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Cytokine expression in the gilt oviduct: Effects of seminal plasma, spermatozoa and extender after insemination

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ABSTRACT

Effects of semen components [fresh semen in extender, spermatozoa in extender (Spz), seminal plasma (SP)], or extender alone (Beltsville thawing solution, BTS) on the expression of selected cytokines [interleukin (IL)-1β, IL-6, IL-10 and transforming growth factor (TGF)-β1)] as well as the presence of cells positive for CD8 or CD25 were studied in the pig oviduct. In addition, cytokines in SP and oviductal flushings were analyzed.

In experiment (Exp) I, groups of gilts were sampled at 5–6 h after insemination with SP, Spz, fresh semen in BTS or only BTS (control). In Exp II, gilts were sampled 35–40 h after insemination with SP, Spz, BTS or only catheter insertion (control).

Most oviductal flushing samples were positive (≥detectable limits) for IL-10 and TGF-β1 but only few for IL-6. The IHC-labelling of IL-6, IL-10 and TGF-β1 was evident, especially in the epithelial cells of the isthmus and infundibulum as well as in the cells of the regional (mesometrial) lymph node. Cilia of the epithelium were positive for IL-6 (strongest in the infundibulum) and TGF-β1 (strongest in the isthmus) but negative for IL-10. There were no consistent differences in IHC-labelling of the cytokines in relation to different treatments, except at 35–40 h after insemination (Exp II), when IL-6 was slightly higher in epithelium of the SP group and IL-10 in the infundibular connective tissue was higher in the SP and Spz groups.

In the isthmus and infundibulum, there were no differences between animals inseminated with BTS (control) and the semen components for any of the cytokine mRNAs at 5–6 h after insemination (Exp I). However, later (35–40 h, Exp II), insemination with SP, Spz and BTS alone appeared to up-regulate TGF-β1 mRNA expression compared with the control group (without any fluid infused). In all treatment groups, the mRNA level for TGF-β1 was higher than for IL-1β, IL-6 and IL-10. Higher mRNA levels of all cytokines were found in the isthmus compared with the infundibulum.

Numbers of CD8-positive cells (both in epithelium and connective tissue) appeared higher in the infundibulum compared with the isthmus and were mostly higher shortly (Exp I) after treatment with SP, SPZ and BTS than later (Exp II) in both segments. CD25-positive cells were few and found solely in the sub-epithelial connective tissue.

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The results indicate that in the porcine oviduct, IL-6, IL-10 and TGF-β1 are endogenous produced and that TGF-β1 may have a more important role for immunomodulation than the other cytokines, especially in isthmus. Differences between isthmus and infundibulum in cytokine mRNA expression and in presence of CD8-positive cells indicate different patterns of immune reactivity in the upper and lower parts of the oviduct.

1. Introduction

At insemination of pigs, semen, i.e. spermatozoa and seminal plasma, is transferred within minutes to the uterotubal junction (UTJ) and the adjacent segment (isthmus), of each oviduct, creating a tubal sperm reservoir where a subpopulation of spermatozoa is stored (review Rodriguez-Martinez et al., 2005). Some small amounts of semen are also directly distributed along the entire oviduct and therefore spermatozoa can be observed 1–2 h after insemination in the ovarian end of the oviduct, the infundibulum (Viring, 1980).

Since the spermatozoa are carrying proteins that are foreign to the female (Beer and Billingham, 1974), successful fertilization depends on a balance between tolerance to paternal alloantigens and immune reactivity against foreign pathogens. In the pig, fertilization takes place in the ampulla–isthmic junction. Thus, environmental modulation of local immune reactivity in the isthmic part appears necessary to support the viability and fertilizing capacity of the spermatozoa. Our earlier study of oviductal sections obtained from sows after insemination showed that the isthmic part was free from neutrophilic granulocytes (Jiwakanon et al., 2006), in contrast to the infundibulum, which contained neutrophils in the sub-epithelial connective tissue about 35–40 h after insemination (Jiwakanon et al., 2006). Also the distribution of lymphocytes and plasma cells differed within the oviduct (isthmus versus infundibulum) after insemination (Jiwakanon et al., 2006).

