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Behavioral Comparisons of Cloned and Non-cloned Pigs

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Abstract: The goal of this study was to evaluate whether there were differences in behaviors of cloned and non-cloned pigs. The objectives were 1) to evaluate maintenance behaviors; 2) to determine the establishment of dominance; 3) to assess learning; and 4) to appraise farrowing and mothering abilities of cloned and non-cloned gilts. Maintenance behaviors recorded at 15-min intervals over a 12-h period were not different in terms of the frequencies of lying, standing, feeding and rooting between groups (clone vs. non-clone). There was a group by time interaction ($P < 0.05$) for aggressive encounters, with cloned gilts more active in late morning. With short term paired feeding tests there were no significant effects of group on attempts to eat. Learning ability was evaluated using a problem-solving maze with a feed reward. There were no differences between groups finding the reward. Total litter weight was not affected by numbers of piglets born alive, farrowing score, mothering score, dominance score, or learning score. Average piglet birth weight was affected ($P < 0.05$) by the number of piglets born, but not by the other independent variables. In general, the behavior of cloned gilts was not different from that of non-cloned gilts.

Key words: Comparisons, cloned, non clone and pigs

Introduction

Genetic manipulation and use of assisted reproductive technologies, including cloning in livestock, have engendered concern of the general public for the welfare of the animals produced with these laboratory procedures. It is important to establish whether cloning procedures per se have any impact on the behavioral characteristics of livestock. While there have been no systematic reports on behavior of cloned animals, some data exist for genetically modified (transgenic) swine and sheep. Pathological conditions have arisen as a result of gene transfer in swine (Pursel *et al.,* 1989). However, when evaluating the behavior of transgenic sheep, there were no differences in the competition for limited quantity of supplementary concentrate, behaviors on pasture and movement order when driven through a crush (Hughes *et al.,* 1996). Additionally, only minor differences were found in normal behavior and competition for a complete diet. The minor differences in behavior were a lower incidence of idling and a delay in the amount of time to attain feed for transgenic sheep compared to controls. Study of animal behavior may include factors such as: abnormal behavior; physiological measures, such as reproductive, ingestive, social, maternal and neonatal behaviors; immune function; disease incidence; production variables; and life expectancy (Broom, 1993; Hunter *et al.,* 1988 and McGlone, 1985 and 1991). The goal of this study was to examine and compare behavior in cloned and non-cloned gilts. The objectives of this study were to: 1) evaluate maintenance behaviors; 2) assess learning; 3) determine social order and the establishment of dominance; and 4) appraise farrowing and mothering abilities of cloned and non-cloned gilts.

Materials and Methods

Animals: The cloned pigs used in these studies were derived from cultured adult somatic cells (granulosa cells), derived from crossbred gilts (1/2 Large White, 1/4 Landrace, 1/4 Duroc). These were the first cloned pigs, five females; all produced using novel nuclear transfer procedures (Polejaeva *et al.,* 2000). The cloned piglets were born on March 5, 2000 and were 7.5 mo of age at the onset of the trials. In some trials only three cloned gilts were used because of temporary lameness. The control gilts were approximately 1 mo younger. The non-cloned gilts were littermates from a contemporary litter with genetic background similar to cloned gilts purchased specifically for comparison.

Maintenance behaviors

Lying, standing, feeding, grooming and aggressive encounters were recorded for the separate group of cloned (8 mo of age) and the separate group of non-cloned (7 mo of age) gilts at 15-min intervals over a 12-h period. Pens (7.3 X 3.0 m) were nearby so that each group had visual and olfactory contact. Recording began at 0700 h. During this observation period the defecation, urination and drinking events were recorded as they occurred. Feed was presented at

0930 h. Lying, standing, feeding and rooting were analyzed by Chi square analyses. Drinking, feeding and aggressive encounters were analyzed within group by analysis of variance using GLM in SAS (1999).

Dominance and Social Order: Short term paired feeding tests were used to establish social order and dominance (Houpt and Wolski, 1980). The test was conducted at 9 mo of age for cloned gilts and 8 mo of age for non-cloned gilts. Gilts were not fed the day of the test (~28 h feed restriction). The testing pen (3' X 3' m) had a single space circular feeder (.3 m diameter) with about 50 g of cracked corn. The test was conducted for 15 min. Prior to entry to the test pen and following the test, sows were evaluated for bites and skin lesions resulting from animal-to-animal agonistic encounters (McGlone, 1985). Skin lesion scores were based on damage to the head, neck, ears, shoulders, body, rump and vulva/tail. The scoring for all areas followed the scale previously established (Martin and Edwards, 1994).

