

## ANIMAL WELFARE

**Title:** What is the optimum age for processing neonatal pigs? – NPB #06-026

**Investigator:** Dr. Stephanie Torrey

**Institution:** Agriculture and Agri-food Canada, Lennoxville

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### II. Industry Summary

Multiple producer groups recommend that piglets be tail docked and ear notched within 24 hours of birth because the procedures are considered less stressful for the piglet at that age. However, there is no research to substantiate this recommendation. Therefore, the objective of this experiment is to compare the effects of processing during the first 24 hours versus 3 days of age on piglet suckling behavior, immune and endocrine status and growth. Piglets were assigned to three treatments (control, sham processed (handled only), and processed). These treatments were applied at either 1 or 3 days of age. Piglet behavior, vocalizations, and growth were examined, and blood was collected on day 5 to assess their immune status. While there were differences between processing and sham processing piglets in vocalizations and behavior, there was no long term effect of processing on suckling behavior or growth, and no consistent effect on health status. While tail docking and ear notching does appear to result in short-term pain and distress, there were no consistent differences between days of treatments, and processing on day 1 appears neither better nor worse than processing on day 3.

### III. Scientific Abstract

Multiple producer groups recommend that piglets be tail docked and ear notched within 24 hours of birth because the procedures are considered less stressful for the piglet at that age. However, there is no research to substantiate this recommendation. Therefore, the objective of this experiment is to compare the effects of processing during the first 24 hours versus 3 days of age on piglet suckling behavior, immune and endocrine status and growth. Six piglets per litter from 20 litters (n=120 piglets) were used in a 3 x 2 complete block design. Each litter comprised a complete block. Piglets were weighed at birth and assigned to one of three treatments (balanced by birth weight): control (C), sham processed (S) and processed (P) (tail docked and ear notched) at one of two ages (1 or 3 days). Vocalizations were recorded during treatment, and piglet behavior was observed immediately after treatment for pain-related behavior. Suckling behavior was observed for six hours on each of days 1-4. Blood samples were collected on day 5 to examine levels of immunoglobulins (IgA and IgG) and insulin-like growth factor-I (IGF-I). Piglet weights were measured at birth and on days 5 and 14. P piglets vocalized at a higher frequency than S piglets ( $P < 0.0001$ ), and produced more high frequency calls than S piglets ( $P = 0.0157$ ). Piglets on day 1 produced more high frequency calls than those on day 3 ( $P = 0.0467$ ). Immediately after treatment, S and P piglets spent less time lying and more time standing than C piglets

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**For more information contact:**

**National Pork Board, P.O. Box 9114, Des Moines, Iowa USA**

800-456-7675, **Fax:** 515-223-2646, **E-Mail:** [porkboard@porkboard.org](mailto:porkboard@porkboard.org), **Web:** <http://www.porkboard.org/>

( $P < 0.001$ ), while P piglets jammed their tail between their legs more than S or C piglets ( $P = 0.0005$ ). Day 1 piglets trembled significantly more than day 3 piglets ( $P = 0.0005$ ), and this was exacerbated by processing ( $P = 0.076$ ). Processing tended to influence IgG, with piglets processed on day 3 having lower plasma IgG concentrations than those processed on day 1. There were no differences between treatments or days of treatment in suckling behavior, weights, growth rates, IGF-I, or IgA concentrations. While tail docking and ear notching does appear to result in short-term pain and distress, there were no consistent differences between days of treatments, and processing on day 1 appears neither better nor worse than processing on day 3.

#### **IV. Introduction**

Neonatal pig management practices, such as tail-docking, teeth clipping and castration, have come under public scrutiny because of the ambiguity regarding their necessity and the lack of research into ways of reducing pain and distress during and immediately after these procedures. Procedures like castration have been shown to be painful to the piglet at any age (Taylor et al., 2001), and seldom, if ever, are anesthetics or analgesics used. Seemingly less invasive procedures such as tail docking, teeth clipping and ear notching also appear to cause pain and distress (Noonan et al., 1994; Prunier et al., 2001), although probably to a lesser degree than castration (in sheep: Grant, 2004).

The National Pork Board recommends that producers perform these procedures (except for castration) within 24 hours of birth because they are considered to be less stressful for the pig at that age (Swine Care Handbook, 2002). However, within the first twenty-four hours after birth, important changes are occurring in piglet suckling behavior. Upon their birth, piglets begin suckling in non-cyclical bouts, consuming invaluable colostrum that is available for a limited time (Devillers et al., 2004). After approximately ten hours, nursing bouts generally become synchronous and cyclical, with most pigs present at the udder for the most of the episodic milk ejections (Lewis and Hurnik, 1985; de Passillé and Rushen, 1989). An increase in nursing bout synchronization occurs throughout the first twenty four hours, with pigs competing for access to productive teats (de Passillé and Rushen, 1989). Processing pigs during this transitional period may be detrimental to the pigs' welfare because they cannot rely on the regular pattern of nutritive and non-nutritive sucking, behavior patterns that may actually help to alleviate pain. Nutritive sucking is comforting to neonatal calves (Veissier et al., 2002) and milk ingestion is both comforting and analgesic to rats (Blass and Fitzgerald, 1988). Pacifier sucking reduces heart rate and cries of infants undergoing painful procedures (Campos, 1994; DiPietro et al., 1994; Corbo et al., 2000), while non-nutritive suckling elicits an analgesic response in neonatal rat pups faced with acute and chronic pain (Anseloni et al., 2004). Additionally, processing at one day of age may negatively impact the piglets' colostrum intake. After castration, neonatal pigs spent less time suckling than non-castrated pigs for up to six hours after the procedure (McGlone et al., 1993). Therefore, pigs that are processed during their first day of life may spend less time suckling and, in so doing, consume less colostrum. Decreased consumption of colostrum is positively related to mortality (Hendrix et al., 1976), due in large part to the relationships between colostrum and immune status (Rooke and Bland, 2002), energy levels (Le Dividich et al., 1994) and thermoregulation (Le Dividich and Noblet, 1981).

