ORIGINAL RESEARCH

Evaluation of segregated early weaning investigating performance, immunologic indicators (CD4, CD8), and herd health status

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Summary

Purpose: *To evaluate the reported benefits of segregated early weaning (SEW) as determined by performance, by immunologic status as indicated by T-lymphocyte differentiation antigens, and by health status.*

Methods: *Experiment I was conducted at a commercial herd with pigs weaned at 11–18 days of age and blocked by age to include 11– to 14-day-old pigs and 14- to 18-day-old pigs. Pigs were randomly assigned to three management groups: 1) onsite, commingled; 2) onsite, sanitized, and 3) offsite, sanitized. Experiment II was conducted in environmentally controlled research chambers. Pigs were weaned at 12–15 days of age and randomly assigned to two management groups: 1) remained in non-sanitized farrowing crates and weaned by removing the sow; and 2) weaned and transferred to a sanitized chamber and crates with access severely restricted.*

Results: *Pigs from both herds used for experiments I and II were found to have* Streptococcus suis *in 77%–88% of the animals tested. Neither performance nor percentages of T-lymphocytes expressing CD2+, CD4+, or CD8+ differentiation antigens were significantly affected by the on- and offsite facility or sanitization, although pigs from the onsite commingled group gained slightly less (*P *<.05) than the on- and offsite sanitized groups. Weaning age also had a significant effect on performance with the later-weaned pigs (14–18 days) gaining significantly (*P *<.05) more than the earlier-weaned pigs (11–14 days).*

Implications: *Production systems practicing good sanitation and conscientious biosecurity measures to establish a minimum pathogen exposure may not demonstrate significant performance results in nursery pigs moved offsite at an early age (<14 days). Also, single T-lymphocyte differentiation antigens are not appropriate indicators of immune status in SEW pigs with minimum pathogen exposure.*

Keywords: *segregated early weaning (SEW), immunologic antigens, growth performance*

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The production performance advantages of segregated early weaning (SEW) of pigs are currently being investigated and applied in many commercial swine production units. In this "movement" technology, pigs are moved away from the sow while immunity from maternal antibodies is still high. Performance results of previous SEW studies have been highly successful in production systems in which performance may have been limited by high pathogen concentrations.[1–5]

Two experiments were conducted in an effort to evaluate the reported benefits of SEW and the effect of nursery site location on average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency, defined as the ratio of feed:gain (F:G). A second goal of these studies was to evaluate the effect of nursery site location on the expression of T-lymphocyte differentiation antigens (CD2+, CD4+, CD8+) to determine immune status. The percentages of T-lymphocytes with the respective differentiation antigens represent functionally different T-lymphocyte subpopulations. A third goal was to determine the effects of weaning age as a part of experiment 1.

Materials and methods

Experiment I: Early weaning on a commercial farm

Experimental design

The first experiment was conducted on a 350-sow farrow-to-finish commercial operation. The second experiment was conducted in the Samuel Brody Climatology Laboratory, located in the Animal Sciences Research Center at the University of Missouri.

Experiment I was conducted at a privately owned farrow-to-finish swine farm with 350 sows. One hundred and seventy-four pigs (Yorkshire × Landrace × Hampshire) were selected from a farrowing of 20 sows. Pigs were weaned at 11–18 days of age and blocked by age to include 11- to 14-day-old pigs (85 pigs) and 14- to 18-day-old pigs (89 pigs). The age blocks were determined by the dates when sows

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farrowed in an effort to create two populations of a relatively equal number of pigs. Baby pigs were processed according to standard husbandry techniques (i.e., 1-cc injections of 50 mg iron given within the first 24 hours after birth, tails docked, teeth clipped, males castrated by 5 days after birth) and no vaccinations were administered. Pigs were randomly assigned to one of the following management groups:

- standard nursery on the farm of origin and managed in a continuous-flow manner (commingled with older pigs in the room but not in the pens);
- standard nursery on the farm of origin and managed in an all-in–all-out (AIAO) manner with stringent sanitization and separate personnel; and
- standard nursery at a site separated from the sow herd by 23 miles and maintained under strict access and sanitization and separate personnel.

