SHORT COMMUNICATION

Reproduction in Domestic Animals **WILEY**

Neither frozen–thawed seminal plasma nor commercial transforming growth factor-β1 infused intra-utero before insemination improved fertility and prolificacy in sows

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Abstract

Seminal plasma (SP) affects reproduction, inducing cell and molecular changes in the female genital tract. A main active component in SP is the modulatory transforming growth factor-β (TGF-β), particularly its TGF-β1 isoform, which affects the synthesis of other cytokines as granulocyte-macrophage colony-stimulating factor, relevant for embryo development and pregnancy. This study evaluated the effect of pooled frozen–thawed SP and commercial TGF-β1 infused during oestrus in sows post-cervically inseminated with liquid extended semen, containing ~4 ml of residual SP, on their fertility and prolificacy. For this, 250 sows in their post-weaning oestrus were used. Sows were randomly assigned to one of the following groups to be post-cervically treated 30 min before insemination: (i) SP group: infused with 40 ml of SP ($N = 57$); ii) Group $TFG_{\beta 1}$: infused with 40 ml of BTS extender containing 3 ng/ml of porcine TGF-β1 ($N = 64$); iii) BTS group: infused with 40 ml of BTS extender ($N = 60$); and iv) Control Group: sows catheterized but not infused prior to AI ($N = 69$). Farrowing rates (range: 86.7% to 91.3%) and numbers of live-born piglets (range: range: 12.8 ± 2.9 to 13.4 ± 3.1) were not affected by any treatment compared with Controls, indicating that neither pre-infusions of SP nor TGF-ß1 30 min before AI influenced subsequent fertility and prolificacy.

KEYWORDS
cytokines, porcine, post-cervical insemination, seminal plasma

1 | INTRODUCTION

The large, >150 ml, boar ejaculate is to 95% acellular, the composite seminal plasma (SP). Most SP is either discarded during the collection of the different ejaculate fractions, or diluted while preparing semen doses for artificial insemination (AI). Despite having low amounts of SP, farrowing rates and litter sizes equivalent to or even greater using AI than those resulting from natural mating are obtained worldwide (Roca et al., 2016), contrasting with the concept that SP plays an important role in sperm viability and transport and (Crawford et al., 2015), and in later events such as ovulation, corpora lutea formation, fertilization, implantation and subsequent pregnancy (Bromfield, 2014; O'Leary et al., 2006; Robertson, 2005, 2007; Waberski et al., 1997; Weitze et al., 1990). Recent studies in pigs indicate that SP administered during oestrus accelerates pre-implantational embryo development and influences endometrial cytokine production six days after

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treatment (Martinez et al., 2021), as well as modifies gene expression in the endometrium and embryos on day 6 of pregnancy, positively regulating genes and pathways associated with embryo development and immune tolerance (Martinez et al., 2019, 2020). These results are important, particularly for some porcine reproductive technologies, such as AI and embryo transfer (ET), since their implementation could improve reproductive efficiency and significantly impact the pig industry sector. However, evidence is scarce on the effect of SP on fertility and prolificacy of the sow. Furthermore, which molecule(s) are responsible for these SP beneficial effects remain unknown. TGF-β is the main active SP-chemokine, particularly its TGF-β1 isoform. Colostrum is the largest known biological source of TGF-β (Robertson et al., 2002), but the SP in mouse (Tremellen et al., 1998), human (Sharkey et al., 2012) and pig (O'Leary et al., 2011) contains similar TGF-β amounts. TGF-β relates to the regulation of the female immune response to SP in mouse and humans (Sharkey et al., 2012; Tremellen et al., 1998). In addition, TGF-β induces production by uterine epithelial cells of the immunoregulatory granulocyte-macrophage colony-stimulating factor (Bromfield, 2014), which increases embryo development and quality in mouse, cattle and humans (Moraes & Hansen, 1997; Robertson et al., 2001; Sjoblom et al., 2002).

