence to plastic. The ability to differentiate into C, A, and O mesodermal lineages after 7 and 14 days was not homogeneous among the samples: ELP cells did not differentiate and PPCE did not differentiate to the A lineage as judged by staining and further confirmed by individual analysis of specific differentiation markers. In all groups, cells proliferated and formed colonies when plated at low density. The best CE was in ELP cells (0.61±0.08; group1) and PPHE (0.64±0.1; group2), whereas PPCE displayed the lowest CE (0.08±0.1) of both groups. Expression of markers (RNA): both groups expressed with varying levels SOX2, OCT4, CD44, and CD117 both in cells and tissue, being higher in the latter. NANOG was not detected at all. Protein: Sox2 in all groups, Oct4 and Cd44 only in group1. All analyses were statistically significant. Seemingly, there are distinct populations of stem/progenitor cells in cow endometria during the estrous cycle, puerperium and endometritis. This detailed characterization of beMSCs had not been published earlier. Funding: Fondecyt1110642 and Conicyt national doctoral scholarship N°21150425, Government of Chile.

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A Comparative analysis of calcium channels in holstein and hanwoo (korean cattle) in the duodenum and kidney

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Transembrane calcium (Ca^{2+}) channels such as transient receptor potential cation channel subfamily V members 5 and 6 (Trpv5/6), Na^{+} /Ca^{2+} exchanger 1 (Ncx1) and plasma membrane Ca^{2+}-transporting ATPase1 (Pmca1b) are known to play an important role in maintaining homeostasis and metabolizing Ca^{2+} ions. Trpv5 and Trpv6 play an important role in Ca^{2+}absorption to the cell, while Ncx1 and Pmca1b play a role in Ca^{2+} excretion from the cell. Holstein is known to provide higher milk production than other cattle breeds but in this respect, it has higher susceptibility to hypocalcaemia that is a risk factor for many of calcium-related diseases such as milk fever. In contrary, Hanwoo (Korean cattle) is relatively strong in the calcium-related diseases. The hypothesis of this study is the differently expressed calcium transport genes in duodenum and kidney results in an increased prevalence of calcium related diseases such as milk fever. Genetic background (or breed) will influence transcript abundance of calcium transport genes in the duodenum and kidney. Expression of Trpv5/6, Ncx1 and Pmca1b was analyzed by realtime polymerase chain reaction (Realtime-PCR), Western blot analysis, and immunohistochemistry. Data were expressed as means ± standard deviations and were analyzed by using a nonparametric one-way ANOVA followed by the Tukey post hoc test (n = 12 per each group). In dairy cows (Holstein), Trpv5 mRNA was greater in kidney and duodenum than in kidney and duodenum of Hanwoo cows. Conversely, Pmca1b mRNA was less in Holstein cows than in kidney from Hanwoo, but no difference in Trpv6 or Ncx1 mRNA expression was observed in duodenum and kidney between the two breeds. Protein expression showed similar patterns in Hanwoo and Holstein cows to those of mRNA expression data. Localization of calcium transporter genes were identified in the glomerulus, proximal and distal convoluted tubules expressed TRPV5, 6, Pmca1b and Ncx1 in the kidney. These four calcium transport genes may play an important role in bovine duodenum, and kidney. But the difference between Holstein and Hanwoo, which show different gene expression patterns, may be helpful in studying diseases associated with calcium metabolism and to develop estartegies to prevent milk fever. This work was supported by a National Research Foundation of Korea (No. 2017R1A2B2005031) grant funded by the Korean government.

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Similarities in endometrial transcriptomic profile between high producing and anestrous dairy cows