At each location, pigs were placed in 5 ft × 7 ft pens with eight pigs per pen. Both age groups were included in each room, although age groups were kept in separate pens. Pigs had free access to water and feed at all times. At weaning pigs were given diet I, at 13 days pigs were given diet II, and at 27 days until the end of the trial pigs were given diet III (Table 1). Body weights were individually determined and feed consumption per pen was determined weekly.

Table 1

Composition of diets I, II, and III
(percentage of basis of components)

Component	Diet I	Diet II	Diet III*
Corn, dent†	27.04	47.49	66.04
Whey, dried	25.00	23.08	
Extruded soy protein concentrate	14.48	6.66	
Lactose	14.47	—	
Spray dried animal plasma	6.75	—	
Soybean meal (48% CP)	5.00	15.00	27.99
Tallow	2.00	2.00	
Spray-dried blood meal	1.75	2.25	
Dicalcium/monocalcium phosphate	1.64	1.88	1.80
Ground limestone	0.69	0.60	0.98
Vitamin premix‡	0.25	0.25	0.25
Carbadox	0.25	0.25	
DL-methionine	0.23	0.11	
L-lysine HCL	0.15	0.15	0.15
Trace mineral premix§	0.15	0.15	0.15
Zinc oxide	0.11	0.11	
Copper sulfate	0.04	0.04	

* Experiment I only.

† For Diet I, crude protein was 8.53%; for Diet II, 7.24%.

‡ Supplies the following per lb: vitamin A, KIU, 2,000; vitamin D, KIU, 200; vitamin E, KIU, 4; vitamin K, 725 mg; vitamin B_{12}, 5.5 mg; riboflavin, 1,500 mg; pantothenic acid, 5,100 mg; niacin, 6,000 mg.

§ Supplies the following per lb: vitamin E, KIU, 4; choline, 100,000 mg; biotin, 40 mg; folic acid, 300 mg.

Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Health parameters

Prior to weaning and at the end of the experiment, pigs were assessed for health status. To be 95% confident of detecting an infected or serologically positive animal with a suspected prevalence of at least 40% in a population of 60 animals, six pigs were tested in each room.[6] Nasal swabs were collected from these 18 pigs for culture of suspected bacterial organisms: *Bordetella bronchiseptica*, *Pasteurella multocida*, and *Streptococcus suis*. Blood samples were obtained by jugular venipuncture and tests were conducted (Oxford Laboratories, Inc., Worthington, Minnesota) for: *Actinobacillus pleuropneumoniae* 1,5,7; Group D *S. suis*; *Haemophilus parasuis*; *Mycoplasma hyopneumoniae*; swine influenza virus (SIV); and porcine reproductive and respiratory syndrome virus (PRRSV). The same tests were conducted at the end of the experiment on the same animals. Animals were observed by a veterinarian once a week.

Antigen determination

The 18 selected pigs were bled by jugular venipuncture just prior to being assigned to their management group and on days 14, 28, and 46 after being placed in respective management groups. The sera was sampled to differentiate T lymphocyte antigens (CD2+, CD4+, CD8+) on lymphocytes in peripheral blood. T-lymphocyte differentiation antigens were isolated and determined as previously described.[7] To review briefly, lymphocytes were isolated from whole heparinized blood using histopaque density gradient (d=1.077) and washed several times in HEPES-buffered RPMI 1640. Isolated cells were adjusted to a concentration of 2×10^6 cells per mL and then incubated with antibodies for the respective T-lymphocyte differentiation antigens. Antibodies used in this experiment were MSA-4 (CD2+), 74–12-4 (CD4+), and 76-2-11 (CD8+).[8,9] These antibodies are specific for single-antibody-positive cells, and dual-antibody-positive cells were not evaluated as a part of this experiment. Cells were then incubated with a fluorescein-isothiocynate-labeled goat anti-mouse IgG antibody. Flow cell cytometry analysis was conducted on an EPICS 753 flow cytometer. Data are represented as percentage of 10,000 cells expressing the respective T-lymphocyte differentiation antigens.

