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Alteration of Reproductive Hormone Levels in Pregnant Sows Induced by Repeated ACTH Application and Its Possible Influence on Pre- and Post-natal Hormone Secretion of Piglets

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Abstract. Prenatal stress has been seen as a reason for reproductive failures in pig offspring mostly originated or mediated by changed maternal functions. Experiments were conducted in pregnant gilts (n=32) to characterize effects of elevated maternal glucocorticoids on the secretion of reproductive hormones (LH, progesterone) during the 1st (EXP 1), 2nd (EXP 2) and 3rd (EXP 3) trimester of pregnancy (TP). Transiently elevated cortisol release was repeatedly achieved by application of 100 IU adenocorticotrophic hormone (ACTH) (Synacthen Depot®) six times every second day beginning either on day 28 (EXP 1), day 49 (EXP 2) or day 75 of pregnancy (EXP 3). Glucocorticoid concentrations were examined in umbilical blood vessels of fetuses which mothers were subjected to ACTH at 2nd and 3rd TP (EXP 4). Furthermore, the pituitary function of newborn piglets of EXP 2 was checked by a LH-RH challenge test. In sows, LH concentrations were at low basal level (0.1–0.2 ng/ml) but with pulsatory release pattern during each TP. The number of LH pulses/6 h (LSM ± SE) of saline treated Controls increased with ongoing pregnancy and decreased to the 3rd TP (1.3 ± 0.2 in EXP 1 vs. 2.0 ± 0.1 in EXP 2 vs. 1.4 ± 0.1 in EXP 3, p<0.05). After ACTH treatment the number of LH pulses left unchanged in Experiments 1 and 2 (1.3 ± 0.2 and 1.5 ± 0.1) and decreased in EXP 3 (0.8 ± 0.2, p<0.05). Differences (p<0.05) were obtained comparing the LH pulse number of ACTH and saline treated sows at the 2nd and 3rd TP. Moreover, areas under the curve (AUC) of each LH pulse and of LH over baseline were significantly reduced by treatment. Levels of progesterone increased (p<0.05) for 150 to 170 min after each ACTH application both in EXP 1 and EXP 2, but not in EXP 3. The mean progesterone concentration was different between trimesters, and ACTH and Controls (1st TP: 30.0 ± 0.9 and 24.4 ± 0.7 ng/ml; 2nd TP: 35.5 ± 0.9 and 29.1 ± 1.0 ng/ml; 3rd TP: 13.6 ± 0.2 and 13.1 ± 0.1 ng/ml; p<0.05). In fetuses (n=87) recovered 3 h after ACTH or saline (EXP 4), the plasma cortisol concentrations were significantly increased in umbilical vein (93.7 ± 5.5 vs. 47.0 ± 5.3 nmol/l) and artery (95.7 ± 5.4 vs. 66.4 ± 5.4 nmol/l), and in periphery (46.8 ± 5.3 vs. 27.1 ± 5.3 nmol/l) compared to controls. Plasma ACTH concentrations, however, did not differ in fetuses of both treatment groups. Postnatal LH-RH challenge tests (1st and 28th day post partum) induced LH surges in female piglets (n=67) both of ACTH and saline treated sows, but did not differ between groups (1st day: 7.2 ± 0.8 vs. 8.1 ± 0.7 ng/ml; 28th day: 10.5 ± 1.7 vs. 13.6 ± 2.2 ng/ml). However, basal LH of piglets whose mothers were submitted to ACTH during 2nd TP was lower on 1st day (1.7 ± 0.2 vs. 2.3 ± 0.2 ng/ml, p<0.05) but not on 28th day (1.0 ± 0.2 vs. 1.1 ± 0.2 ng/ml). However in both groups, the basal LH was always higher on 1st as on 28th day (p<0.05). Thus, chronic intermittent ACTH administration is able to influence the release pattern of maternal reproductive hormones. However, these findings demonstrate that these effects are dependent on the stage of pregnancy. Furthermore, it was shown that maternal cortisol can cross the placenta during gestation and thus may affect maternal-fetal interactions and, as a result, reproductive function of offspring.

