Lymphocyte immunotherapy performed in pregnant Gilts and Sows did not affect preweaning mortality in the corresponding litters.

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Summary

This study was designed to test if lymphocyte immunotherapy (LI) of pregnant Gilts and sows could improve preweaning mortality rates. In a commercial farm, Gilts (n=61) first (n=57) or second parity Sows (n=42) were selected and random assigned to treatment with saline (control group) or LI with lymphocytes obtained from multiparous sows, Boar, or growth-fattening pigs donors (alloimmunized groups). Treatments were performed at day 85 of pregnancy. Litter size and weight were evaluated by multifactor analysis of variance, whereas farrowing and preweaning mortality rates were analyzed by Mann-Whitney test. All traits evaluated at farrowing and at weaning did not significantly differ between groups (P>0.05); when data from all immunized groups were altogether compared with control group (P>0.5). Enteritis tended to be the most common cause of morbidity (P = 0.053). Mortality rate tended to increase in piglets from alloimmunized females (P = 0.12). The results suggest that LI of pregnant cross-bred Gilts, first or second-parity sows did not affect preweaning mortality in piglets from subsequent lactation.

Key Words: Farrowing, Litter Size, Litter Weight, Mortality, Swine, Weaning.

Introduction

Farrowing and preweaning mortality of infectious etiology affects the economy of pig industry worldwide (38, 40). Several efforts have been made to reduce these problems which include management schedules (12, 38), both specific (14) and non-specific immunizations of pregnant dams and immuno-stimulation of suckling piglets (1, 4, 10, 11, 34, 42). Litters born from sows inoculated with the K88 Escherichia coli antigen had significantly more colostrum-derived anti-K88 specific antibodies in serum than control litters, and antibody titers significantly correlates with low mortality rates (10). Similar results have been achieved by immunization of pregnant sows with crude or purified Transmissible Gastroenteritis Virus (TGE) antigens or its antigenic-related Porcine Respiratory Coronavirus (PRC) antigens (1, 4, 11). Based on evidence on the effect of adoptive transfer of immunity (31) -for a detailed review see Maldonado et al. 1997 (20), we hypothesize that lymphocytes from adult healthy pigs could in some way affect the immunological competence of piglets born from LI-treated dams, resulting in decreased preweaning mortality (17). Thereafter, the purpose of the present study was to evaluate if LI should improve preweaning mortality rates when inoculated to pregnant first and second parity Gilts and Sows.

Materials and methods

This study was carried out in a commercial farm located 15 Km from Medellín, in the state-like of Antioquia (Colombia) located at 2000 m over sea level. One hundred and sixty pregnant cross-bred Gilts, first and second parity Sows, were selected and randomly assigned to experimental groups, with the inclusion criteria of 85 days of pregnancy.

Experimental groups. Sixty-one F1 Gilts from 14 different genetic background (predominantly Landrace) were assigned to receive a subcutane-
ous inoculation with: Group 1, LI with lymphocytes obtained from multiparous Sows (n=16); Group 2, LI with lymphocytes obtained from boars (n=15); Group 3, LI with lymphocytes obtained from growth-fattening pigs (n=16); and Group 4, inoculation with saline solution (n=15).

The same schedule was performed with first parity - Group 1, n=14; Group 2, n=14; Group 3, n=14; and Group 4, n=14; or second parity Sows - Group 1, n=14; Group 2, n=14; Group 3, n=14; and Group 4, n=14.

Pregnant Gilts and Sows allocated in individual pregnancy stalls were inoculated with their respective treatment at 85 d of pregnancy, by a subcutaneous inoculation in the posterior ear fold. Pregnancy was allowed until completion and females were moved to farrowing barns at 105 days of pregnancy. Litter size and weight at farrowing and at weaning and mortality were recorded and further evaluated.

Peripheral Blood Mononuclear Cell (PBMC) Isolation. Multiparous Sows, Boars, or Growth-fattening pigs from the same farm were used as PBMC donors. Blood (50mL) was collected in sterile-heparinized glass vacutainers (Monoject, Sherwood, St Louis, MO) by jugular puncture with 19G x 1 1/2" sterile needle, and transported to the laboratory at 4°C. PBMC were obtained by gradient (Hystopaque, D=1077; Sigma, St. Louis, MO) centrifugation as previously described (19). Individual doses of 1 ml of pooled PBMC (8 x 10^6 - 1.5 x 10^6 cells) from each donor specification were packed out in individual eppendorf vials and transported to the farm under refrigeration at 4°C until inoculation of the females, the next day following sampling.

