

# Litter Characteristics of Gilts Artificially Inseminated with Transforming Growth Factor- $\beta$

Michelle Rhodes, Joel H. Brendemuhl, Peter J. Hansen

Department of Animal Sciences, University of Florida, Gainesville, FL, USA

## Keywords

Transforming growth factor- $\beta$ , pigs, litter size, pregnancy, fertility

## Correspondence

Peter J. Hansen, Department of Animal Sciences, University of Florida, PO Box 110910, Gainesville, FL 32611-0910, USA.  
E-mail: hansen@animal.ufl.edu

Submitted October 06, 2005;  
accepted October 31, 2005.

## Citation

Rhodes M, Brendemuhl JH, Hansen PJ. Litter characteristics of gilts artificially inseminated with TGF- $\beta$ . *Am J Reprod Immunol* 2006; 56:153–156

doi:10.1111/j.1600-0897.2006.00423.x

## Problem

Semen is a rich source of transforming growth factor- $\beta$  (TGF- $\beta$ ) and it has been proposed that this molecule promotes embryonic survival by modifying immune responses to promote tolerance toward paternal antigens and by inducing release of cytokines that promote embryonic development. The role of TGF- $\beta$  was tested using pigs by evaluating whether its addition to washed sperm improves conceptus survival and fetal growth.

## Methods of study

At estrus, gilts were artificially inseminated twice at 12-hr intervals with 100 mL of either washed semen resuspended in a commercial semen extender supplemented with 2 mg/mL of gelatin or washed semen in the same extender containing 65 ng/mL of TGF- $\beta$ 1. Three boars were used as semen donors. At day 80 ( $\pm$ 4 days) of gestation, gilts were sacrificed and reproductive tracts harvested.

## Results

Treatment had no effect ( $P > 0.10$ ) on total or live fetuses per litter, implantation rate, fetal survival or percentage of corpora lutea resulting in live fetuses at day 80. Insemination with TGF- $\beta$ 1 also did not affect total or average fetal weight or total placental weight. There was a tendency ( $P = 0.09$ ) for average placental weight of live fetuses to be lower for pregnancies established in gilts treated with TGF- $\beta$ 1. Also, placental efficiency (mass of fetus/mass of placenta) was greater ( $P < 0.05$ ) for pregnancies established in gilts treated with TGF- $\beta$ 1. The high fertility in control gilts (80% implantation rate and 11.5 live fetuses per litter) is indicative that soluble seminal factors are not necessary for the establishment of pregnancy.

## Conclusions

Within the ranges tested, concentration of TGF- $\beta$  in the fluid phase of the inseminate is not an important determinant of conceptus survival or fetal and placental growth to day 80 of gestation in the pig.

## Introduction

Regulatory molecules in semen have been proposed to play an important role in modifying the environment of the female reproductive tract. Among the proposed actions are promotion of maternal immune

tolerance toward the conceptus, activation of macrophages involved in tissue remodeling and implantation, release of cytokines that stimulate embryonic development, and long-term actions that affect fetal growth.<sup>1</sup> Transforming growth factor- $\beta$  (TGF- $\beta$ ) is one component of seminal plasma that has been

implicated in regulating maternal physiology. The growth factor is present in human semen at total concentrations of about 300 ng/mL, primarily in the latent, inactive form, with  $\beta 1$  and  $\beta 3$  being present in approximately equal concentrations and  $\beta 2$  being less abundant.<sup>1</sup> In mice, TGF- $\beta$  increases the release of granulocyte-macrophage colony-stimulating factor (GM-CSF) from cultured endometrial epithelial cells and, following intrauterine deposition, accumulation of GM-CSF in uterine fluids and macrophages and eosinophils in endometrium.<sup>2</sup> Treatment of human endometrial epithelial cells with TGF- $\beta$  increased mRNA for interleukin (IL)-1 $\beta$ , IL-6 and leukemia inhibitory factor.<sup>3</sup> TGF- $\beta$  also regulates lymphocyte function by inhibiting T-cell proliferation, blocking differentiation of Th1 and Th2 lymphocytes, and promoting differentiation of Treg cells.<sup>4</sup> Maturation of dendritic cells is also inhibited by TGF- $\beta$ .<sup>5</sup> These actions of TGF- $\beta$  could perhaps block maternal immune responses against sperm and embryonic antigens in common with semen antigens. Actions of TGF- $\beta$  to remodel tissues<sup>6</sup> could conceivably participate in the preparation of the endometrium for implantation.