Key words: Luteinizing hormone, Progesterone, adenocorticotrophic hormone (ACTH), Pregnancy, Stress, Fetus, Pig

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Stressful stimuli activate in mammals the hypothalamic-pituitary-adrenal (HPA) axis which leads to the release of corticotrophin releasing factor (CRF), adenocorticotrophic hormone (ACTH), and cortisol. Corticoids (corticosterone and cortisol) are the main stress response with permissive, suppressive, stimulatory or preparatory effects [review: 1]. Besides these effects, cortisol plays an essential role for cell cycle regulation in many organs, binds to the aldosterone receptor (MC-R) in mineralocorticoid-sensitive tissues [2], and is essential during inflammation preventing an excessive activation of the immune system [3]. Cortisol is a responsible steroid in swine as well. During pregnancy stress may influence the function of the porcine placenta and corpora lutea, which are steroidogenic tissues involved in the maintenance of pregnancy [4, 5]. Moreover, prenatal stress may exert lasting effects on gonadal function, behaviour, regulation of the HPA axis and on immuno-competence of the offspring [6–9]. Stress related effects on developing fetuses and on coming reproductive function of offspring may differ between various periods of the pregnancy and the ongoing differentiation of the fetal reproductive tract [10–12]. Despite comprehensive research the mechanisms by which stressors affect reproductive function in swine are not fully known [13].

Although single or repeated acute stress (e.g. restrain of snout, electric foot shock, transportation or jugular puncture) induces elevated cortisol levels, it may not result in deleterious effects [14–16]. Studies with different models of stress imitation have demonstrated that both administration of ACTH or cortisol impaired reproductive function in female pigs when given in a way which resulted in a sustained elevation of cortisol in plasma [17–19]. Furthermore, cortisol was shown to affect the LH secretion preferentially by reducing the frequency and amplitude of GnRH pulses [20, 21], and by effects at pituitary level [22].

In pregnant pigs, the concentration of maternal LH is low and with minor pulsatile secretion [23–26]. But all in all, only little information is available on gonadotropin concentration during porcine pregnancy and during stressful conditions of pregnancy.

The objective of this study was to characterize the secretion pattern of LH and progesterone (P₄) throughout the 1st, 2nd and 3rd trimester of

pregnancy (TP), and under the influence of elevated cortisol levels induced by repeated ACTH injections. Further, the effects of elevated maternal glucocorticoids on cortisol concentrations in umbilical blood vessels of fetuses was evaluated, and the pituitary function of newborn piglets checked by a LH-RH challenge test.

Materials and Methods

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministerial Department of Mecklenburg-Vorpommern, Germany.

Animals, hormonal treatment and blood sampling

Altogether 32 pregnant primiparous Landrace sows were used in four experiments (EXP 1. n=8, EXP 2. n=8, EXP 3. n=10, EXP 4. n=6). Sows were held in individual pens beginning on day 11 after A.I. (= day 11 of pregnancy). They were fitted with indwelling jugular catheters [27] under ketamin/xylazin anaesthesia (Ursotamin®, Xylazin®, Serumwerk Bernburg, Germany) either between days 24–25 (EXP 1; 1st trimester of pregnancy-TP), days 46–47 (EXP 2; 2nd TP) or days 83–84 of pregnancy (EXP 3; 3rd TP). Sows were fed a commercial food for pregnant sows and had free access to water.

Six i.m. injections, each of 100 IU ACTH (Synacthen® Depot, Novartis Pharma, Brussels, Belgium) or saline, were given at 08:00 every two days beginning on day 28 (EXP 1), 49 (EXP 2) or 85 of pregnancy (EXP 3), respectively. In each experiment, the half of animals received ACTH or saline (Controls). Blood samples were drawn on treatment days immediately before and every 10 min after ACTH administration from 08:00 to 14:00.

In EXP 4, only one injection of 100 IU ACTH (n=3) or saline (n=3) was given on day 65 of pregnancy at 08:00. Three hours post-ACTH injection, all fetuses were recovered by Caesarean section *lege artis* under ketamin/xylazin anaesthesia. In total, 42 fetuses from ACTH-treated and 45 fetuses from Control sows were recovered. Blood samples were obtained 3 h after ACTH or saline application via venipuncture from the external jugular vein of the sow, and from the umbilical vein and artery of each fetus. Additionally, blood samples were obtained from each fetus after decapitation. Cortisol

concentrations were also measured in umbilical vein and artery of 24 fetuses (each 2 male and 2 female) from 6 ACTH and saline treated sows of EXP 3 on day 105 of pregnancy.