Two studies, both published in technical reports, have compared the effect of age at processing on mortality and growth of piglets. In one study, commercially-raised piglets processed at one day of age had lower pre-weaning mortality and higher 21-day weights than pigs processed at two days of age, although the results were confounded by handling (Kober and Thacker, 1999). A second study examined the growth and mortality of pigs processed at either one or three days of age (Nicholson and McGlone, 1992). Pigs processed at three days of age tended to have lower mortality than those processed at one day of age. The authors suggested that this difference may be the result of colostrum intake. However, no study has examined the influence of age at processing on piglet behavior or immune status. Therefore, the objective of this experiment is to compare the effects of processing during the first 24 hours versus 3 days of age on piglet suckling behavior, passive immunity and growth. The results will help us determine the optimum age for processing neonatal pigs.

## V. Objectives

The overall objective of this research project is to evaluate swine well-being during routine production procedures. Specifically, this project will determine the optimum age at which to process pigs by:

1. Determining if pigs processed within 24 hours of birth exhibit more behavioral and vocal signs of pain and distress during processing than pigs processed at three days of age
2. Determining if pigs processed within 24 hours of birth experience more disruption in their suckling behavior than pigs processed at three days of age
3. Determining if pigs processed within 24 hours of birth have decreased passive immunity and growth rates compared to pigs processed at three days of age

## VI. Materials and Methods

### 1. Experimental Design

One hundred and twenty piglets from 20 litters were used in this experiment, in a 3 x 2 complete block design with each litter being a complete block. Piglets were weighed at birth, individually identified and assigned to one of three treatments (blocked by birth weight): 1. Control (no handling); 2. Sham (handling as if processed); 3. Processed (tail docked and ear notched). The three treatments were applied either at Day 1 ( $15.3 \pm 5.1$  hrs after birth) or Day 3 ( $64.1 \pm 6.5$  hrs after birth). Time 0 occurred with the birth of the first piglet in the litter. Only litters that had six viable experimental piglets (birth weight > 1 kg) born within 4.5 hours of Time 0 were included in the experiment.

Immediately upon their birth and prior to initial suckling, piglets were removed from the farrowing crate and kept in a heated box until the six experimental of the litter have been born. Colostrum samples were collected from unsuckled teats after the birth of at least six viable piglets. At this time, all six experimental piglets were returned to the sow, in close proximity to the udder, to permit all to begin suckling simultaneously. Four additional piglets per litter served as non-experimental piglets to standardize litter size to 10 piglets. Up to twelve piglets were permitted on the sow until the time of first processing, when supernumerary piglets were fostered from the experimental sows. There were 54 gilts and 66 boars used in the experiment. Boars were castrated after the termination of the experiment on day 14.

For litters with Time 0 (birth of first piglet) between 10:00 and 23:59, day 1 treatments were applied at 07:30 the following morning. For litters with Time 0 between 00:00 and 09:59, day 1 treatments were applied at 15:00. For all litters, day 3 treatments were applied at 07:30 to facilitate management. The sham and processed piglets were removed from the farrowing pen and brought by cart to the hallway outside a separate room. One piglet was brought into the room at a time, with the door closed to minimize noise disturbance. The order of treatment (sham and processed) was alternated between litters of piglets and ages within litters. Tail docking and ear notching of piglets was performed by trained handler, and done without the use of anesthesia or analgesia. Piglets were held between the knees of the handler for ear notching (Figure 1). Standard stainless steel ear notchers (CMDV, St. Hyacinthe, Quebec) were used for notching piglets ears. All processed piglets received one notch in the middle of each side of each ear (two notches per ear). Piglets were then placed upright on a stainless steel surface for tail docking. Side-cutter pliers (CMDV, St. Hyacinthe, Quebec) were used for tail docking, and tails were docked to one third the original length. Sham processed piglets were held in the same manner as processed piglets, with the ears and tails manipulated with the fingers. Processed and sham processed piglets were handled for  $30.33 \pm 0.39$  seconds.

### 2. Vocalizations and Behavior

During the application of the treatment, piglets' vocalizations were recorded. Vocalizations were analyzed using the Raven Pro 1.2.1, a sound analysis software program. The frequency (Hz) of each vocalization was

determined as the frequency with the highest energy (amplitude), and calls were classified as either high ( $\geq 1000$  Hz) or low ( $< 1000$  Hz) frequency calls as used in previous published work (Weary et al., 1998) (Figure 2).

Live observations were performed immediately after the treatment was applied to quantify the acute behavioral effect of the processing procedure. Three piglets of each litter (control, sham, processed for either day 1 or 3) were observed every 20 seconds for 10 minutes for general behavior (suckling, standing, lying alone, lying huddled with other piglets, playing/fighting, sitting, and other), tail movement (no tail movement, tail jammed between legs, tail wagging, attempting to scratch with back legs), and head/body movements (trembling, shaking head, attempting to scratch with front legs). If a suckling bout occurred during the ten minute observation period, observations were extended for the duration of time equivalent to the duration of the suckling bout.

Piglet behavior was video-recorded for six hours immediately after treatment, and for six hours on the days following treatment (i.e., observations for six hours on days 1, 2, 3, 4). Behavior of all six experimental piglets was observed on each day during and between nursing bouts. During nursing bouts (when greater than 50% of the litter was active at the udder), each piglet's behavior was observed every 10 seconds, and between nursing bouts, each piglet was observed every five minutes, to determine if they were actively suckling/massaging the udder or performing any other behavior away from the udder.

### 3. Growth and immune and endocrine measures

Piglets were weighed at birth and on days 5 and 14 of age. Growth rates were determined for the first 5 days, and for the entire experiment.

