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Housing Pregnant Sows in Turnaround Stalls During Gestation Impacts Behavior, Immune and Well-Being

Ashley DeDecker, Michael Mandru and Janeen L. Salak-Johnson

Department of Animal Sciences, University of Illinois, Urbana, Il USA

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Corresponding Author:
Janeen L. Salak-Johnson
Oklahoma State University
Department of Animal and Food Sciences, Stillwater, OK, USA
Email: janeen.johnson@okstate.edu

Abstract: This study aimed to determine the effects of housing gestating multiparous sows in alternative turn-around stalls on selective measures of well-being. Sows were randomly assigned to either a Turn-Around Stall (TAS) or standard Straight Stall (STS) from gestational day 6 until 110. Behavior was registered on days 6, 30, 45, 65, 90 and 110 of gestation and immune and cortisol on days 30 and 90. Social rank and directional orientation of the sow's head (forward or backward) was only determined for sows housed in the TAS. On gestational days 6 and 30, duration of stand and oral-nasal-facial behaviors were greater ($p<0.05$) for sows in TAS compared to sows in STS. Regardless of gestational day, sows in TAS spent more ($p<0.01$) time standing and eating than sows in STS. Plasma cortisol and B-cell induced proliferative index and neutrophil chemotaxis were greater ($p<0.05$) for sows in TAS, whereas, natural killer cell cytotoxicity ($p<0.05$) and lymphocytes ($p=0.07$) were greater for sows in STS. Lesion scores were more ($p<0.0001$) severe and backfat depth less ($p<0.001$) for sows in TAS compared to those in STS. Moreover, sows in TAS were facing forward 70% of the time on days 6 and 110, but facing backward 50 to 60% of the time on all other days ($p<0.05$). Socially, dominant sows in the TAS were more aggressive, won more encounters and shoved the gate more often ($p<0.05$) and had heavier litters ($p<0.05$) when compared to their submissive counterparts. These data imply that housing sows in turn-around stalls during gestation can positively and negatively impact measures of well-being including behavior and immune function. The behavioral and physiological responses found indicate there may be a cost associated with turning and that sow social rank may affect the outcome.

Keywords: Behavior, Gestation, Housing, Immune, Turn-Around Stalls

Introduction

Despite the positive aspects associated with an individual gestation stall, including reduced aggression and injuries, control of feed intake and individual care, its acceptability concerning animal welfare has been in question for some time. Primarily due to the inability of sows to turn-around and restricted space allowance which hinders postural changes and other behaviors. Early work by McFarlane *et al*. (1988) found that as the width of a flared gestation crate was increased from 112 cm to 122 cm, the average turns performed by pregnant gilts increased from 8.6 to 12.9 turns per day. Bergeron *et al*. (1996) reported that gilts housed in turn-around stalls turned on average 75 times per day which is much higher than rates of 11.2 (McFarlane *et al*., 1998) and 23.6 (Johnson *et al*., 1990) for gilts and sows weighing between 132 and 164 kg. Others reported that sows housed in a 2.4 m wide pen had 200 turning movements per day, but when the width was reduced to 60% of the body length of the sow, they turned less than 36 times (Bøe *et al*., 2011). Also, providing stall-gilts enough space to turn-around resulted in reduced cortisol, but had no effect on cell-mediated response to Phytohemagglutin (PHA) as assessed by skinfold thickness (Bergeron *et al*., 1996).

Moreover, in the US, acceptable requirements for the use of the conventional stall for dry sows focuses on adequate stall space, which allows the sow to easily lay down in full lateral recumbency without simultaneously touching both sides of the stall. The current stall is long and wide enough to accommodate the majority of sows while standing, but it does not adequately accommodate larger-bodied sows while laying (McGlone *et al*., 2004). Providing sows more stall space affects postural changes (Anil *et al*., 2006), especially when stall width is adjusted to accommodate large sows (Zverina *et al*., 2015) and resulted in reduced lesion severity scores (Zverina *et al*., 2015; Salak-Johnson *et al*., 2015). But, increasing the width of a stall does not provide enough space for the average sow to reorient her body 180°. Limited data exist for the minimum individual pen width required for sows to turn-around and affect sow behavior, but no data exist on the effects of turning-around by pregnant sows has on long-term wellbeing. Therefore, this study aimed to evaluate the effects of housing pregnant multiparous sows throughout gestation in an alternative turn-around stall on behavior, immune function and well-being compared to sows in standard straight stall.

