Uterine immune reaction and reproductive performance of sows inseminated with extended semen and infused with pooled whole dead semen

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ABSTRACT: The objective of this study was to investigate the effect of infusing whole dead semen (WDS) after AI with diluted commercial semen on uterine inflammatory reaction and embryonic survival rate in gilts. Sixty Yorkshire-Landrace gilts were assigned at their second estrus to one of the following AI treatments: 1) commercial semen adjusted to $1 \times 10^9$ sperm cells (S1) per dose, followed by an infusion of 80 mL of WDS (S1-WDS); 2) S1 followed by an infusion of 80 mL of Beltsville Thawing Solution (S1-BTS); 3) commercial semen adjusted to $3 \times 10^9$ sperm cells (S3) per dose, followed by an infusion of 80 mL of BTS (S3-BTS); and 4) a negative control group, in which gilts received two infusions of 80 mL of BTS (BTS). Two days after the first AI, eight gilts from Groups 1, 2, and 4 were slaughtered and reproductive tracts were collected. One horn was cut open longitudinally along the antimesometrial aspect and endometrial samples were taken and immediately frozen for analysis of messenger RNA (mRNA) abundance for inflammatory cytokines and growth factors. The other horn was flushed with 20 mL of PBS, and the contents of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β1) were determined by ELISA. On d 25 after AI, gilts from Groups 1, 2, and 3 were slaughtered and their reproductive tracts were collected to evaluate the number of fetuses and corpora lutea. On d 2 after the first AI, only TGF-β1 was detected in the flush of all gilts, and no difference was observed between S1-WDS, S1-BTS, and BTS gilts. Endometrial levels of IFN-γ and interleukin (IL)-6 mRNA were marked in all gilts, but they were not affected by the AI treatments, whereas the mRNA abundances for IL-1 and IL-2 were negligible. Infusions of WDS or BTS after a fertile AI did not affect IGF-I, IGF-I receptor, or IGF-II mRNA levels compared with gilts infused with BTS only, whereas the mRNA abundance for the IGF-II receptor was decreased ($P < 0.05$) in WDS-infused gilts. In gilts inseminated with S1 doses, infusion of WDS did not affect the number of live embryos. Although infusions of WDS did not affect the mRNA level and secretion of the cytokines measured and did not improve embryonic survival rates, further studies are needed to better understand the influence of semen composition on the uterine response after mating.

Key Words: Artificial Insemination, Cytokines, Pigs, Seminal Plasma, Uterus


Introduction

In Canada and in many other countries, using three billion spermatozoa per dose of AI is recommended. Several attempts have been made to decrease the amount of spermatozoa per dose of AI to 1 billion, but the effect on fertility rate is generally detrimental (Pedersen, 1991; Woelders, 1991). New strategies that are based on the physiology of sperm transport and other events within the female tract are under development to minimize sperm losses (Rath, 2002). However, the dilution of semen to 1 billion spermatozoa or less per dose also reduced the concentration of seminal plasma, which contains factors that can influence not only the potential of the spermatozoa to fertilize the eggs, but also the regulation of uterine immune reactions against spermatozoa.

In swine as in other mammals, deposition of semen into the female reproductive tract induces a cascade of cellular and molecular changes that, in many aspects,
resembles a classic inflammatory response (Bischof et al., 1994; Robertson et al., 1994). Within hours after mating, leukocyte infiltration occurs in the endometrium of sows (Bischof et al. 1994; Rozeboom et al., 1998). The presence of seminal plasma appears to be essential to regulate uterine immune reactions after mating. For instance, it has been shown that porcine seminal plasma suppresses in utero the migration of granulocytes into the uterus of gilts after breeding (Rozeboom et al., 1999), and in vitro inhibits neutrophil chemotaxis (Rozeboom et al., 2001a). In mice, it was reported that semen factors act on uterine cells to induce the expression of growth factors and cytokines such as interleukin (IL)-6 and IGF (Simmen et al., 1992; Robertson et al., 1994).

In this study, the influence of diluting semen and infusing whole dead semen (WDS) at AI on uterine immunological changes and embryonic survival rate was investigated by measuring messenger RNA (mRNA) levels of different cytokines and growth factors and the number of alive embryos in the gilt reproductive tract.