On day 5, blood samples were collected via jugular venipuncture from all experimental piglets. Blood and colostrum samples were analyzed for insulin-like growth factors I (IGF-I). Concentrations of IGF-I were determined with a previously described RIA (Aribat et al. 1993). The IGF-I was extracted using the formic acid-acetone method. Inter-assay coefficient of variation (CV) for plasma samples was 2.82%, and intra-assay CV was 5.46%. Intra-assay CV for colostrum was 5.83%. Blood and colostrum samples were analyzed for IgA using an ELISA Quantification Kit (Bethyl Laboratories, Inc., Montgomery, Texas, Catalog No. E100-102). Intra-assay CV for IgA in plasma was 6.29% and in colostrum, 5.43%. Blood samples were also analyzed for IgG using an ELISA Quantification Kit (Bethyl Laboratories, Inc., Montgomery, Texas, Catalog No. E100-104). Intra-assay CV for IgG was 4.4%. Blood and colostrum samples were analyzed for IFN- $\gamma$ ; however, cytokine levels in both were below the minimum detectable level (7.5pg/ml).

### 4. Statistics

Data were analyzed using a mixed model procedure in SAS. Main effects tested were treatment and day of treatment and litter was used as random effect. For suckling behavior and growth rates, data were analyzed as repeated measures. Birth weight was used as a covariate for all data. For analyses of IgA and IGF-1 in piglet plasma, colostrum concentrations were used as a covariate.

## VII. Results

### 1. Vocalizations

For the 80 processed and sham processed piglets, 2620 vocalizations were recorded during treatment application. Fifteen vocalizations were discarded due to background interference. Piglets performed an average of 33.2 vocalizations during handling or processing, for a vocalization rate of 1.09 vocalizations/second (range: 0.18 to 1.88 vocalizations/second). There was no difference in call rate between treatments ( $P = 0.51$ ; sham:

1.06 ± 0.06 calls/sec; processed: 1.11 ± 0.06 calls/sec) or ages (P = 0.95; day 1: 1.09 ± 0.06 calls/sec; day 3: 1.09 ± 0.06 calls/sec). Vocalizations were classified as high if their frequency was greater than 1000 Hz; otherwise, they were classified as low (Figure 2). Three processed piglets did not perform any vocalizations above 1000 Hz, while 11 sham processed piglets did not perform high frequency vocalizations. The average frequency of all vocalizations was 1174 ± 67 Hz. There was a significant difference between processing treatments (P < 0.0015) and no difference between ages (P = 0.57) (Figure 3).

With vocalizations separated into high and low calls, processed piglets produced more high frequency calls than sham processed piglets (P = 0.0157), and piglets at day 1 produced more high calls than those on day 3 (P = 0.0467) (Table 1). In contrast, low frequency calls tended to be more frequent in sham processed piglets and piglets on day 3 (P = 0.0979 and P = 0.1095, respectively) (Table 1).

## 2. Behavior

### 2.1 Immediate effects of processing

Processed and sham processed piglets spent more time standing and less time huddling than control piglets during the ten minutes immediately after treatment (Table 2). There was no difference in time spent suckling, lying alone, playing or sitting. On day 1, all piglets (including control, sham and treatment) spent more time suckling, less time standing and less time playing than piglets on day 3 (Table 2).

There was a statistical interaction between processing treatment and day of treatment for time spent lying alone after treatment (P = 0.07). On day 1, processed piglets spent more time lying alone than either control or sham processed piglets (Processed: 11.4 ± 2% of time lying; Control: 3.5 ± 2% of time lying; Sham: 1.1 ± 2 % of time lying). However, on day 3, all piglets spent a similar amount of time lying alone after the treatment (Processed: 4.3 ± 2% of time lying; Control: 6.2 ± 2% of time lying; Sham: 6.7 ± 2% of time lying).

There was a significant effect of treatment (P = 0.0005) and a trend towards an interaction between treatment and day of treatment (P 0.0839) on tail jamming (Figure 4).

There was no effect of processing treatment on tail wagging (P = 0.56; control: 2.0 ± 0.8% of scans; sham: 1.5 ± 0.8%; processed: 1.0 ± 0.8%), but 3 day old piglets tended to wag their tail more than 1 day old piglets (P = 0.0603; day 1: 0.8 ± 0.6% of scans; day 3: 2.3 ± 0.6%).

Processing and day of processing significantly affected trembling (Figure 5). Piglets trembled more on day 1 than on day 3 (P = 0.0004), and those processed on day 1 trembled significantly more than any piglet on day 3 (P < 0.05).

### 2.2 Effects on suckling behavior

During nursing bouts, there was no effect of processing treatment or day of treatment on suckling behavior overall (Table 3) or on any specific day. There was also no difference between treatments or day of treatment in suckling behavior performed between suckling bouts (Table 3).

## 3. Mortality and growth rates

Two piglets died during the course of the experiment. One sham processed piglet (treatment on day 1) died on day 3. One processed piglet (treatment on day 3) died on day 8. There was no other mortality through 14 days of age.

Average birth weight of the experimental piglets was 1.47 ± 0.03 kg (range: 1.035 kg to 2.225 kg). Piglets weighed 2.26 ± 0.06 kg at 5 days of age (range: 1.45 kg to 3.48 kg) and 4.75 ± 0.13 kg at 14 days of age (range:

1.47 kg to 7.11 kg). Piglets gained an average of  $235 \pm 9$  g/day (range: 15 g/d to 384 g/d). There was no difference in weights or growth rates between processing treatments or day of treatment (Table 4).