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High milk production (HMP) dairy cows present a profound increase in feed intake which has been associated with higher steroid metabolism and lower concentration of plasma P_4 and E_2 compared with medium milk production (MMP) cows. Low P_4 may alter endometrial gene expression, reducing the capacity of the uterus to support conceptus development. However, the effect of low P_4 concentrations on endometrial gene expression is not well known yet. The aim of this study was to compare endometrial gene expression among HMP, MMP, superovulated (SO) and anestrous (ANE) cows during diestrus using RNA-seq technology. Grazing lactating dairy cows (~50 DIM) without any clinical disease were allocated to four groups: HMP (~10,000 kg/305d, n=3), MMP (~7,500 kg/305d, n=3), ANE (follicles <8 mm, without CL, <0.3 ng/mL P_4; n=3) and SO (P_4 =90 ng/mL; n=3). Cows from HMP, MMP and SO groups were synchronized with a Presynch-Ovsynch protocol. The SO cows also received a superovulation protocol. Blood was obtained on days 0, 4 and 9 of the estrous cycle to measure P_4 and E_2 concentrations by chemiluminescence (Immunoanalyzer Elecsys and Cobas e, Roche). Endometrial samples were collected using a biopsy instrument on day 9 and stored at -80ºC until RNA extraction. The cDNA libraries for RNA-seq were constructed using the TruSeq Stranded mRNA and sequenced using the HiSeq 1500 platform 2x150pb (Illumina Inc. CA, USA). Resulting sequence reads were aligned to the bovine reference genome (bosTau8), using the Rsubread package (R-software). Multidimensional scaling (MDS) was performed to determine similarities between samples according to gene expression. Differentially expressed genes (DEG, FDR<0.05) in HMP compared to MMP, and in ANE compared to SO, were determined by genewise statistical analysis (EdgeR package). The resulting up or down-regulated DEG were compared by Pearson's chi-square test to determine relatedness between the same significant genes determined in both HMP vs MMP and ANE vs SO (overlapping DEG). Enrichment analysis of the significant overlapping genes was performed with DAVID database. The MDS analysis showed that samples from MMP and SO cows clustered apart from HMP and ANE cows' samples. The numbers of upregulated DEG were 353 and 897, while down- regulated DEG were 324 and 764, for HMP versus MMP and ANE versus SO, respectively. There were 124 overlapping genes for the up-regulated DEG ($P=1.8x10^{-88}$) and they were enriched for MHC Class I (P=0.0000002), innate immunity ($P=3.3x10^{-8}$) and inflammatory response ($P=4.7x10^{-6}$). For the down-regulated DEG, the 40 overlapping genes ($P=1.8x10^{-5}$) were enriched for the GnRH signaling pathway (P=0.01), oxytocin signaling pathway (P=0.03) and calcium signaling pathway (P=0.004). In conclusion, HMP cows in diestrus and ANE cows present a similar endometrial transcriptomic profile showing enriched with up-regulated genes involved in the immune system and inflammatory response. This work was supported by a PICT 2014-0414 grant to LVM, by UNLP Incentive Program V11/230 grant to RLS and by ARPECOL grant to RLS. Keywords: RNA sequencing, dairy cows, milk yield, endometrial expression.

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Expression of immunological markers by bovine endometrial stem cells after priming with PGE_2

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The aim of this research was to characterize, at the mRNA level, the response of bovine endometrial mesenchymal stem cells (beMSCs) to an inflammatory illness-like environment after priming with PGE_2. For this, we used previously tested beMSC lines from the late luteal phase of the estrous cycle, which showed an altered expression of immune response genes after PGE_2 priming (Lara *et al*., 2017. Stem Cells International. Doi.org/10.1155/2017/4297639). All cell lines were cultured in DMEM-F12 medium supplemented with 10%FCS,1X AAM solution, 1 mM sodium pyruvate and 2 mM L-glutamine, at 5%CO_2, 39°C and 100% humidity. The beMSCs showed fibroblastoid-like morphology, adherence to plastic, and the ability to differentiate into chondrogenic(C), adipogenic(A) and osteogenic(O) mesodermal lineages when treated for 7 and 14 days with lineage specific inducers. The expression of immunological markers was measured after priming of beMSC cultures with 0 (mock), 1, 3 or 10 μM of PGE_2, pH 6.8 (Caymann Chemical) in the presence of serum. All experiments were repeated three times. After 28 hours of priming, the cells were scraped, and subjected to RT-PCR. For this, RNA extraction and synthesis of cDNA was performed using EZNA Total RNA Kit I (Omega Biotek) and M-MLV Reverse Transcriptase enzyme (Thermo Fisher), respectively. We used DDCT RT-qPCR, to measure the relative expression of the following genes: IL6, IL10, PGES, TNFa, IFNg, TLR2, TLR4 and COX2; ACT B expression was used as housekeeping gene. Multiple comparison statistics was performed comprising fold change of expression of individual genes in all cell lines and doses of PGE_2 priming. For this, ANOVA and Fisher LSD post-hoc test were used. Values of $P<0.05$ were considered as statistically significant. After priming with PGE_2, statistically significant, dose dependant over expression was found among all evaluated dosages in relation to untreated, for all genes except COX2. For IL6, expression was different for all dosages except 1 μM of PGE_2 and for TNFa at 10μM. In conclusion, priming of beMSC with PGE_2 induces a shift in the expression of important inflammation-related genes which can be associated to the response observed in uterine disease. Apparently 1 μM is sufficient to induce such response. These results confirm at the individual gene level our previous transcriptomic findings. No such data are available in the literature for beMSC. Our findings might have an impact on the development of PGE2-based strategies to manage inflammation in uterine disease such as endometritis in cows. Funding: CORFO-INNOVA national funding for undergraduate thesis code EM.TES-67609, Government of Chile.

