Animal Reproduction

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SUPEROVULATION, OPU-IVP, AND EMBRYO TRANSFER

Long-term blood plasma biochemical analysis in pigs derived from assisted reproductive technologies.

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Rising livestock demand has increased use of assisted reproductive technologies (ART). Evidence indicates long-term effects of ART on molecular physiology and metabolism. This study explores ART's impact on biochemical parameters in a colony of pigs from 1 to 5 years of age. Animals were born after artificial insemination (AI) and transfer of embryos *in vitro* produced with (RF-IVP) or without (C-IVP) oviductal and uterine fluids during *in vitro* fertilization and embryo culture media (París-Oller et al., J Anim Sci and Biotechnol, 2021). Pigs were maintained under identical conditions and sampled every six months. Number of animals in each group decreased throughout life: 33 to 9 (AI); 22 to 10 (C-IVP); and 14 to 4 (RF-IVP). The number of blood samples was the same as the number of animals and were collected via jugular venipuncture in lithium heparin tubes and immediately transported to laboratory. Plasma was obtained through centrifugation (1200 g, 20 min, 4ºC, Eppendorf 5810 R) of blood collected via direct jugular venipuncture in lithium heparin tubes. Plasma was assessed using a chemistry analyzer (Olympus AU400, Japan) for creatinine (CREA), urea, amylase, creatine kinase (CK), cholesterol, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lipase, total protein (TP), albumin (ALB), globulin (GLOB), triglycerides (TRIGL), and total bilirubin (TBIL). Data were analyzed by mixed-effects model and likelihood-ratio test to determine effects of age, group and sex on the variables. Post-hoc test for multiple comparisons Tukey's method was used. Statistical significance was set at $P < 0.05$. Age significantly influenced all variables. Glucose, cholesterol, lipase, CK, ALP, AST, ALT were not affected by any other variable. TP, GLOB, UREA and TRIGL showed progressive elevation with age, with peak values between 4-5 years. ALB, cholesterol, CK, ALP, AST, GGT exhibited characteristic juvenile decline, with ALP and AST showing reduction during early development (155 to 52 U/L and 121 to 37 U/L respectively) followed by stabilization, while ALT displayed oscillatory changes. Glucose and TBIL showed initial elevation peaking at 2 years, before decreasing. CREA and amylase fluctuated irregularly. Critical developmental transitions occurred at 1.5-2 years and 4-5 years, representing key physiological maturation milestones. ALBU showed the highest mean concentration in the RF-IVP group and lowest in the AI group, while GGT showed the opposite pattern. ALBU, GLOB and urea were influenced by sex, with females higher ALBU and urea levels, while males showed elevated GLOB values. Long-term results confirm slight persisting biochemical differences in naturally and artificially conceived pigs, although the clinical relevance is unnoticeable.

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