Experiment II: Simulated early weaning in a controlled research facility

Experimental design

Experiment II was conducted at the Samuel Brody Climatology Laboratory located in the Animal Sciences Research Center at the University of

Table 2

Performance of weaned pigs in experiment I

	Onsite commingled	Onsite sanitized	Offsite sanitized
Weaning weight (lb ± SEM)	**9.1 ±0.09**	**10.1 ±0.26**	**9.4 ±0.20**
Final weight (lb ± SEM)	**51.6 ±0.77[a]**	**54.1 ±1.10[b]**	**55.0 ±0.85[b]**
ADG (lb)			
Week 1	0.6	0.6	0.6
2	0.7	0.9	1.0
3	0.8	0.6	0.9
4	0.9	1.0	1.0
5	1.3	1.5	1.3
6	1.5	1.5	1.6
ADFI (lb)			
Week 1	0.7	0.8	0.9
2	1.0	1.1	1.2
3	1.1	1.0	1.2
4	1.5	1.6	1.7
5	2.3	2.3	2.3
6	2.8	2.8	3.1
F:G			
Week 1	1.2	1.2	1.6
2	1.4	1.3	1.3
3	1.4	1.9	1.5
4	1.6	1.7	1.8
5	1.7	1.5	1.7
6	1.9	1.9	1.9

a,b Numbers in the same row with different superscripts are significantly different ($P < .05$).

Table 3

Performance of weaned pigs from two different age groups in experiment I

	11–14 days old	14–18 days old
Weaning weight (lb ±SEM)	**9.12 ±0.1**	**10.06 ±0**
Final weight (lb ±SEM)	**51.58 ±0.7[a]**	**54.09 ±0.9[b]**
ADG (lb)		
Week 1	0.5	0.7
2	0.8	0.9
3	0.7	0.8
4	0.9	1.1
5	1.3	1.5
6	1.5	1.6
ADFI (lb)		
Week 1	0.7	0.8
2	1.0	1.2
3	1.1	1.2
4	1.5	1.7
5	2.1	2.5
6	2.8	3.0
F:G		
Week 1	1.5	1.2
2	1.4	1.3
3	1.5	1.6
4	1.8	1.6
5	1.6	1.7
6	1.9	1.9

ab Difference between age groups ($P < .05$).

Missouri-Columbia. This facility has rooms that are controlled for temperature and humidity, regulated photoperiod, and 100% fresh air with 15 air changes per hour. A "buffer room" precedes entry to each of these rooms to facilitate sanitization control.

Ten crossbred sows (Yorkshire × Landrace × Duroc) were obtained from the University of Missouri Swine Research Complex for this experiment. Each chamber housed five sows. Sows were brought into the facility 10 days before farrowing. Pigs were weaned at 12–15 days of age. As with experiment I, standard husbandry practices were used in baby pig processing and no vaccinations were administered. At weaning, the sows were removed from the facility. Half of the pigs (n = 40) remained in nonsanitized farrowing crates (5 × 7 ft, eight per crate, with the actual crate part removed), which were not cleaned once the sows were removed. The other half of the pigs (n = 40) were transferred to another chamber that had been sanitized and only restricted personnel observing stringent sanitization procedures were allowed to care for these pigs. Number of crates, space per crate, and number of pigs per crate were equal in both chambers.

Pigs were fed diets I and II as in experiment I (Table 1). Body weights and feed consumption were determined as in experiment I. Serology tests and immunologic determinations for T-lymphocyte differentiation antigens were conducted as in experiment I, except pigs sampled for immunologic parameters were bled prior to management group assignments and at 10, 24, and 38 days after weaning. Eighteen pigs (nine from each chamber) were used to assess health status, while 10 pigs (n = five per treatment) were used for immunologic determinations.

Statistical analysis

Data for both experiments were analyzed using the general linear models procedure of SAS.[10] SAS uses a two-tailed T-test to determine probability significance. For performance, the model included environment (nursery sites for experiment I and sanitized versus nonsanitized for experiment II), week, and the interaction of the two. Age was included in the model for experiment I only. For the immunologic data, the model included environment, bleed, and the interaction of the two. Significant differences among means were determined by least significant differences test with a 95% confidence interval.

Results

Pigs from both herds used for experiments I and II were found to have *S. suis* in 77%–88% of the animals tested. Other pathogens tested for but not detected included: PRRSV, *M. hyopneumoniae*, swine influenza, and *A. pleuropneumoniae*.