Materials and Methods

Animals

This experiment was conducted at the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada in Lennoxville. Sixty nulliparous Yorkshire-Landrace gilts (provided by Génétiporc Inc., St-Bernard, QC, Canada) was used. The gilts were fed 2.8 kg of a basal diet (50% corn, 20% barley, 20% wheat bran, and 5% soybean; as-fed basis) once daily and checked twice daily for estrus after contact with intact boars. At the first estrus, gilts were housed individually in a pen and their weight ranged between 110 and 125 kg. All gilts were cared for and slaughtered according to practices approved by the Animal Care Committee of the Dairy and Swine Research and Development Center and according to the recommended code of practice of the CCAC (1993).

Fresh Semen and Pool Whole Dead Semen Used at AI

Porcine semen used in this experiment was provided by the Centre d’Insémination Porcine du Quebec (CIPQ Inc., St-Lambert, QC, Canada). Two groups of three Duroc boars of known fertility were collected for this experiment. Semen was rejected when more than 20% of sperm cells were abnormal and/or motility score was low (on a subjective scale of 1 to 5, 1 being very motile and 5 being not motile; an ejaculate with a score of 4 or 5 was rejected). A group of boars was selected to provide commercial semen that required a dilution ratio of approximately 1:10 to adjust the quantity of motile spermatozoa to $3 \times 10^8$ per 80-mL dose using Beltsville thawing solution (BTS). Semen samples were sent to the research center three times per week. When necessary, semen was further diluted with BTS to adjust quantity of spermatozoa to $1 \times 10^9$ per dose just before use.

Fifteen Duroc Boars were used to build a pool of WDS. Semen from each boar was collected using the gloved-hand technique and immediately frozen at $-80\,^\circ\text{C}$. To constitute the pool of WDS, frozen semen was thawed and equal proportions of total volume of semen collected from each boar were mixed. Pooled WDS aliquots of 80 mL were frozen at $-80\,^\circ\text{C}$ again until being used in AI treatment.

Experimental Design

At the second estrus, gilts were assigned to one of the four AI treatments as follows: 1) 20 gilts were inseminated with commercial semen adjusted to a concentration of $1 \times 10^9$ sperm cells per 80-mL dose (S1) followed by an infusion of 80 mL of WDS (S1-WDS), 2) 20 gilts were inseminated with S1 followed by an infusion of 80 mL of BTS (S1-BTS), 3) 12 gilts were inseminated with commercial semen as provided at a quantity of $3 \times 10^9$ spermatozoa per 80-mL dose (S3), followed by an infusion of 80 mL of BTS (S3-BTS), and 4) a control negative group, in which eight gilts were infused twice with 80 mL of BTS. The first AI was performed 12 h after estrus detection, and the second, 12 to 18 h later.

Two days after the first AI, eight gilts from Groups 1, 2, and 4 were slaughtered to evaluate the influence of AI treatments on uterine immune reactions. This 2-d period after first AI was chosen based on available published data (Bischof et al., 1994; Rozeboom et al., 1998) and on development of inflammatory immune reactions, which are usually well established 48 h after activation of the immune system. Reproductive tracts were collected, placed on ice, and brought to the laboratory within 20 min following slaughter. Both horns were separated from connective tissues. One horn was cut open longitudinally along the antimesometrial aspect and endometrial tissue samples (approximately 1 cm$^2$) were taken and immediately frozen in liquid nitrogen for gene expression analysis. All tissue samples were stored at $-80\,^\circ\text{C}$. The other horn was flushed with 20 mL of PBS, pH 7.2, according to Laforest et al. (1992). The liquid collected was called uterine flush and it was frozen at $-20\,^\circ\text{C}$ for further cytokine analysis. Measurements in uterine flush were expressed as total amounts (concentration $\times$ total volume of uterine flush analysis).

