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Effect of weaning age on cortisol release in piglets

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ABSTRACT. The effect of weaning age on the adrenal cortex, which plays a vital role in the stress response, is currently unknown. Therefore, plasma adrenocorticotropic hormone (ACTH) and cortisol levels, weights and relative weights of adrenal glands, and steroidogenesis-related protein and enzyme expression levels in piglets weaned on different days were determined. Piglets weaned at 35 days had significantly lower ACTH levels than those weaned at 14 or 21 days, and cortisol levels of piglets weaned at 21, 28, and 35 days were significantly lower than those of piglets weaned on day 14. Adrenal gland weights of piglets weaned at 28 and 35 days and relative adrenal gland weights of piglets weaned at 35 days were significantly lower than those of piglets weaned at 14 days. However, no significant difference was detected in the expression of melanocortin-type 2 receptor mRNA, which is associated with weaning age. Steroidogenic acute-regulatory (StAR) mRNA and cholesterol side-chain cleavage cytochrome P450 mRNA

expression levels in piglets weaned at 28 and 35 days were significantly lower than in those weaned at 14 or 21 days, and P450 11β mRNA expression levels in piglets weaned at 28 and 35 days were significantly lower than in those weaned at 14 days. Therefore, early-weaned piglets exhibited increased adrenal gland weights and StAR and steroidogenic enzyme expression, all of which contributed to high cortisol levels. The high plasma ACTH and cortisol levels in early-weaned piglets indicate that these animals would be greatly affected by stress.

Key words: Weaning age; Piglet; Adrenal cortex; Cortisol; Adrenal gland weight; Steroidogenic enzyme

INTRODUCTION

Glucocorticoids (GCs) affect a plethora of biological functions in most organs and tissues, and maintain basal and stress-related energy homeostasis. Whereas physiological levels of GCs are required for proper metabolic control, endogenous GC deficiency or excess has been linked to a variety of metabolic diseases, including type II diabetes and obesity (Witchel and DeFranco, 2006; Drake et al., 2007; Vegiopoulos and Herzig, 2007; Zhang et al., 2015). Cortisol is the natural GC in pigs, as it is in humans.

Cortisol secretion from the adrenal cortex involves multistep reactions, which are generally regarded as being under the control of adrenocorticotropic hormone (ACTH). ACTH binding to the melanocortin-type 2 receptor (MC2R) on adrenocortical cells ultimately results in an increase in cyclic adenosine monophosphate (cAMP), a second messenger that activates protein kinase A (PKA). PKA phosphorylates cAMP response element binding protein (CREB), which then activates the transcription of genes involved in steroidogenesis, such as steroidogenic acute regulatory-(StAR) mRNA gene (Richards, 2001) to mediate the delivery of cholesterol from the outer to the inner mitochondrial membrane for cortisol synthesis (Clark et al., 1994; Waterman, 1995). The six-carbon side chain is removed from cholesterol to form pregnenolone, which is catalyzed by cholesterol side-chain cleavage cytochrome P450 (P450scc), which is an inner mitochondrial membrane protein (Hall, 1986). After the conversion of pregnenolone to progesterone by 3β-hydroxysteroid dehydrogenase (Abbaszade et al., 1995), hydroxylation by 17α-hydroxylase cytochrome P450 and 21-hydroxylase cytochrome P450 yields 11-deoxycortisol. The 11-deoxycortisol is further hydroxylated by 11β-hydroxylase cytochrome P450 ($P450_{11\beta}$) to form cortisol. The chronic response to ACTH in the adrenal cortex involves increased transcription of the genes that encode these steroidogenic enzymes (Sewer and Waterman, 2001).