#### 4. Endocrine and immune measurements

The concentration of IGF-I in colostrum was  $395.24 \pm 21.66$  ng/mL (range 228.95 ng/mL to 549.14 ng/mL), and the concentration in piglet plasma was  $97.88 \pm 2.63$  ng/mL (range: 36.81 ng/mL to 167.92 ng/mL). The concentrations in colostrum were not correlated with concentrations in piglet plasma ( $P = 0.27$ ). There was no effect of treatment ( $P = 0.30$ ) or day of treatment ( $P = 0.99$ ), but a tendency for an interaction between treatment and day (Figure 6).

The concentration of IgG in piglet plasma was  $28.73 \pm 1.28$  mg/mL (range: 6.23 mg/mL to 78.64 mg/mL). There was a significant difference between ages ( $P = 0.018$ ) and a tendency for a difference between treatments ( $P = 0.09$ ) in IgG concentration (Figure 7).

The concentration of IgA in piglet plasma was  $3.53 \pm 0.19$  mg/mL (range: 0.36 mg/mL to 10.82 mg/mL). In colostrum, IgA concentrations were 19.56 mg/mL (range: 8.47 mg/mL to 45.16 mg/mL). There was a significant correlation between IgA concentrations in colostrum and plasma ( $P < 0.0001$ ). There was no significant difference between treatments ( $P = 0.65$ ; control:  $3.55 \pm 0.43$  mg/mL; sham:  $3.63 \pm 0.43$  mg/mL; processed:  $3.39 \pm 0.44$  mg/mL) or day of treatment ( $P = 0.49$ ; day 1:  $3.59 \pm 0.42$  mg/mL; day 3:  $3.45 \pm 0.42$  mg/mL).

### VIII. Discussion

Currently, organizations such as the National Pork Board and the Canadian Agri-Food Research Council recommend that producers tail dock, ear notch, and teeth clip neonatal piglets when they are less than 24 hours old, while the American and Canadian Veterinary Medical Associations recommend that the procedures be performed within the first week of piglets' lives. However, data to support either of these recommendations was lacking. Therefore, this experiment was designed to compare the vocalizations, behavior, growth, and immune status of piglets tail docked and ear notched at one and three days of age to piglets that were handled on those days, or unhandled control piglets.

Two previous studies have compared the effect of age at processing on mortality and growth of piglets. Nicholson and McGlone (1992) found that piglets processed on day 3 tended to have a higher survival rate than those processed on day 1. They attributed this result to processing interfering with colostrum intake in younger piglets. However, they did not measure immune status or suckling behavior. A second study (Kober and Thacker, 1999) compared the processing of piglets at days 1 and 2. They found a higher mortality rate in piglets processed on day 2; however, these piglets had been handled twice rather than once like the younger piglets. In our study, we acknowledged that handling and birth weight may influence our other measures. We compared piglets that were not handled to those that were handled as if processed and those that were actually processed. We also chose to use piglets born at a viable weight (greater than 1kg) and balanced birth weight between treatments and days of treatment. We had a very low mortality rate (2 piglets out of 120), but were able to confidently compare behavior, growth and immune status without being confounded by differences in birth weight.

During treatment application, processed piglets vocalized at a higher average frequency than piglets that were sham processed and produced more high frequency vocalizations (calls greater than 1000 Hz) than sham processed piglets. This finding corresponds with two studies on castration that found castrated piglets produce more high frequency vocalizations than sham castrated piglets (Weary et al., 1998; Taylor and Weary, 2000). Because of their findings, Weary and colleagues (1998) suggested that the rate of high frequency vocalizations is a reliable indicator of pain. White and colleagues (1995) found that the piglets castrated without lidocaine

vocalized at a higher frequency (Hz) than those castrated with the anesthetic, and the increased frequency corresponded with increased heart rate. Using castration as a model, Horn et al. (1999) also found changes in vocalizations to correlate with changes in physiological stress parameters. Taken together, these studies on castrated piglets indicate that increases in high frequency vocalizations, and an overall higher vocalization frequency are indicative of pain. As such, our study provides evidence that tail docking and ear notching are painful procedures for neonatal piglets.

We found some differences in the behavior of piglets immediately after treatment application. Processed piglets jammed their tail between their legs at a higher rate than sham handled or control piglets and also tended to tremble more after the procedures. Two other studies also found processed piglets to jam their tail at a significantly higher rate than non-processed piglets through two minutes after treatment (Noonan et al., 1994; Rand et al., 2002). Although all piglets on day 1 trembled more than piglets on day 3, a difference most likely due to the younger piglets' lack of brown fat stores and inability to thermoregulate (Mitchell et al., 2001), this difference was exacerbated by processing, with piglets processed on day 1 trembling significantly more than those on day 3.

In terms of general behavior immediately after processing, processed piglets did not differ from sham processed piglets. Changes in standing and huddling behavior can be attributed to the removal of the piglets from their home pen and handling. The effect of day on suckling and standing after processing reflect changes in the dynamics of suckling during the first two days after birth. During and immediately after parturition, milk flow is continuous, and gradually becomes synchronous and cyclical (Fraser, 1980). As a result, piglets on day 1 are spending more time at the udder and less time performing other behavior such as standing and playing, than piglets on day 3. From the video observations of suckling behavior, there was no effect of treatment or day of treatment in time at the udder during or between nursing bouts.

There was no effect of treatment or day of treatment on body weights or growth through two weeks of age. Because we used only 10 piglets per litter and litter size was generally larger than 10 piglets (average size was 12 piglets), we were able to exclude the lowest, and occasionally the highest, birth weight piglets. Processing treatments do not appear to be traumatic enough to induce changes in growth rates compared to average.