Materials and Methods

Animals and Housing

All experimental procedures used in this study were approved by Illinois State University Animal Care and Use committee (Normal, IL). A total of 40 multiparous sows (parities 1 to 4) were randomly assigned to either a conventional Straight Stall (STS) or an alternative Turn-Around Stall (TAS) from gestational day 6 till 109±2. The outside dimension of the STS and TAS was 0.69 m wide ×2.13 m long. Sows in the TAS could increase the width of the rear of the stall up to 1.73 m by pushing the hinged and movable gate which separated the sow pair. But, if a sow did turnaround, it was at the expense of the adjacent (or neighboring) Sow.

Sows were artificially inseminated within 24 h after estrus onset and again 24 h later. Pregnancy was confirmed at day 27±2 post-mating using ultrasound machine (Bantam, IE Medical Imaging) for trans-abdominal examinations. Sows were individually fed at 0800 h a fiber modified diet mainly of shelled corn and soybean meal with a portion of these ingredients being replaced with wheat middlings (15%) and soybean hulls (30%) as described in DeDecker *et al*. (2014). All nutrients were present at concentrations that met or exceeded nutrient requirements (NRC, 1998). Each sow had access to an individual water nippler.

Behavior

Live behavioral observations were made using GV-1240 (Geovision, Inc., Irvine, CA) video capture combo card and Web-Cam remote capability with Geovision Remote Monitoring Software (Geovision, Inc., Irvine, CA) and video-records were also captured. Behaviors were observed on days 6, 30, 45, 65, 90 and 110 of gestation and registered. Continuous-sampling was used to register eat, lay, stand, sit and oral-nasal-facial behaviors for sows kept in either STS or TAS by a trained observer during 3 h (time periods: 0800-0900 h; 1200-1300 h; 1600-1700 h). Additional behaviors were registered from video-records for sows kept in TAS to determine directional (forward or backward direction) preference of lying or standing within the stall as well as social rank. For social rank, these behaviors included aggressive encounters, fights (won or loss) and gate-shoves between the neighboring pair of sows in the TAS.

Skin Lesion Scores

Skin lesions were scored as previously described by Salak-Johnson *et al*. (2007) and modified by DeDecker *et al*. (2014). Scoring was on a 0 to 6 scale, with 0 being normal (no lesions) and 6 being a severe open wound and could receive a score ranging from 0 to 6 or a combined score for any location on any particular day. For example, a sow might have at a certain location: Redness + swelling (2), swelling + callus (3) and marked wound/fresh scratch (5), for a total combined lesion score of 10.

Blood Collection and Assays

On gestational d 30 and 90, sows were nose-snared and 10 mL of blood was collected by jugular vena-puncture using vacutainers that contained either sodium heparin or EDTA. All blood samples were obtained within ≤ 2 min. Total White Blood Cell counts (WBC) were made electronically using a Coulter Z1 particle counter (Beckman Coulter, Indianapolis, IN). Whole blood smears were made, fixed in methanol, stained with Hema-3® staining system (Fisher Scientific, Houston, TX) and viewed under a light microscope to determine leukocyte differential by counting 100 cells per slide. Plasma was collected and stored at −20°C. Total plasma cortisol was measured using a validated commercial radioimmunoassay (Coat-A-Count®, Los Angeles, CA) and intra- and inter-assay CV was 4.5% and 7.62%, respectively.

Whole blood was diluted with Roswell Park Memorial Institute medium (RPMI; Gibco, Carlsbad, CA) layered over Histopaque® -1077, (density = 1.077 g/mL; Sigma Aldrich, Saint Louis, MO) and -1119 (density = 1.119 g/mL; Sigma Aldrich) and centrifuged at 700 × g for 30-min at 25°C for isolation of immune cells. Lymphocytes were removed from the 1077 layer, washed twice in RPMI, re-suspended and counted. Neutrophils were removed from the 1119 layer, washed once and red blood cells were lysed and then cells were washed once again.