Weaning is stressful for piglets, and has considerable negative effects on their productivity, well-being, and welfare. Most studies that have investigated cortisol levels in piglets have only examined levels at a single-weaning age (Moeser et al., 2007; Campbell et al., 2013; Wu et al., 2013; Fang et al., 2014; Liu et al., 2014; Wang et al., 2014). A few have explored the effects of different weaning ages on cortisol levels (Colson et al., 2006; Jarvis et al., 2008), but none have studied the effect of weaning age on the adrenal cortex. The present study evaluated the effects of weaning age on piglet adrenal gland plasma ACTH and cortisol secretion, mRNA expression levels of the ACTH receptor (MC2R), StAR and two related steroidogenic enzymes (P450scc and $P450_{11\beta}$), and adrenal gland weight in order to reveal the

molecular mechanisms involved in the effect of weaning age on cortisol levels, and to provide a theoretical reference for guiding piglet production.

MATERIAL AND METHODS

Animals and treatments

Twenty-four crossbred (Duroc x Landrace x Yorkshire) piglets of a similar age and body weight from eight litters were randomly assigned to four groups with six piglets in each, and were weaned at 14, 21, 28, or 35 days of age.

Feeding management and experimental diets

The piglets were vaccinated after birth according to standard procedures. Two-stage feed for nursery pigs (nutritional composition listed in Table 1) was provided from the age of 8 days. All piglets were allowed free access to water and sow milk before weaning, and to creep feed after weaning. The health of the piglets was recorded throughout the experiment.

Table 1. Diet composition and nutrient levels (air-dried).

Component	Composition (%)		Nutrient	Nutrient levels (%)	
	Diet I	Diet II		Diet I	Diet II
Extruded soybean	34.90	31.40	DE/(MJ/kg)	15.09	14.15
Corn	37.60	50.00	Crude protein	23.78	20.45
Whey powder	15.00	5.50	Ca	0.93	0.69
Fish meal	8.00	6.70	TP	0.79	0.65
Soybean oil	-	0.90	AP	0.61	0.39
Wheat bran	2.00	3.50	Na	0.62	0.72
$CaHPO_4$	0.85	0.60	Lys	1.86	0.29
Limestone	0.65	0.40	Met + Cys	0.79	0.68
Premix[a]	1.00	1.00	Thr	0.99	0.85
Total	100	100			

[a]Premix provided the following (per kg): 150 mg Fe, 150 mg Cu, 50 mg Mn, 150 mg Zn, 1 mg Co, 0.5 mg Se, 0.5 mg I, 12,000 IU vitamin (V) A, 5000 IU VD_3, 40 IU VE, 26 mg VB_{12}, 5 mg VB_2, 12 mg VB_3, and 28 mg niacin. DE = digestive energy.

Sample collection

At 42 days of age, all experimental piglets were killed by jugular bloodletting after a 12-h fast. Blood samples were collected in heparinized tubes and immediately placed on ice. Plasma was separated by centrifugation at 1300 *g* and 4°C for 15 min. Plasma samples were stored at -20°C until used for analysis. The adrenal tissues were immediately harvested, rapidly frozen in liquid nitrogen, and stored at -80°C until they were used for analysis. All of the experimental procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of Tianjin Agricultural University. The experimental protocol was approved by the Department Animal Ethics Committee of Tianjin Agricultural University.

Radioimmunoassay for plasma ACTH and cortisol

Plasma concentrations of ACTH and cortisol were measured in duplicate using commercially available ^{125}I-RIA kits (Beijing Research Institute of Biotechnology, China) according to the manufacturer guidelines. The kits were validated for measuring porcine plasma samples, and the detection limits were 5 pg/mL and 2 ng/mL for ACTH and cortisol, respectively. The intra- and inter-assay coefficients of variation were, respectively, 6 and 12% for ACTH and 7.6 and 8.7% for cortisol.

Adrenal gland weight

Both adrenal glands of each piglet were weighed, and these weights were summed. The relative adrenal gland weight was calculated by dividing this summed weight by the weight of the piglet.

Reverse transcription (RT) and polymerase chain reaction (PCR)

Total RNA was extracted using a TRIzol Total RNA Kit according to the manufacturer instructions (Ambion Biotech Co. Ltd., Shanghai, China). The total RNA content was then quantified at 260 nm using a photometer (Eppendorf Biophotometer). The ratios of absorption (260/280 nm) were between 1.8 and 2.0. Electrophoresis of the RNA samples was conducted on 1.4% agarose formaldehyde gel to verify their integrity.

