Comparison of Salivary and Peripheral Cortisol Before and After Farrowing in Swine

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Introduction

Measuring stress hormones in periparturient sows is challenging

- Jugular vein puncture requires snaring, which is stressful..
- Before and after parturition, cortisol (Figures 1 and 2) is released to help regulate and prepare the sow's body for tissue damage (Amdi et al., 2015).
- Cortisol concentrations are more variable among periparturient sows than early-gestation sows (Kansen et al., 2017; Figure 3).

Refinement of methods and reducing animals needed in stress-research

- Variation from sow saliva or ear-vein blood cortisol samples can be used to estimate the optimal amount of periparturient sows needed for future projects (Li et al., 2017).

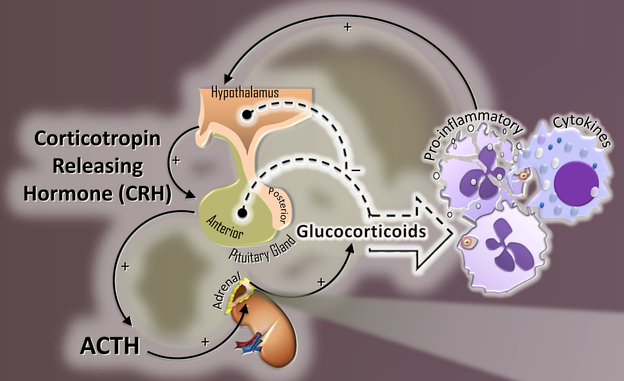
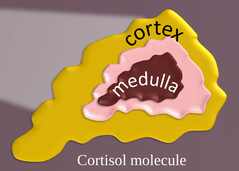


Figure 1A. Adrenal gland Within milliseconds of a stimuli, acute stress causes the sympathetic nervous system (SNS) to release catecholamines (i.e. adrenalin) release from the adrenal medulla and nerves. Glucocorticoids are the main regulatory hormone of a full stress response which are secreted from the adrenal cortex minutes after the stimuli.



Cortisol molecule structure

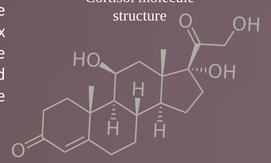


Figure 1B. After the SNS stimulates catecholamine release, the hypothalamopituitary adrenal (HPA) axis is activated. First, CRH stimulates the corticotrophs of the anterior pituitary gland to release adrenocorticotropic hormome (ACTH). This hormone stimulates the release of glucocorticoids (GC; i.e. cortisol, corticosterone) from the cortex of the adrenal. Glucocorticoids are important because they provide homeostatic negative feedback. Tissue damage caused by parturition and immune cells activate the HPA; GC then regulate inflammation (Figure adapted from Hulbert and Moisà, 2016)

Objectives

- ✓ Determine the coefficient of variation (CV%) of periparturient sows and calculate the number of samples needed per treatment.
- ✓ Determine which sample type (saliva vs. low-volume ear vein) will detect the change of cortisol that occurs after parturition.

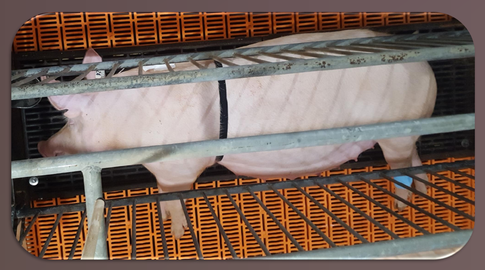


Figure 2. Low-stress cortisol collection is important for sow stress-related and behavior projects. Left panel is an overhead view of a sow lying in the lateral recumbent position during labor. She is wearing heart rate (black strap), lie-position (blue leg wrap), and head-movement monitors (white patch). Right panel is neonatal (day 0) piglets marked with tape by their birth order. Jugular venipuncture requires restraint, and will disrupt behaviors and add stress to both sows and piglets.

Materials and Methods

Animals (IACUC # 3896)

- 10 multiparous sows (DNA Genetics; K-State SRC) were used
- Samples were collected 1 day before and after farrowing.

Salivary Collection

- Sows chewed on a 51 cm cotton-rope for 5-10 min.

Peripheral Collection

- 300 uL of blood was collected after marginal venipuncture of the ear.
- 26 gauge needle and syringe treated with heparin was used.

Measuring Cortisol Concentrations

- Samples were analyzed using a competitive-binding, enzyme-linked immunosorbent assay (ELISA; Detect X; Arbor Assays, Anne Arbor, MI; Figure 3).

