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Seminal fluid signaling in the female reproductive tract: Lessons from rodents and pigs¹

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ABSTRACT: Seminal fluid contains potent signaling agents that influence female reproductive physiology to improve the chances of conception and pregnancy success. Cytokines and prostaglandins synthesized in the male accessory glands are transferred to the female at insemination, where they bind to receptors on target cells in the cervix and uterus, activating changes in gene expression that lead to modifications in structure and function of the female tissues. The consequences are increased sperm survival and fertilization rates, conditioning of the female immune response to tolerate semen and the conceptus, and molecular and cellular changes in the endometrium that facilitate embryo development and implantation. Male-female tract signaling occurs in rodents, livestock animals, and all other mammals examined thus far, including humans. In mice, the key signaling moieties in seminal plasma are identified as members of the transforming growth factor- β family. Recent studies indicate a similar signaling function for boar factors in the pig, whereby the sperm and plasma fractions of seminal fluid appear to syner-

gize in activating an inflammatory response and downstream changes in the female tract after insemination. Seminal plasma elicits endometrial changes, with induction of proinflammatory cytokines and cyclooxygenase-2, causing recruitment of macrophages and dendritic cells. Sperm contribute by interacting with seminal plasma factors to modulate neutrophil influx into the luminal cavity. The cascade of changes in local leukocyte populations and cytokine synthesis persists throughout the preimplantation period. Exposure to seminal fluid alters the dynamics of preimplantation embryo development, with an increase in the number of fertilized oocytes attaining the viable blastocyst stage. There is also evidence that seminal factors influence the timing of ovulation, corpus luteum development, and progesterone synthesis. Insight into the molecular basis of seminal fluid signaling in the female reproductive tract may inform new interventions and management practices to ensure maximal fertility and reduce embryo mortality in pigs and, potentially, other livestock species.

Key words: pig, pregnancy, reproduction, rodent, seminal fluid, uterus

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INTRODUCTION

Seminal plasma is generally viewed as a transport and survival medium for sperm. However, its role is now recognized to extend beyond this to targeting the female tissues after insemination. Studies in rodents, livestock species, and humans show that introduction of semen into the female reproductive tract elicits molecular and cellular changes, with the physiological con-

sequences bearing on conception and pregnancy. Factors in seminal plasma and associated with sperm that have potential biological effects in the female include cytokines, sex hormones, and prostaglandins (Mann, 1964; Aumuller and Riva, 1992; Maegawa et al., 2002). These molecules bind to target cells in the cervix, uterus, and oviduct, activating changes in gene expression that impact the function of the tract and the likelihood of successful establishment of pregnancy (Robertson and Sharkey, 2001; Robertson, 2005).

In this short review, we summarize our current understanding of the physiological significance of seminal fluid signaling in rodent and porcine species, which share the attribute of in utero insemination and the capacity for seminal fluid targeting of the upper region of the female reproductive tract. A better knowledge of this relatively unexplored aspect of reproductive biol-

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ogy might bring new insight to the events of early pregnancy and have implications for farm practice in the pig breeding industry.

THE INFLAMMATORY RESPONSE TO INSEMINATION

The immediate response to insemination in mammals is a rapid and dramatic influx of inflammatory cells into the site of semen deposition. This inflammatory reaction to semen was first observed in rabbits (Taylor, 1982) but has since been described in several other species (Robertson, 2005). Studies in rodents provide a comprehensive understanding of the molecular and cellular regulation of the inflammatory cascade and have informed experiments in pigs and other animals, as well as in humans. In mice, the response is initiated when seminal proteins interact with estrogen-primed cervical and uterine epithelial cells to activate synthesis of proinflammatory cytokines, including granulocyte-macrophage colony-stimulating factor (**GM-CSF**), IL-6, macrophage chemotactic protein-1, IL-8, and an array of other chemokines (Sanford et al., 1992; Robertson et al., 1996; Robertson et al., 1998). Within hours of insemination, the surge in proinflammatory cytokine release causes inflammatory leukocytes, including macrophages, dendritic cells, and granulocytes, to traffic from the blood and accumulate in the subepithelial stromal tissue. During the initial acute phase, large populations of neutrophils migrate through the epithelial surface and efflux into the uterine luminal cavity (De et al., 1991; McMaster et al., 1992; Robertson et al., 1996). The response is short-lived and is resolved before embryo implantation, in parallel with the decline in inflammatory cytokine release as rising progesterone levels inhibit synthesis of GM-CSF and proinflammatory chemokines (Robertson et al., 1996, 1998).