Postnatal LH-RH challenge tests (EXP 5; injection of 5 µg Gonavet®, Veyx Pharma, Schwarzenborn, Germany) was done in 67 female piglets, whose mothers were subjected to repeated ACTH application during the 2nd TP, on day 1 and day 28 post partum (p.p.). Blood samples (1 ml) were drawn via venipuncture from the external jugular vein immediately before and 30, 60, 120 and 180 min after GnRH injection.

The blood samples were collected in ice-cooled plastic syringes containing EDTA-Kalium as anti-coagulant (KABE, Germany). Samples were centrifuged at 2,000 to 4,000 rpm for 15 min at 4 °C, plasma portioned in aliquots and stored until analysis at -20 or -80 °C.

Hormone analyses

LH was determined by a non-isotopic electrochemiluminescence immunoassay (ECLIA). A sandwich type assay was performed according to the method for the determination of bovine LH [28]. The ECLIA was carried out in 12 × 75 mm polypropylene tubes (Minisorb PE; Nunc, Roskilde, Denmark). An *N*-hydroxy-succinimide ester of ruthenium(II)-tris-bipyridine chelate (Ru-ester) was used to label the monoclonal antibody 517 B7 against bovine LH (b-LH) as recommended by the manufacturer (IGEN, Gaithersburg, MD). The standard porcine-LH (p-LH, iodination grade) and polyclonal rabbit-anti pLH were provided from Biotrend (Cologne, Germany). The Ru-labelled monoclonal antibody (25 µl) and polyclonal antibody (50 µl) were allowed to bind to the hormone (standard/sample, 20 µl). Determination of the standard curve was started at 12.8 ng/ml and diluted throughout 0.025 ng/ml. After overnight incubation 50 µl/tube of secondary antibody, sheep anti-rabbit IgG coupled to magnetic beads (50 µl; Dynal, Oslo, Norway), was added and mixed. Finally, the intensity of chemiluminescence was measured using an Origen™ 1.5 analyser (IGEN). The ECLIA ran for approximately 1 h per carousel sample changer fitted with 50 samples. The sensitivity of the method was calculated to be 0.03 ng/ml. The intra- and interassay CV were 6.1 and 8.7%, respectively.

The peripheral plasma P₄ level was analyzed

using a competitive single-antibody ³H-RIA without extraction [28]. The tracer, [1,2,6,7-³H] progesterone, was purchased from Amersham Biosciences Europe (Freiburg, Germany). The antibody was raised in rabbits by immunisation with a 11-hydroxy-progesterone conjugate and further purified by affinity chromatography on protein A superose (Amersham Pharmacia Biotech). As result, cross-reactivities to related steroids, e.g. pregnenolone, androstendione, cortisol, were lower than 0.4%. The CV for intra-assay and inter-assay were 7.7 and 10.1%, respectively.

Plasma cortisol concentrations were measured in duplicates using a commercial available ¹²⁵I-RIA kit (DRG International Inc., Mountainside, NY) according to the manufacturer's guidelines. The antibody for this assay was highly specific with less than 5% cross-reactivity with any potentially competing plasma steroids (DRG International). The test sensitivity was 0.3 µg/dl, and intra- and interassay coefficients of variation were 5.3 and 9.8%, respectively.

ACTH concentrations were determined in duplicate in 200 µl plasma with an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) that has been validated for animal specimens (Nichols Institute Diagnostics, Geneva, Switzerland) and has no cross-reactivities with α-MSH, β-MSH, β-endorphin or β-LPH [29]. The intra- and interassay coefficients of variance were 3.2 and 7.8%, and the assay sensitivity was 1.6 pg/ml.

