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## Effects of neonatal surgical castration or immunocastration on the adrenal axis of males pigs

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To explore the effect of the suppression of testicular hormones on the adrenal axis, we compared entire pigs (E, n = 18), pigs castrated early in life (SC, surgical castration at 5-6 days of age, n = 14) and pigs submitted to immunocastration around puberty (IC, vaccination against Improvac® at 88 and 117 days of age, n = 16). Saliva samples were collected at 112 and 147 days of age at various times during the day (9:00, 11:00, 17:00, 23:00, 5:00 and 9:00). Blood samples were collected just before each vaccination and at 151 days of age. At slaughter (152 days of age), adrenals were collected, freed from surrounding tissue and weighed. For 11-12 pigs/experimental group, the adrenal cortex was frozen in liquid nitrogen and stored at -80°C until transcriptional activity analysis by the fluidigm technology (qPCR on cDNA).

Plasma testosterone level was lower in SC than in E and IC at 88 and 117 days of age and was lower in SC and IC pigs than in E pigs at 151 days of age ( $P < 0.05$ ). A similar result was obtained for plasma estradiol except that level was similarly low in all groups at 88 days of age. Plasma cortisol and ACTH levels did not differ between groups at the three ages ( $P > 0.1$ ). Salivary cortisol level was influenced by the time of day at both ages ( $P < 0.001$ ) with the highest value at 6:00 and the lowest at 23:00. The treatment effect was not significant at 112 days ( $P > 0.1$ ) contrarily to 147 days ( $P < 0.01$ ) with a lower mean salivary cortisol level in E than in IC ( $P < 0.04$ ) and C ( $P < 0.09$ ) pigs. Adrenals were lighter in E than in SC pigs ( $P < 0.05$ ) and intermediate in IC pigs. The levels of transcriptional activity of 11 genes essential to produce corticoids, DHEA or androstenedione (STAR, CYP11A1, CYP17, HSD3B1, CYP21, CYP11B), to control the cortisol-cortisone conversion (HSD11B1 and HSD11B2) and, of 3 cofactors (CYP5A, CYP5B, CYB5R3) were evaluated in the adrenal cortex. Transcript level of CYP11A1 was lower in E than in other pigs ( $P < 0.05$ ) and that of CYB5R3 in IC than in other pigs ( $P < 0.05$ ). A trend for a lower transcript level of STAR was observed in E compared to IC pigs. Therefore, the transcription of the CYP11A1 gene seems to be permanently influenced by the suppression of testicular hormones whereas the transcription of some genes (CYB5R3, STAR) is only transiently influenced and that of other genes (8 out of 11) is not influenced.

Overall, our data indicate a lower activity of the adrenal axis in entire than in surgically or immunologically castrated pigs, suggesting an inhibitory effect of sex hormones on the adrenal axis. The effect of sex hormones removal seems to depend on the time elapsed from castration.

Keywords: cortisol, testosterone, oestradiol, boar, castration