As in other species examined, exposure to semen induces leukocyte infiltration into the reproductive tract of the pig.<sup>7-9</sup> Cytokine production in the reproductive tract was enhanced upon exposure to seminal plasma<sup>9</sup> (although not to whole semen with killed sperm<sup>10</sup>) and there is evidence that mating induces an immune response in the lymph nodes draining the uterus.<sup>7</sup> Increasing litter size remains an important economic goal of the swine industry. Regulation of embryonic survival represents one means to do so because the greatest occurrence of pregnancy loss is prior to day 30 of gestation.<sup>11</sup> The potential exists for increasing litter size in pigs by modifying immune responses against sperm because increases in litter size have been obtained by intrauterine exposure of gilts to semen with killed sperm before mating.<sup>12</sup> The objective of the present study was to test whether the addition of TGF- $\beta$  to semen extender increases litter size, placental weight, and fetal weight in artificially inseminated gilts.

## Materials and methods

### Animals

This experiment was performed at the University of Florida Swine Research Unit using a group of 20

pre-pubertal gilts. Gilts were maintained in five groups, separated by weight and handled similarly in all respects. When all members of a group reached approximately 150 days of age or 80 kg, animals were injected subcutaneously with 5 mL of PG600 (400 IU of pregnant mare's serum gonadotropin and 200 IU of human chorionic gonadotropin; Intervet, Millsboro, DE, USA). Gilts were then exposed to two boars daily until standing estrus was observed.

At least 14 days after the onset of estrus, gilts were moved to individual pens and began dietary supplementation for 14 days of 15 mg/day of altrenogest (Matrix, Intervet). Following altrenogest withdrawal, gilts were returned to group housing and estrus was checked twice daily. At the next observed estrus, gilts were artificially inseminated twice, once every 12 hr beginning at either 12 hr ( $n = 16$ ) or 24 hr ( $n = 4$ ) after observation of estrus, with 100 mL of either washed semen from one of the three boars suspended in a BTS boar semen extender (Continental Plastic Corp., Delavan, WI, USA) supplemented with 2 mg/mL of enzyme immunoassay (EIA) grade gelatin from pig skin (Bio-Rad, Richmond, CA, USA), or washed semen suspended in BTS boar semen extender containing 2 mg/mL of gelatin and 65 ng/mL of human recombinant TGF- $\beta 1$  (R&D Systems, Minneapolis, MN, USA). The treatment each gilt received was determined randomly ( $n = 11$  controls and 9 treated with TGF- $\beta 1$ ). Each gilt was inseminated using semen with the same treatment and from the same boar on both occasions.

### Semen Handling Procedure

Fresh boar semen was collected via the gloved hand method. The ejaculate was measured for initial volume and was diluted 1:1 (v/v) with BTS boar semen extender. All subsequent steps were performed at room temperature. Diluted ejaculate was centrifuged at  $400 \times g$  for 30 min. The cellular fraction was resuspended in extender and the supernatant was diluted further with extender and centrifuged again at  $400 \times g$  for 30 min. The two cell pellets were pooled, washed again in extender, and then resuspended in extender supplemented with 2 mg/mL of EIA grade gelatin from pig skin. The volume varied from 200 to 600 mL to ensure sufficient 100-mL inseminate doses for all gilts to be inseminated with that ejaculate. The final concentration and motility of sperm were determined

on a Hamilton Thorn Sperm Motility Analyzer (Beverly, MA, USA) and confirmed via visual observation. The number of sperm per inseminate averaged  $6.8 \times 10^9$  sperm (range  $3 \times 10^9$ – $17 \times 10^9$ ) and was not different between treatments. Semen not used immediately after washing was stored at 18°C and discarded after 72 hr. When added, the TGF- $\beta$ 1 was added to the inseminate immediately before insemination.

### Data Collection at Slaughter

At day 80 of gestation ( $\pm 4$  days), gilts were sacrificed and reproductive tracts harvested. Maternal dressing weight and number of corpora lutea (CL) were recorded for each gilt. For each fetus, placental tissues were dissected from fetuses and weights were determined for individual fetuses and placental tissues. From these measurements, the following calculations were made:

Live fetuses = number of total fetuses – number of mummified fetuses

Implantation rate =  $100 \times (\text{number of total fetuses} / \text{number of CL})$

Fetal survival =  $100 \times (\text{number of live fetuses} / \text{number of total fetuses})$

Percent of CL yielding live fetuses =  $100 \times (\text{number of live fetuses} / \text{number of CL})$

Placental efficiency = weight of live fetuses / weight of placentae of live fetuses

### Statistical Analyses

All statistical analyses were performed by least-squares analysis of variance using the GLM procedure of the Statistical Analysis System for Windows 9.0 (SAS Institute, Cary, NC, USA). Data are presented as least-squares means  $\pm$  standard errors.

## Results

### Litter Size and Conceptus Survival

The number of CL per pig averaged 14.2–14.3. Of these presumed ovulations, 80–84% resulted in an implantation and 79% resulted in a live fetus at day 80 of gestation. There were no significant differences between groups in total fetuses per litter, live fetuses per litter, implantation rate, fetal survival or percentage of CL resulting in live fetuses at day 80 (Table I).