Statistics

Statistical analysis was done with SAS®, release 8.2. The model used for the analyses was a general linear model $Y = X\beta + \varepsilon$, where Y denotes the vector of observations of a investigated trait, X the known design matrix of the vector β of the fixed effects on this trait (total mean, levels of the factors group (control, experiment), phase of pregnancy (1st, 2nd and 3rd trimester), day of experiment within the pregnancy phase and all interactions between these factors), and ε is the vector of random errors, with expectation $E(\varepsilon) = 0$ and covariance matrix $V(\varepsilon) = R$. Repeated measures on the animals (day of experiment within the pregnancy phase) were taken into account by the repeated statements of the procedures mixed (continuous data) or Genmod (count data; Poisson distribution) in SAS/

STAT software (SAS Institute Inc., 1999). The significance of each of the main effects and the interactions in the model was tested [type III F-tests (proc mixed); Wald statistic (proc Genmod)]. The model for the cortisol concentrations in fetuses included the fixed factors treatment, gender, blood vessel, the corresponding interactions and the factor sow nested within treatment as a random variable. For each variable, LSMeans and SE were calculated to display the results. Additionally, all pairwise differences between the LS-means were tested using the PDIF-option in the LSMEANS-statement of proc Mixed and proc Genmod. The calculation of pulses and their further characterization was performed by the PULSAR program [30].

Results

All sows have continued pregnancy till Caesarean section or parturition, respectively. The procedure of catheterization at the 1st, 2nd and 3rd TP, and the frequent blood sampling every 10 min throughout six hours on each treatment day did not affect the progress of pregnancy. The number of fetuses/piglets (13.7 ± 0.6 in ACTH treated vs. 13.1 ± 0.6 in Control sows), the number of intact fetuses/liveborn piglets (12.7 ± 0.6 vs. 12.7 ± 0.5), and the mean fetus/piglets weight (1.10 ± 0.02 vs. 1.20 ± 0.09 kg) were obviously not influenced by repeated ACTH application.

The application of ACTH to sows caused a significant increase of plasma cortisol concentrations after 30 min lasting up to 360 (3rd TP) and 480 min (2nd TP; effect of treatment, and treatment by sampling time, $p < 0.001$). Maximum cortisol levels (247.0 ± 18.8 to 276.5 ± 17.2 nmol/l)

were reached between 120 and 180 min. As an example, Figure 1 demonstrates plasma cortisol levels after ACTH and saline application at 3rd TP.

The basal LH levels ranged in all sows between 0.11 and 0.16 ng/ml with differences between trimester of pregnancy and treatment groups (Table 1). The highest basal LH level was found during the 2nd TP in ACTH treated and during the 2nd and 3rd TP in Control sows.

The LH amount released (AUC over baseline) was higher in the 3rd compared to 1st and 2nd trimester of pregnancy both for ACTH and saline treated animals, and differences were obtained between ACTH and Controls at the 2nd and 3rd TP. A pulsatile LH release pattern was evident throughout each trimester. The number of LH pulses/6 h of saline treated Controls increased with ongoing pregnancy and decreased to the 3rd TP

Fig. 1. Plasma cortisol concentrations before and after ACTH or saline injection (↓) in pregnant gilts during 3rd trimester of pregnancy. Data represent LSMeans $\pm$ SE. * and ***: $p < 0.05$ and 0.001 , respectively.

Table 1. Basal LH level, LH amount over baseline, LH pulse number, LH amount in pulses and progesterone level (LSMeans $\pm$ SE) in ACTH treated ($n=13$) and Control sows ($n=3$) at 1st, 2nd and 3rd trimester of pregnancy

Parameter	1 st trimester		2 nd trimester		3 rd trimester	
	ACTH	Control	ACTH	Control	ACTH	Control
LH basal (ng/ml)	$0.14 \pm 0.01^{a,A}$	$0.11 \pm 0.01^{b,D}$	$0.16 \pm 0.01^{a,B}$	$0.15 \pm 0.01^{b,E}$	$0.11 \pm 0.01^{a,C}$	$0.16 \pm 0.01^{b,E}$
LH over baseline (ng/ml)	0.5 ± 0.1^A	0.6 ± 0.1^D	$0.4 \pm 0.1^{a,A}$	$1.3 \pm 0.1^{b,E}$	$1.2 \pm 0.2^{a,B}$	$2.3 \pm 0.2^{b,F}$
Number of LH pulses	1.3 ± 0.2	1.3 ± 0.1^D	$1.5 \pm 0.1^{a,A}$	$2.0 \pm 0.1^{b,E}$	$0.8 \pm 0.2^{a,B}$	$1.4 \pm 0.1^{b,D,F}$
LH pulse (ng/ml)	0.3 ± 0.1^A	0.5 ± 0.1^D	$0.3 \pm 0.1^{a,A}$	$0.6 \pm 0.1^{b,D}$	$1.1 \pm 0.1^{a,B}$	$1.7 \pm 0.1^{b,E}$
Progesterone (ng/ml)	$30.0 \pm 0.9^{a,A}$	$24.4 \pm 0.7^{b,D}$	$35.5 \pm 0.9^{a,B}$	$29.1 \pm 1.0^{b,E}$	$13.6 \pm 0.2^{a,C}$	$13.1 \pm 0.1^{b,F}$

^{a,b} within a row denotes significant differences ($p < 0.05$) between ACTH and controls within each trimester.