An inflammatory cascade regulated by similar processes occurs after insemination in the pig uterus. Neutrophils are recruited into the uterine lumen within hours of insemination (Lovell and Getty, 1968; Claus, 1990; Rozeboom et al., 1998), and this is accompanied by accumulation of macrophages and dendritic cells, granulocytes, and lymphocytes in the endometrial stroma (Bischof et al., 1994, 1995; Engelhardt et al., 1997). Sperm and seminal plasma contribute to this response; experiments with vasectomized boars show that seminal plasma constituents can mediate an increase in endometrial macrophage numbers (Bischof et al., 1994; O'Leary et al., 2004), whereas insemination with washed sperm caused a greater influx of neutrophils into the luminal cavity than seminal plasma or whole semen (Rozeboom et al., 1999).

Studies with gilts administered pooled seminal plasma by transcervical catheter showed that specific factors present in seminal plasma interact with uterine cells to induce expression of GM-CSF, IL-6, and monocyte chemoattractant protein-1, and that neuroendocrine stimulation resulting from the physical act of mat-

ing is not necessary for this response (O'Leary et al., 2004). These inflammatory factors have chemotactic properties and are implicated in regulating macrophages and dendritic cell recruitment into the endometrial stroma. Although the luminal neutrophil response is resolved within 24 h (Rozeboom et al., 1998), the inflammatory cells infiltrating the endometrium appear to persist and undergo differentiation in the tissue for several days, expanding local activated macrophage and dendritic cell populations for the duration of the preimplantation period (O'Leary et al., 2004). This transition in leukocyte phenotype is likely to be driven by the more rapid termination of inflammatory cytokine synthesis that occurs in gilts exposed to seminal plasma (O'Leary et al., 2004).

INFLUENCE OF SEMINAL PLASMA ON FEMALE REPRODUCTIVE EVENTS

The inflammatory response to seminal fluids has a central role in female tract processing of seminal material and recovery of tissue homeostasis after mating. In addition, there is mounting evidence to suggest that exposure to seminal fluids can proactively influence subsequent events in the female tract to promote conception and progression of pregnancy. Although the success of AI shows that seminal constituents other than sperm are not mandatory for pregnancy, there are data showing that reproductive success and quality of the outcome can be compromised if females are not exposed to seminal plasma. Depending on the species, effects ranging from reduced fertilization and embryo implantation to altered growth of the placenta and fetus are observed when seminal signaling is perturbed.

The most compelling information comes from rodents and shows that seminal fluid can influence sperm survival and competence, development of the preimplantation embryo, and receptivity of the uterine endometrium. Experiments in which the seminal vesicle, prostate, or coagulating glands are surgically removed from mice, rats, or hamsters before mating each show that seminal vesicle fluid is the most vital nonsperm component of the ejaculate (Pang et al., 1979; Queen et al., 1981). In the absence of seminal plasma, impaired fertility appears to result from a reduction in the forward motility and survival of sperm, resulting in reduced fertilization (Peitz and Olds Clarke, 1986); in hamsters, this is accompanied by a slower cleavage rate in preimplantation embryos and greater fetal loss after implantation (O et al., 1988). In mice, embryo transfer protocols generally employ recipients exposed to seminal plasma by mating to vasectomized males, but fetal loss and abnormality is considerably greater when embryos are transferred after pseudopregnancy achieved without exposure to male fluids (Watson et al., 1983). Confirmation that the effects of seminal plasma are mediated, at least partly, through promoting receptivity in the female tract is provided by studies showing that when recipient females are mated with seminal vesicle-

deficient males, transferred embryos implant normally but give rise to fetuses with retarded growth trajectories and placental development (Bromfield et al., 2004). In rats, implantation rates and fetal growth are similarly impaired unless females are inseminated before embryo transfer (Carp et al., 1984).