Neutrophil chemotaxis was measured using an assay previously described by Salak *et al*. (1993) to determine the ability of cells to migrate toward assay medium or recombinant human complement-5a (10−7 *M*; Sigma Aldrich) and recombinant human IL-8 (100 µg/mL; Sigma Aldrich). Neutrophil phagocytosis was measured using a flow cytometry-based assay as previously described by Jolie *et al*. (1997) with minor modifications as described by Niekamp *et al*. (2006). Fluorescent beads were pre-incubated for 30 min with non-heat-inactivated porcine serum, then beads were added at a 10:1 (beads-to-neutrophils) ratio and samples were incubated for 45 min. The percentage of engulfment of beads by cells was evaluated using flow cytometry.

Mitogen-induced lymphocyte proliferation assay was performed using a CellTiter 96® nonradioactive cell proliferation assay (Promega, Madison, WI) following the manufacturer's protocol with minor modification as previously described by Sutherland *et al*. (2005) to determine T- and B-cell mitogen induced lymphocyte proliferation. Lymphocytes were placed in triplicate into a sterile 96-well flat-bottom plate at a cell concentration of 5×10^6 cells/mL and Concanavalin A (CONA; Sigma Aldrich) and Lipopolysaccharide (LPS; Sigma Aldrich) were used as mitogens (0, 0.2, 2.0 and 20 µg/mL) to stimulate T and B cells, respectively. Plates were read using a microplate reader (BIO-TEK Instruments, Inc., Winooski, VT) at wavelength 550 nm with reference wavelength 690 nm and results expressed as a proliferation index: Optical density$_{(550/690\ nm)}$ stimulated cells ÷ Optical density$_{(550/690\ nm)}$ non-stimulated cells.

Natural killer (NK) cell cytotoxicity was measured using a commercially available nonradioactive cytotoxicity detection kit (Roche Diagnostics, Indianapolis, IN) as described previously by Sutherland *et al*. (2005). Lymphocytes were used as effector cells at a concentration of 1×10^7 cells/mL and K-562 chronic human myelogenous leukemia cells (ATTC, Manassas, VA) adjusted to a constant 10,000 cells/well as target cells. Samples were run in triplicate at effector (lymphocytes) to target cell (K-562) ratios of 12.5:1, 25:1, 50:1 and 100:1, respectively. Plates were read using a microplate reader (BIO-TEK Instruments) at wavelength 490 nm and reference wavelength 690 nm and percent cytotoxicity was calculated as described by Lumpkin and McGlone (1992).

Statistical Analysis

Statistical analyses were performed using SAS (SAS Inst. Inc., Cary, NC). All traits were tested for departures from a normal distribution. A natural logarithmic transformation was applied to all traits deviating from a normal distribution. A linear mixed effects model was used to analyze the physiological measurements. The model included the fixed effects factors of stall type (2 levels: STS or TAS) and day of measurement (days vary depending on the measure) and all interactions. The behavior model also included the hour of measurement. Estimates were obtained using the PROC MIXED of SAS. Significance was set at ($p\leq0.05$).

Results

Interactive effect of stall type × gestational day occurred for cortisol and NK cytotoxicity. On d 30, sows kept in TAS had greater ($p=0.03$) plasma cortisol concentration compared to sows kept in STS (47.3±3.0 ng/mL Vs. 39.6±2.9 ng/mL, respectively). Sows kept in STS had greater ($p=0.05$) NK cytotoxicity compared to those kept in TAS (70.3±7.8% Vs. 47.0±6.7%). However, NK cytotoxicity (37.3±6.3%) was less ($p<0.05$) on d 90 for sows kept in the STS compared to d 30, whereas, NK cytotoxicity was similar on d 30 and 90 (40.3±5.7%) for sows kept in TAS.