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Abductive analysis of metabolic interactions in bovine mammary epithelial cells

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Studying the regulation and interactions of different pathways of amino acid (AA), carbohydrate (CHO), lipid, and energy (E) metabolism in mammalian cells is important to understand the mechanisms of milk protein synthesis. An abductive type analysis was carried out, to provide an approximation to a description of the possible metabolic relationship of the proteome in an in vitro model of bovine lactocytes. Primary bovine mammary epithelial cells (PBMEC) were cultured for 6 days after reaching confluence in lactogenic DMEM. Following lysis of cells, cellular total protein concentration was measured by BCA (Pierce®). Extracts of total protein were subjected to label-free, quantitative proteomics using liquid chromatography separation and mass spectometry analysis (ISOQuant®). To study the metabolic pathway, a multi-fasta file was created with all protein sequences and submitted to BlastKOALA in KEGG. KEGG pathways (n=44) were grouped into metabolism of AA, CHO, lipids and E. Using the KEGG results, a data matrix was created with the metabolic pathways as columns, the proteins identified within these pathways as rows, and the values of the proteins as absent or present. To better understand the dynamics underlying the metabolic pathways, and as a first data exploration, a P-value matrix of chi-squared distances was calculated and a pictogram was created including values equal to or less than 0.1. To eliminate proteins and metabolic pathways that were not significant ($P>0.05$), multiple correspondence analysis was used, and the R^2 of each metabolic pathway was calculated to determine its relevance in the analysis. An algorithm was designed that iterative conduct multiple correspondence analysis and select significant variables between each iteration, eliminating routes that were not significant to the first dimension or component (protein synthesis, PS). This procedure was performed until there were no metabolic pathways to eliminate. Finally, a cluster analysis was performed using the resulting data matrix from which the possible simultaneous relationships between the proteins were detected. The groups of routes corresponding to the metabolism of AA and of CHO, represent the greatest number of interactions, 19 and 26 respectively. The algorithm took five iterations until the number of non-significant metabolic pathways was equal to zero. The metabolism of tryptophan was the route that explained most of PS variance (70%), while the biosynthesis of arginine explained the least (20%). The cluster analysis resulted in 9 groups of proteins (n=36) that possibly interact in 9 pathways: ascorbate and aldarate metabolism; fatty acid degradation; glycerolipid metabolism; alanine, aspartate and glutamate metabolism; valine, leucine and isoleucine degradation; lysine degradation; arginine biosynthesis; histidine metabolism; and tryptophan metabolism. We conclude that the PBMEC model is a useful tool to study metabolic interactions in the complex milk protein synthesis pathway. Acknowledgement to University of Antioquia for granting ZT R-C a postdoctoral commission 2016-17; to the College of Agriculture and Life Sciences David R. and Margaret Lincicome Endowment at Virginia Tech for partial support of ZT R-C as Visiting Scientist, and to Dr. Loor for cell donation.

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Effect of maternal ability of Corriedale and Corriedale Pro ewes on lamb growth

