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Adding serum of cows supplemented with b-carotene during bovine in vitro embryo culture has no effect on embryo development

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Elevated serum NEFA concentrations, typically present in negative energy balance (NEB) cows, are known to compromise bovine *in vitro* oocyte and embryo quality and developmental competence. These observations seem to be associated with oxidative stress. Therefore, antioxidant (AO) supplementation such as beta-carotene (bC) can be a promising solution to ameliorate embryo quality and survival. However, little is known about the possible neutralizing effect of bC on NEB compromised embryos. Accordingly, we hypothesize that bC can overcome the potential negative effects of metabolic conditions associated with NEB on embryo development. To investigate this we aimed to evaluate the effect of serum from bC supplemented positive EB (PEB) or NEB cows on embryonic developmental competence.

5 non-lactating Holstein Friesian cows were subjected to 4 consecutive dietary treatments, 28 days each: 1) 1.2x maintenance (M) (=PEB-bC), 2) 1.2xM with daily 2000mg bC (Rovimix 10% bC, DSM) (=PEB+bC), 3) 0.6xM + bC (=NEB+bC) and 4) after a 6 week acclimatization period 0.6xM (=NEB-bC). Serum was collected 72h after ovulation, pooled per dietary treatment and heat inactivated during 30min at 56°C. In total 1404 bovine slaughterhouse grade 1 cumulus oocyte complexes were serum-free matured (4 repeats), routinely fertilized and cultivated for 6.7 days with the addition of 10% serum of the 4 different treatments. Cleavage (48h post insemination (pi)), blastocyst rates (7.7 days pi) and the rates of blastocysts from cleaved zygotes were calculated. Developmental competence data were compared between the four treatments using a binary logistic regression model taking replicate, treatment and the interaction of both factors into account. NEFA and bC data were analyzed using a paired samples T-test (IBM SPSS Statistics 20). Bonferroni correction was applied. Serum NEFA concentrations were significantly elevated in NEB compared to PEB (0.36 ± 0.18mM vs. 0.21 ± 0.11mM; P=0.011). BC supplementation drastically increased bC concentrations in serum in NEB (0.44 ± 0.18µg/ml vs. 3.28 ± 0.78µg/ml; P<0.001) as well as in PEB (1.02 ± 0.91µg/ml vs. 3.04 ± 1.28µg/ml; P<0.001). Unexpectedly no significant differences were found on cleavage rates (on average 81%), subsequent development until blastocyst stage (on average 29%) nor blastocyst rates from cleaved zygotes (on average 36%). Briefly, our model was not able to indicate any negative effect of NEB serum on *in vitro* embryo development compared to PEB and hence no extra beneficial effects of bC could be observed on the outcome. In conclusion, these data show that more research is needed to optimize this model to investigate the effect of specific dietary strategies on pre-implantation embryo quality.