Using Serum Cortisol to Distinguish Between Acute Stress and Pain Response Following Castration in Piglets

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Summary and Implications

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. To minimize the stress it has been recommended that this procedure should be conducted in the first wk of life. Therefore, the objective of this study was to evaluate plasma cortisol differences between a stressful (manual restraint) and painful (castration) event under controlled conditions. One hundred cross-bred male pigs 9 to 11 d of age were chosen at random from 24 litters. Fifty piglets were randomly assigned to be castrated (**CAST**) with the remainder sham castrated (**SHAM**). Processing tie was different between pre and post samples ($P < 0.0001$). Pre treatment blood samples was 38.8 min and post treatments blood samples was 45.5 min respectively. Processing times for SHAM and CAST pre ($P = 0.98$) and post ($P = 0.46$) treatment blood samples were not different. Pre-castration values were not different ($P > 0.05$) between CAST and SHAM groups. The post-castration mean value of cortisol for the SHAM group increased from 73.5 nmol/L to 145.3 nmol/L ($P < 0.0001$). The CAST group serum cortisol increased from 75.4 nmol/L to 357.3 nmol/L ($P < 0.0001$). Post-castration values were different ($P < 0.0001$) between the SHAM (145.3 nmol/L) and CAST group (357.3 nmol/L). This study measured a distinct difference between piglets that experienced stress due to restraint and blood collection and piglets that experienced those stresses plus castration.

Introduction

In the EU Directive 91/630/EEC as amended by Directive 2001/88/EC and Directive 2001/93/EC notes that *"Castration is likely to cause prolonged pain which is worse if there is tearing of the tissues. Those practices are therefore detrimental to the welfare of pigs, especially when carried out by incompetent and inexperienced persons. As consequence, rules should be laid down to ensure better practices."* The Directives also note that for all procedures intended as an intervention carried out for other than therapeutic or diagnostic purposes or for the identification of the pigs in accordance with relevant legislation and resulting in damage to or the loss of a sensitive part of the body or the alteration of bone structure shall be prohibited with the following exceptions: 1) A uniform reduction in of corner teeth of piglets by grinding or clipping not later than the seventh day of life of the piglets leaving an intact smooth surface; 2) boars' tusks may be reduced in length where necessary to prevent injuries to other animals or for safety reasons, 3) docking of a part of the tail, 4) ***castration of male pigs by other means than tearing of tissues,*** and 5) nose ringing only when the animals are kept in outdoor husbandry systems and in compliance with national legislation. 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 however there is a need for a robust, repeatable physiological indicator of pain to objectively assess how production practices ie castration impacts both short and long term well-being of the male piglet. Castration includes several events likely to be painful: scrotal incision, extraction of the testes, and severing of the spermatic cords. 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 wk of age compared to pre-weaned piglets (< 20 d). One study by Taylor et al., (2000) reported behavioral differences between castrated and handled only piglets. In the first 2 h immediately following 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 h. 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. 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. Plasma cortisol has been seen to increase in cases of stress caused by only handling where no procedure or pain was involved. Cortisol has been shown to have significant increases in response to pain or stress independently. Therefore, the objective of this study was to

evaluate plasma cortisol differences between a stressful (manual restraint) and painful (castration) event under controlled conditions.

Materials and Methods

Animals and location: The study was conducted at a 3800 head sow farm located in the Midwest. One hundred cross-bred male pigs 9 to 11 d of age were chosen at random from 24 litters. Within a single farrowing room, each piglet was pre-treated with a shot of Excede (Pfizer®, New York, NY) by the farm manager and female piglets were then returned to the sow.

Treatments: Male piglets were numbered uniquely and returned to the sow. Fifty piglets were randomly assigned to be castrated (**CAST**) with the remainder sham castrated (**SHAM**). Two trained technicians carried out the procedure. One held the piglet, and the second draw blood. Castration was via an open surgical technique without local analgesia or anesthesia. The scrotum was incised sharply with a scalpel blade disinfected in dilute chlorohexidine diacetate solution. The testes and spermatic chords were exteriorized and were removed by blunt dissection. The wound was then sprayed with dilute chlorohexidine diacetate solution and the pig returned to the sow. The average time for a castration was 30 s. The sham castration was performed following the procedure described in Taylor et al. (2000). 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. The average time for a sham castration was 20 s.

Blood withdrawal: 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 45 min post castration sample time was determined based off of a similar study with cattle, in which the maximum cortisol response was 45 min post castration (Coetzee et al., 2008). 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. In order to keep this as controlled as possible, the time of all sample collection, handling and treatment events were recorded including time of placement on dry ice, and in ultralow freezer. Blood sample times were taken as the needle exits the pig. Castration time was taken as when the disinfectant was sprayed on the wound, and beginning of processing was when the centrifuge was started. Three timers were started simultaneously at the beginning of the study to ensure that all times recorded were the same.

1. Completion of a blood sample defined as the needle exited the piglet.
2. Castration time defined as when the disinfectant was sprayed on the wound.
3. Beginning of processing time defined as when the centrifuge was started.

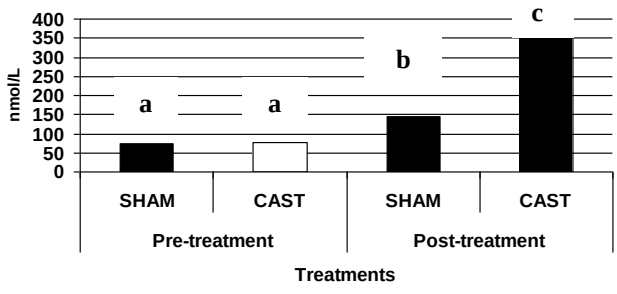
In addition the time that blood was placed on dry ice and in ultralow freezer was recorded. Plasma cortisol concentrations were determined by use of a solid-phase competitive chemiluminescent enzyme immunoassay and an automated analyzer system (Devane, 2005). Laboratory technicians who analyzed the samples were blinded to the treatment group.

Statistical analysis: The piglet was the experimental unit (n = 100 or 50 piglets per treatment). Data was analyzed using variance (ANOVA) with appropriate pair wise comparisons. The mean values and the Standard Error of the Mean (SEM) at each time point were calculated and the results are presented graphically.

Results

Processing time was different between pre and post samples ($P < 0.0001$). Pre treatment blood samples was 38.8 min and post treatments blood samples was 45.5 min respectively. Processing times for SHAM and CAST pre ($P = 0.98$) and post ($P = 0.46$) treatment blood samples were not different. Pre-castration values were not different ($P > 0.05$) between CAST and SHAM groups. The post-castration mean value of cortisol for the SHAM group increased from 73.5 nmol/L to 145.3 nmol/L ($P < 0.0001$). The CAST group serum cortisol increased from 75.4 nmol/L to 357.3 nmol/L ($P < 0.0001$). Post-castration values were different ($P < 0.0001$) between the SHAM (145.3 nmol/L) and CAST group (357.3 nmol/L; Figure 1).

Figure 1. Cortisol Levels Pre and Post-Castration. Columns with matching superscripts differ ($P < 0.05$).



Discussion

Processing times between treatments at pre and post were not different. Both SHAM and CAST post-treatment had a significant increase in plasma cortisol versus pre-treatment. When comparing pre-treatment SHAM to post treatment SHAM cortisol increased 1.9 times and when comparing pre-treatment CAST to post treatment CAST cortisol increased 4.7 times. 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. In contrast to previous studies, these findings suggest that cortisol, under tightly controlled experimental conditions, does distinguish between the stress of handling and painful events. Post-treatment samples had a longer period of time.