^{A,B,C} and ^{D,E,F} within a row denotes significant differences ($p < 0.05$) between trimesters of ACTH and controls, respectively.

Fig. 2. Plasma progesterone concentrations before and after ACTH or saline injection ($\downarrow$) in pregnant gilts at the 1st (A), 2nd (B) and 3rd (C) trimester of pregnancy. Data represent LSMeans $\pm$ SE. * $p < 0.05$.

($p < 0.05$). After ACTH treatment the number of LH pulses left unchanged in the 1st and 2nd trimester and decreased to the 3rd one. The LH pulse number was lower ($p < 0.05$) after ACTH at the 2nd and 3rd TP compared to saline treated sows. Furthermore, the LH amount released in a pulse was higher in Controls as in ACTH treated sows at the 2nd and 3rd

TP. While LH pulse number was decreasing to the 3rd TP in each treatment group, the LH amount of each pulse was increasing, however.

The analysis of plasma P₄ did not reveal signs of a pulsatile release. The mean P₄ level increased from the 1st to 2nd TP and was lower at the last trimester of pregnancy (Table 1, $p < 0.05$). In ACTH treated sows, the average progesterone concentrations were always higher as in Controls. Progesterone significantly increased after each ACTH injection commencing within 20 min and remaining elevated for 150–170 min during the 1st and 2nd, but not during the 3rd trimester of pregnancy. This effect was not observed in Controls (Fig. 2).

In fetuses ($n=87$) recovered 3 h after ACTH (2nd TP, day 65; EXP 4), the plasma cortisol concentrations were significantly increased in umbilical vein and artery, and in periphery compared to controls (Table 2). Such effect was not observed in fetuses ($n=24$) which mothers were subjected to repeated ACTH injection from day 85 to 101 and recovered on day 105 of pregnancy. Plasma ACTH concentrations in the umbilical vein did not differ in fetuses of both treatment groups during 2nd TP (34.5 ± 10.2 vs. 48.0 ± 7.6 pg/ml).

Postnatal LH-RH challenge tests on day 1 and day 28 p.p. induced LH surge in female piglets both of ACTH and saline treated sows, but surge parameters did not differ between treatment groups (Table 3). Only Control piglets had both a higher maximum LH concentration and LH amount released comparing day 28 and day 1 p.p. The basal LH of piglets whose mothers were submitted to ACTH during 2nd TP was lower as Controls on day 1, but not on day 28 p.p. However in both treatment groups, the basal LH was always higher on day 1 as on day 28 p.p.

Discussion

One result of our study is that repeated stimulation of pregnant sows with ACTH during different trimesters of pregnancy causes adrenocortical activation with reiterable cortisol release. This effect was not observed in saline treated sows. The repeated administration of saline does not affect the pituitary-adrenocortical activity compared to untreated controls, indicating that the injection itself is not stress-inducing [31]. The

Table 2. Plasma cortisol concentrations in sows (n=12), and plasma cortisol and ACTH concentrations (LSMeans $\pm$ SE) in fetuses (n=111) on days 65 and 105 of pregnancy following single or repeated ACTH application

	day 65 of pregnancy		day 105 of pregnancy	
	ACTH	Control	ACTH	Control
Cortisol in sows (nmol/l)	402.2 $\pm$ 21.3 ^a	72.6 $\pm$ 21.3 ^b	230.8 $\pm$ 39.7	166.6 $\pm$ 39.7
Cortisol in fetuses (nmol/ml)				
umbilical vein	93.7 $\pm$ 5.5 ^a	47.0 $\pm$ 5.3 ^b	214.9 $\pm$ 50.2	309.0 $\pm$ 50.2
umbilical artery	95.7 $\pm$ 5.4 ^a	66.4 $\pm$ 5.4 ^b	242.8 $\pm$ 55.8	347.6 $\pm$ 55.8
fetal peripheral plasma	46.8 $\pm$ 5.3 ^c	27.1 $\pm$ 5.3 ^d	356.5 $\pm$ 91.3	451.1 $\pm$ 91.3