Well-designed studies to evaluate seminal fluid exposure in pigs are scarce. Reports on fertility rates using AI compared with natural mating are difficult to reconcile across studies, with considerable variation in reproductive performance reflecting different breeding practices including the inseminator's experience, the method of estrous detection, and the insemination protocol. Comparisons made when AI was first implemented suggested that conception rates and litter sizes were compromised by AI compared with natural mating (Baker et al., 1968; Skjervold, 1975; Claus, 1990). However, with current AI practice in modern, large-scale facilities, farrowing rates and litter sizes are comparable to natural service. Pregnancy rates of >90%, farrowing rates of >85%, and litter size of >9 are routinely achieved after AI in pigs using a single dose of the sperm-rich fraction of semen diluted 20 to 30 times (Vazquez et al., 2005). This suggests that seminal fluid stimulation of the female tract is not essential, particularly when insemination occurs close to the time of ovulation, and when sperm are deposited high in the reproductive tract to minimize physical loss (Vazquez et al., 2005).

In herds with poorer reproductive performance or a less than optimal breeding practice, seminal plasma can improve reproductive outcomes even after natural mating with an intact boar. Increases in farrowing rates from 70 to 81% were reported when seminal plasma was administered before natural service, and seminal plasma treatment increased farrowing rate and litter size when given with AI plus natural service (Flowers and Esbenshade, 1993). Similar results were reported when mating with an intact boar was supplemented by seminal plasma provided by a vasectomized boar (Mah et al., 1985). Uterine infusion with heat-killed semen during the previous estrus can also increase litter size and improve farrowing rate (Murray et al., 1983, 1986), and similar effects result from mating with vasectomized boars in previous estrous periods (Flowers and Esbenshade, 1993), suggesting that some benefit persists into subsequent cycles. Similarly, when AI is impaired by a less than optimal female condition, seminal plasma may promote pregnancy success. When tracts were previously exposed to an adverse inflammatory treatment, improvements in conception rate and farrowing rate resulted from addition of seminal fluid to sperm at AI (Rozeboom et al., 2000).

TRANSFORMING GROWTH FACTOR- β AND OTHER ACTIVE FACTORS IN SEMEN

Depending on the animal species, the proinflammatory signals in seminal fluid have been associated with

both sperm and seminal plasma. Experiments with mice from which the accessory glands were surgically removed showed that in this species the active inflammation-inducing moieties are derived from the seminal vesicle, where the majority of seminal fluid is produced (Robertson et al., 1996). Using protein chromatographic techniques and neutralizing antibodies, transforming growth factor- β (TGF- β) was identified as the principal trigger for induction of uterine inflammatory responses (Tremellen et al., 1998; Robertson et al., 2002). Seminal vesicle TGF- β synthesis is testosterone-dependent, with a severe reduction evident after castration, and partial recovery after administration of exogenous testosterone (Robertson et al., 2002). The majority of the TGF- β present in male seminal fluids is synthesized in the latent form and appears to be activated in the female reproductive tract by plasmin and other enzymes after insemination (Robertson et al., 2002).

In pigs, it appears likely that different active fractions associated with sperm and seminal plasma can interact with each other and with female tract cells to attenuate the quality and duration of the uterine inflammatory response. Seminal plasma attenuation of the inflammatory infiltrate (Rozeboom et al., 1999, 2001a) is mediated by seminal plasma inhibition of boar sperm-induced activation of chemotactic components in plasma (Rozeboom et al., 2001b). However, the precise nature of the active constituents remains to be identified. Although TGF- β cytokines have been detected in high concentrations in boar seminal fluid (O'Leary et al., 2002), and immunosuppressive activity characteristic of TGF- β is associated with protein fractions of the appropriate size in boar seminal fluid (Claus, 1990), whether this cytokine family contributes to regulation of the female inflammatory response in gilts is unproven.

A recent study failed to find any beneficial effect of recombinant TGF- β 1 administered at AI on total or live implantation rate at d 80 of pregnancy (Rhodes et al., 2006); however, with only 9 to 11 gilts per treatment, this study was not powered to detect the small changes in litter size expected on the basis of previous studies with seminal plasma. Estrogens are another candidate mediator because boar seminal plasma is a rich source of estrogens, and transcervical administration of estrogen can elicit some improvement in farrowing rate (Flowers and Esbenshade, 1993) and mimic some aspects of the physiological response to insemination (Claus, 1990). Prostaglandins and oxytocin have also shown promise in eliciting farrowing rate and litter size increases (Flowers and Esbenshade, 1993).