Every combination of pairs within a group was separately tested twice and the dominant animal defined as the one that spent the majority of time feeding during each test period. Dominance rank was the number of wins each gilt achieved divided by the total number of tests. Behaviors monitored during the testing trials include: 1) bite targets - head, ear(s), neck/shoulders, body, rump; 2) head butts; and 3) the gilt that ate first.

Data were analyzed for variance using GLM and Wilcoxon nonparametric statistics in SAS (1999). For the analysis of variance model, the dependent variables were the amount of time spent eating, attacking, fleeing and the number of attempts to eat the feed. Gilts and competition pair were considered fixed effects. Analyses were conducted within group. In separated models, scratches and bites given and received and head butts were dependent variables with group and gilts as independent variables. The Wilcoxon test was used for rankings.

Learning: A problem solving exercise using a maze was developed to evaluate learning with a feed reward at different locations in the maze (Fig. 1). Individuals were tested initially at 8.5 mo of age in random order. As each pig entered the maze, a timer began to record the amount of time needed to reach the feeder (location A). A maximum of 10 min was allowed to find the feed reward. The amount of time to gain access to the feed in the feeder was recorded, as was the number of wrong turns taken. The maze trials were replicated at location A and a second set of trials was conducted at a second feed reward location (location B). All testing was conducted within a one-month period.

Data were analyzed by analysis of variance using GLM, PROC Mixed and Wilcoxon nonparametric statistics in SAS (1999). For the Mixed model the dependent variables were the amount of time elapsed to attain feed and the number of wrong turns taken before finding the feed. Gilts were considered random, while group (clone vs. non-clone), feed location and replicate were fixed effects. The model also included all interactions. Gilts within group were used to test for group differences. The Wilcoxon test was used for rankings.

Farrowing Ease, Mothering Abilities, Suckling Behavior: All gilts were synchronized by oral administration of 18 to 20 mg Regu-Mate® (Altrenogest, Hoechst) mixed into the feed for 14 d. They received 250 μg of Estrumate® (Bayer), i.m., on the last day of progestogen treatment. Human chorionic gonadotropin (500 U; Intervet America) was administered, i.m., 96 h after the last progestogen treatment and gilts were bred by AI with the same semen on January 11, the day after gonadotropin treatment (Polejaeva *et al.,* 2000). All five of the cloned gilts and 3 of the 5 non-cloned gilts were diagnosed pregnant at 30 d. One non-cloned gilt farrowed on day 114, one cloned gilt farrowed on day 117 and the remaining gilts farrowed on day 115. The ease of farrowing, mothering score and the suckling order were assessed and recorded for each gilt. Piglets were weighed at birth. Farrowing ease was assessed using a scoring system (Jones, 1986). The number of piglets born, piglet size, the uniformity of the litter, their overall appearance and the gilt's behavior (temperament) were used to determine mothering scores. Mothering scores were as follows: 5 = a very healthy litter with high survival rate, mother has adequate milk and there was a successful transfer of colostrum to the litter; 4 = moderately healthy litter, a greater distribution in physical size of piglets (i.e. noticeable runts) and mother has adequate milk transfer; 3 = health of the litter is questionable (more than 25% of the litter is sick and/or underweight) and there are problems with milk transfer to piglets; 2 = 50% of the litter does not survive the first week and there is little or no transfer of colostrum; 1 = 75% or more of litter does not survive the first week and there is no transfer of colostrum. Nursing behavior was assessed on d 10 and 11 at midday. Piglets were identified with indelible ink and nursing order recorded. Data were analyzed by analysis of variance using GLM procedures in SAS (1999). For the analysis of variance model the dependent variables were litter weight, average piglet weight, farrowing score, mothering score, teat being suckled and change in teat being suckled. Depending on the dependent variable, independent variables included group (clone vs. non-clone), number born alive, dominance rank, learning rank, replicate and piglet. Pearson correlation coefficients were used to assess various relationships. Wilcoxon nonparametric statistics were used to evaluate piglet dominance scores within gilts. Dominance scores were assigned relative to suckling the most anterior teats.