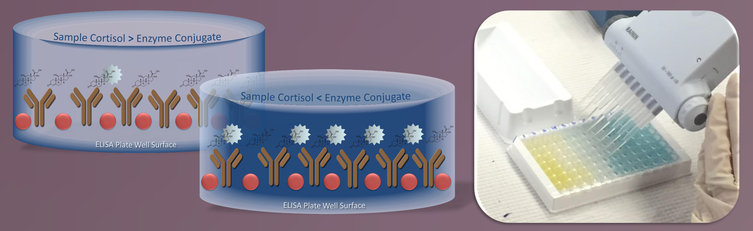
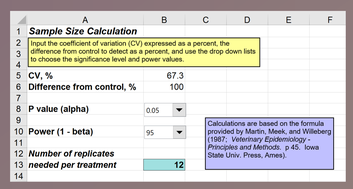


Figure 3. Cortisol concentrations of samples were measured using a competitive-binding ELISA. Capture antibodies (Y) bind with high affinity to the wells of the plate. The cortisol molecules in the sample compete with a known amount of cortisol that is conjugated to an enzyme that produces color (blue) upon activation with a substrate. Samples with high concentrations of cortisol appear clear and with low concentrations appear dark-blue. Then, An acidic "stop solution" is added, turning the wells to varying colors of yellow. Then a plate reader is used to measure the color. Standards are used to create a fitted-curve, and the curve's formula is used to calculate cortisol concentrations for each sample.

Data were analyzed

- Descriptive statistics and CV% were calculated using the Proc Univariate method with SAS (version 9.4; SAS Inc., Raleigh NC).
- CV% were used to calculated sample size (Figure 4) at $P = 0.05$, Power = 0.90 for expected differences of 25, 50, and 100% between samples from control and treated sows.
- Proc mixed in SAS was used to compare the sows cortisol concentrations before and after farrowing.

Figure 4. Once variation is calculated , it can be used in a sample size calculator to determine how many animals (experimental units if single-housed) are needed for future experiments. Future experiments will involve treatments that can cause small (25%), moderate (50%), and great changes in cortisol secretion. Sample size calculator was downloaded from M. Galyean's webpage.



	A	B	C	D	E	F
1	***Sample Size Calculation***					
2	Input the coefficient of variation (CV) expressed as a percent, the difference from control to detect as a percent, and use the drop down lists to choose the significance level and power values.					
3						
4						
5	**CV, %**	67.3				
6	**Difference from control, %**	100				
7						
8	**P value (alpha)**	0.05				
9						
10	**Power (1 - beta)**	95				
11						
12	***Number of replicates***					
13	***needed per treatment***	12				
14						

Calculations are based on the formula provided by Martin, Meek, and Willeberg (1987; *Veterinary Epidemiology - Principles and Methods*. p 45. Iowa State Univ. Press. Ames).

Results

Qualitative assessment of sample collection

- Periparturient sows were reluctant to chew on cotton rope and sows preferred to change from lying to sitting during collection.
- Ear vein collection was rapid and disruption of behaviors was minimal.

Salivary and blood cortisol pre vs. post Farrowing

- Sows tended to have higher circulating cortisol on day 1 after farrowing than day -1 before farrowing ($P = 0.06$; Figure 5). No differences ($P > 0.10$) were detected in saliva.

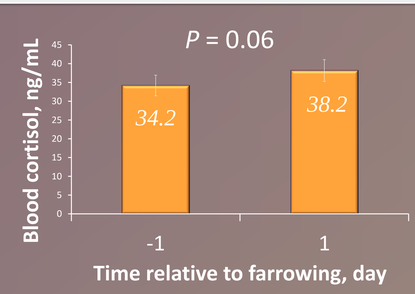
Results

CV% and sample size calculator

- Salivary cortisol was detected in low concentrations (pg/mL) and had greater variation (Table 1) than plasma cortisol.
- Less sows are needed to detect expected treatment differences from mild (25%), moderate (50%) and severe (100%) stressors (Table 1).

Table 1. Descriptive statistics of all the samples collected from each sow (n = 10). CV% was used in sample size calculator: *P* value significance = 0.05, Power = 0.90. Δ= expected difference from a control (no stress) and stress-treated sows.

	Salivary cortisol, pg/mL	Plasma cortisol, ng/mL
Mean	228.8	38.1
Median	211	41.4
±SD	154.1	9.5
CV%	67.3	24.8
Min	69.0	19.2
Max	614.0	55.5
Δ	n =	n =
25%	188	26
50%	47	6
100 %	12	3



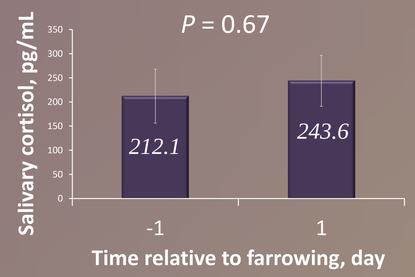


Figure 5. Top panel: Plasma cortisol from marginal ear vein blood. Bottom panel: Salivary cortisol.

Implications

- **Marginal ear-vein collection of blood will be the preferred method for projects that involve periparturient sows because:**
  - Less sows are needed for even mild stressor treatments than saliva
  - "Stealthy" collection allows the sow to remain in a resting position, which is less likely to disrupt nursing bouts
- **Salivary collection may not be an appropriate method because the periparturient state decreases motivation to eat and chew** (Hansen et al., 2017).

References

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