The RT reaction mix for first-strand cDNA synthesis included 1X RT buffer, 10 mM deoxynucleotide triphosphates (dNTPs), 10 U RNase inhibitor, 100 U Moloney murine leukemia virus reverse transcriptase (M-MLV RT), 2.5 μM random hexamer primers, and 2 mg total RNA, in a final 25-μL volume. RNA samples were denatured at 70°C for 5 min and placed on ice for 5 min with the random hexamer primers and dNTPs before RT. The tubes were incubated for 1 h at 37°C, 5 min at 95°C, and then chilled to 4°C.

The RT reaction mix (2 μL) was used for a PCR in a final 25-μL volume that contained 0.5 U *Taq* DNA polymerase (Promega, Shanghai, China), 10 mM NaCl, 5 mM Tris-HCl, pH 9.0, 0.1 mM DL-Dithiothreitol, 5% (w/v) glycerol, 0.1% (w/v) Triton X-100, 0.01 mM ethylenediaminetetraacetic acid, 0.2 mM each dNTP, 1.0-2.0 mM $MgCl_2$, and 0.2-0.5 μM each forward and reverse primers. Primers for MC2R, StAR, P450scc, $P450_{11\beta,}$ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed according to porcine sequences published in GenBank (Table 2) using Primer Premier 5.0. The PCR products of the target genes were sent to Haojia Biotech Co. Ltd., China, to verify the sequencing. The reported sequences exactly matched the published sequences in GenBank.

Various controls were used to avoid and monitor any possible contamination of genomic DNA and environmental DNA during both RT and PCR. A pooled sample generated by mixing an equal quantity of total RNA from all the samples was used for optimizing the PCR conditions and normalizing the intra-assay variations. The number of PCR cycles was determined to ensure that the amplifications were terminated within the linear range for quantitation. All of the samples were included in the same run of RT-PCR, which was repeated at least three times. Both RT and PCR were performed using a GeneAmp® PCR System 9600 (Perkin Elmer, USA).

Table 2. Primer sequences used for pig gene amplification.

Target gene	Product length (bp)	Sequence (F, forward; R, reverse)	Reference
MC2R	206	F: 5'-TGTTCCCGCTGATGCTGGTGTT-3' R: 5'-GGGGTCAGCTGGGCAGAGTGTC-3'	AF450083
StAR	395	F: 5'-CTGGACATCCTCAGCAACCA-3' R: 5'-GCTCTGATGACCCCCTTCTG-3'	U53020
P450scc	213	F: 5'-TTTACAGGGAGAAGCTCGGCAAC-3' R: 5'-TTACCTCCGTGTTCAGGACCAAC-3'	X13768
$P450_{11\beta}$	198	F: 5'-GTGGCGTGTTCTTGCTAA-3' R: 5'-AGATGCTGGGCTTGATGT-3'	D38590
GAPDH	285	F: 5'-TACATGGTCTACATGTTCCAGTATG-3' R: 5'-CAGGAGGCATTGCTGACAATCTTG-3'	AF017079

MC2R = melanocortin-type 2 receptor; StAR = steroidogenic acute-regulatory gene; P450scc = cholesterol side-chain cleavage cytochrome P450; $P450_{11\beta}$ = 11β-hydroxylase cytochrome P450; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

The PCR products (18 μL) were separated using 2% agarose gel electrophoresis. The band intensities were analyzed with the Image Lab™ software and Analysis System 120 (Bio-Rad Co. Ltd., USA). The ratio of the target gene mRNA intensity relative to that for GAPDH was used to represent the abundance of mRNA expression.

Statistical analysis

All statistical analyses were performed using SPSS 12.0 for Windows. All data are reported as means ± SE. The general linear model procedure was used to determine treatment effects using one-way analysis of variance. $P < 0.05$ was considered statistically significant for all analyses.

