volume of milk produced by TF (200 mL in the peak of lactation), the overall hG-CSF production was high (10.8 mg/day). As expected, the hG-CSF were absent in all milk and serum of NTF. The SCC ranged from 22.7 to 426.1 k/μL in the milk of TF, while for NTF samples were within the normal range (0–1.4 k/μL, Pugh, 2002). Finally, the profile of serum hG-CSF did not match with the milk levels and two peaks (7.39 and 3.47 ng/mL) were detected in the first half of the lactation. Since serum hG-CSF was low (<1.0 ng/mL) in most of period and was lower than 0.1 ng/mL or null outside of lactation, it is possible that detected peaks have been produced by the hormonal treatment. In conclusion, the transgenic founder was able to produce great quantities of hG-CSF in the milk consistent with its use as bioreactor.

Use of two methods for embryo recovery in an embryo transfer program from a founder transgenic goat for human granulocyte colony-Stimulating Factor (hG-CSF)

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In a transgenesis program, after obtaining the founder, its breeding is fundamental to create a transgenic herd secreting the recombinant protein. This study aimed to compare the embryo recovery method (surgical vs. transcervical) to expedite the production of transgenic progeny from a founder transgenic doe for hG-CSF. It was used a transgenic Canindé doe, two non-transgenic Canindé bucks and nine undefined breed recipients. Four in vivo embryo production sessions were performed: two by surgical (laparotomy) and two by transcervical method. The embryo donor superovulated using a hormonal treatment consisting of vaginal sponges impregnated with progestagen coupled to pFSH and cloprostenol injections. From the second session, it was used flunixin-meglumine in the prevention of premature regression of corpora lutea (PRCL). The recipients received progestagen and cloprostenol associated with an eCG injection. Donor mating was performed at estrus onset and 24 h later. The embryo recovery was performed 7 days post-estrus and, just before, a laparoscopy was done to assess the number and quality of CL. The transcervical recovery was performed with a circuit and catheter for small ruminants and the donor received a cloprostenol injection at 12 h before collection. The transgenic kids were identified by PCR. During one surgical session and one transcervical, the collection was not performed due to the occurrence of PRCL and follicular cysts, respectively. In total, it was verified 24 CL and the recovery rate was 69.2% (9/13) and 72.7% (8/11), using surgical and transcervical method, respectively. Sixteen embryos were transferred to the recipients and eight kids were born. Two males (surgical method) and 1 female (transcervical method) were identifying as transgenics. In summary, embryo recovery (surgical or transcervical), coupled with embryo transfer, expedited the production of progeny from a transgenic founder doe.

Effects of cAMP modulation on meiotic progression and developmental competence of in vitro matured sheep oocytes

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Successful oocyte in vitro maturation (IVM) can facilitate the production of large numbers of embryos in a precisely controlled environment for research involving transgenics, cloning, and stem cells. However, IVM oocytes are significantly less viable than those matured in vivo. Removal of oocytes from their follicular environment results in spontaneous resumption of meiosis independent of normal signaling events. Controlling the onset of meiotic resumption via maintenance of elevated cAMP with adenylyl cyclase activation and phosophodiesterase inhibition, and subsequent hormone stimulation improved developmental competence in bovine and murine IVM oocytes (Albuz et al., Human Reprod 2010). This study evaluated the use of cAMP modulation in a sheep model. Changes in oocyte cAMP were quantified during the first 2 h of oocyte maturation in control or cAMP modulatoring medium. A transient drop in cAMP was observed 15 m after oocyte collection, which could be prevented by cAMP modulation. The effect of cAMP elevation on meiotic resumption was measured by completion of germinal vesicle breakdown (GVBD) and progression to metaphase II (MII). Cumulus-oocyte-complexes (COCs) were matured for 8 h in control or modulating medium and immunostained for Lamin A/C. Fewer modulated oocytes underwent GVBD than controls (30.19% vs. 80.13%, respectively). To measure progression to MII, COCs were matured for 20 and 28 h and their DNA visualized with Hoechst staining. Progression to MII was slowed in modulated versus control oocytes at 20 h (55.15% vs. 72.26%), but the effect was transient and modulated oocytes reached 81.34% MII by 28 h. Lastly, the effects of modulation on blastocyst formation were measured. Oocytes were parthenogenetically activated 24 or 28 h post maturation, and cultured to blastocyst. cAMP modulation did not improve blastocyst formation at either time-point (24 h: 19.11% vs. 19.41%; 28 h: 17.24% vs. 25.78%). Thus, while cAMP modulation delays meiotic progression, it does not improve developmental competence of IVM oocytes in sheep.

Profiling of bacteria from the milk of human lysozyme transgenic goats and control goats at early lactation

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Previously, our lab developed transgenic goats which are hemizygous for the human lysozyme gene in efforts to increase the anti-microbial properties of goat's milk to nearer human levels. They express lysozyme in their milk at approximately 270 μg/mL. The presence of lysozyme at more than 1,000 times the levels found in healthy goat milk has the potential to have significant effects on the commensal microbiota of the udder and the bacteria found in the milk. We employed 16S rDNA