

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)**OPU - IVF and ET**

Niacin supplementation during oocyte maturation improves bovine in vitro fertilization rate after ICSI

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Intracytoplasmic sperm injection (ICSI) has effectively been used in both human and animal fertility laboratories. Reactive oxygen species production above its critical levels has been shown to detrimentally affect the oocyte competence. Optimal condition for the in vitro oocyte maturation is one critical step for the occurrence of normal fertilization. We hypothesized that the addition of nicotinic acid (niacin), a potent antioxidant, to the oocyte maturation media can improve the oocyte fertilization during ICSI. Therefore, the present experiment was designed to assess the effects of niacin treatment during in vitro maturation on the fertilization rates of the bovine oocytes after ICSI procedure. Bovine ovaries were collected from the local abattoir and then transported to the IVF laboratory in less than 2 hours. Then, good and excellent quality cumulus-oocytes complexes (COCs) were recovered and randomly divided in four groups: in group 1 (control), the COCs were matured and then fertilized (n=168) in a standard maturation medium (Azari et al., Veterinary Research Communication. 41, 49-56, 2017). In group 2, the COCs were matured in a standard maturation medium with no niacin and then fertilized (n=85) using a standard ICSI procedure (Ashibe et al., Theriogenology, 133, 71-78, 2019) under an inverted microscope (Olympus, IX71, Japan). In group 3, the COCs were matured in a standard maturation medium supplemented with 1mM niacin and then were fertilized (n=88) using a standard ICSI procedure as performed in group 2. A semen with high fertility was used in all replicates of IVF and ICSI (n=5). The motile spermatozoa were separated using a swim-up method. In group 4, the COCs were matured in a standard maturation medium with no niacin and then were chemically activated using calcium ionophore A23187 and ethanol 7% with no sperm injection (n=38) for the parthenogenetic division (Bevacqua et al., Theriogenology, 74, 922-931, 2010). Oocytes maturation protocol was basically similar in all groups by using TCM-199 medium supplemented with 10% FCS, 5 IU/mL hCG (Karma, Germany), 10 ng/ml EGF (Sigma, USA), and 0.1 IU/ml human FSH (Follitrope, South Korea). In all maturation media, 50µg/ml Gentamicin (Sigma, USA) was also added. Groups of 30-50 COCs were cultured for 24 h in a 500 µl culture media at 38.5°C in 5% CO₂. Assessment of oocyte fertilization was performed using aceto-orcein staining method. The percentage of normal fertilization rates among the groups were compared using chi-square test. The percentage of normally fertilized oocytes in group 1 (control IVF group) was higher than that of the group 2 (ICSI with no niacin) (59.6 vs. 39.0%, p<0.05). However, there was no difference in the percentage of normally fertilized oocytes when the ICSI procedure was performed on the matured oocytes supplemented with niacin (group 3) as compared to group 1 (50.9 vs. 59.6%, p>0.05). The percentage of fertilized oocytes in group 4 (the chemically activated oocytes) was lower (8.%) than those of the other experimental groups (p<0.05). The results of present study demonstrated that the addition of niacin to the maturation culture media can increase fertilization rate of bovine oocytes after ICSI.

Keywords: niacin, ICSI, bovine