The immune and endocrine measures we chose to examine are general indicators of colostrum intake and immune status. Insulin-like growth factor-I was measured in colostrum from unsuckled teats and in piglet plasma at day 5. Our results are within the normal range of previous reports in colostrum (Farmer et al., accepted for publication) and neonatal piglet plasma (Young Lee et al., 1991). Although there was a tendency for a statistical interaction between treatment and day of treatment, differences between single treatment and day combinations were not statistically or biologically significant. The IgG concentrations in piglet plasma were also similar to previously reported values for neonatal piglets (Bland et al., 2000; Martin et al., 2005). Piglets processed on day 3 had the lowest level of IgG, on average 7 mg/mL lower than all other piglets. The lower IgG concentrations may possibly be due to the injuries to the ears and tails at an age when colostrum-derived IgG is naturally decreasing (Martin et al., 2005).

In conclusion, tail docking and ear notching appears to cause pain and distress to newborn piglets, resulting in changes to vocalizations and behavior above handling alone. However, in our study, these procedures did not have any detrimental effect on suckling behavior or growth, and results from endocrine and immunological measures are equivocal. In addition, there doesn't appear to be any concrete evidence that processing piglets on day 1 is better or worse than on day 3. Further studies are needed to examine ways to reduce the pain and distress caused by these routine management procedures.

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## **X. Acknowledgements**

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A. Sham ear notching

B. Tail docking

Figure 1. Processed and sham processed piglets were handled in the same manner. Ear notching/sham ear notching was performed first, followed by tail docking/sham tail docking. During ear notching/sham ear notching (A), the piglet was placed between the handler's legs. Then, the piglet was placed on a flat surface for tail docking/sham tail docking (B).

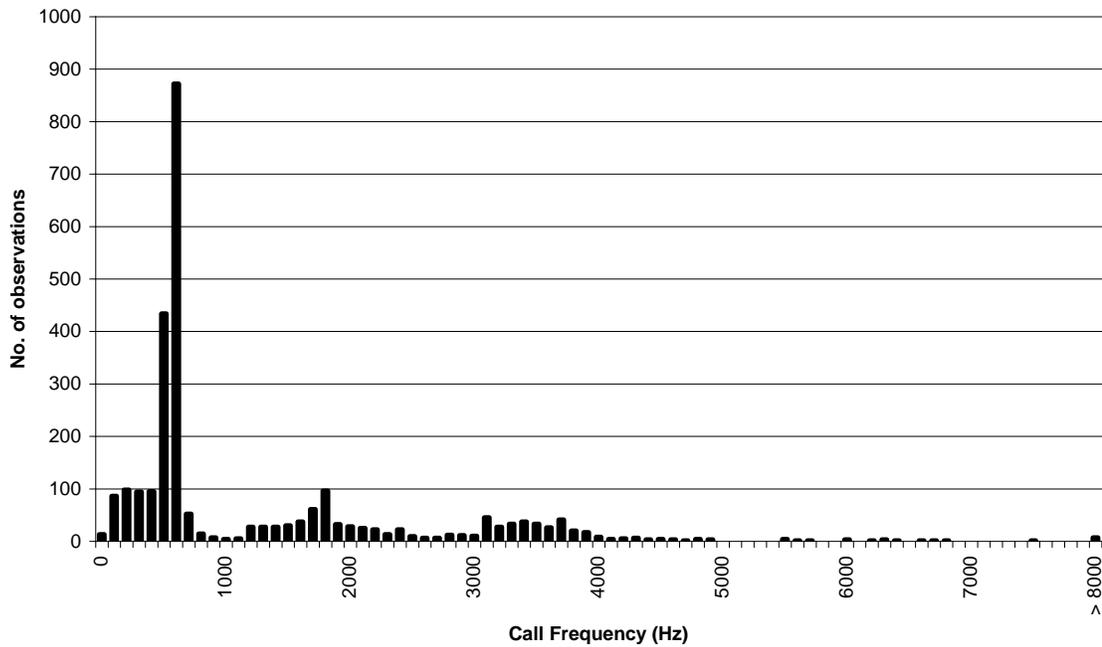


Figure 2. Histogram of all piglet vocalizations. Vocalizations were tri-modal: with peaks at approximately 500-600 Hz, 1500-1700 Hz, and between 3000-4000 Hz. Similar to Weary and colleagues (1998), a trough occurred at 1000 Hz, and vocalizations in this experiment were classified as low if they had a frequency of less than 1000 Hz and high if their frequency was higher than 1000 Hz.

