Using serum cortisol to distinguish between acute stress and pain response following castration in piglets

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Abstract
In the United States swine industry, castration is essentially universal and only a select number of male pigs are left intact as potential breeder boars. Pain and distress inflicted by castration is an animal well-being concern in livestock production. Castration in pigs has been shown to cause a stressful and painful response. The need for a robust, repeatable physiological indicator of pain for use in the assessment of production practices designed to minimize these impacts has been recognized.

Disciplines
Agriculture | Animal Sciences | Large or Food Animal and Equine Medicine | Veterinary Physiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments
Using serum cortisol to distinguish between acute stress and pain response following castration in piglets

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Introduction
In the United States swine industry, castration is essentially universal and only a select number of male pigs are left intact as potential breeder boars. Pain and distress inflicted by castration is an animal well-being concern in livestock production. Castration in pigs has been shown to cause a stressful and painful response.¹ The need for a robust, repeatable physiological indicator of pain for use in the assessment of production practices designed to minimize these impacts has been recognized.²⁻³

Castration includes several events likely to be painful: scrotal incision, extraction of the testes, and severing of the spermatic cords.⁴⁻⁷ This procedure has been recommended to be conducted in the first week of life. There is some evidence showing a greater effect of castration on weaned piglets aged 7 to 8 weeks of age compared to pre-weaned piglets (< 20 d).⁵⁻⁸ One study by Taylor et al.⁹ reported behavioral differences between castrated and handled only piglets. In the first 2 hours immediately preceding castration, piglets spent more time sitting or standing inactive and less time lying. They also spent more time at the teat and less time lying in the first 22 hours.

In relation to events expected to be painful, acute cortisol response has been used to determine the extent and duration of distress associated with painful procedures in pigs.¹⁰⁻¹² In order to distinguish levels of pain response in pigs and identify less painful management procedures, it is necessary to have a validated, objective measurement. Acute cortisol response is involved in other processes excluding pain such as a diurnal rhythm, homeostasis and stress, so it is not perceived to reliably discriminate between a painful and a stressful response.¹³ Plasma cortisol has been seen to increase in cases of stress caused by only handling where no procedure or pain was involved.¹¹⁻¹⁶ Cortisol has been shown to have significant increases in response to pain or stress independently. This study evaluated plasma cortisol differences between a stressful (manual restraint) and painful (castration) event under controlled conditions.

Materials and methods
The study was conducted at a 3800 head sow farm located in the Midwest. One hundred cross-bred male pigs 9 to 11 days of age were chosen at random from 24 litters. All animals appeared healthy at the time of selection for the study.

Male piglets were numbered uniquely and returned to the sow. Fifty pigs were randomly assigned to be castrated (CAST) with the remainder sham castrated (SHAM). All pigs were restrained and blood was drawn for the time zero, pre-treatment blood sample. Immediately afterwards the pig was castrated or sham castrated and returned to the sow. The pig was then re-captured and restrained 45 minutes after its treatment and a post-treatment blood sample was collected.

The time of all sample collection, handling and treatment events were recorded including time of placement on dry ice and in an ultralow freezer.

Piglets were castrated following the farm's standard operating procedure and procedures were performed by trained personnel. The sham castration was performed following the procedure described in Taylor et al.⁹ The blunt edge of the scalpel blade was run along the scrotum. The testes were then handled as if castration was being carried out and the scrotum sprayed with dilute chlorhexidine diacetate solution.

Four milliliters (mLs) of blood was collected for each time point, for a total of 8 mls collected per animal. Immediately following collection, all blood samples were gently mixed with EDTA and placed on ice.
Samples were immediately processed on farm. During processing they were centrifuged at 1,600 G for 10 minutes. Following centrifugation, plasma was then transferred using a transfer pipette to two separate falcon tubes which were immediately placed on dry ice. All blood samples remained on ice throughout processing and were frozen by one hour and 4 minutes post sample collection.

Plasma cortisol concentrations were determined by use of a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system as described previously. Laboratory technicians who analyzed the samples were blinded to the treatment group.

The mean values and the Standard Error of the Mean (SEM) at each time point were calculated and the results are presented graphically. Pre and post castration samples were analyzed using analysis of variance (ANOVA) with appropriate pairwise comparisons.

Results
The difference between the mean castration time (30 seconds) and the sham castration time (20 seconds) was analyzed and was significant ($P < .0001$). This means that castrated animals may have a larger increase in cortisol due to a longer handling period, pain, or a combination of both.

To eliminate any breakdown in cortisol between sample collection and processing, all times recorded were analyzed using ANOVA comparisons. It is important for the serum to be fully processed and frozen as quickly as possible, so the time recorded (referred to as processing time) was the time from pre-treatment blood collection to time of placement on dry ice. The processing time for all pig's pre-treatment blood samples was 38.81 minutes. The mean time for all pig's post-treatment processing time was 45.48 minutes. This significant difference ($P < .0001$) means that the post-treatment samples had a longer period of time to breakdown. The magnitude of cortisol increases is underestimated.

Comparisons were made between processing times for SHAM and CAST pre-treatment blood samples which were not different ($P = 0.98$). The comparison for post-treatment blood samples between SHAM and CAST pigs was also not different ($P = 0.46$). Therefore no bias was introduced as a consequence of processing time differences.

Pre-treatment values were not significantly different between CAST and SHAM pigs. The post-treatment mean
value of cortisol for the SHAM pigs increased significantly from 73.5 nmol/L to 145.3 nmol/L ($P < 0.0001$). The CAST pigs serum cortisol increased significantly from 75.4 nmol/L to 357.3 nmol/L ($P < 0.0001$). Post-treatment values were significantly different ($P < 0.0001$) between the SHAM (145.3 nmol/L) and CAST pigs (357.3 nmol/L).

**Discussion**

Both SHAM and CAST groups had a significant increase in plasma cortisol versus pre-treatment. Additionally, the CAST pigs had 3.92 times the SHAM group increase when post-treatment means were compared. The most likely explanation is that there was a difference in amount of pain experienced by the two groups. This study measured a distinct difference between animals that experienced stress due to restraint and blood collection and animals that experienced those stresses plus the pain of castration. These results suggest that cortisol, under controlled experimental conditions, does distinguish between the stress of handling and stress versus pain. Relative to previous studies, these results show a cortisol response to pain of significantly greater magnitude compared to handling stress alone in the same environment. The most likely explanation for the difference between these results and previous cortisol research is the sample size combined with meticulous sample handling. These results suggest that cortisol may be a useful diagnostic tool to distinguish handling and stress versus pain.

**References**


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