Twenty-five days after the first AI, 12 pregnant gilts from Groups 1, 2, and 3 were slaughtered and their reproductive tracts were collected to evaluate the embryonic mortality rate that occurs in the first month of gestation. The embryonic mortality rate was calculated as follows:

$$100 - \frac{\text{number of alive embryos}}{\text{number of corpus lutea}} \times 100$$

Conception rate in this experiment was 97%; 37 gilts were used to allow collection of data on a total of 36 gilts (12 gilts per treatment) at 25 d of gestation.
Messenger RNA Expression in Endometrial Tissue

A semiquantitative reverse transcription-PCR was used to determine levels of mRNA coding for genes of interest (Table 1). Total RNA was extracted from endometrial tissue samples using 2 mL of TRIzol reagent per 160 to 180 mg of tissue according to the manufacturer’s procedure (Gibco-BRL, Burlington, ON, Canada). The extracted RNA was resuspended in 50 μL sterile water, quantified spectrophotometrically at 260 nm, and stored at −80°C. For each RNA sample, an aliquot was analyzed by electrophoresis in a 1% (wt/vol) agarose gel to verify its integrity. The cDNA was generated using a Superscript preamplification system according to the manufacturer’s instructions (Gibco-BRL). The primers and conditions used for PCR amplification are given in Table 1. Primers were designed with PC/gene 6.85 (Intelligenetics Inc., University of Geneva, Geneva, Switzerland) using available porcine complementary DNA (cDNA) sequences. The identity of PCR products was determined by the analysis of amplified products after restriction enzyme digestion.

To ensure that PCR amplifications were in the exponential range, three aliquots (1, 2 and 4 μL) were amplified at the fixed number of cycles.

For each gene, a 2-μL aliquot of the reverse transcriptase product from endometrial tissue was amplified by PCR using a PTC-200 DNA engine (MJ Research, Waltham, MA). The 50-μL PCR reaction contained 200 μM dNTPs (2’deoxy-nucleotide 5’triphosphate), 1.5 mM MgCl₂, and 1.25 U of Taq DNA polymerase (Amersham Pharmacia Biotech, Baie d’Urée, QC, Canada). The PCR profile consisted of an initial denaturing step at 95°C for 2 min, an appropriate number of cycles of denaturing at 95°C for 15 s, annealing at the corresponding temperature for 1 min (Table 1), elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. In each reaction, a negative control without cDNA was performed for each gene. The PCR-amplified products were run on a 2% (wt/vol) agarose gel to verify its integrity. The cDNA was generated using a Superscript preamplification system according to the manufacturer’s instructions (Gibco-BRL). The primers and conditions used for PCR amplification are given in Table 1. Primers were designed with PC/gene 6.85 (Intelligenetics Inc., University of Geneva, Geneva, Switzerland) using available porcine complementary DNA (cDNA) sequences. The identity of PCR products was determined by the analysis of amplified products after restriction enzyme digestion.

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The relative densities of transcript were expressed as arbitrary optical units. A ratio of the optical density of each gene transcript standardized with glyceraldehyde-3-phosphate deshydrogenase (GAPDH) transcript was calculated before statistical analysis was performed to correct for differences in cDNA synthesis. Each PCR amplification was performed in duplicate.

**Cytokines and PGE₂ in the Uterine Flush**

Commercial ELISA kits were used as recommended by the manufacturers to measure the amount of transforming growth factor-β₁ (TGF-β₁) (Emax Immunoassaysystem; Promega, Madison, WI), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (Cytoscreen immunoassay kit for swine TNF-α and IFN-γ; BioSource International, Camarillo, CA) in the uterine flush. The sensitivities of the ELISA for TGF-β₁, TNF-α, and IFN-γ were 32, 6, and 2 pg/mL, respectively. Before performing TGF-β₁ ELISA, all samples were acidified at pH <3.0 using 1 N HCl for 10 min at room temperature, and then they were neutralized at pH 7.0 by adding 1 N NaOH. Results were expressed as the total amount of cytokines in uterine flush (cytokine concentration × total volume of uterine flush collected). All samples were measured in triplicate.

Concentrations of PGE₂ were determined by RIA using manufacturer’s instructions (ICN Biomedicals Inc., Costa Mesa, CA). The sensitivity of the assay was 150 pg/mL. Each sample was measured in triplicate.

**Characterization of Pooled Whole Dead Semen**

The concentration of TGF-β₁ and PGE₂ was measured in the pooled semen by ELISA as described above. The IGF-I concentration was determined by RIA as described previously (Abridat et al., 1990). Sensitivity of the assay was 62.5 pg/mL.