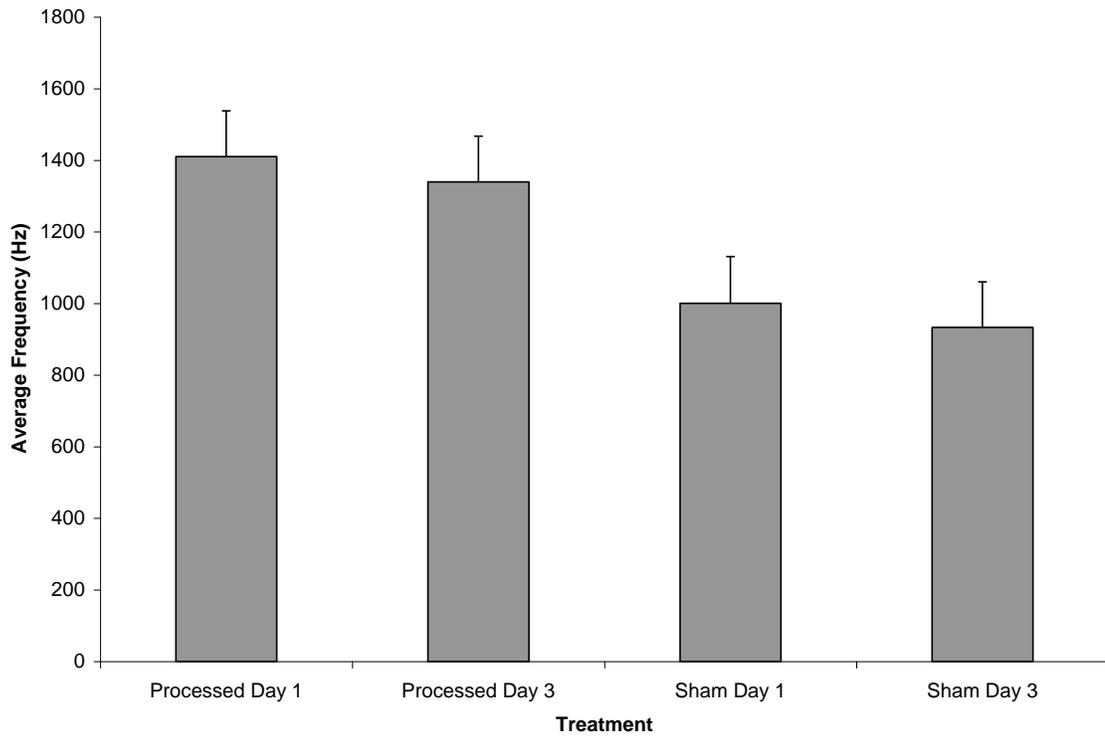


Figure 3. Mean frequency of vocalizations (mean frequency  $\pm$  standard error). Processed piglets vocalized at a higher frequency than sham processed piglets ( $P < 0.0001$ ). There was no difference between days 1 and 3 ( $P = 0.57$ ).

Table 1. Vocalizations during processing treatment (means  $\pm$  standard error). For treatments, Sham D1 represents piglets that were sham processed on day 1. Proc represents processed piglets. Because control piglets were not handled, there are no vocalization data for these piglets.

	Treatments				P value	
	Sham D1	Sham D3	Proc D1	Proc D3	Trt	Day
<b>Call rate (calls/sec)</b>						
High calls	0.35 $\pm$ 0.08	0.20 $\pm$ 0.07	0.52 $\pm$ 0.07	0.38 $\pm$ 0.07	0.0157	0.0467
Low calls	0.71 $\pm$ 0.08	0.91 $\pm$ 0.08	0.64 $\pm$ 0.08	0.71 $\pm$ 0.08	0.0979	0.1095
<b>Avg call frequency (Hz)</b>						
High calls	2320 $\pm$ 137	2879 $\pm$ 137	2450 $\pm$ 114	2826 $\pm$ 122	0.71	< 0.001
Low calls	513 $\pm$ 15	526 $\pm$ 14	513 $\pm$ 14	545 $\pm$ 14	0.17	0.35

Table 2: General behavior immediately after processing. SE is the standard error of the mean. For treatments, C = control, S = sham processed and P = processed. There were no interactions between treatment and day of treatment.

	Treatment					Day			
	C	S	P	SE	P value	1	3	SE	P value
Suckling	24.9	30.2	26.7	5.3	0.72	36.0	18.9	4.5	0.0017
Standing	23.5	37.1	37.1	4.4	0.013	26.2	38.8	3.8	0.0037
Lying alone	5.8	3.4	7.0	1.7	0.31	4.7	6.1	1.4	0.50
Huddling	39.4	22.5	24.4	5.2	0.01	29.4	28.2	4.5	0.82
Playing	3.2	3.9	1.5	1.0	0.15	1.0	4.7	0.9	0.0004
Sitting	2.7	2.5	2.7	0.7	0.97	2.1	3.2	0.6	0.16

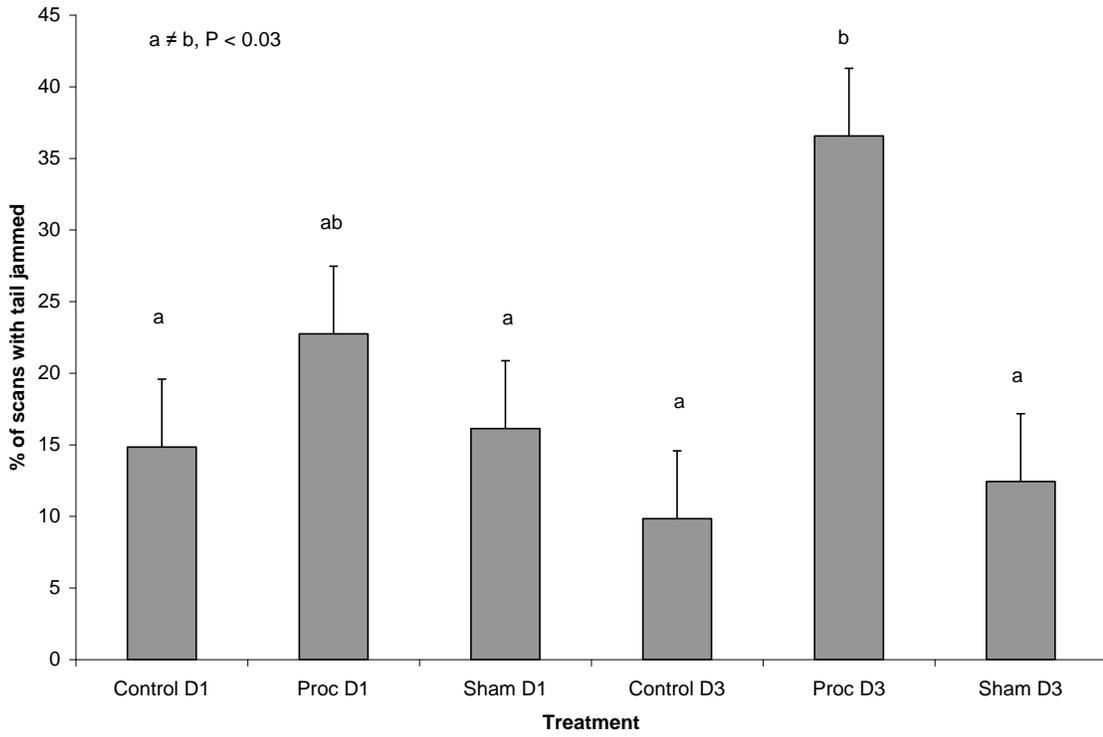


Figure 4. Tail jamming during the ten minutes after treatment application (mean % of scans  $\pm$  standard error). There was a significant effect of processing treatment ( $P = 0.0005$ ) and a trend towards an interaction between treatment and day of treatment ( $P = 0.0839$ )