RESULTS

Plasma ACTH and cortisol levels

As shown in Figure 1A and B, plasma ACTH and cortisol levels gradually decreased with a delay in weaning. The ACTH levels in piglets weaned at 35 days were significantly lower than those in piglets weaned at 14 or 21 days ($P < 0.05$). Cortisol levels in piglets weaned at 21, 28, and 35 days were significantly lower than those in piglets weaned at 14 days ($P < 0.05$), and cortisol levels in piglets weaned at 35 days were also significantly lower than in piglets weaned at 21 or 28 days ($P < 0.05$; Figure 1B).

Adrenal gland weight and relative adrenal gland weight

As shown in Figure 2A and B, adrenal gland weights and relative adrenal gland weights also gradually decreased with a delay in weaning age. The adrenal gland weights of piglets weaned at 28 and 35 days were significantly lower than those of piglets weaned at 14 days ($P < 0.05$). In addition, the relative adrenal gland weights of piglets weaned at 35 days were significantly lower than those of piglets weaned at 14 days ($P < 0.05$; Figure 2B).

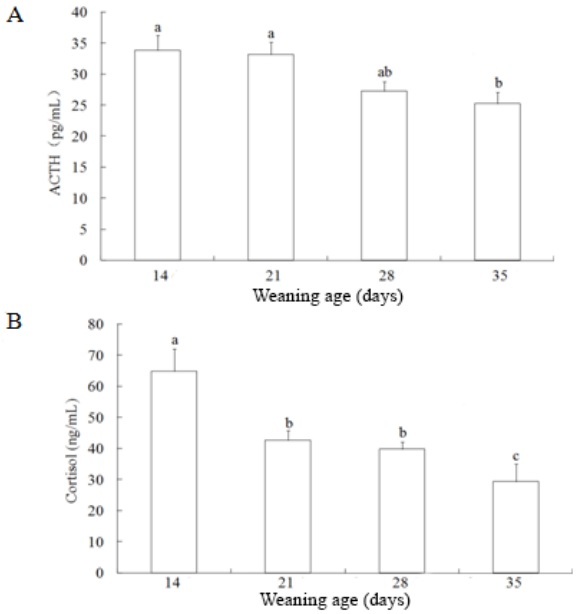

Figure 1. Effect of weaning age on plasma adrenocorticotropic hormone (ACTH) and cortisol levels in piglets. **A.** ACTH levels. **B.** Cortisol levels. Values are reported as means ± SE. Mean values without a common superscript differed significantly between different weaning age groups ($P < 0.05$).

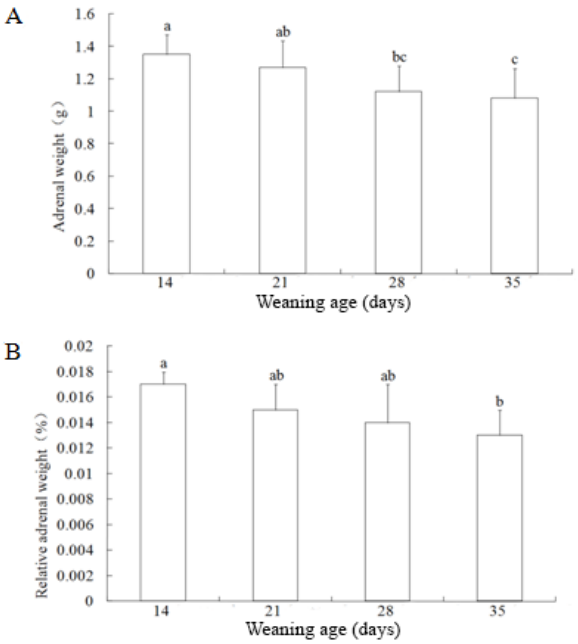

Figure 2. Effect of weaning age on adrenal gland weight and relative adrenal gland weight of piglets. **A.** Adrenal gland weight. **B.** Relative adrenal gland weight. Values are reported as means ± SE. Mean values without a common superscript differed significantly between different weaning age groups ($P < 0.05$).