Shown in Table 1, neutrophil chemotaxis toward C5a ($p<0.05$) and IL-8 ($p<0.05$) and LPS-induced lymphocyte proliferation ($p=0.05$) responses were greater for sows in TAS compared to sows in STS. Sows in STS had greater ($p<0.05$) NK cytotoxicity and lymphocyte numbers ($p=0.07$) than did sows in TAS (Table 1).

Shown in Table 2, durations of standing ($p<0.01$) and eating ($p<0.001$) behaviors were greater for sows kept in the TAS than for those kept in the STS. Percentage of eating was also greater ($p<0.01$) for sows in the TAS than for those in STS (Table 2). Sows in STS had greater backfat depth (17.3 Vs. 16.1 cm, $p<0.0001$) and less severe lesion score (20.0 Vs. 23.4, $p<0.0001$) compared to sows in the TAS.

As shown in Fig. 1, on gestational d 6 and 110 sows spent ≥ 65% of time facing forward in the TAS ($p<0.001$); whereas, on all other days, time spent facing forward decreased to ≤ 50%. As shown in Fig. 2, time of day affected the direction sows were facing, during 0800-0900 h sows spent 90% of their time facing forward; whereas, during 1200-1300 and 1600-1700 h sows spent 50% and 30% of time facing forward, respectively ($p<0.001$).

Socially, dominant sows won more ($p<0.05$) aggressive encounters than did submissive sows (5.8 Vs. 0.0, SE=1.7, respectively) and shoved gate more often ($p<0.05$) than did submissive sows (5.0 Vs. 1.7, SE=1.0, respectively). Socially, dominant sows also had more ($p<0.05$) bouts of aggression compared to submissive sows (16.8 Vs. 0.88, SE=3.1, respectively). Dominant sows had heavier litters at birth (38.6 Vs. 30.1, SE=2.3, respectively; $p<0.05$), but all other litter-related traits, including numbers born alive, stillborn and weaned and litter weaning were all similar between dominant and submissive sows.

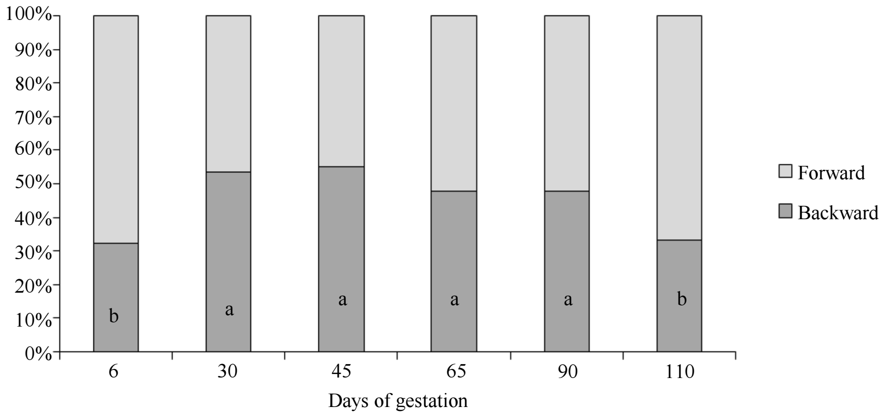

Fig. 1: The day of gestation affects the direction the sow faces in the turn-around stall; [a,b]Across days of gestation, means without a common superscript letter differ (p<0.001)