Results

Maintenance Behaviors: Maintenance behaviors were compared for three cloned and five non-cloned weight/age matched gilts. Two of the five cloned gilts were temporarily lame during this segment of the testing. Chi square analyses revealed no significant ($P > 0.05$) differences in the frequencies of lying, standing, feeding and rooting behaviors between groups (clone vs. non-clone; Table 1). Feeding and rooting activities are a subsample of standing behavior. Analysis of variance revealed no effect of group, gilt, time or time by group interaction on drinking behavior. However, the analysis of aggressive encounters revealed a significant group by time interaction ($P < 0.01$) that is portrayed in Fig. 2. Generally, most encounters occurred before midday and the cloned gilts were more active in late morning. The non-cloned gilts had more aggressive encounters during the period of 1330 to 1430 h. Defecation and urination activities were few in numbers. Feeding activity was significantly affected by time ($P < 0.01$), with the greatest activity between 0930 and 1130 h and about half of the gilts fed again between 1300 and 1430 h.

Dominance: There were no significant effects of group (clone [C] vs. non-clone [NC]) on attempts to chase away (data not shown), fleeing activity (data not shown), or attempts to eat during the paired tests. However, there was a significant difference ($P < 0.01$) for the amount of time each gilt spent eating during the dominance testing (Table 2). Also, there was a significant pair effect ($P < 0.05$) for the amount of time spent eating within the non-cloned gilts (data not shown). Generally, pair combinations of NC3 and NC2, NC4 and NC5 resulted in shorter eating times (< 1 min) during the testing period than other matches. Within the clones, C2 spent the most time eating and had the highest percentage of winning events. On the other hand, NC3 was the most dominant non-cloned gilt, winning 100 % of her pairings and ranking first on the Wilcoxon test (Table 2). Assessment of gilt variances within groups resulted in no differences ($P > 0.05$), suggesting homogeneity. There were no significant differences between the number of scratches before the dominance encounters and afterward among groups (data not shown). However, there were significant differences for ear, shoulder,

Table 1: Distribution of maintenance behavioral activities for cloned and non-cloned gilts

Group	Activity							
	Lying	Standing	Feeding	Rooting	Drinking*		Defecation*	Urination*
Cloned						(No)	8	22
%	54.4	45.6	37.3	41.8				
(Time)	(6.5h)	(5.5h)	(2.1h)	(2.3h)	10.7		2.7*	7.3*
Non-cloned						(No)	8	23
%	60.0	40 .0	38.8	31.6				
(Time)	(7.2h)	(4.8h)	(1.9h)	(1.5h)	7.8		1.6*	4.6*

*mean per 12 h

Table 2: Dominance variables observed during paired testing for cloned (C) and non-cloned (NC) gilts

Gilt	Time Eating (min)	Wilcoxon Rank	Percentage of Wins	# Attempts to Eat
C1	1.38 ± 0.44^a	4 (2)	50	0.50 ± 0.52
C2	3.00 ± 0.44^b	1 (1)	100	0 ± 0.52
C4	0 ± 0.44^c	8 (3)	0	0 ± 0.52
NC1	0 ± 0.44^c	7 (5)	0	1.33 ± 0.34
NC2	1.06 ± 0.29^a	6 (4)	37.5	0.56 ± 0.34
NC3	2.69 ± 0.31^b	2 (1)	100	0 ± 0.37
NC4	1.56 ± 0.31^a	3 (2)	75	0.50 ± 0.37
NC5	1.10 ± 0.31^a	5 (3)	50	0.38 ± 0.37

^{abc}Means + SE with different superscripts differ at $P < 0.05$; (n) Wilcoxon rank within group

Table 3: Overall least squares means ± SE for the time taken to find feed and the number of wrong turns taken going through the maze by group

Group	Time elapsed (min)			Number of Wrong Turns		
Cloned	3.00	±	2.15	3.00	±	1.77
Non-Cloned	3.02	±	1.66	3.35	±	1.37

Table 4: Least squares means ± SE for time elapsed and number of wrong turns for gilts navigating through the maze to assess learning

Gilt	Time Elapsed (min)	Wilcoxon Rank	Number Wrong Turns	Wilcoxon Rank
C1	6.67 ± 0.93	7	5.00 ± 1.43	7
C2	1.45 ± 0.93	5	2.25 ± 1.43	4
C4	0.88 ± 0.93	2	1.75 ± 1.43	3
NC1	0.66 ± 0.93	3	1.25 ± 1.43	2
NC2	10.00 ± 0.93	8	9.50 ± 1.43	8
NC3	1.89 ± 0.93	6	2.25 ± 1.43	5
NC4	1.92 ± 0.93	4	3.00 ± 1.43	6
NC5	0.61 ± 0.93	1	0.75 ± 1.43	1

body and vulva and tail scratches among gilts ($P < 0.01$) before and after the encounters (data not shown). There were no differences ($P > 0.05$) between bites (head bites – 0.39 ± 1.21) or head butts (1.11 ± 1.50) given or received during the dominance testing either within groups or gilts.