^{a,b} p<0.01; ^{c,d} p=0.09.**Table 3.** Basal and maximum LH concentrations, and AUC of LH surge (LSMeans $\pm$ SE) of piglets (n=67) on days 1 and 28 post partum (p.p.)

	day 1 p.p.		day 28 p.p.	
	ACTH	Control	ACTH	Control
Maximum LH (ng/ml)	7.2 $\pm$ 0.8	8.1 $\pm$ 0.7 ^C	10.5 $\pm$ 1.7	13.6 $\pm$ 2.2 ^D
AUC of LH surge (ng/ml)	23.5 $\pm$ 2.7	28.6 $\pm$ 2.9 ^C	34.8 $\pm$ 5.1	42.8 $\pm$ 4.9 ^D
Basal LH (ng/ml)	1.7 $\pm$ 0.2 ^{a,A}	2.3 $\pm$ 0.2 ^{b,C}	1.0 $\pm$ 0.2 ^B	1.1 $\pm$ 0.2 ^D

^{A,B} and ^{C,D} within a row denotes significant differences (p<0.05) between days p.p. of ACTH and controls, respectively.^{a,b} within a row denotes significant differences (p<0.05) between ACTH and controls within each day p.p.

exogenous ACTH treatment, which can be considered as a simplified “stress model”, ascertains ACTH- and cortisol-related effects, and avoids stress-related effects of other systems, i.e. endogenous opioide peptides, catecholamines and prolactin. ACTH application has been shown to increase cortisol levels in swine [32–34]. The adrenocortical response to ACTH was consistent during each trimester of pregnancy and significantly increased cortisol levels were found at all days. No signs of habituation or sensitization were observed. Although maternal cortisol was elevated after repeated ACTH application by 100–200% for more than 6 h, no deleterious effects on pregnancy were found. Razdan *et al.* [35] found as well no influence of stress-like situation (food deprivation, ACTH application) on early fetal development.

Serious stress in sows' pregnancy may affect, however, placental metabolism and maternal blood levels of reproductive hormones, and impair fetal development. Studies have shown that hormones of the HPA axis are able to suppress gonadal function by inhibiting GnRH release [36]. CRF, ACTH and cortisol act on the hypothalamus to

restrain the GnRH secretion or directly on the pituitary to inhibit gonadotropin responsiveness to GnRH. Furthermore, an involvement of endogenous opioid peptides (EOP) and some other compounds on LH secretion has been documented [37–39, reviews: 40, 41].

In pregnant sows, the concentration of LH has been reported to be low (between 0.25–0.50 ng/ml), without differences throughout the entire pregnancy, but with some pulsatile fluctuation [23–26, 42, 43]. To our knowledge, there are, only few data on the pattern of LH pulsatility throughout pregnancy in the pig [26] and, moreover, on its stress-induced changes.

The present results on the course of LH concentration during early, mid- and late pregnancy showed uniform basal levels between 0.11 and 0.16 ng/ml, which are in the range reported previously [26, 42]. More LH (AUC over baseline) was released in controls with ongoing pregnancy but LH secretion was suppressed in ACTH treated sows commencing at the 2nd TP. Nevertheless, a modest pulsatile release pattern was found in each period of pregnancy. LH pulse frequency increased from the 1st to 2nd TP and

decreased to late pregnancy. Kraeling *et al.* [26] reported also a higher number of LH peaks on days 30–70 of pregnancy compared with later period. Whereas ACTH did not affect the LH pulse frequency at the 1st TP, the pulse number was decreased during the mid- and late pregnancy. The lack of ACTH-related influence on LH pulse secretion during the 1st TP may be due to the transient elevation of estrogen level during early pregnancy having a strong negative feedback on LH release [44]. Therefore, an additional inhibition of LH pulse secretion by ACTH is omitted. Furthermore, the amount of LH released at each pulse was reduced of about 50% by ACTH treatment during the 2nd and 3rd trimester.