Adrenal cortex MC2R, StAR, P450scc, and $P450_{11\beta}$ mRNA expression

The abundance of MC2R, StAR, P450scc, and $P450_{11\beta}$ mRNA in the adrenal cortex was analyzed by RT-PCR (Figure 3). Electrophoresis gels for the above genes are shown in Figure 3A. There was no significant difference in the level of MC2R mRNA expression between piglets weaned on different days (Figure 3B). In contrast, the abundance of StAR, P450scc, and $P450_{11\beta}$ adrenal cortical mRNA gradually decreased with a delay in weaning (Figure 3C, D, and E). The levels of adrenal cortical StAR and P450scc mRNA in piglets weaned at 28 and 35 days were significantly lower than those in piglets weaned at 14 or 21 days ($P < 0.05$; Figure 3C and D). The abundance of $P450_{11\beta}$ mRNA in piglets weaned at 28 and 35 days was significantly lower than that in piglets weaned at 14 days ($P < 0.05$; Figure 3E).

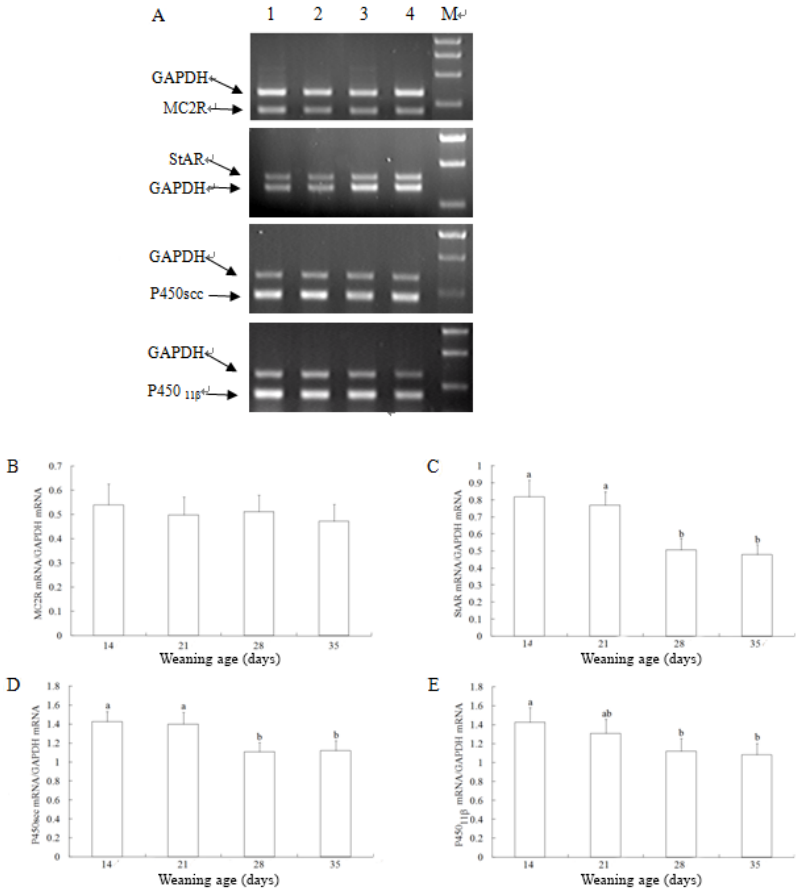

Figure 3. mRNA expression in the adrenal cortex of piglets. **A.** Representative electrophoresis gels for adrenal melanocortin -type 2 receptor (MC2R), steroidogenic acute-regulatory mRNA (StAR), cholesterol side-chain cleavage cytochrome P450 (P450scc), and 11β-hydroxylase cytochrome P450 ($P450_{11\beta}$) mRNA expression. *Lanes 1*, *2*, *3*, and *4* = samples from piglets weaned at 14, 21, 28, or 35 days, respectively; *lane M* = a DNA ladder (DL2000). **B. C. D.** and **E.** Show the relative abundances of adrenal cortical MC2R, StAR, P450scc, and $P450_{11\beta}$ mRNA, respectively. Values are reported as means ± SE. Mean values without a common superscript differed significantly between different weaning age groups ($P < 0.05$).