The objective of this study was to evaluate the effects of frozen–thawed, pooled SP and commercial TGF-β1 of pig platelets infused into the uterine body of sows 30 min before AI with liquid, extended semen on their subsequent fertility and prolificacy.

2 | MATERIALS AND METHODS

2.1 | Animals

All procedures were ratified by the Ethics Committee for Animal Experimentation of the University of Murcia (22,072,015) and by the Ministry of Water, Agriculture and Environment of the Region of Murcia (No. A13160604).

In this study, weaned Landrace x Large-White hybrid sows (2–7 parity) and mature (2- to 3-year-old) Pietrain boars housed in a production farm (Porcisan SA, Murcia, Spain).

2.2 | Experimental design

To determine the effects of SP and TGF-β1 on fertility and prolificy, 250 sows were, in their post-weaning oestrus, randomly assigned to one of the following groups based on the intrauterine infusion administered 30 min before each AI: (1) SP group: infused with 40 ml of SP ($N = 57$); (2) TGF-β1 group: infused with 40 ml of BTS (Pursel & Johnson, 1975) supplemented with 3 ng/ml of commercial porcine platelet-derived TGF-β1 (R&D Systems, Inc. Minneapolis, USA; $N = 64$); (3) BTS group: infused with 40 ml of BTS extender ($N = 60$); and (4) Control Group: sows that did not receive any infusion prior to AI ($N = 69$). Pregnancy diagnosis was performed 24–28 days post-AI, and those pregnant remained to account for farrowing rates and

prolificacy. All sows included in the study were weaned at interval days 21–24 post-partum, and only those sows with a weaning to oestrus interval of 4 to 5 days were included in the experiment. Sows were selected according to an optimal reproductive history (averaging fertility >90% and litter size >10 piglets) and adequate body condition (2.7 to 3.2 on a five-point scale on the day of weaning) following the criteria previously (Nohalez et al., 2017).

2.3 | Oestrus detection and AI

Oestrus was detected twice a day, beginning 1 day after weaning, in the presence of vasectomized boars. The ejaculate sperm-rich fraction was manually collected once a week. Semen was extended with BTS extender at 35°C to produce AI-doses with 30×10^6 sperm/ml. Sows were post-cervically inseminated twice at 6 and 24 hr after the onset of oestrus, with doses of 1.5×10^9 sperm/40 ml of BTS and ~4 ml of residual SP. All collected ejaculates fulfilled the standards of quantity and sperm quality thresholds for the preparation of AI-semen doses (> 200×10^6 spermatozoa/ml, 70% of them motile and 75% depicting normal morphology). In each replicate, all sows from each group were inseminated with seminal doses from one and the same boar.

2.4 | Preparation of SP

Sixteen boars, different from those used for the preparation of AI-doses, were used for SP collection. The ejaculates were centrifuged (3 times) at $1,500 \times g$ at 17°C for 10 min. The non-existence of spermatozoa in the supernatant was microscopically confirmed after the last centrifugation. The SP from the 16 boars was pooled, and 40 ml aliquots of the SP pool were prepared and stored at −20°C. Before AI, SP aliquots were thawed at 37°C for 20 min and infused directly into the uterus using a post-cervical AI catheter.

2.5 | Statistical analysis

Data were analysed with the statistics package IBM SPSS 24.0 (IBM, Chicago, Il, USA). Data (seven replicates) were compared using the mixed ANOVA model and the Bonferroni post hoc test or the Fisher's exact test, as appropriate. Differences were considered significant at $p < .05$.