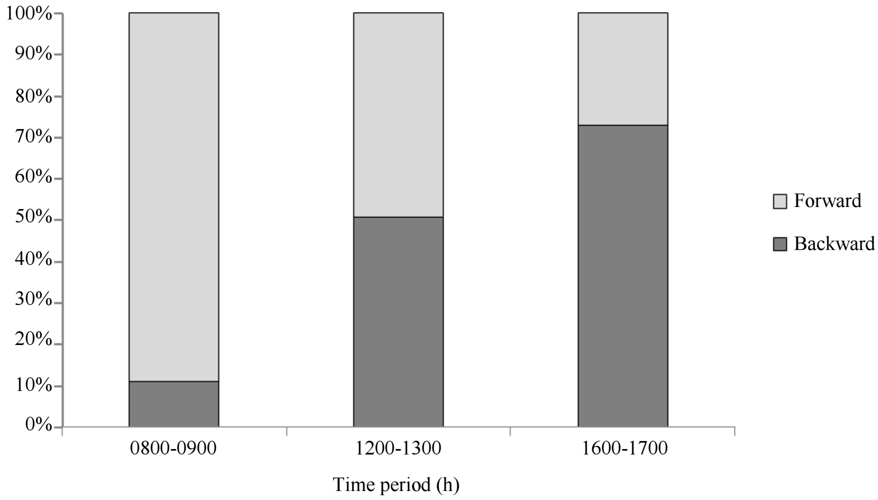

Fig. 2: Effect of time period on direction chosen by sows kept in TAS throughout gestation; [a,b,c]across time periods, means without a common superscript letter differ (p<0.001)

Table 1: Main effects of stall type gestating sows were housed in throughout gestation on mean immune measures and cortisol (least squares means ± SE).

Immune trait[1]	Standard	Turn-around	P-value
Total WBC, 10^8/mL	2.1±0.1	2.2±0.1	0.72
Lymphocyte, 10^7/mL	3.1±0.1	2.6±0.1	0.07
Neutrophils, 10^6/mL	5.8±0.3	5.5±0.3	0.18
Neutrophils, %	37.0±1.4	37.9±1.4	0.59
Lymphocytes, %	54.4±1.3	53.4±1.3	0.58
Monocytes, %	5.1±0.4	4.6±0.4	0.44
Eosinophils, %	3.7±0.3	3.7±0.3	0.29
Neutrophil-to-Lymphocyte ratio	0.77±0.1	0.82±0.1	0.58
Phagocytosis, %	55.3±1.4	54.6±1.4	0.43
LPS-induced proliferation index	1.1±0.02	2.0±0.02	0.05
ConA-induced proliferation index	1.4±0.02	1.2±0.02	0.57
NK cytotoxicity, % 50:1	58.3±5.1	43.8±5.4	<0.05
C5a Chemotaxis, no./4 fields	64.5±6.7	89.1±5.9	<0.05
IL-8 Chemotaxis, no./4 fields	51.5±4.3	70.0±3.9	<0.05
Plasma cortisol, ng/mL	27.7±2.2	42.3±2.2	<0.05

[1]Means of immune measures taken on gestational days 30 and 90

Table 2: Effect of stall type on the behavior of gestating sows throughout gestation (least squares means ± SE)

Behavior	Stall type: Standard	Stall type: Turn-around	P-value
Lay			
Duration, min/bout	24.4±2.1	23.7±2.0	0.81
Percentage, %	52.2±1.6	50.5±1.5	0.38
Sit			
Duration, min/bout	1.0±0.2	1.2±0.2	0.48
Percentage, %	3.0±0.7	3.4±0.7	0.64
Stand			
Duration, min/bout	20.9±0.9[b]	25.7±0.9[a]	<0.01
Percentage, %	40.7±1.5[d]	46.0±1.5[c]	0.09
Eat[1]			
Duration, min/bout	27.1±0.8[b]	30.7±0.8[a]	<0.001
Percentage, %	46.0±1.2[b]	51.7±1.3[a]	<0.01
ONF			
Duration, min/bout	11.4±0.8	12.4±0.7	0.44
Percentage, %	27.4±1.4[d]	32.0±1.4[c]	0.06

[a,b] Within a row, means without a common superscript letter differ ($p \le 0.05$);
[c,d] Within a row, means without a common superscript letter differ ($p < 0.10$);
[1] eat behavior only registered during time period 1 (0800-0900)

