Long-term Phenotypical Implications of Assisted Reproductive Technologies in Cattle

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Assisted reproductive technologies (ART) are regularly used in cattle breeding to produce animals of high genetic value. In 2022, over 1 million *in vitro*-produced embryos were transferred worldwide. However, recent studies suggest that *in vitro*-produced animals may differ in health, milk production, fertility, and gestational length compared to those derived *in vivo*. Currently, the long-term effects of ART on growth have not been studied. We aimed to investigate whether there are differences in IGF-1, T4 and cortisol levels, and their interactions with growth parameters (body weight, withers height, thoracic circumference and body length), between cattle of *in vivo* and *in vitro* origin.

The 19 animals included in the study were either derived from artificial insemination (AI; 5 males and 2 females) or from embryos produced *in vitro* with either reproductive fluids as source of protein (RF-IVP; 4 males and 1 female) or BSA (C-IVP; 4 males and 3 females). Growth parameters were assessed at 0, 3, 7, 15, every 15 days until day 360, 550, 750, 900, 1100, 1300 and 1500 days of age. Blood samples were collected at 75, 150, 360, 550, 900, 1100 and 1500 days of age and centrifuged. Plasma hormone concentrations were determined using a solid-phase, enzyme-labeled competitive chemiluminescent enzyme immunoassay. Data were analyzed using a linear mixed-effects model. Benjamini-Hochberg was used for *p-value* correction. Data were considered significant when $p < 0.05$.

IGF-1 levels were different between both *in vitro* groups, being 1.6 folds higher in RF-IVP than in C-IVP, but no differences were found between the *in vitro* groups and the AI group. There was no correlation between IGF-1 and weight. Still, there was a positive correlation between IGF-1 and the rest of the growth parameters studied. Higher IGF-1 concentrations were correlated with higher height at withers (0.078 ± 0.029 cm/$\frac{ng}{mL}$), and higher body length (0.16 ± 0.04 cm/$\frac{ng}{mL}$). Additionally, in the RF-IVP group, higher IGF was associated with higher thoracic circumference (0.28 ± 0.12 cm/$\frac{ng}{mL}$).

T4 levels were reduced with age, and this reduction was smaller in males than in females. Additionally, in the AI group, T4 was 1.6 folds higher in females than in males at 75 days of age. With regard to the growth parameters, T4 showed a negative correlation with weight (-13.26 ± 6.49 Kg/$\frac{\mu g}{dL}$), a positive correlation with thoracic circumference in the RF-IVP group (5.06 ± 2.67 cm/$\frac{\mu g}{dL}$), and no correlation with either withers height or body length.

In males, a small but significant increase in cortisol level was observed with age. Additionally, a positive correlation was found between cortisol and weight in the AI

group (34.11 ± 25.78 kg / $\frac{\mu g}{dL}$). On the contrary, there was a negative correlation between cortisol and body length in males, and bigger height at withers tended to be associated with lower cortisol concentration (p=0.06). There was no correlation between cortisol and thoracic circumference.

In conclusion, we determined for the first time the relationship between growth parameters and IGF-1, T4 and cortisol, from birth to 4 years of age in cattle from *in vivo* and *in vitro* origins. It was found that i*n vitro* embryo production supports the development of healthy animals, in terms of growth parameters and hormonal regulation, with no clinically significant differences compared to AI animals.

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