DISCUSSION

The present study demonstrated that weaning age significantly affects plasma ACTH and cortisol levels, adrenal gland weight, and MC2R, StAR, and related steroidogenic enzyme mRNA expression levels in piglets.

A vital function of the adrenal cortex is to respond to stress. Elevation of the plasma cortisol concentration in stressful situations is an adaptation response of the organism to a changing environment (Jarvis et al., 2006; Rutherford et al., 2006; Li et al., 2008a,b). Activation of the hypothalamus-pituitary-adrenal (HPA) axis, as indicated by the secretion of hypothalamic corticotropin-releasing hormone and pituitary ACTH, and adrenal cortical steroidogenesis, is the main regulatory mechanism that underlies elevated plasma cortisol levels in response to stress. Cortisol, in turn, may act at the level of the hippocampus and hypothalamus to inhibit HPA activation, thereby establishing a regulatory feedback loop (Jarvis et al., 2006; Rutherford et al., 2006). Although this feedback loop can guarantee a return of the HPA axis to basal homeostasis, multiple factors at several levels may affect the basal plasma cortisol level or the set point of the HPA system. We found that differences in plasma ACTH levels at different weaning ages were similar to those observed in plasma cortisol levels; both ACTH and cortisol levels gradually decreased with a delay in weaning age. This result suggests that piglets weaned at a young age may be more affected by stressful stimuli than those weaned at older ages, as the higher levels of ACTH released from pituitary cells would lead to increased levels of cortisol secreted by the adrenal cortex in the piglets weaned earlier.

The adrenal gland is one of the most important endocrine organs in regulating body function, and is the main site for cortisol synthesis (Wang et al., 2014). Our results indicate that both the weight of the adrenal gland and its weight relative to that of the piglet increase more in piglets that are weaned earlier than in piglets that are weaned later. This is consistent with the finding that the ACTH level in piglets weaned at a younger age was higher than in piglets weaned at an older age. The main physiological role of ACTH is to stimulate adrenal gland growth and hormone synthesis by adrenal cortical cells (Fragoso et al., 2015). In the present study, the elevated plasma ACTH levels in the early-weaned piglets could have caused adrenal gland hyperplasia, increasing the weight of the adrenal gland, compared with that in the late-weaned piglets, and increasing the size or number of the adrenal cortical cells that synthesize cortisol, resulting in higher cortisol levels in piglets weaned at an earlier age.

Steroidogenesis in the adrenal gland is under the control of ACTH through the mediation of its receptor, MC2R. MC2R is a G protein-coupled receptor belonging to the melanocortin receptor superfamily. ACTH activity is mediated by MC2R via the cAMP/PKA signaling pathway (Handler et al., 1988). Induced steroidogenic enzyme expression and the secretion of GCs from the adrenal cortex are molecular and physiological indicators of normal adrenal gland function (Weber and Clark, 1994). To our knowledge, the patterns of adrenal gland gene expression involved in ACTH signaling have not been characterized in piglets weaned at different ages. We found no significant association between adrenal cortical MC2R mRNA expression and weaning age. However, it remains unclear whether the protein levels of this receptor differ with weaning age, and this awaits further investigation.

The initial step of steroidogenesis in the adrenal gland is the transport of cholesterol from the outer to the inner mitochondrial membrane, where P450scc is localized (Stocco, 2001). This transport step is a rate-limiting step of steroidogenesis, and is regulated by StAR,

which is abundant in the adrenal cortex and gonads. Previous studies have shown that StAR mRNA and protein expression are directly correlated with basal and ACTH-induced cortisol secretion (Kallen et al., 1998; Le Roy et al., 2000). In the present study, the expression of StAR mRNA in early-weaned piglets was higher than that in late-weaned piglets, suggesting greater cholesterol transport and cortisol secretion in early-weaned piglets. In addition, $P450_{11\beta}$ mRNA expression in the early-weaned piglets was also higher than in late-weaned piglets. Our findings suggest that the enhanced mRNA expression of cytochrome P450 hydroxylases may be responsible for high cortisol levels in early-weaned piglets.