Seminal plasma (SP) is a complex mixture of secretions from testes, epididymidis and accessory glands and in the boar especially from the seminal vesicles (Ekhlasi-Hundrieser et al., 2002; Manaskova and Jonakova, 2008). At mating/insemination, SP may exert an immunoregulatory role in the female reproductive tract. Data from human beings and rodents have shown that SP can modulate a variety of immunological functions (see review Alexander and Anderson, 1987; Thaler, 1989; Kelly and Critchley, 1997; Robertson and Sharkey, 2001). Also boar SP appears to contain fractions that either suppress (Stanek et al., 1985; Veselsky et al., 1991; Dostal et al., 1997) or stimulate the immune response (Leshin et al., 1998; Yang et al., 1998) and a dose-dependent suppressive effect of SP was observed in vitro, on the chemotaxis of blood-derived PMNs (Rozeboom et al., 2001b).

A major variable influencing the ensuing immune response in a tissue is the expression of cytokines (see review Constant and Bottomly, 1997; Santana and Rosenstein, 2003; Kaiko et al., 2008). In accordance, seminal plasma can induce expression of mRNA for the pro-inflammatory cytokine interleukin (IL)-6, but not for IL-1β, in porcine endometrium 34 h after intra-uterine infusion (O'Leary et al., 2004) and stimulate mRNA expression of the suppressive cytokines IL-10 and TGF-β in endometrial cells at 3 h after intra-uterine infusion (Taylor et al., 2008). Even if only small amounts of seminal plasma reach the oviduct, it may be sufficient to regulate the cytokine production in this tissue and thereby affect the local immune reactivity. Expression of TGF-β isoforms and the TGF-β type II receptors at both the mRNA and protein levels has been described in the human oviduct (Zhao et al., 1994), and a presence of TGF-β1 and the TGF-β type II receptor in the porcine isthmus and ampulla has been indicated by immunohistochemistry (Buhi et al., 1997). However, effects of SP on cytokine production in the porcine oviduct are to our knowledge not described.

CD8-positive T cells are the dominant T cell population in both human (Boehme and Donat, 1992) and equine (Brinsko and Ball, 2006) oviductal mucosa. Theoretically, after insemination paternal antigens present in semen could prime the recruitment of anti-paternal cytolytic CD8-positive T cells, as discussed by Seavey and Mosmann (2008). Effect of TGF-β on the CD8 expression of leukocytes has been shown in vitro (Ouellette et al., 1999). However, there is no available data on the effect of different semen components on CD8-positive cell distribution in the porcine oviduct. In addition, expression of CD25 indicates lymphocyte activation and is therefore of interest to examine in combination with the presence of CD8-positive cells.

The present study therefore aimed to analyze effects of SP, spermatozoa and extender on the presence of cytokines regarded as pro-inflammatory [interleukin (IL)-1β and IL-6] or suppressive [IL-10 and transforming growth factor (TGF)-β1] on both mRNA and protein levels, shortly (at 5–6 h) and later (at 35–40 h) after insemination in gilts. In addition, the distribution of cells expressing CD8 or CD25 was examined by immunohistochemistry. Also pooled SP and oviductal flushings were analyzed for their content of cytokines.

2. Materials and methods

Two experimental studies, approved by the Ethical Committee for Experimentation with Animals, Uppsala, Sweden, were performed. In the first experiment (Exp I), gilts were inseminated with 100 ml of seminal plasma (SP, $n = 4$), spermatozoa in extender [Beltsville thawing solution, BTS (Pursel et al., 1973), $n = 4$], fresh semen in extender (BTS, $n = 4$) or extender (BTS) alone as control ($n = 4$) and slaughtered 5–6 h after insemination. In the second experiment (Exp II), gilts were inseminated with 100 ml SP ($n = 4$), spermatozoa in BTS ($n = 4$) or BTS ($n = 4$) alone. In four control gilts only the disposable catheter (Goldenpig™, IMV,