FEMALE TRACT RESPONSES ACTIVATED BY SEMINAL FLUID

The inflammatory response stimulated by seminal fluid affects several reproductive processes by virtue of the wide range of biological downstream effects of the leukocytes recruited into the endometrial and cervical

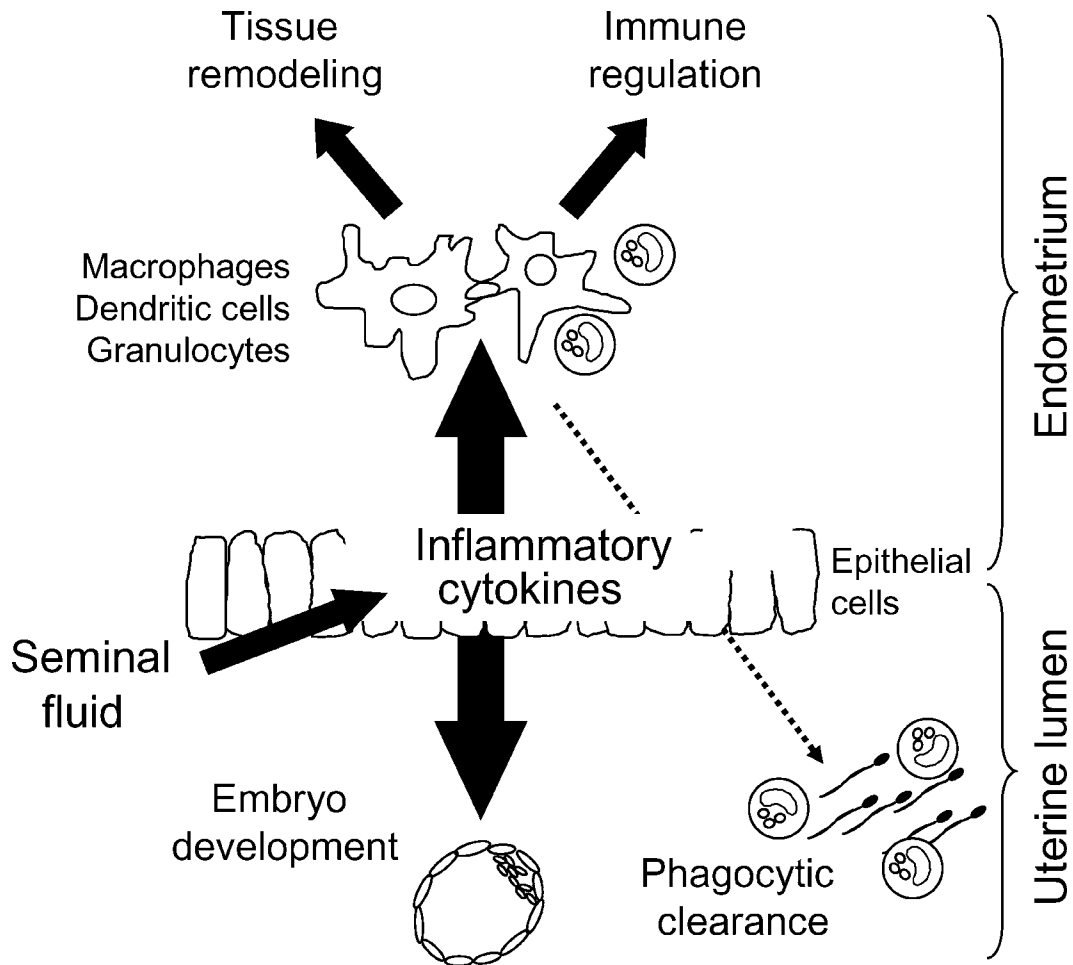


Figure 1. Schematic diagram illustrating the actions of seminal fluid in the female reproductive tract. Studies in mice and pigs show that active moieties in seminal plasma and associated with sperm interact with epithelial cells in the cervix and uterus of the female reproductive tract at mating to induce synthesis of proinflammatory cytokines. These cytokines cause the recruitment and activation of inflammatory cells in the uterine endometrium, including macrophages, dendritic cells, and granulocytes. The macrophages and dendritic cells have roles in remodeling of the endometrial tissue and in activating maternal immune tolerance of pregnancy. Neutrophils traversing the endometrial epithelium into the lumen act to clear seminal debris and maintain uterine sterility. Epithelial cytokines activated by seminal plasma are also secreted into the luminal fluid, where they exert trophic actions on the developing preimplantation embryo.

