We propose to develop the pig as a model for studying the role of NAADP and target the TPC2 gene in pig embryos using CRISPR guide RNAs. DNA constructs were designed to target the TPC2 gene and express Cas9 protein to insert a disabling mutation. To select the most effective constructs, they were tested in an immortalised pig mesenchymal stem cell line. In vivo derived pig zygotes were obtained 24 h post insemination (hpi). Genital tracts were collected at slaughterhouse and zygotes were collected by oviducts flushing. Cas9 mRNA and sgRNA were injected directly into the cytoplasm of pig zygotes. After microinjection, zygotes were transferred to culture medium NCSU-23 for up to 7 days. Then, embryos were frozen for DNA extraction, high resolution melting analysis (HRMA) and DNA sequencing. Embryo development was reduced after embryo injection in comparison to controls (15.21 vs. 43.5% blastocyst formation rate at day 7, $p < 0.05$). In 10 of 28 (35.71%) of injected embryos a mutation was detected by DNA sequencing, confirming that TPC2 KO pig embryos had been produced. It is the first time in our knowledge that a TPC2 KO pig embryo generation has been reported. The efficiency of the methodology must be implemented optimizing embryo manipulation and injection process to produce TPC2 KO piglets after embryo transfer. (Supported by Royal Society IE140324, MINECO AGL2015-66341-R and Fund. Seneca 20040/GERM/16)

How ram epididymal and electroejaculated sperm differ in their behavior during capacitation: impact on cleavage and blastocyst development

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Sperm origin, even within the same individual, may cause a different response to capacitation. To explore these events, ram epididymal (PM) and electroejaculated (EE) spermatozoa, from the same males, were incubated for 0, 15, 30 and 90 min under capacitation conditions (SOF supplemented with 10% estrous sheep serum at 38.5°C in 5% CO_2) previously to perform an in vitro fertilization test (IVF). Sperm capacitation marker such as protein tyrosine phosphorylation was evaluated at each incubation time by Western-Blot, as well as their fertilization ability and early embryo development. A Linear Mixed Model was carried out and p-value ≤0.05 was considered as statistical significance. Achieved results point out significant higher tyrosine phosphorylation for PM samples, regardless incubation times. Thus, proteins around 70 (3924.77 vs. 387.44) and 60 kDa (1511.22 vs. 131.42) at 0 min; 70 (2257 vs. 23.15), 65 (4232.30 vs. 85.61) and 22 kDa (154662 vs. 469.80) at 15 min; 95 (2887.32 vs. 435.60), 70 (1214.52 vs. 80.47) and 50 kDa (1649.97 vs. 171.92) at 30 min and protein around 50 kDa (507.91 vs. 0) at 90 min, were highly expressed for PM samples in comparison with EE ones. The highest cleavage rate for PM samples was observed at 0 min although at this time the lowest blastocyst rate was obtained. Similar trend is also observed for EE samples but at 30 min. The optimal incubation time, based on cleavage and blastocyst rates, for both samples, was 15 min. However, EE spermatozoa rendered better results compared to PM ones. It seems that those proteins which were expressed in a significantly different way between samples are not keys for an IVF system. Further isolation and identification of these proteins will be necessary to know their role during ram sperm fertilization.

NGF system is differentially expressed in the ovary, oviduct and uterus of rabbit does although independent of serum hormonal levels

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Both NGF and its receptors (TrkA and p75) have been demonstrated to be present in the female tract of some species. Our goal was to study if NGF and its receptors showed a differential expression in the reproductive tract in follicular (FP) and luteal phase (LP) and its relationship with blood estradiol (E2), progesterone (P4) and NGF serum concentrations in rabbit females. Ovary, uterus and oviduct of eCG treated animals were removed 48 h later (FP) or 7 days after GnRH injection (LP) and fixed. Blood samples were recovered at the same points and hormones determined by ELISA. Immunohistochemistry of paraffined samples with the ABC method was performed for NGF, TrkA and p75. NGF was expressed in oocytes of smaller follicles, and in granulose and theca cells in secondary follicles, stronger stained in the LF. Corpus luteum (CL) showed strong immunoreaction. Both oviduct and uterus expressed NGF in both phases. TrkA was highly expressed in all the structures studied, slightly more intense in the LP. However, p75 only was found in secondary and preantral follicles in the vascular layer between the thecas, and in the CL. Signal in the uterus and oviduct was scarce. Vessels were very immunoreactive for all the NGF system. Similar levels of E2 were found in both phases (44.85 ± 8.2 vs.45.70 ± 6.02 pg/ml) whereas P4 concentration was higher in the LF (0.67 ± 0.11 vs.22. 28 ± 3.73 ng/ml; $p < 0.05$). NGF concentration was maintained during the cycle (345.42 ± 77.45 vs.301.72 ± 69.66 pg/ml) and no correlation was found with serum steroid levels. In conclusion, NGF system seems to have an important role during both phases in the rabbit female tract and is independent of hormonal environment. (Funds by AGL2015-65572-C2-2-R Grant and Predoctoral Contract UCM-Santander.)