**Statistical analysis**

Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) according to a completely randomized design in a one-way structure of AI treatment for each day of slaughtering. On d 2, the following comparisons were used in the ANOVA to evaluate AI treatment effects on parameters: S1-WDS vs. S1-BTS and (S1-WDS and S1-BTS) vs. BTS. In slaughtered gilts on d 25, the following comparisons were used in the ANOVA: S1-WDS vs. S1-BTS and S1-WDS vs. S3-BTS. A logarithmic transformation was applied to satisfy the ANOVA requirements when needed.

**Results**

**Characterization of Pooled Dead Semen**

Boar WDS used for the infusion contained 2,100 ng/mL of TGF-β₁, 74.2 ng/mL of IGF-I, and no detectable amount of PGE₂.
with semen extender. These results indicate that both cytokines are produced in the first 2 d after estrus, either in unmated or mated gilts. However, the uterine inflammatory reaction was not characterized by the release of IFN-γ and TNF-α in the uterine lumen regardless of infusion with either WDS or BTS based on the results obtained with the ELISA kits used to measure their concentration. The failure to detect IFN-γ and TNF-α in the uterine flushes may be due to a shortcoming of assay sensitivities, which were in the range of 10 pg/mL.

The results also indicate that the type of immune reaction induced after mating did not induce mRNA transcripts for cytokines, such as IL-1β and IL-2, normally involved in activation of immune responses. These results contrast with those who reported an increase in CD8+ cells of low fluorescence in the uterine lymph nodes 2 to 4 d after seminal plasma exposure (Bischof et al., 1994). This increase in the CD8 subpopulation was mirrored by an increase in the proportion of cells expressing IL-2 receptor and upregulation of the class-II major histocompatibility complex expression, indicating lymphocyte and macrophage activation within the uterine lymph nodes. However, in this study, measurements were made on cells isolated from the endometrium, and immune reactions that take place in this tissue may differ from those that occur in the lymph node.

In other studies done in mice or human (McMaster et al., 1992; Yeaman et al., 1998; von Wolff et al., 1999; Robertson, 2000), it has been demonstrated that somatic cells and/or leukocytes in endometrial tissues produce IL-1, TNF-α, GM-CSF, and IFN-γ after mating, although most activations occur within the first few hours after mating. Therefore, it is possible that the slaughter time chosen in the present experiment, which was at least 24 h following the second AI, was not in the window period of major inflammatory reaction occurring very shortly after AI. Seminal plasma appears to play an important role in the duration and magnitude of uterine immune reaction (Engelhart et al., 1997; Rozeboom et al., 1999). For instance, it has been shown that spermatozoa diluted in PBS caused an acute influx of polymorphonuclear neutrophils into the uterine lumen of sows that persisted for more than 24 h, whereas dilution of spermatozoa in seminal plasma caused a transient inflammatory response in uterine sows that lasted less than 12 h following AI (Rozeboom et al., 1999). Thus, the discrepancy between the present results and those reported previously could be explained by the time of sampling after insemination. Therefore, further studies are necessary to better characterize the immune reactions that develop in the first 24 h after AI.

The porcine WDS used to infuse gilts at AI was rich in TGF-β1. The TGF-β family of proteins comprises pleiotropic secretory molecules with potent immunoregulatory properties (Letterio and Roberts, 1998). For instance, in inflammatory responses, TGF-β initially acts as a proinflammatory molecule causing recruitment