tissues (Figure 1). These inflammatory changes are likely to act in concert with the direct effects of seminal fluid to coordinate insemination with the events of ovulation and maximize the chances of conception and initiation of pregnancy. Four categories of effector function are likely: (1) clearance of superfluous sperm and microorganisms introduced into the uterus at mating; (2) activation of female immune responses specific to paternal transplantation proteins and other antigens present in semen; (3) tissue remodeling associated with preparation of endometrial receptivity; and (4) activation of expression of cytokines and growth factors implicated in preimplantation embryo development. Together, these seminal plasma-induced changes in the female tissues act to assist in delivery of the most competent male gametes to the oocyte and maximize the

opportunity for fertilization, embryo development, and successful implantation.

IMPACT ON SPERM SURVIVAL AND TRANSPORT

Seminal plasma is a diluent and vehicle for sperm that supports sperm viability through nutritive and protective activities (Mann, 1964). In pigs, seminal plasma facilitates delivery of sperm to the upper reproductive tract through direct effects on uterine contractility mediated by estrogen, which acts through inducing $\text{PGF}_{2\alpha}$ release immediately after insemination (Claus, 1990). Inflammatory cells recruited in response to seminal fluid further influence the capacity of sperm to reach the oocyte, particularly the abundant popula-

tions of neutrophils that infiltrate the uterine lumen to remove superfluous sperm, microorganisms, and seminal debris. The upper reproductive tract is normally sterile, with intromission providing the opportunity for access by commensal microorganisms originating from male and female tissues, or sexually transmitted pathogens. A male contribution to maintenance of a sterile female tract would assist sperm survival and increase the likelihood of a mating event resulting in pregnancy. At least in humans, there is evidence of selective sperm phagocytosis acting to filter morphologically abnormal spermatozoa (Tomlinson et al., 1992). Through differential resistance to phagocytosis, individual sperm could thus be selected for fertilization competence. There is evidence that apparently viable and morphologically normal spermatozoa are also targeted, suggesting selection may occur on the basis of morphological or antigenic parameters other than the ability to fertilize (Taylor, 1982; Roldan et al., 1992). This raises the prospect of the inflammatory response providing a mechanism for active female tract selection of male gametes permitted to advance to fertilization of oocytes, although such a mechanism remains hypothetical until a molecular basis for leukocyte discrimination between sperm is identified.

The physiological significance of seminal plasma in uterine clearance and immune regulation might be particularly important in species where rapid resolution of the uterine inflammatory response is linked with reproductive success. In swine, AI into an inflamed uterus impairs conception rate and successful progression to farrowing, but coadministration of seminal plasma alleviates the effect of a hostile environment (Rozeboom et al., 2000). This study concluded that seminal plasma acts largely through protecting sperm from neutrophil attack by reducing the numbers of neutrophils accessing the luminal compartment (Rozeboom et al., 1999). Together with the direct effects of seminal plasma on neutrophils (Rozeboom et al., 2001a), seminal plasma regulation of uterine chemotactic factors is likely to contribute. Exposure to seminal plasma initially activates endometrial expression of cytokines and chemokines, but this is followed by more rapid termination of GM-CSF, monocyte chemoattractant protein-1, and cyclooxygenase-2 (COX-2) mRNA synthesis than in tracts exposed to carrier alone (O'Leary et al., 2004). Furthermore, it is important to note that the activation phenotypes of inflammatory cells are highly variable and responsive to microenvironmental signals (Hunt and Robertson 1996), so that the immune regulatory cytokines induced by seminal plasma are likely to attenuate the functional properties of the inflammatory leukocytes recruited into the female tract.

ACTIVATION OF MATERNAL IMMUNE TOLERANCE OF PREGNANCY

In rodents and pigs, macrophages and dendritic cells comprise the major populations of inflammatory cells

retained in the endometrial stromal tissue after exposure to semen (Robertson et al., 1996, O'Leary et al., 2004) because the neutrophil response is short-lived, with the majority of these cells eliminated via the uterine lumen within 24 h (McMaster et al., 1992; Rozeboom et al., 1998). Macrophages and dendritic cells are professional antigen processing and presenting cells that take up and transport seminal antigens to draining lymph nodes, resulting in activation of immune responses to paternal major histocompatibility complex (MHC) and other antigens in semen. The female immune response to seminal antigens is characterized in mice by hypertrophy of lymph nodes draining the uterus and evidence of lymphocyte activation (Beer and Billingham, 1974; Johansson et al., 2004).