**Table I** Effect of Transforming Growth Factor- $\beta$ 1 (TGF- $\beta$ 1) in the Inseminate on Litter Size, Implantation Rate and Fetal Survival

	Control	TGF- $\beta$
Number of corpora lutea (CL)	14.3 $\pm$ 0.7	14.2 $\pm$ 0.6
Litter size, all fetuses	12.0 $\pm$ 0.6	11.6 $\pm$ 0.6
Litter size, live fetuses	11.5 $\pm$ 0.7	11.5 $\pm$ 0.6
Implantation rate (%)	80.1 $\pm$ 3.0	84.3 $\pm$ 2.9
Fetal survival (%)	97.6 $\pm$ 2.4	97.3 $\pm$ 2.5
CL yielding live fetuses (%)	79.1 $\pm$ 3.0	79.3 $\pm$ 2.6

**Table II** Effect of Transforming Growth Factor- $\beta$ 1 (TGF- $\beta$ 1) in the Inseminate on Fetal and Placental Weight Considering Live and Dead Fetuses

	Control	TGF- $\beta$
Fetal weight, total (g)	5215 $\pm$ 348	4971 $\pm$ 315
Fetal weight, average (g)	439 $\pm$ 19	428 $\pm$ 17
Placental weight, total (g)	2556 $\pm$ 163	2256 $\pm$ 148
Placental weight, average (g)	215 $\pm$ 8	197 $\pm$ 8

**Table III** Effect of Transforming Growth Factor- $\beta$ 1 (TGF- $\beta$ 1) in the Inseminate on Fetal and Placental Weight, Considering Only Live Fetuses

	Control	TGF- $\beta$
Fetal weight, total (g)	5202 $\pm$ 353	4964 $\pm$ 320
Fetal weight, average (g)	446 $\pm$ 17	435 $\pm$ 15
Placental weight, total (g)	2546 $\pm$ 165	2246 $\pm$ 149
Placental weight, average (g)	219 $\pm$ 9	199 $\pm$ 8 $\dagger$
Placental efficiency	2.0 $\pm$ 0.1	2.2 $\pm$ 0.1*

$\dagger P = 0.09$ ; \* $P < 0.05$ .

### Fetal and Placental Characteristics

Insemination with TGF- $\beta$ 1 did not affect total or average fetal weight or total placental weight, regardless of whether data were calculated for all fetuses (Table II) or live fetuses only (Table III). There was a tendency ( $P = 0.09$ ) for average placental weight of live fetuses to be lower for pregnancies established in gilts treated with TGF- $\beta$ 1; average weights were 219  $\pm$  8.5 g for controls and 199  $\pm$  8 g for gilts treated with TGF- $\beta$ 1 (Table III). As fetal weights were not affected by treatment despite the reduced placental weights, placental efficiency (mass of fetus/mass of placenta) was greater ( $P < 0.05$ ) for pregnancies established in gilts treated with TGF- $\beta$ 1 (Table III).

## Discussion

Results from this study failed to support the idea that TGF- $\beta$  or other components of seminal plasma are important determinants of conceptus survival and growth in pigs.

The concentration of TGF- $\beta$  in the inseminate used for control gilts was likely to be very low with the only source of TGF- $\beta$  being molecules that were absorbed to the surface of ejaculated sperm.<sup>13</sup> Despite the low concentrations of TGF- $\beta$  deposited with insemination in these gilts, as well as low concentrations of other soluble seminal plasma factors, fertility was high in control gilts as indicated by the 80% implantation rate and a litter size of 11.5 fetuses.

It is possible that the high fetal survival rates in the control gilts prevented a beneficial effect of TGF- $\beta$  because uterine capacity was reached in controls. The concentration of TGF- $\beta$  added to washed sperm in this experiment (65 ng/mL) is within the physiological range of concentrations found in semen, but it cannot be ruled out that the addition of TGF- $\beta$  at higher concentrations or using a different subtype might have been beneficial. TGF- $\beta$  in boar seminal plasma is mostly in the biologically active form and ranges in concentration from 28 to 495 ng/mL with about equal amounts of TGF- $\beta$ 1 and - $\beta$ 2.<sup>14</sup> In any case, fertility is clearly not compromised by low concentrations of TGF- $\beta$  in the inseminate (or other factors in seminal plasma) as indicated by the 80% implantation rate and litter size of 11.5 live fetuses occurring in control gilts.

One positive effect of TGF- $\beta$  was an increase in placental efficiency for live fetuses. Specifically, placentae were slightly smaller in the TGF- $\beta$  group even though fetal size was unaffected. This result could reflect an action of TGF- $\beta$  to increase transplacental transport, so that the placenta did not need as much growth to meet the needs of the fetus, or an action of TGF- $\beta$  to reduce placental growth with the result that efficiency of transplacental transport was increased to meet the demands of the conceptus.

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