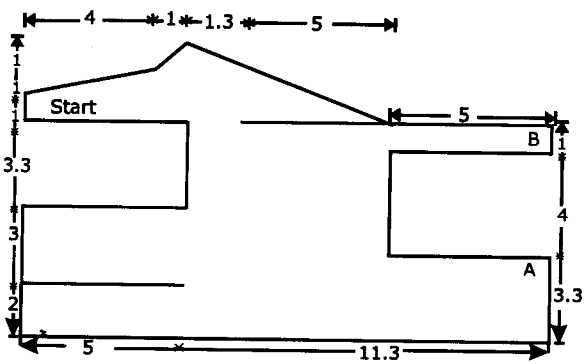

Fig. 1: Diagrammatic sketch of the maze. Gilts were timed from when they entered the maze to reach feed rewards at **A** (first test location) or **B** (second test location). Also, the number of wrong turns was recorded until the time the gilt achieved the feed reward. Dimensions are in meters

Learning: There was no group effect in the maze study (Table 3). That is, there was no difference ($P > 0.05$) between the cloned and non-cloned gilts going through the maze. There was no significant gilt effect ($P > 0.05$); showing that the time elapsed and number of wrong turns were not different. The interaction of feed location by replicate for number of wrong turns approached significance ($P = 0.10$; 2.6 ± 1.5 vs. 3.0 ± 1.5 for replicates 1 and 2 in location A, respectively and 5.5 ± 1.5 vs. 1.6 ± 1.5, respectively, in location B), suggesting some frustration finding the reward in the second location. The reduction in wrong turns for the second replicate at the second location suggests short term learning. The gilt effects approached significance ($P = 0.053$) for time elapsed through the maze and the Wilcoxon Ranking ($P < 0.05$) is shown to infer differences in learning (Table 4). NC2 had the greatest amount of time elapsed (10 min) going through the maze. There was no difference in Wilcoxon scores for group for either variable ($P > 0.05$), suggesting that cloning did not affect learning.

Farrowing and Mothering: Litters averaged 6.9 ± 0.8 piglets total with 6.5 ± 0.7 alive. Cloned gilts had a range of 4 to 9 piglets, while non-cloned gilts had a range of 4 to 8 piglets. Litter weights were 7.5 ± 0.6 kg and ranged from 3.4 to 8.9 kg for cloned gilts, with the range of 7.0 to 12.3 kg for non-cloned gilts. Gilt C2 gave birth to 7 piglets, but because of violent behavior toward the piglets had only 3 by the end of the first week. Two piglets from gilt C2 were cross-fostered to gilt NC5 right after birth. Gilt C3 had 5, 2 died the first week and the remaining 3 were not seen nursing during the suckling study. Gilt C4 gave birth to 6 piglets, all died the first week. There were minimal losses with the non-cloned gilts during the first week, 1 piglet.

Total litter weight was not affected ($P > 0.05$) by numbers of piglets born alive, farrowing score, mothering score, dominance score, or learning score. Average piglet birth weight was affected ($P < 0.05$) by the number of piglets born, but not by the other independent variables. The average piglet weight was 1.2 ± 0.1 kg. There was a correlation between the number of piglets per litter and total litter weight ($r = 0.96$; $P < 0.01$). Farrowing score was not affected by