Reproductive Performance

Two days after AI treatment, uterine weight did not differ between gilts inseminated with semen adjusted at 1 × 10⁹ spermatozoa and those infused with BTS only. The average uterine weights of S1-WDS-, S1-BTS-, and BTS-treated gilts were 483 ± 64, 449 ± 32, and 458 ± 62 g, respectively. On d 25 of pregnancy, uterine weight, the number of live embryos, and the rate of embryonic mortality did not differ (P > 0.10) in gilts inseminated with either 1 or 3 × 10⁹ spermatozoa, although embryo number and embryonic mortality were numerically higher and lower, respectively, when AI was performed with 3 × 10⁹ spermatozoa. In gilts inseminated with 1 × 10⁹ motile spermatozoa, WDS infusion did not affect the number of live embryos and the rate of embryonic mortality compared to those infused with BTS (Table 2).
and activation of monocytes, whereas later it acts to suppress macrophage activation (Ashcroft, 1999). Moreover, an antagonistic relationship between IL-12, IFN-γ, and TGF-β has been shown. These results indicate that TGF-β serves as a growth and differentiation factor for T regulatory cells (Letterio and Roberts, 1998). The dampening effect of TGF-β on proinflammatory cytokine expression in other lymphocyte lineage is reinforced by the inhibitory effect of TGF-β on IFN-γ and TNF-α synthesis in natural killer cells (Bellone et al., 1995). Therefore, the high TGF-β1 content in the WDS may be responsible, in part, for the inhibition of inflammatory reactions after mating as proposed in previous studies in mice (Nocera and Chu, 1995; Robertson et al., 2002). These immune-deviating effects of seminal TGF-β in the female tract could be also amplified by locally synthesized TGB-β (Robertson et al., 2002). In the present work, TGF-β1 was found in uterine fluid of gilts infused with the BTS extender only, showing that TGF-β1 was also produced in the uterus of unmated gilts after estrus. However, based on the results in this study, it cannot be concluded with certainty that seminal TGF-β1 suppressed secretion of TNF-α and IFN-γ in the gilt uterine tract after mating. However, previous results (Rozeboom et al., 2000) demonstrated that the induction of an inflamed uterine environment by infusion of lipopolysaccharide or killed spermatozoa prior to fertile insemination without seminal plasma significantly reduces conception and farrowing rates of sows compared with those observed when insemination was performed with 3 billion motile spermatozoa suspended in seminal plasma. Their results showed that fertility of spermatozoa is increased by seminal plasma when semen is deposited into an inflamed uterine environment, suggesting that seminal components play a role in regulating inflammatory reactions. Therefore, seminal TGF-β1 may influence in a positive way uterine reaction to AI by suppressing inflammatory reaction and creating a uterine environment that is favorable for fertilization and early embryonic survival.

In the present study, the mRNA transcript coding for GM-CSF, a chemotactic factor, was not detected in endometrial tissues of the gilts 2 d after insemination. This result contrasts from those previously reported in mice, where GM-CSF release was induced in estrogen-primed uterine epithelial cells after mating (Robertson and Seamark, 1990). Specific factors in seminal plasma, including TGF-β1 (Tremellen et al., 1998), could be responsible for the infiltration and the activation of GM-CSF-responsive leukocytes (Robertson et al., 1997). In those studies, an increased GM-CSF secretion mainly occurs within the first day postmating, and its presence was not detected in uterine fluid of mated mice sacrificed on d 2 to 6. In swine, exposure to semen also induces neutrophil recruitment (Rozeboom et al., 1999) and increases the abundance of macrophages and lymphocytes in the stromal tissues (Engelhardt et al., 1997). In the presence of seminal plasma, this influx of immunocompetent cells into the uterus lasts less than 12 h following AI (Rozeboom et al., 1999). They also demonstrated that the in vitro chemotactic response of blood-derived polymorphonuclear neutrophils was suppressed when they were cultured in the presence of seminal plasma compared with cells incubated with blood plasma (Rozeboom et al., 2001b). Therefore, discrepancies between our results and those obtained by Robertson and Seamark (1990) in mice could be due to the time of gilt endometrial tissue sampling to measure mRNA transcript of chemotactic factors such as GM-CSF. The reaction to semen could also vary from one species to another.

During the estrous cycle and pregnancy, morphological and functional differentiation occurs in the uterus. These physiological changes are characterized by an increased level of mRNA transcripts for IGF, IGF-R, and IGFBP (Simmen et al., 1992; Badinga et al., 1999; Correia-da-Silva et al., 1999). In this study, the abundance of IGF-II mRNA was higher in BTS-infused gilts than in those inseminated with fertile semen, whereas the expression of IGF-IR was similar in all groups. These results suggest that the presence of seminal factors may influence the regulation of the IGF system through the expression of IGF-II. The type-II receptor contains distinct binding sites for two ligands, IGF-II and mannose-6-phosphate-bearing molecules such as TGF-β1 (Jones and Clemmons, 1995). This receptor does not appear to transduce mitogenic signals