We found that plasma ACTH and cortisol levels in piglets weaned at a relatively young age were higher than in those weaned at an older age; consequently, early-weaned piglets probably experience a more pronounced stress response. Piglets weaned at a relatively early age also exhibited heavier adrenal glands and increased StAR and steroidogenic enzyme mRNA expression levels, which contributed to their higher cortisol levels than piglets weaned at an older age.

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REFERENCES

Abbaszade IG, Clarke TR, Park CH and Payne AH (1995). The mouse 3 beta-hydroxysteroid dehydrogenase multigene family includes two functionally distinct groups of proteins. *Mol. Endocrinol.* 9: 1214-1222.

Campbell JM, Crenshaw JD and Polo J (2013). The biological stress of early weaned piglets. *J. Anim. Sci. Biotechnol.* 4: 19. http://dx.doi.org/10.1186/2049-1891-4-19

Clark BJ, Wells J, King SR and Stocco DM (1994). The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). *J. Biol. Chem.* 269: 28314-28322.

Colson V, Orgeur P, Foury A and Mormède P (2006). Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. *Appl. Anim. Behav. Sci.* 98: 70-88. http://dx.doi.org/10.1016/j.applanim.2005.08.014

Drake AJ, Tang JI and Nyirenda MJ (2007). Mechanisms underlying the role of glucocorticoids in the early life programming of adult disease. *Clin. Sci.* 113: 219-232. http://dx.doi.org/10.1042/CS20070107

Fang CL, Sun H, Wu J, Niu HH, et al. (2014). Effects of sodium butyrate on growth performance, haematological and immunological characteristics of weanling piglets. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 98: 680-685. http://dx.doi.org/10.1111/jpn.12122

Fragoso MCBV, Alencar GA, Lerario AM, Bourdeau I, et al. (2015). Genetics of primary macronodular adrenal hyperplasia. *J. Endocrinol.* 224: R31-R43. http://dx.doi.org/10.1530/JOE-14-0568

Hall PF (1986). Cytochromes P-450 and the regulation of steroid synthesis. *Steroids* 48: 131-196. http://dx.doi.org/10.1016/0039-128X(86)90002-4

Handler JD, Schimmer BP, Flynn TR, Szyf M, et al. (1988). An enhancer element and a functional cyclic AMP-dependent protein kinase are required for expression of adrenocortical 21-hydroxylase. *J. Biol. Chem.* 263: 13068-13073.

Jarvis S, D'Eath RB, Robson SK and Lawrence AB (2006). The effect of confinement during lactation on the hypothalamic-pituitary-adrenal axis and behaviour of primiparous sows. *Physiol. Behav.* 87: 345-352. http://dx.doi.org/10.1016/j.physbeh.2005.10.004

Jarvis S, Moinard C, Robson SK, Sumner BE, et al. (2008). Effects of weaning age on the behavioural and neuroendocrine development of piglets. *Appl. Anim. Behav. Sci.* 110: 166-181. http://dx.doi.org/10.1016/j.applanim.2007.03.018

Kallen CB, Arakane F, Christenson LK, Watari H, et al. (1998). Unveiling the mechanism of action and regulation of the steroidogenic acute regulatory protein. *Mol. Cell. Endocrinol.* 145: 39-45. http://dx.doi.org/10.1016/S0303-7207(98)00167-1

Le Roy C, Li JY, Stocco DM, Langlois D, et al. (2000). Regulation by adrenocorticotropin (ACTH), angiotensin II, transforming growth factor-β, and insulin-like growth factor I of bovine adrenal cell steroidogenic capacity and expression of ACTH receptor, steroidogenic acute regulatory protein, cytochrome P450c17, and 3β-hydroxysteroid dehydrogenase. *Endocrinology* 141: 1599-1607.