Gilts and Sows were fed a conventional dry sow diet which met or exceeded all nutrient requirements. At day 110 of gestation sows were fed an additional 1 kg of feed daily. After farrowing, sows were fed a lactation diet which met or exceed all nutrients requirements for lactating Sows when the daily lactation ration was divided into two meals, fed at 0800 and 1600 hours.

Recover of litter data. Data from total litter and alive litter, stillborn and mummies, as well as total weight from alive litter were registered immediately after farrowing. During lactation only intra-treatment cross-fostering was performed. At weaning, data included lactation length, number of weaned piglets, and total litter weight.

Preweaning Mortality. Care was taken to record preweaning mortality and its possible causes. This resulted in classification of mortality according to clinical signs and postmortem evaluation of died piglets with diarrhea, respiratory disease, heart failure, or non-infectious etiology.

Statistical analysis. Farrowing and preweaning mortality rates were analyzed by Mann-Whitney test. Litter size and weight at farrowing and at weaning were analyzed by multifactor analysis of variance including all factors that have an effect on litter size and weight at farrowing and at weaning as previously reported for Gilts (21, 22, 24) first-parity (8, 23) or multiparous Sows (9). The least squared means model proposed by Harvey (1975), was used to evaluate the differences between groups (18). The following adjustments and categorization of variables were performed for the analysis:

1. Length of previous lactation: Group 1, females until 30 days of lactation; and Group 2, females with more than 31 d of lactation.
2. Weaning-to-conception interval: Group 1, sows until 5 days; and Group 2, females with more than 5 days.
3. Litter size at previous weaning: Group 1, females that weaned up to 8 piglets; and Group 2, females that weaned more than 8 piglets.
4. Running season at farrowing: Group 1, females farrowed between March and July (running season); and Group 2, females farrowed between August and September (heat season).
5. Age at farrowing (for Gilts): Group 1, females farrowed until 334 days old; Group 2, females farrowed within 335 to 365 days old; and Group 3, females farrowed with more than 365 days.
6. The genetic background of females was classified as follows: Group 1, German Landrace (L); Group 2, Large White (LW); Group 3, Belgium Landrace (LB); Group 4, Pietrain (P); Group 5, Duroc (D); Group 6, L x P; Group 7, LW x D; Group 8, L x LW; Group 9, L x D; Group 10, 1/4 L x 1/4 LW x 1/4 D x 1/4 P; Group 11, 1/4 LW x 1/4 L x 2/4 D; Group 12, 1/2 Ham x 1/2 LB; Group 13, P x D; and Group 14, Dekalb background.
7. The possible cause of mortality during lactation was classified as: Group 1, non-infec-
tious etiology; Group 2, Diarrhea; Group 3, Respiratory symptoms; Group 4, Heart failure.

Results

A total number of 160 pregnant females were included in the study between March to November, 1996. All Gilts and Sows finally succeed in a litter with at least one piglet born alive. Upon observation no Gilts or Sow aborted or experience any other sign of complications.

Because no statistically significant differences were observed between sources of allogeneic lymphocytes (multiparous sows, Boars or Growth-fattening pigs), data from these treatment groups were pooled (Lymphocyte immuno-therapy, LI) and compared with control group (Saline, SSS). No statistically significant differences (P>0.05) were observed between LI and SSS groups for any of the traits evaluated (Table 1).

<table>
<thead>
<tr>
<th>Parity</th>
<th>treatment’</th>
<th>n</th>
<th>Total litter</th>
<th>Alive litter</th>
<th>Litter weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>LI</td>
<td>47</td>
<td>9.98 ± 0.5</td>
<td>8.56 ± 0.47</td>
<td>12.08 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>10.68 ± 0.71</td>
<td>9.46 ± 0.7</td>
<td>12.14 ± 0.57</td>
</tr>
<tr>
<td>Second</td>
<td>LI</td>
<td>43</td>
<td>9.20 ± 0.45</td>
<td>8.72 ± 0.34</td>
<td>12.65 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>8.58 ± 0.67</td>
<td>8.79 ± 0.59</td>
<td>13.58 ± 0.64</td>
</tr>
<tr>
<td>Third</td>
<td>LI</td>
<td>34</td>
<td>9.83 ± 0.41</td>
<td>9.16 ± 0.39</td>
<td>14.20 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8</td>
<td>9.02 ± 0.78</td>
<td>8.79 ± 0.78</td>
<td>16.39 ± 0.81</td>
</tr>
</tbody>
</table>

*The values represent the pooled data from females that were inoculated with PBMC from multiparous Sows, Boars and growth-fattening piglets.