Neither performance nor percentages of T-lymphocytes expressing CD2+, CD4+, or CD8+ differentiation antigens were significantly affected by the on- and offsite facility or sanitization (Tables 2–4). Pigs from the onsite commingled group gained slightly less ($P < .05$) than the on- and offsite sanitized groups, although feed efficiencies were similar to those in the on- and offsite sanitized groups. Interestingly, weaning age had a significant effect on performance (Table 3) with the later-weaned pigs (14–18 days) gaining significantly ($P < .05$) better than the earlier-weaned pigs (11–14 days). No difference in feed efficiency was observed.

As in experiment I, no differences were found on performance or expression of T-lymphocyte differentiation antigens in sanitized versus nonsanitized conditions in experiment II (Tables 5–6). All pigs were weaned between 12-15 days of age so no age effects were tested.

Table 4

Percentage of lymphocytes expressing cluster of differentiation (CD) antigens CD2+, CD4+ and CD8+ in Experiment I

	Onsite commingled	Onsite sanitized	Offsite sanitized	*P* Age	*P* Room	*P* Age × room
CD2+						
Day 1	50.4 ± 5.3	40.2 ± 5.1	44.0 ± 5.1	.0001	.6437	.3789
14	44.5 ± 1.6	52.4 ± 4.2	50.5 ± 7.4			
28	62.3 ± 4.4	64.9 ± 2.4	68.2 ± 4.5			
46	45.0 ± 4.1	52.2 ± 3.8	50.3 ± 4.7			
CD4+						
Day 1	20.6 ± 3.6	14.9 ± 2.5	11.9 ± 1.1	.0035	.9686	.3216
14	19.8 ± 2.7	19.2 ± 2.6	20.0 ± 3.7			
28	21.4 ± 3.3	25.1 ± 2.6	26.1 ± 3.4			
46	21.8 ± 2.9	22.9 ± 3.6	24.4 ± 2.7			
CD8+						
Day 1	25.3 ± 4.6	21.1 ± 4.7	28.6 ± 6.7	.0003	.2969	.7531
14	22.0 ± 3.0	29.6 ± 2.6	30.1 ± 8.0			
28	39.6 ± 6.9	39.5 ± 4.3	47.8 ± 3.8			
46	31.1 ± 5.1	36.7 ± 6.9	31.9 ± 4.8			
CD4:CD8						
Day 1	0.9 ± 0.2	1.0 ± 0.4	0.6 ± 0.2	.0712	.3131	.6387
14	1.0 ± 0.3	0.7 ± 0.1	0.9 ± 0.2			
28	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.0			
46	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1			

Table 5

Performance of weaned pigs in experiment II

	Nonsanitized	Sanitized
Weaning weight (lb)*	**10.4 ±0.1**	**10.1 ±0.1**
Final weight (lb)*	**48.9 ±0.8**	**48.9 ±0.5**
ADG (lb)[a]		
Week 1	0.7 ±.0	0.8 ±.0
2	0.9 ±.0	1.0 ±.0
3	1.1 ±.1	1.2 ±.0
4	0.9 ±.1	0.8 ±.0
5	1.0 ±.0	1.0 ±.0
ADFI (lb)[a]		
Week 1	1.0 ±.1	1.0 ±.1
2	1.2 ±.0	1.3 ±.1
3	1.9 ±.0	1.8 ±.1
4	1.6 ±.1	1.5 ±.1
5	1.7 ±.1	1.6 ±.1
F:G		
Week 1	1.4 ±.2	1.3 ±.2
2	1.3 ±.1	1.3 ±.1
3	1.7 ±.1	1.5 ±.1
4	1.8 ±.2	1.9 ±.1
5	1.7 ±.2	1.6 ±.1

* Mean ± SEM.

Discussion

In the studies reported here, no significant differences were observed in ADG, F:G, and ADFI among pigs moved to offsite nurseries or in pigs maintained in a strictly sanitized environment as compared to pigs reared in an onsite sanitized environment, or in an environment where the sows are moved away from the pigs with the pigs remaining in the same crates in which they were farrowed. In both of these studies the pigs carried *S. suis* pathogens to all rooms and sites as determined by nasal swab surveillance conducted at the beginning and end of the studies. There was no evidence of other pathogens observed clinically or serologically. Clark, et al., isolated *S. suis* from pigs in all groups at all ages suggesting that pigs are exposed to and carry *S. suis* organisms possibly from day 1 of life.[11] This exposure most likely originates with the sow.