In the pig, LH is the main luteotropic factor from early to mid-gestation [23, 45, 46]. The low-frequency pulsatile LH release found in our study and previously [23] seems to be crucial for proper function of porcine corpora lutea, which are the main source of progesterone and necessary for maintaining pregnancy [5, 47]. Data summarized by Knight [4] indicate that the porcine placenta produces also large quantities of progesterone throughout gestation which are utilized locally and/or rapidly metabolized through androgens to estrogens. LH binding to uterine receptors may be involved in placental progesterone production [review: 48]. The lower LH availability after ACTH detected at the 2nd and 3rd TP could result the amount of secreted luteal progesterone. The results, however, are opposing. We found elevated steroid levels for about 3 h after ACTH application both during the 1st trimester, where LH was not affected by ACTH, and during the 2nd TP, where LH was influenced. The source of this additional progesterone is assumed to be the adrenals [49]. This supposition is supported by likewise elevated concentrations of 17 α -OHP, a related steroid, after each ACTH administration (44). No ACTH-related progesterone increase was found during the 3rd TP, although LH and LH frequency were influenced. It remains open whether the lacking progesterone increase after ACTH is the result of lower capacity of the adrenals, corpora lutea and/or placenta. However, it has to keep in mind that a complex of luteotropic components (LH, prolactin, endogenous opioids) and its interaction with progesterone is involved in late gestation in the sow [42, 50–52].

It is well established that exogenous ACTH

effects an increase in cortisol. At pregnancy, the placenta has a changing capacity to convert corticoids and thus to protect fetuses [53–56]. In stressful situation, excess of cortisol may force this protective mechanism. It remains speculative if transiently elevated progesterone levels, as observed in our study, contribute to increased cortisol levels by blocking cortisol-transforming activities of 11 β -hydroxysteroid-dehydrogenase-2 (11 β -HSD-2) [57] or by displacement from plasma protein binding [58]. Varying activities for 11 β -HSD are also present in corpora lutea [58, 59]. Thus, the function of the ovary in pregnant sows could be affected by exogenous ACTH.

A significant increase of cortisol concentrations in the umbilical vein and artery of fetuses was detected 3 h after ACTH application to sows on day 65 of pregnancy compared to Controls. This may lead to a higher exposure of cortisol in the fetuses. Generally, umbilical venous cortisol levels were lower compared to maternal plasma cortisol concentrations both in treated and control animals, demonstrating the mechanism to inactivate cortisol by the placenta [60, 61]. The higher cortisol concentrations in umbilical artery in controls fetuses may also reflect the ability of the fetal adrenal to release cortisol at this time. The fetal adrenal is able to respond to ACTH at 75 days, suggesting an active HPA axis at this time [60, 62]. However, in fetuses which mothers were subjected to repeated ACTH treatment during the 3rd trimester, no differences were found both of umbilical cortisol between treated and control fetuses and of maternal and fetal cortisol on day 105 of pregnancy. One explanation is that the mature fetal adrenal secretes enhanced cortisol in the peripartal period.

It was shown that stress can alter aspects of immune function in pigs [6, 63–65] but to our knowledge, stress-related gonadotropin alteration in neonate piglets are missing. In neonate female piglets at the age of 6–7 days the mean LH concentration was 2.3 ± 0.2 ng/ml [66]. Similar results were obtained in female Control piglets on day 1 p.p. in our study. However, those from ACTH treated mothers had a lower LH level. It is still speculative if ACTH-related steroids feedback actions are involved. On day 28 p.p. no differences were found between ACTH and Control piglets, but in both groups the basal LH concentrations were decreased compared to day 1. On the other

side, all piglets demonstrate a LH surge after LH-RH challenge (LH surge amount: 23.5 to 42.8 ng/ml) with no differences between treatments. Consequently, neonate female pigs are able to release LH from the pituitary and as demonstrated by Ponzilius *et al.*, [24] in a pulsatile manner as well. Our results were obtained with a limited number of piglets and pigs which mothers were subjected to repeated ACTH during the 2nd TP, only. Nevertheless, an impact of ACTH on LH system may be evident leading to reproductive alterations

during lifetime.

In summary, results of this study showed that repeated administration of ACTH ("stress-like simulation") to pregnant sows is able to influence the release pattern of maternal reproductive hormones, and these effects are dependent on the stage of pregnancy. Furthermore, it was shown that maternal cortisol can cross the placenta during gestation and thus may affect maternal-fetal interactions and, as a result, reproductive function of offspring.

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