Abstract P117

Cap Dependent Translation Regulation in Early Stage Porcine Zygotes

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Mammals rely on the regulated translation of selected maternal mRNAs to control oocyte maturation and embryogenesis. These transcripts usually remain silent until their translation is temporally and spatially required during early development. Different translational regulatory mechanisms have evolved to assure coordinated initiation of development. We have focused in this study on expression and phosphorylation of mRNA cap-binding protein-translation initiation factor eIF4E and hyperphosphorylation of its regulatory, 4E- binding protein 1 (4E-BP1), as well as Mnk1 kinase, which phosphorylates eIF4E, as markers of cap-dependent translational processes in pig oocytes from metaphase II/mitosis transition. We examined expression and phosphorylation of the above mentioned proteins in porcine oocytes after parthenogenetic activation by 10 μm Ionomicine and 6-dimethylaminopurine. Cells collected every 2 h after activation were subjected to Immunoblotting analysis and to protein kinase assay. Our results show that the expression of eIF4e, 4E-BP1 and Mnk1 do not significantly change during first 12 h after activation, after 15 h the expression level of 4E-BP1 decreases, as does the level of eIF4E in two cell stage embryos. On the other hand, phosphorylation of eIF4E gradually decreases from 4 to 15 h post-activation, which is in good correlation with the similar decrease in activity of its kinase, Mnk1. Similarly, phosphorylation of 4E-BP1 also decreases during this period, which suggests its increased binding to eIF4e, leading to down regulation of cap-dependent translation during the first embryonic cell cycle.

Abstract P118

Sperm Source and Sperm Treatment Affect Penetration and Sperm Decondensation Rates in Pig IVF

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Epididymal spermatozoa appear to be able to reach capacitation and to fertilize eggs *in vitro* much easier than ejaculated spermatozoa do (Yanagimachi, 1988) although it is possible that this affirmation could be affected by capacitation treatments. The aim of this study was to determine if different sources (ejaculated vs epididymal spermatozoa) and sperm capacitating methods, usually employed in porcine IVF, could affect penetration (PEN) and sperm decondensation rates (SD). The capacitation treatments (both for ejaculated and epididymal sperm) were: washing in DPBS supplemented with 0.1% BSA (BSA), unwashed (UW), or washed on a Percoll gradient (PG). The IVF was carried out as described previously by Matás et al. (Reprod. 2003; 125:133–141) with 2 and 4 h of coculture. 2 h after insemination, the PEN rate was affected by sperm treatment ($p < 0.01$), with the highest PEN rate for ejaculated-PG spermatozoa (38.2%). At 4 h after insemination, epididymal spermatozoa (independently of treatment) reached similar PEN percentage as ejaculated-PG spermatozoa. This group also showed higher values for PEN (78.4%) and SD (89%) compared to ejaculated-UW (27.4%, 60.8%; respectively for PEN and SD) and ejaculated-BSA (30.1% and 42.9% respectively for PEN and SD). Sperm source as well as sperm treatment affect the penetration and sperm decondensation rates. Four hours after insemination, epididymal spermatozoa achieve IVF results comparable to ejaculated sperm washed in a Percoll gradient.

Abstract P119

Short Gamete Co-incubation Times during Porcine IVF does not Increase Monospermic Fertilization

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A reduction in coincubation time has been suggested as an alternative method to reduce polyspermic fertilization. The aim of this study was to evaluate the effect of short periods of gamete co-incubation during pig *in vitro* fertilization. A total of 2518 *in vitro* matured oocytes were inseminated with thawed spermatozoa and co-incubated during 0.25, 1, 2, 3, 7, 10 min and 6 h. The oocytes from 0.25 to 10 min groups were washed three times in mTBM medium (Abeydeera and Day, 1997; Biol Reprod 57:729–34) to remove spermatozoa not bound to the zona and transferred to the same medium (containing no sperm) until 6 h were completed. After 6 h, presumptive zygotes from each group were cultured in NCSU-23 medium (Peters and Wells, 1993; J Reprod Fertil 48:61–73) for 12–15 h to assess fertilization parameters. After each period of co-incubation, some oocytes were stained with hoechst-33342 to count zona bound sperm. Data from three replicates were analysed by anova using the MIXED procedure of SPSS. Although the number of zona bound sperm increased ($p < 0.01$) with the co-incubation time, no increase ($p > 0.05$) was seen in penetration rates among groups from 2 min to 6 h (53.5 ± 2.8 – $61.3 \pm 2.6\%$). The reduction of coincubation time did not affect the monospermy rate either (range 71.3 ± 3.4 – $80.2 \pm 3.8\%$). These results show that, although high penetration rate can be obtained with coincubation times as short as 2 min, monospermy can not be improved using this strategy [Supported by SENECA, CARM (2I05SU0012), MEC (AGL2004-07546) and CDTI (04-0231)].

Abstract P120

Vitrification of Two-to-four Cells Porcine Embryos Cultured *In Vitro*

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Although porcine morulae and blastocysts have been successfully vitrified by Open Pulled Straw (OPS), results achieved with early stages of development are very poor. The aim of this study was to evaluate the effect of vitrification on the survival rate of 2–4 cells embryos as well as blastocysts derived from *in vitro* cultured 2–4 cells embryos. Embryos were collected from weaned sows ($n = 12$) on day 2 of the cycle (D0 = onset of estrus). A total of 63, 2–4 cells embryos were vitrified on the collection day (V2) and the other embryos were cultured in NCSU-23 for 96 h to obtain blastocysts, and then vitrified (VB; $n = 65$). Vitrification and warming were performed as described by Cuello et al. (2004; Theriogenology 62:353–61) using superfine OPS-straws. After warming, V2 embryos and VB were cultured for 144 h and 48 h, respectively, in NCSU-23 at 39°C, in 5% CO_2 in air. All embryos were evaluated during and at the end of the culture period. 81.7% of fresh 2–4 cells embryos developed into blastocysts *in vitro*. However, only 3.2% of the V2 embryos developed to the blastocyst stage ($p < 0.01$). The survival rate (embryos developing after warming) in V2 was lower ($p < 0.01$) than that from VB (9.5% vs 72.3%, respectively). These results show that porcine 2–4 cells embryos can be vitrified using this protocol with a very low efficiency. However, if 2–4 cells embryos are cultured *in vitro* up to the blastocyst stage, these embryos can be successfully vitrified [Supported by MEC (AGL2004-07546) and SENECA (01287PD04) and CARM (2I05SU0012)].