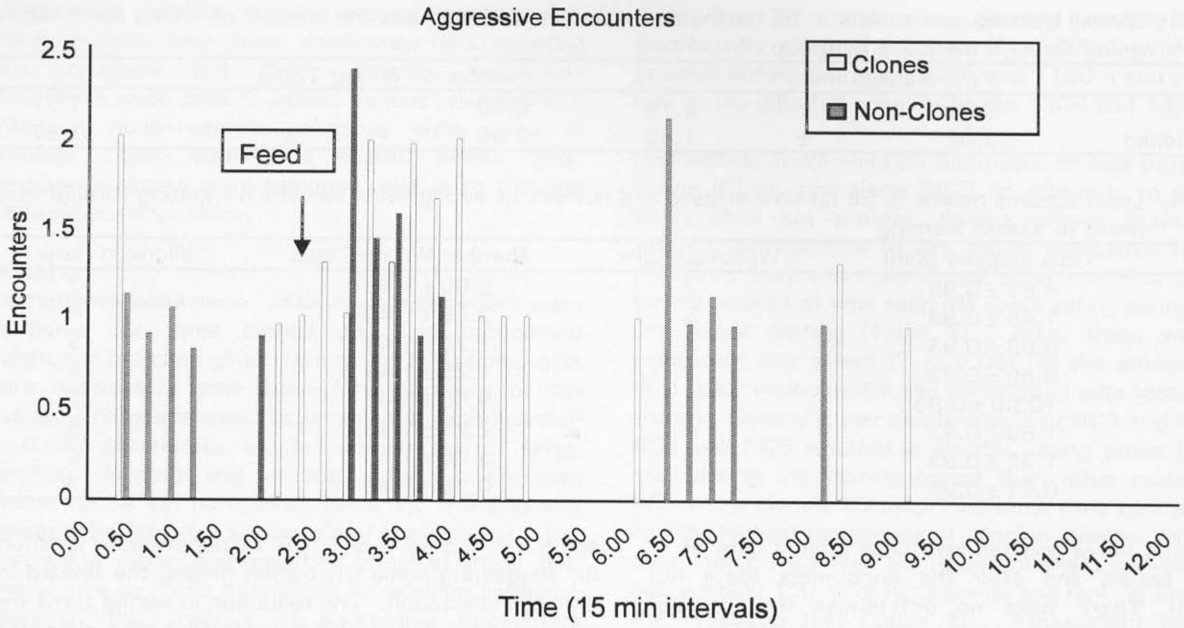

Fig. 2: Observations of aggressive encounters for cloned and non-clone gilts throughout a 12-h period. Data represent means at 15-min intervals

group (P > 0.05) and was 4.6 ± 0.1 for cloned gilts, while non-cloned gilts scored 4.3 ± 0.1. Farrowing score was affected by dominance ranking (P < 0.05) and there was a correlation of r = - 0.76 (P < 0.05), showing that more dominant gilts had higher farrowing scores. Learning rank did not affect farrowing scores (P > 0.05). Mothering score was not affected (P > 0.05) by group, dominance or learning and was 2.7 ± 0.9 for cloned gilts, while non-cloned gilts scored 5.0 ± 0.9.

Teat suckling position of piglets was affected by gilts within group (P < 0.01) and piglets within gilt (P < 0.01), but not affected by the main effects of group or replicate, or the replicated by group interaction (P > 0.05). The change in suckling position was not affected (P > 0.05) by the variables tested. Wilcoxon rankings for suckling order were significant (P < 0.05) for all gilts except C1. By d 10 and 11 postpartum all piglets of gilt C4 had died. Also, the suckling behaviors of piglets for gilt C3 were not included because they were not observed suckling during the study period.

Discussion

Maintenance Behaviors: Maintenance behaviors generally were not different between groups. The feeding activity pattern was closely associated with the time feed was first made available with our once daily feeding schedule. Group housed pigs tend to be synchronized with regard to feeding and have highest frequency of using electronic feeding systems between 0600 and 0900h (Young and Lawrence, 1994). In another study diurnal feeding patterns were found with electronic feed recording equipment with the highest feeding between 0600 and 1100 h (Hyun and Ellis, 2001). The amount of time spent eating is in close agreement with their reported 2 h time spent for groups of 2 to 4 pigs in electronic feeders. They also report that their young pigs spent about 80% of the day lying, 6 to 10% of the day standing and 2% of the day sitting. Based on a 12-h observational period, our gilts spent considerably less time lying and more time standing. Sows fed once per day had a relatively constant feeding order that was correlated with parity and social hierarchy was linear (Broom, 1993).

Dominance: Dominance orders are beneficial in livestock production systems, because they reduce energy expenditure, reduce injuries due to agonistic encounters and establish priorities for feed and water. The introduction of new animals into established groups causes excitement and results in re-establishment of a new social order (McGlone, 1985). Paired feeding tests have been used to determine dominance (Houpt and Wolski, 1980).