If the farm of origin is able to attain high-health status, which in these studies implies one primary bacterial pathogen with no endemic viral exposure, the advantages of site separation may not be observed. This places an increased emphasis on attaining a minimum level of pathogen exposure in the sow herd and making a management commitment to sound sanitation practices.

The results of the second experiment suggest that it may be possible to remove the sows from the crates at a set date to assure a consistent weaning age (14 days) and then transport pigs to the nursery all on the same day to fill the sanitized nursery room. There were no performance differences between pigs that were weaned into strictly sanitized nursery pens and those that remained in the same crates in which they were farrowed for the duration of the experiment. Further studies are required to determine the effects on pig performance when sows and litters remain in adjoining crates where pigs have been weaned.

Stahly, et al., have used T-lymphocyte differentiation antigens to support the classification of pigs into high- and low-immune stimulation categories.[3] In these studies high-immune stimulated pigs were

weaned at 19–21 days of age and remained in onsite nursery facilities that were close to the sow herd. Low-immune stimulated pigs were weaned at 10–14 days of age into an offsite nursery away from other pigs and away from the sow herd. The results of those studies suggested that pigs that maintain a low immune stimulation perform better than the high-immune stimulated pigs as indicated by ADG, ADFI, and F:G. In those studies, pigs were identified as having a low immune stimulation if the percentages of T-lymphocytes bearing CD4+ were 33.9% and those bearing CD8+ were 48.1%. Pigs were identified as having a high immune stimulation if the percentages of T-lymphocytes bearing CD4+ were 42.4% and those bearing CD8+ were 16.8%. In trying to establish an indicator of immune status, the ratio of CD4+:CD8+ was calculated. For the pigs identified as low immune stimulated, the CD4+:CD8+ ratio was 0.7, for the pigs identified as high immune stimulated, the ratio was 2.5.[14,15] These ratios have been used to suggest that a higher level of suppressor cells (CD8+) in the low-immune stimulation pigs indicates a low level of immune stimulation and the higher level of helper cells (CD4+) suggests immune stimulation in the high immune stimulation pigs.[12,13] The ratios remained consistent in those hogs until they reached a slaughter weight of 109 kg (240 lb).

Using T-lymphocyte differentiation antigens, which represent functionally different subpopulations, has been proposed as a means of determining a high- versus low-stimulated immune system.[3] The ability to classify populations of T-lymphocytes as helper (CD4+) or cytotoxic/suppressor (CD8+) cells using monoclonal antibodies and flow cytometry have served to further our understanding of the functional differences of the various cell populations of the immune system.[14, 15]

Table 6

Percentages of T-lymphocytes expressing CD2+, CD4+ and the CD4:CD8 ratio in experiment II

					P	
	Nonsanitized	Sanitized	PSE	E	W	E x W
CD2+						
Week 1	49.0^{ab}	54.4^{ab}	2.9	.01	.01	.01
2	46.8^{ab}	45.2^{a}				
3	44.6^{a}	45.3^{a}				
4	49.8^{ab}	69.2^{c}				
CD4+						
Week 1	19.1	21.9	1.3	.50	.11	.38
2	15.5	14.6				
3	22.6	17.6				
4	18.9	17.9				
CD8+						
Week 1	17.7^{d}	22.0^{d}	2.8	.02	.01	.02
2	23.0^{d}	19.1^{d}				
3	16.6^{d}	22.7^{d}				
4	24.3^{d}	49.8^{e}				
CD4:CD8						
Week 1	1.2	1.0	.1	.08	.10	.28
2	0.7	0.8				
3	1.5	0.8				
4	0.9	0.4				

a-e Values with different superscripts differ significantly ($P < .05$).
PSE pooled standard error.
E environment, non-sanitized vs sanitized.
W week.
E×W environment by week interaction.