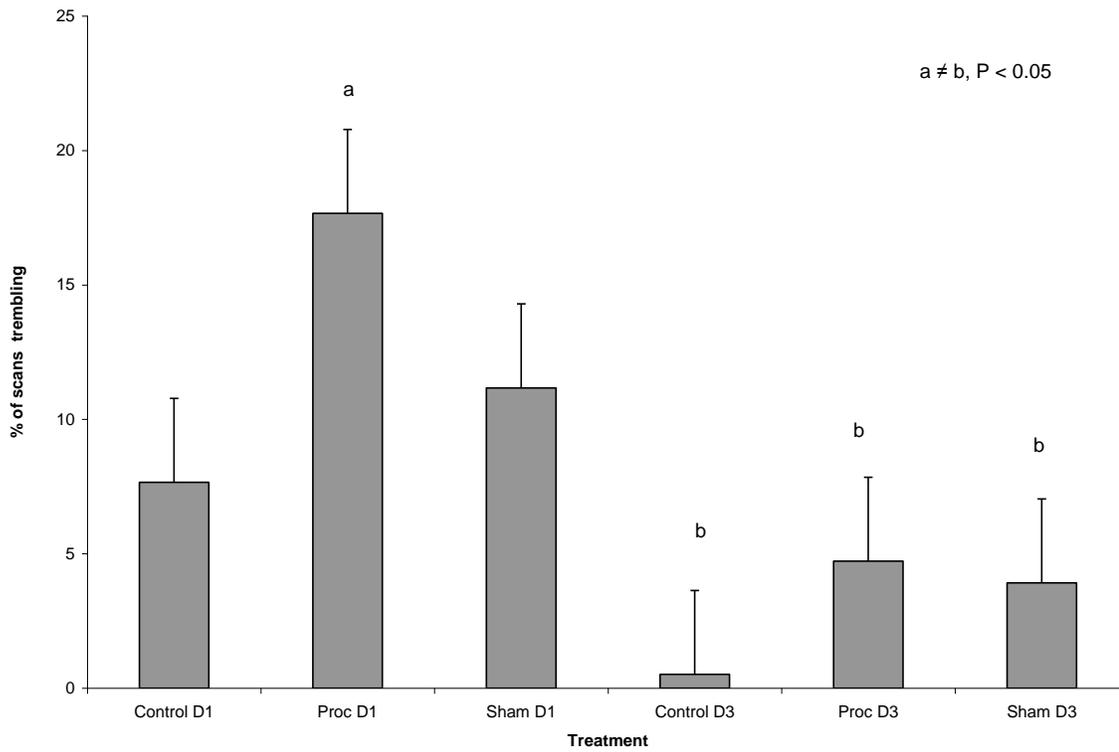


Figure 5. Trembling during the ten minutes after treatment application (mean % of scans  $\pm$  standard error). There was a significant effect of day of treatment ( $P = 0.0005$ ) and a trend toward a significant effect of treatment ( $P = 0.076$ ).

Table 3. Suckling behavior during and between suckling bouts (mean percent of scans  $\pm$  standard error). During suckling bouts, piglets were classified as either suckling or massaging the udder, at the udder but not suckling or massaging (includes searching for or fighting over a teat), or away from the udder. Between nursing bouts, piglets were classified at either at the udder or away from the udder.

	During suckling bouts			Between suckling bouts	
	Suckling	At udder	Away	At udder	Away
<b>Treatment</b>					
Control	65.3 $\pm$ 1.6	18.7 $\pm$ 1.4	16.0 $\pm$ 1.2	19.5 $\pm$ 1.1	80.5 $\pm$ 1.1
Sham	66.2 $\pm$ 1.6	17.1 $\pm$ 1.4	16.7 $\pm$ 1.2	20.0 $\pm$ 1.1	80.0 $\pm$ 1.1
Processed	69.1 $\pm$ 1.6	15.1 $\pm$ 1.4	15.8 $\pm$ 1.2	20.0 $\pm$ 1.1	80.0 $\pm$ 1.1
P value	0.12	0.11	0.73	0.54	0.54
<b>Day</b>					
1	66.8 $\pm$ 1.4	17.1 $\pm$ 1.2	16.1 $\pm$ 1.0	19.6 $\pm$ 1.0	80.4 $\pm$ 1.0
3	67.0 $\pm$ 1.4	16.8 $\pm$ 1.3	16.2 $\pm$ 1.0	20.3 $\pm$ 1.0	79.7 $\pm$ 1.0
P value	0.89	0.84	0.94	0.34	0.34

Table 4: Weights and average daily gain (ADG) (means  $\pm$  standard error). ADG represented the entire two week trial. Weights on day 14 were not available for three litters due to technical problems.