First-parity Sows. Total litter did not significantly differ (p>0.05) between LI or SSS groups (Figure 1). Sows with German Landrace and Large White background had the greater value for total born piglets (p<0.05). Sows that first farrowed before 335 days of age gave birth significantly (P<0.05) less piglets than sows farrowed having more than 335 days old. Litter alive did not significantly differ between groups (P>0.05), and the only factor that had effect in the model for this parameter (P< 0.05) was epoch at farrowing (Figure 2).

Epoch (P = 0.13) and genetic background of the sow (P = 0.15) tended to affect litter weight at farrowing. Litters from parity 1 sows had the lowest weight whereas litters from parity 3 sows were the heaviest, with not statistically significant differences (P>0.05). Similarly, not statistically significant differences (P>0.05) between treatments were observed for litter size and weight at weaning (Figure 3).
Second-parity Sows. Total litter (Figure 1) alive litter (Figure 2) and litter weight (Figure 3) did not significantly differ (P>0.05) between groups. Number of weaned piglets in previous lactation tended (P = 0.06) to affect total litter, whereas no effect were observed for alive litter. In addition, no significant differences were observed for litter size (Figure 4) or weight at weaning (Figure 5) between groups. However, number of weaned piglets in previous lactation (P<0.01) and epoch of farrowing (P<0.05) had a significant effect in the model. Weaning weight of previous lactation tended to have effect in the model (P = 0.08). Litter weight at farrowing was significantly affected by litter alive (P>0.01), epoch of farrowing (P<0.05), and tended to be affected by weaning weight in previous lactation (P = 0.056).

Third-parity sows. No statistically significant differences were found for total litter (Figure 1) and alive litter (Figure 2) in third-parity Sows (P>0.05). Factors with a significant effect in the model for total litter and alive litter were weaning weight in previous lactation (P<0.01), and epoch of farrowing (P<0.05). Not statistically significant differences between groups were found for litter weight (Figure 3). Factors with a significant effect in the model were genetic background of the Sow (P<0.05), alive litter (P<0.01) and previous lactation length (P = 0.07). Females from Landrace background had heavier litters (16.2 ± 0.6 Kg) than females form the remaining genetic background (14.1 ± 0.6). No statistically significant differences (P>0.05) were found for litter size (Figure 4) and weight (Figure 5) at weaning.

Preweaning mortality rates. No statistically significant differences (P>0.05) between groups were found for preweaning mortality rates, neither in first nor in second or third-parity Sows (Figure 6) with first-parity Sows having the greatest mortality rate without statistically significant differences (P>0.05). However litters form alloimmunized females tended to have greater mortality (2.58 %) than litters from control females (1.62 %).

Causes of preweaning mortality. Because not statistically significant differences were found between LI and SSS groups, data from LI and SSS first, second and third-parity females were analyzed altogether. The results indicated that diarrhea tended (P = 0.053) to cause the greater mortality rate (3.07 ± 0.36%) rather than respiratory (1.5 ± 0.53%) or non-infectious (1.72 ± 0.74) diseases.
Discussion

The results of the present study indicate that, when administered subcutaneously to pregnant Gilts, first-parity or multiparous Sows, lymphocyte immunotherapy do not affect neither litter size and weight at farrowing and at weaning nor preweaning mortality rates. Diarrhea is one of the most common cause of mortality in piglets, severely affecting pig industry in our country (26, 29, 40) as well as others (3). Several management schedules have been tested in order to reduce the detrimental effect of this pathology, including conventional preventive immunizing protocols (36) and booster of pregnant females with pathogen-specific antigens (1, 3, 14). Because of the high capability of the late pregnancy-immune system to increase immunoglobulin-producing plasma cells in mammary gland (32, 33, 35), immunizing protocols performed during the last third of pregnancy try to improve the maternal humoral immune responses in order to confer a high protective calostrol immunity to the litters.