Swine are unique in that the relative percentages of CD4+ and CD8+ cells are reversed from those reported for humans; the expression of CD8+ is greater than that of CD4+.[8] This is further reflected in the CD4+:CD8+ ratio, which has been reported to be 0.6 in mature miniature pigs, compared with 1.5 to 2.0 in humans.[9,16]

It is also interesting to note from one experiment that the percentages of CD4+ and CD8+ were similar in the thymus; however, the relative percentages changed significantly after the cells migrated to the blood and spleen.[7] In that particular experiment the CD4+:CD8+ ratio did not change substantially with age, but did differ with organ ($P < .0001$). In another experiment conducted by Becker and Misfeldt that looked at the effects of hot environments on the immune systems of sows and litters, the ratio of CD4+:CD8+ was three times higher ($P < .0001$) in the litters than in the sows.[17] Since these litters were healthy, the ratio was not considered an indicator of pathological conditions, as seen in humans. Although this ratio may be a good indicator of maturation in neonates, our data suggest that it may be an inappropriate indicator of immune status in SEW pigs.

In experiment I, we detected a difference due to age in the expression of T-lymphocyte differentiation antigens CD2+, CD4+, and CD8+ ($P < .05$). Overall, the percentages of T-cell lymphocytes expressing CD4+ and CD8+ tended to change over time and the ratio of CD4+:CD8+ tended to stabilize by the end of the experiment at 0.8. These findings are consistent with those of Becker and Misfeldt and suggest a maturation of the T-lymphocyte differentiation antigens on T-cell lymphocytes in pigs occurring over a 6–7 week period.[7,17] The porcine immune system is not fully developed at birth, and the expression of T-lymphocyte differentiation antigens seems to occur before the lymphocytes have the ability to respond to mitogens.

In these studies, we found no detectable differences in percentages of T-lymphocytes expressing differentiation antigens CD2+, CD4+, CD8+, or the ratio of CD4+:CD8+ due to the effects of room or environment or their interaction. This may be due to the fact that pathogen exposure and endotoxin exposure was minimal in both studies, realizing that the stimulation of these T-lymphocyte subpopulations occurs as a result of various stimuli and microorganisms.[18] In an experiment conducted by Richerson and Misfeldt, it was demonstrated that environmental influences in laboratory animals can influence host immunologic responses.[19] This experiment also stated that exposure of an animal to microbial, viral, or parasitic agents may increase natural killer granular lymphocytes. Pathogens appear to have the capacity to induce cytokines which can result in the modulation of the host's immune

system. It is possible that T-lymphocyte differentiation antigens are not the method of choice for determining levels of immune status in the pig. Other methods to determine in-vivo immune status should be pursued.

The results reported here suggest that production systems that practice good sanitation and conscientious biosecurity measures to establish a low pathogen exposure may not demonstrate significant performance results in nursery pigs moved offsite at early ages (<14 days). These results also suggest that using single-cell surface antibodies to differentiate T-lymphocyte antigens are not appropriate indicators of immune status in SEW pigs with minimum pathogen exposure. The use of dual positive antigens may have resulted in different findings, as pigs have a high percentage of dual-positive cells from single-positive CD4 and CD8. In swine, T lymphocytes consist of CD2+ CD4+ CD8+, CD2+ CD4+ CD8-, CD2+ CD4- CD8+, and CD2+ CD4- CD8- subpopulations.[20,21]

Implications

- In a swine production unit that has minimized pathogen exposure, the advantages of production improvements may not exceed the additional capital and labor required to move the nursery phase of production offsite.
- The use of T-lymphocyte differentiation antigens (CD4+, CD8+) and the ratio of these antigens may not be proper indicators of immune status in herds able to attain a minimum level of pathogen exposure.
- The results from the first experiment suggest that pigs weaned at 14–18 days of age gained significantly better than those pigs that were weaned at 11–14 days of age.
- Pigs may be weaned from sows while remaining in the crates with no adverse effects on performance if all the sows are removed at the same time. Further studies are required to determine the effects on pig performance if sows and litters remain in adjoining crates.
- Further studies are required in the application of T-lymphocyte differentiation antigens in the pig, as there are several differences between the expression of these antigens in pigs versus humans. The normal maturation process of these differentiation antigens in pigs and the effects of disease on this maturation process will require further studies.

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