Weight (kg)	ADG (g/d)
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	Day 0	Day 5	Day 14	
<b>Treatment</b>				
Control	1.50 ± 0.05	2.31 ± 0.08	4.92 ± 0.17	244 ± 10
Sham	1.53 ± 0.05	2.33 ± 0.08	5.06 ± 0.17	252 ± 10
Processed	1.52 ± 0.05	2.27 ± 0.08	4.96 ± 0.17	244 ± 10
P value	0.79	0.68	0.81	0.80
<b>Day</b>				
1	1.51 ± 0.04	2.30 ± 0.07	5.00 ± 0.14	248 ± 8
3	1.52 ± 0.04	2.31 ± 0.07	4.96 ± 0.14	245 ± 8
P value	0.77	0.83	0.81	0.79

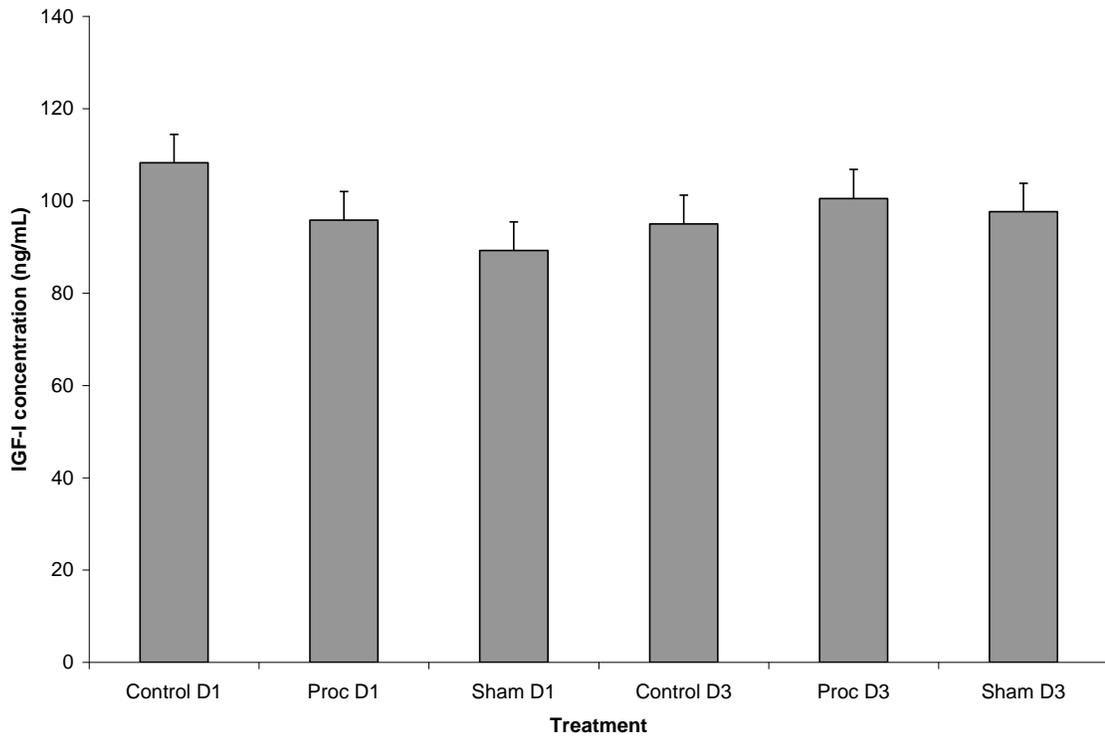


Figure 6. Insulin-like growth factor-I concentrations in piglet plasma (ng/mL  $\pm$  standard error). There was tendency for an interaction between treatment and day of treatment ( $P = 0.0896$ ).

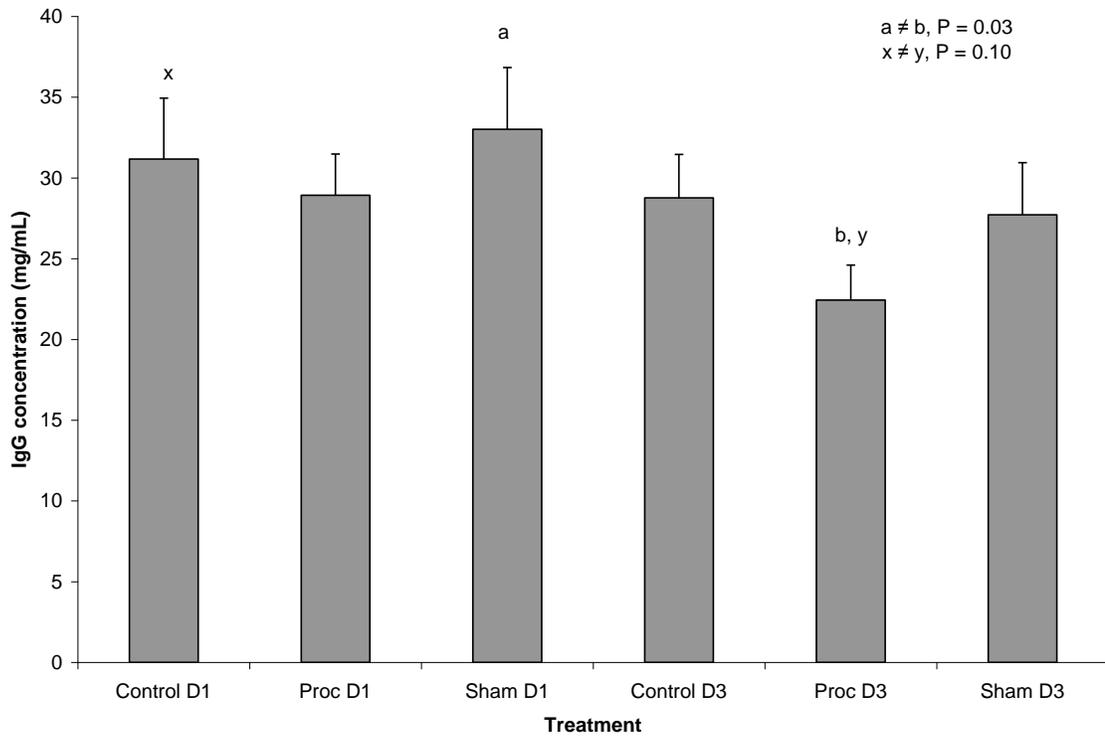


Figure 7. IgG concentrations in piglet plasma (mg/mL  $\pm$  standard error). There was a significant effect of treatment ( $P = 0.090$ ) and day of treatment ( $P = 0.0183$ ).