3 | RESULTS

There were no significant differences in parity, lactation length, weaning to oestrus interval and reproductive history of the sows between groups. Table 1 shows the fertility and prolificacy obtained in each of the experimental groups. Farrowing rates (range: 86.7% to 91.3%), mean number of total piglets born (range: 14.6 ± 2.9 to 15.3 ± 3.0), mean number of piglets born alive (range: 12.8 ± 2.9 to

	SP	TFG-ß1	BTS	Control
Number of sows	57	64	60	69
Pregnancy rate [n (%)]	53 (93.0)	61 (95.3)	55 (91.7)	64 (92.7)
Abortion rate [n (%)]	1 (1.9)	0 (0.0)	1 (1.8)	0 (0.0)
Farrowing rate [n (%)]	50 (87.1)	58 (90.6)	52 (86.7)	63 (91.3)
Total piglets born[a]	14.6 ± 2.7	15.3 ± 3.0	14.6 ± 2.9	15.0 ± 2.9
Born alive[a]	12.8 ± 2.9	13.3 ± 2.9	13.1 ± 2.7	13.4 ± 3.1
Stillborns[a]	1.8 ± 1.6	2.0 ± 1.9	1.5 ± 1.4	1.6 ± 1.4

TABLE 1 Farrowing rates and litter sizes of weaned sows infused with seminal plasma (SP), BTS extender containing porcine TGF-β1 (TGF-β1) or BTS extender (BTS) 30 min before a post-cervical insemination with liquid semen

Note: Controls were non-infused sows.

[a]Means and standard deviation of seven replicates.

13.4 ± 3.1) or mean number of piglets born dead (range: 1.5 ± 1.4 to 2.0 ± 1.9) remained similar (n.s.) between groups. In total, 27 out of 250 sows inseminated did not farrow. Of those 27 sows, two aborted and 25 returned to oestrus between 22- and 38-days post-insemination. There was no relationship between treatment and regular and irregular returns to oestrus.

4 | DISCUSSION

Pre-infusion of frozen–thawed SP or porcine TGF-ß1 at the concentrations used in our study 30 min before each AI with liquid, extended semen did not have significant effects on the fertility and prolificacy of the sows. Here, 40 ml of SP were used to mimic commercial volumes of seminal doses for post-cervical AI. Such volume is clearly lower than that introduced into the sow's reproductive system during natural mating (~150 ml), but much higher than that introduced in a normal dose of post-cervical AI (~4 ml) (Caballero et al., 2004). Several factors can confound the SP-infusion ineffectiveness: the use of frozen–thawed SP, the volume used (40 ml) or the residual SP in the AI-doses. Likewise, porcine TGF-$_{ß1}$ infusions also failed to affect the outcomes, agreeing with previous studies, using higher concentrations (60 ng/ml) of human recombinant TGF-ß1 which, although increasing placental efficiency, did not affect embryo survival, foetal or placental growth at least until day 80 of gestation (Rhodes et al., 2006). Here, the TGF-ß1 ineffectiveness could result from the dose, TGF-ß1 source (porcine platelets), reconstitution for the infusions and/or administration route, calling for further research.

The high reproductive performance of the control group could also mask possible beneficial effect of the infusions, as suggested in other studies (Flowers & Esbenshade, 1993; Rhodes et al., 2006), because the AI- doses contained a certain amount of SP (~4 ml). Possibly, positive effects of pre-infusions of SP and/or TGF-ß1 could be seen when reproductive performance is low, as when only one AI/or mating per oestrus is done. Under such regime, SP-infusions before natural mating or AI indeed improved farrowing rates (Flowers & Esbenshade, 1993).

In conclusion, neither frozen–thawed SP nor TGF-ß1 infused during oestrus, at the concentrations used, influenced fertility or prolificacy of sows after AI.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

IP, HR-M and EAM conceived and designed the study. IP, CC, MAG and EAM performed the experiments. HR-M and EAM analysed and interpreted the data. IP and EAM wrote the primary manuscript. IP, HR-M, CC and MAG gave critical suggestions and contributed to the editing of the manuscript. All authors revised and approved the manuscript for publication. EAM, MAG and HR-M obtained the funding to carry out the study.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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