Discussion

One of the major criticisms of the conventional straight gestation stall is the restricted space which hinders freedom of movement including turning around and the ability to interact socially; whereas, the alternative turn-around stall allows for greater mobility and interaction with the adjacent (or neighboring) animal. Although we did not quantify the number of turns, we did assess the percentage of time sows spent facing forward or backward when housed in turn-around stalls. During early and late gestation, sows spent the majority of their time facing forward but during mid-gestation they spent more time facing backward, thus implying that sows will turn around, but their preference for direction in the stall can be affected by gestational stage. Sow behavior also differed between sows housed in turn-around stalls versus sows in standard stalls during gestation. We found that sows in turn-around stalls were more active overall. Sows in turn-around stalls stood more and performed more oral-nasal-facial behaviors than the sows that were housed in the standard stalls. These findings were similar to Bergeron *et al*. (1996) who reported that gilts housed in turn-around stalls stood more frequently than gilts in standard stalls; whereas, others found no differences in the frequency of standing behavior for either gilts or sows housed in turn-around stalls (McFarlane *et al*., 1988; Johnson *et al*., 1990). Moreover, Bergeron *et al*. (1996) reported that gilts were often nosing/licking the stall bars as well as manipulating the chain when housed in turn-around stalls, they hypothesized that the smaller size of these gilts may have facilitated movements since the behavior of each gilt was highly correlated with the neighboring gilt. We speculate that sows in our study used their snout not only as a means to investigate their environment but to turn-around. The design of the turn-around stall required them to manipulate components of the swinging gate, especially the chain, to be able to increase the space in the rear of the stall to perform turning movements. Once they turned around these components were more accessible thus increasing oral-nasal-facial behaviors which may have contributed to the higher percentage of facing backward especially post-feeding.

This study reveals the impacts of housing multiparous sows in turn-around stalls for the entire gestational period on sow behavior, immune function and overall well-being. But, more interestingly, sow social rank may also affect the outcome even when housed in a turn-around stall. Some have reported lower cortisol concentrations for gilts housed in turn-around stalls but no difference in immune function between gilts housed in either stall type (Bergeron *et al*., 1996). The lower cortisol concentrations among gilts housed in turn-around stalls may be partly explained by the fact that these gilts were able to move more and may have adapted to their environment since Becker *et al*. (1989) found increased cortisol among those that could turn around but not move freely. Conversely, we found that sows housed in the turn-around stalls had greater plasma cortisol and less NK cytotoxicity, but these sows had greater chemotaxis and B-cell lymphocyte proliferation than sows in a standard stall. It is highly plausible that the increased cortisol among sows in the turn-around was partly due to the increased aggression between the pair as well as the design of the stall. The dominant sow among the pair was more aggressive and shoved the gate more often, but the submissive sow also shoved the gate which may have intensified the aggressive encounters between the pair. Researchers frequently observed that the swinging gate provided more opportunity for frequent and severe bouts of aggression to occur between the sows in the turn-around.

This increased aggression amongst sows in the turn-around may have contributed to the increased activity as well as the greater levels of cortisol and reduced immune function until the sows adapted to some of the potential aversive stimuli including establishing a social hierarchy within the pair. Dawkins (1988) hypothesized that the demand curve for a specific behavior is non-linear such that the animal will decrease the “demand” for a certain behavior as the costs increases, but may pay the cost if it is highly motivated to perform the behavior. McFarlane *et al.* (1988) observed that increasing the "cost" of turning by reducing the width of the gestation stall resulted in a reduction in turning. Even though the need for turning does not seem to be very strong there is still a possibility of performing this behavior for some sows may still be essential to ensure well-being, but not without a cost. Because some welfare metrics were increased while others were decreased among the sows housed in the turn-around stalls we cannot definitively conclude that turning-around is or is not important to the gestating Sow.

Conclusion

The present study revelaed that housing pregnant sows in turn-around stalls for the entire gestational period had both positive and negative effects on sow behavior, immune and productivity. Moreover, social status of the pregnant sow and the design of the turn-around stall may also partially explain the impact of housing sows in turn-around stalls for the entire gestational period.

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Author's Contributions

Janeen L Salak-Johnson: Conceived and designed the experiments, received the funding for the work, re-analyzed data and interpreted results and prepared the manuscript.

Ashley DeDecker: Executed the experiments and collected and analyzed data.

Michael Mandru: Collected behavior data to determine social status and aggression.

Ethics

All experimental procedures used in this study were approved by Illinois State University Animal Care and Use committee (Normal, IL).

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