Lymphocyte immunotherapy is an immunological protocol designed for the treatment of Recurrent Spontaneous Abortion (RSA) of alloimmune ethiology in humans, with a wide range of beneficial results represented as healthy newborns (2, 6, 25, 28, 37). The protocol has also been used in animal models (5). However, the mechanisms of LI still yet to be elucidated (7, 16, 30). On the other hand, in animal models it has been established that the protective immune response against several kind of pathogens is mediated by both the activation of type 1 helper T-Cells (Th1 cells), required for the induction and activation of T cell-mediated effector mechanisms, and the activation of type 2 helper T-Cells (Th2 cells) required for the induction of humoral immune responses (13, 15). Because lymphocytes actively pass from the mother to the piglets throughout calostrum (39, 41), we considered the possibility to test if LI could be a new alternative to induce a calostrum-mediated protective immune response in suckling piglets. Our working hypothesis proposed that lymphocytes from allogeneic source -multiparous sows, boars, or growth fattening piglets, would differ in their immunizing potential because of differences in the exposure of each group of donors to antigenic challenge. Accordingly, adult healthy animals exhibit a resistant status against the common pathogens of the farm. Also their peripheral lymphocytes would function as an appropriate chal-

enge to confer protective immunity to the suckling piglets by previous immunization of the pregnant dam.

However, the results of the study showed that LI did not affect preweaning mortality rates. Because of several non consistent results that would indicate a beneficial or detrimental effect of LI in some of the traits evaluated, we conclude that this immunological approach did not function as an effective way to reduce morbidity or mortality rates in suckling piglets.

Total litter, litter alive and litter weigh at farrowing was measured only with routine procedures because it was less probable that this parameter could be affected by LI when inoculated at the last third of pregnancy except for a possible effect on mortality rates at farrowing. However, no complications were observed between experimental groups, in such a way that we can conclude this immunological approach is not harmful for the dam or its litter.

On the other hand the immune response of the females or the suckling piglets after the immunization schedule was not evaluated, will be an important indicator of the response to the challenge to be evaluated. Furthermore experiment controlled studies will stress the possibility to confer adoptive transfer of immunity by using specific antigens that could be controlled, and evaluated by in vitro protocols evaluating the induced immune response.

We have performed a set of experiments designed to evaluate the effect of LI on reproduction in pigs. We used different protocols of immunization including the subcutaneous or intraterine route of administration, previous to mating challenge of Gilts and Sows, but none of these studies consistently showed an effect of the treatment on reproductive performance in pigs (20-24, 27).

Conclusion

We conclude that LI do not affect preweaning mortality of the corresponding litter when administered to pregnant Gilts, first or second-parity Sows, from commercial swine herds. The protocol should be tested under experimental farm conditions, and the evaluation of specific immune responses against selected specific pathogens, or a set of conventional antigens, should be made.
Acknowledgments

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Resumen

El presente estudio se diseñó para evaluar si la terapia con linfocitos alogénicos (LI) aplicada en cerdas gestantes podía reducir las tasas de mortalidad en lactancia de sus respectivas camadas. En una granja porcicola comercial se seleccionaron cerdas gestantes (80-90 días) de primera (n = 61), segunda (n = 57) y tercera (n = 42) gestación, y se asignaron al azar al tratamiento con 1) linfocitos alogénicos (grupos inmunizados) obtenidos de cerdas gestantes, machos reproductores o cerdos de engorde; o 2) con solución salina (grupo control). Los datos del tamaño y el peso de la camada al nacer y al destete de registraron y evaluaron por análisis de varianza multifactorial mientras que la mortalidad al nacer y en la lactancia se evaluaron por prueba de Mann-Whitney. Ninguno de los parámetros evaluados de la camada al nacer y al destete, presentaron diferencias estadísticamente significativas entre los grupos aloinmunizados y el grupo control (P>0.05). La enteritis mostró tendencia a ser la mayor causa de mortalidad en lactancia (P = 0.053). La mortalidad presentó tendencia a ser mayor en los lechones de las cerdas aloinmunizadas (P = 0.12). Los resultados sugieren que la terapia con linfocitos alogénicos, administrada en cerdas gestantes de primera, segunda o tercera gestación, no afecta la mortalidad predestete de la subsecuente camada.

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