### Table 2. Physiological uterine variables measured in gilts on d 25 after insemination and infusion with or without whole dead semen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine wt, g±</th>
<th>No. of corpora lutea</th>
<th>No. of embryos</th>
<th>Embryonic mortality rate, %±</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1-WDS</td>
<td>2481±109</td>
<td>16.3±0.5</td>
<td>12.4±0.9</td>
<td>22.0±0.5</td>
</tr>
<tr>
<td>S1-BTS</td>
<td>2369±109</td>
<td>15.8±0.5</td>
<td>12.2±1.0</td>
<td>22.8±1.0</td>
</tr>
<tr>
<td>S3-BTS</td>
<td>2411±158</td>
<td>16.5±0.5</td>
<td>13.3±1.0</td>
<td>19.8±5.0</td>
</tr>
</tbody>
</table>

*aT1-WDS = AI performed with semen adjusted at 1×10⁹ spermatozoa per dose followed by infusion with whole dead semen; S1-BTS = AI performed with semen adjusted at 1×10⁹ spermatozoa per dose followed by infusion with Beltsville thawing solution; S3-BTS = AI performed with semen adjusted at 3×10⁹ spermatozoa per dose followed by infusion with Beltsville thawing solution; BTS = sows infused with Beltsville thawing solution only.^

bValues are means±SEM. Twelve gilts per group was used.

c100 – (number of embryos/number of corpora lutea) x 100.
(Moats-Staat et al., 1995). Instead, its role would be to sequester IGF-II from IGF-IR, which mediates most of the biological effects of IGF-I and IGF-II (Nisley and Lopacynski, 1991). In the IGF system, activation of latent TGF-β appears to be regulated by the binding of the cation-independent mannose 6-phosphate to the IGF-II (Dennis and Rifkin, 1991). These results indicate that interactions between TGF-β and mannose 6-phosphate/IGF-II play an important role in regulating IGF-I and IGF-II functions (Melnick et al., 1998; Ghahary et al., 2000). Therefore, the presence of TGF-β1 and IGF-I in boar semen could have a significant effect on the remodeling of uterine tissue after insemination and during pregnancy through the IGF system.

Based on published results (Bishof et al., 1994; Rozeboom et al., 1999) and those from the present study, it appears unlikely that dead sperm in WDS increased the immunological reaction. In fact, infusion of WDS did not affect the immune response. However, if an activation of the inflammatory reaction by WDS had occurred, it would have been important to further evaluate the role of dead sperm in the activation of the immune reaction of the uterus, especially the possible involvement of membrane changes following the freezing-thawing process and the immune reaction.

Finally, inseminated gilts with semen doses containing one-third to many spermatozoa as those inseminated with commercial doses (1 billion vs. 3 billion) had similar reproductive performances regardless of the infusion of whole dead semen. These results suggest that 1 billion motile spermatozoa per dose of AI are sufficient to reach approximately 80% of viable embryos. However, numerical values on the reproductive performances at 25 d indicate an average diminution of 1.1 embryos and an increase of approximately 15% (19.8 to 22.8) in embryonic mortality in gilts inseminated with 1 billion spermatozoa per dose compared with those inseminated with 3 billion. These values are comparable to those for a larger number of animals reported previously by Pedersen (1991), who obtained a reduction of 1.4 born piglets using doses of 1.4 billion vs. 2.8 billion spermatozoa at AI. On the other hand, Alexopoulos et al. (1996) and Mercat et al. (1999), using doses similar to those used by Pedersen (1991) to inseminate sows, did not observe a difference in the total number of born piglets per litter. In the present study, increased concentration of seminal plasma through the infusion of WDS did not affect reproductive performance. The results indicate that even when seminal plasma represents less than 3% of seminal plasma (as seen in doses of 1 billion), it seems to be sufficient to maintain reproductive performances. Nevertheless, a physiological role for seminal plasma during AI in the pig cannot be precluded, especially in the search of strategies to optimize fecundity potential of extended semen containing less than 1 billion spermatozoa. The minimum amount of seminal plasma needed to control uterine immune reactions and to optimize reproductive performance remains to be determined.

**Implications**

Increasing the amount of seminal plasma by infusing whole dead semen at artificial insemination does not seem to modulate uterine inflammatory reaction (36 h after the first artificial insemination) or to affect the success of artificial insemination performed with extended boar semen. Nevertheless, the potential role of seminal plasma, which contains a high concentration of transforming growth factor-β1, to regulate uterine immune reactions in the first 24 h after artificial insemination and to create an optimal uterine environment for the development of embryos remains to be defined, especially in new artificial insemination strategies using less than 1 billion spermatozoa per dose.

**Literature Cited**