Percentage of wins in the papir encounters within groups was reflected almost exactly in the amount of time that the dominant gilt spent eating the test feed. The Wilcoxon Ranking reflected this measure of dominance. Attempts to eat reflected dominance in the non-cloned group, where NC1 the most submissive gilt had the numerically highest number of attempts. In both groups the dominant gilts did not attempt to eat, they ate. Time spent eating was negatively correlated with attempts to eat ($r = -0.36$; $P < 0.01$), showing that dominant gilts displaced subordinate ones. Interestingly, there was a positive correlation between the Wilcoxon ranking for learning based on time elapsed through the maze and dominance ($r = 0.29$; $P < 0.05$), suggesting that learning may be related to dominance and supporting arguments that aggressive behavior may be learned (Houpt and Wolski, 1980). Our data agree with (Hunter *et al.,* 1988; Martin and Edwards, 1994) who found that dominance rank was correlated to duration of time spent eating at a single space feed trough. Agonistic behaviors and consequences of them (scratches and cuts) were relatively minor and may be due to early establishment of hierarchical order within groups (Curtis, 1981). There were few changes in bites and scratches from before to after the testing period. The limited agonistic behaviors agree with other reports, which found most activity directed toward the front end of the recipient sow (Martin and Edwards, 1994). The patterns of bites and scratches to the ears, neck, shoulders and face support work of others (McGlone, 1985) who found that these were the predominate sites where aggressive interactions take place. While none of the dominance variables were different by group, there appear to be strain differences in defeat-induced behaviors in mice (Siegfried *et al.,* 1984).

Learning: Learning involves redirecting behavior so that the animal becomes more efficient at attaining motivational goals (Lawrence and Terlouw, 1993). When learning a new association, such as in this maze study, the information gets processed at a more cognitive level to allow alteration of the association more easily. The problem with the delivery of a reward, such as food, is that the animal, not the experimenter, determines when reward will be delivered and it is difficult to control when each learning episode occurs (Pearce and Bouton, 2001).

Two measures were used to assess learning of gilts. These measures were speed at negotiating a maze to attain a food reward and the number of wrong turns gilts took as they navigated the maze. It appeared that learning occurred as there was a reduction in the number of wrong turns from the first to second replicate as testing was conducted at the second reward location and there was no difference due to group background. Our data, showing individual differences as opposed to group differences support the memory process involvement in habituation (Contet *et al.,* 2001).

Maternal and Piglet Behaviors: The average number of piglets born per litter was slightly lower than the 7.9 to 12.5 reported by others (Bradshaw and Broom, 1999; Leenhouwers *et al.*, 2001; McGlone and Fullwood, 2001). Additionally, average weight of piglets born and litter weight were lower than they reported, but stillbirths were less than the reported 0.8 to 0.9 (McGlone and Fullwood, 2001). It has been reported that there is a genetic component for piglet survival from the onset of farrowing to weaning that consists of a maternal component and a direct genetic component, with the maternal component influencing piglet survival through uterine quality and mothering ability (Leenhouwers *et al.,* 2001). While we found no statistical differences between groups for the variables we measured, there were numerical differences in litter size, weight and maternal support between our cloned and non-cloned gilts. Additionally, two of the cloned gilts did not provide good piglet support to d 10 postpartum. The survival rate at d 10 was 65% for cloned gilts and 94% for non-cloned gilts, which blankets the 84% found for piglet survival in farrowing crates (Bradshaw and Broom, 1999). Clone 4 had a maternal score of 1 and lost all piglets before the nursing evaluation. Also, C3 had three of four very thin and unhealthy piglets at the time of the nursing assessment.

Teat order for suckling was affected by sow and this was because sows suckled different numbers of piglets. Suckling position appeared to be well established by d 10 and 11 postpartum and did not change during the observation periods, in agreement with others (Nielsen *et al.,* 2001). It has been reported that suckling position is established during the first day of life and that the more dominant pigs suckle more anterior glands (Curtis, 1981). The rankings established the dominance order that was maintained in order to minimize strife within the litter. While milk yield was greater in the more anterior glands (Garst *et al.,* 1999) and may be related to piglet growth rate, gland position suckled had no impact on piglet daily gain in primiparous sows (Nielsen *et al.,* 2001).

In conclusion, there were no significant differences in the frequencies of lying, standing, feeding and rooting behaviors between groups. When dominance was tested, there were no significant effects of group on attempts to chase away, fleeing activity, or attempts to eat during the paired tests. When learning was evaluated, there was no difference between the cloned and non-cloned gilts navigating their way through the maze. Farrowing score was not different across groups. Total litter weight was not affected by numbers of piglets born alive, farrowing score, mothering score, dominance score, or learning score. Mothering scores were not affected by group, dominance or learning scores. In general, the behavior of cloned gilts was not different from that of non-cloned gilts.

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