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Maternal ability of ewes of two biotypes and its effect on growth and development of their lambs were evaluated under grazing conditions in Uruguay. The biotypes used were Corriedale (C) and Corriedale PRO (C-PRO). The latter is a prolific biotype composed of 25% Finnish Landrace (FL), 25% East Friesian (EF) and 50% C. All the lambs were C-PRO, either from C-PRO ewes sired by rams from the same biotype or C ewes sired by 50% FL and 50% EF rams. From 132 ewes, 205 lambs were born (117 male and 88 female lambs); 52 ewes were single bearing (34 C-PRO and 18 C) and 80 were twin bearing (41 C-PRO and 39 C). The ewes were kept on native pasture and 45 days before parturition twin bearing ewes (as diagnosed by ultrasound) were moved to a cultivated pasture for the rest of the experimental period. Lamb weight was recoded at birth (BirthWt) and on average at days 26, 49 and 62 after lambing had peaked (LW1, LW2 and LW3, respectively) and at weaning (WW, day 110). Statistical analysis for BirthWt included the main effects of ewe biotype (C, C-PRO), ewe age (2, 3, or more than 3 years), sex of lamb (male, female), number of fetuses gestated (NFG; one, two) and ewe biotype*NFG. For LW1, LW2, LW3 and WW the effects included in the model were ewe biotype, ewe age, sex of lamb, lamb age in days at recording (LAR) as covariate, NFG*number of lambs reared (NLR; one, two) and ewe biotype*NFG*NLR. For BirthWt, ewe biotype*NFG interaction was statistically significant ($P<0.05$), with higher BirthWt in lambs from C-PRO ewes gestated as singles. Significant effects on LW1, LW2 and LW3 were sex (higher weights for male lambs), NFG*NLR interaction and LAR. Lambs gestated and reared as singles had the highest body weight, followed by lambs gestated as twins but reared as singles, and last the lambs gestated and reared as twins. Ewe age affected only LW2. Ewe biotype*NFG*NLR had a significant effect on WW, with lambs gestated and reared as singles from both biotypes heavier than twin lambs reared as twins or singles. Ewe biotype only affected BirthWt in favor of C-PRO ewes, but not any of the other weights, which suggests no differences in milk production between biotypes in spite of the EF component of C-PRO. Twins had lower BirthWt than singles, which was maintained throughout weaning. The NLR affected initial weights (LW1 and LW2); twins reared as singles had higher weights than when reared as twins, however, no differences were found in LW3 and WW. Authors kindly thank the Refugio stud and the Echeverría family for the use of their flock.

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Quantification of PAG genes in semen of high and low fertility sires using droplet digital PCR

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Substantial variation in pregnancy rates and pregnancy loss exist among sires that are deemed satisfactory in traditional semen fertility evaluations. Developing techniques to identify or predict the field fertility of a sire can help improve sire selection and reproductive efficiency. The objective of this experiment was to evaluate genomic PAG profiles in semen samples from sires classified as having high (n = 3) or low (n = 3) pregnancy loss in a previous experiment (Franco et al., 2018, JAS, 96, 632-640). Extraction of DNA was performed using the Chelex-100 method described by Manuja et al., (2010) and absolute quantification of gene copies was performed using QX100™ Droplet Digital™ PCR System (ddPCR; Bio-Rad, Pleasanton, CA). A pool of DNA collected from 2 Holstein bulls were included in each gene experiment for data normalization. Approximately 16,000 droplets were generated in each well using droplet generator (Bio-Rad QX200) and cycled droplets were read individually with the QX200 droplet-reader (Bio-Rad). Reads were analyzed with QuantaSoft droplet reader software 1.3.2.0 (Bio-Rad) where concentration estimates were based on the fraction of droplets which amplification has occurred. It was modeled as a Poisson distribution and the normalization of the abundance of the query sequence to the control sample. PAG genes from modern group (PAGs 6, 7, 16, 18, 19, 20, 21) and ancient group (PAGs 2, 8) were analyzed and difference in concentration were calculated using PROC GLM on SAS 9.4. Overall, *Bos indicus* sires (Nelore) had numerically increased concentrations of all genes analyzed, but only PAG 21 was statistically different (3.2 vs 1.8 copies/uL; P = 0.06) when compared to *Bos taurus* sires. No statistical difference in individual genes was observed between sires classified as high or low pregnancy loss (P = 0.15). Additionally, there was no statistical difference when genes were grouped by phylogenetic groups. However, it was interesting to observe variance among groups, where ancient PAGs genes were higher in high pregnancy loss samples while modern PAG genes were increased in low pregnancy loss samples (P = 0.1). These differences could be explained by the fact that the assay used to classify the sires was developed targeting modern PAG genes. Although a low number of samples prevented statistical significance from being achieved, variation in PAG gene copy number between sires encourages further exploration of the PAG profile in sires to address differences in fertility and pregnancy loss.