Li LA, Xia D, Bao ED, Wei S, et al. (2008a). Erhualian and Pietrain pigs exhibit distinct behavioral, endocrine and biochemical responses during transport. *Livest. Sci.* 113: 169-177. http://dx.doi.org/10.1016/j.livsci.2007.03.008

Li LA, Xia D, Wei S, Hartung J, et al. (2008b). Characterization of adrenal ACTH signaling pathway and steroidogenic enzymes in Erhualian and Pietrain pigs with different plasma cortisol levels. *Steroids* 73: 806-814. http://dx.doi.org/10.1016/j.steroids.2008.03.002

Liu J, Yu B, Mao X, Huang Z, et al. (2014). Effects of maternal folic acid supplementation and intrauterine growth retardation on epigenetic modification of hepatic gene expression and lipid metabolism in piglets. *J. Anim. Plant Sci.* 24: 63-70.

Moeser AJ, Klok CV, Ryan KA, Wooten JG, et al. (2007). Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292: G173-G181. http://dx.doi.org/10.1152/ajpgi.00197.2006

Richards JS (2001). New signaling pathways for hormones and cyclic adenosine 3',5'-monophosphate action in endocrine cells. *Mol. Endocrinol.* 15: 209-218.

Rutherford KMD, Haskell MJ, Glasbey C and Lawrence AB (2006). The responses of growing pigs to a chronic-intermittent stress treatment. *Physiol. Behav.* 89: 670-680. http://dx.doi.org/10.1016/j.physbeh.2006.08.006

Sewer MB and Waterman MR (2001). Insights into the transcriptional regulation of steroidogenic enzymes and StAR. *Rev. Endocr. Metab. Disord.* 2: 269-274. http://dx.doi.org/10.1023/A:1011516532335

Stocco DM (2001). StAR protein and the regulation of steroid hormone biosynthesis. *Annu. Rev. Physiol.* 63: 193-213. http://dx.doi.org/10.1146/annurev.physiol.63.1.193

Vegiopoulos A and Herzig S (2007). Glucocorticoids, metabolism and metabolic diseases. *Mol. Cell. Endocrinol.* 275: 43-61. http://dx.doi.org/10.1016/j.mce.2007.05.015

Wang L, Chen X, Li X, Shu G, et al. (2014). Evaluation of adrenocorticotropin regulated glucocorticoid synthesis pathway in adrenal of different breeds of pigs. *Livest. Sci.* 169: 185-191. http://dx.doi.org/10.1016/j.livsci.2014.08.007

Waterman MR (1995). A rising StAR: an essential role in cholesterol transport. *Science* 267: 1780-1781. http://dx.doi.org/10.1126/science.7892600

Weber A and Clark AJL (1994). Mutations of the ACTH receptor gene are only one cause of familial glucocorticoid deficiency. *Hum. Mol. Genet.* 3: 585-588. http://dx.doi.org/10.1093/hmg/3.4.585

Witchel SF and DeFranco DB (2006). Mechanisms of disease: regulation of glucocorticoid and receptor levels--impact on the metabolic syndrome. *Nat. Clin. Pract. Endocrinol. Metab.* 2: 621-631. http://dx.doi.org/10.1038/ncpendmet0323

Wu X, Xie C, Yin Y, Li F, et al. (2013). Effect of L-arginine on HSP70 expression in liver in weanling piglets. *BMC Vet. Res.* 9: 63-68. http://dx.doi.org/10.1186/1746-6148-9-63

Zhang J, Yu HW, Li J, Zhu YK, et al. (2015). Reduced cortisol in the absence of bacterial infection in patients with hepatitis B virus cirrhosis. *Genet. Mol. Res.* 14: 7957-7963. http://dx.doi.org/10.4238/2015.July.17.3