Concentration of procaine and exposure time influence in vitro fertilization rate in the equine

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CONCENTRATION OF PROCAINE AND EXPOSURE TIME INFLUENCE *IN VITRO* FERTILIZATION RATE IN THE EQUINE

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Most wild equids are currently endangered or threatened, as mentioned in the Red List of the International Union for the Conservation of Nature and several domestic horse breeds are at risk of extinction. Genome resource banking requires cryoconservation of semen, oocytes and/or embryos. Embryo production in equids is limited *in vivo*, since routine induction of multiple ovulation is still ineffective. Embryo production *in vitro* allows the production of several embryos per cycle that could easily be frozen owing to their small size. Intracytoplasmic Sperm Injection (ICSI) has been widely adopted to generate horse embryos *in vitro*, however ICSI is time-consuming and requires expensive equipment and expertise in micromanipulation. We have established an efficient *in vitro* fertilization (IVF) technique in the equine (Ambruosi et al., 2013 Reproduction, 146: 119-133) but IVF zygotes have a low developmental competence. Incubation of gametes with procaine, necessary for induction of sperm hyperactivation, may have a deleterious effect on embryos quality. Our objective was to increase the developmental competence of the IVF zygotes by decreasing procaine concentration or exposure time. Immature cumulus-oocyte complexes were collected from slaughtered mares in a local slaughterhouse, cultured for 26 hours in an *in vitro* maturation medium and pre-incubated for 30 minutes in oviductal fluid collected from slaughtered females. Fresh sperm was collected, diluted to $10x10^6$ spermatozoa/ml, incubated for 5 hours in a capacitating medium and diluted to $1x10^6$ spermatozoa/ml. Spermatozoa were then added procaine (1mM or 5mM) and co-incubated with oocytes for 2, 4 or 18 hours. Zygotes were cultured in DMEM-F12 for 48 hours post-IVF, fixed and analyzed. In experiment 1, spermatozoa were added 5mM procaine and co-incubated with oocytes for 2 hours *vs* 18 hours. The percentage of zygotes 48 hours post IVF was higher for 18 hours co-incubation (62%, 13/21) than for 2 hours (0%, 0/22) (Chi2 test $p<0.05$). In experiment 2, spermatozoa were added 5mM procaine and co-incubated with oocytes for 4 hours *vs* 18 hours. The percentage of zygotes 48 hours post IVF was similar for 18 hours (44%, 7/16) and 4 hours co-incubation (32%, 6/19) (Chi2 test $p>0.05$). In experiment 3, spermatozoa were added 5mM *vs* 1mM procaine and co-incubated with oocytes for 18 hours. The percentage of zygotes 48 hours post IVF was higher for 5mM procaine (48%, 13/27) than for 1mM (19%, 5/26) (Chi2 test $p<0.05$). In the 3 experiments, zygotes contained at least 2 highly decondensed pronuclei, pronuclei decondensation being the first step of embryo development. We also observed 2 cleaved embryonic structures in the group 5mM during 18 hours, but the quality of these embryos was poor.

In conclusion, decreasing procaine concentration or exposure time influence IVF rate and doesn't improve equine embryo quality.