Hostile immune responses to seminal antigens would be incompatible with fertility by preventing the female tract from tolerating future semen exposures. There would also be consequences for pregnancy because the conceptus shares paternal antigens with those in semen (Thaler, 1989). However the immune activation elicited by semen does not cause rejection of male antigens, due to the presence in seminal plasma of several powerful immunoregulatory molecules, such as PGE and TGF- β , that dampen potentially destructive type-1 (cell-mediated) immune responses and instead skew the immune response toward regulatory lymphocyte activation (Letterio and Roberts, 1998; Weiner, 2001). Consistent with the expected actions of PGE and TGF- β , the female tract immune response to semen results in a state of functional immune tolerance to male antigens (Robertson et al., 1997). Because there are similarities between the MHC and other antigens carried by semen and the conceptus, the female immune response to ejaculate antigens might also confer immune protection to the conceptus (Robertson et al., 1997).

There are few studies to evaluate the existence of homologous immune events in large animal species; however, their occurrence is likely given the presence of the necessary antigenic elements and immune-deviating compounds in semen. The endometrial inflammatory response after insemination in gilts is accompanied by expression of activation markers (MHC class II and IL-2 receptor) expression in draining lymph nodes (Bischof et al., 1994; Waberski et al., 2006), reflecting lymphocyte activation in response to seminal antigens and the possibility of immunological consequences for an ensuing pregnancy. Observations that administration of pooled leukocytes to the AI dose can improve embryo viability (Almlid, 1981) are also consistent with semen providing an antigenic stimulus that activates the female immune response in readiness for pregnancy. A mechanism involving immune priming to seminal antigens would also explain the beneficial effects of uterine treatments with seminal or leukocyte antigens at previous estrous events (Murray et al., 1983, 1986; Flowers and Esbenshade, 1993).

REGULATION OF ENDOMETRIAL TISSUE REMODELING AND UTERINE RECEPTIVITY

Leukocytes can exert effects in their local milieu by secreting an array of potent enzymes and signaling molecules that affect extracellular matrix turnover and the behavior of endothelial cells in the endometrium. The leukocytes recruited in response to semen, particularly macrophages, are implicated in restructuring the endometrial environment to facilitate implantation and placental development (Hunt and Robertson, 1996).

Regulation of angiogenesis is the major potential avenue for macrophage effects on implantation. Activated macrophages have the capacity to influence each phase of the angiogenic process, including alterations of the local extracellular matrix, induction of endothelial cells to migrate and proliferate, and formation of capillaries (Sunderkotter et al., 1994). Consistent with a proangiogenic role for semen-induced inflammatory changes, vascular endothelial growth factor mRNA abundance in hamsters is reduced after mating with accessory gland-deficient males (Chow et al., 2003). An additional target for macrophage-secreted products is the extracellular matrix of the endometrial stroma, which is remodeled before and during the decidual transformation, with breakdown of the existing matrix and the deposition of new components (Aplin, 2002). Matrix metalloproteinases (MMP) regulate this process after coordinated increases in their transcription, secretion, and proteolytic activation, along with their regulatory proteins, the tissue inhibitors of metalloproteinases. Macrophages are a major source of a broad range of MMP under the influence of cytokines, extracellular matrix, and PG (Goetzl et al., 1996). Consistent with a role for semen-induced inflammatory cells in MMP regulation is the finding in golden hamsters that the absence of male accessory gland fluids is associated with reduced expression of MMP-2 in the implantation site (Chow et al., 2003).

Macrophage mediators potentially also target the luminal epithelial cells involved in embryo attachment during the initial phases of implantation (Kosaka et al., 2003). Macrophages interdigitate between epithelial cells in the endometrium (Hunt and Robertson, 1996), where they would be well-positioned to influence integrin expression at the paracrine level. In pigs, an increase in the number of endometrial glands is one of the most striking changes induced by exposure to a vasectomized boar (Bischof et al., 1994), indicating that macrophages recruited after seminal exposure might release cytokines that promote epithelial cell proliferation. Evidence of increased endometrial vascularity and edema was observed during the acute phase of the inflammatory response to seminal plasma in gilts (O'Leary et al., 2004), but whether any seminal fluid-induced changes in vascular or matrix structure persist through the preimplantation period remains to be evaluated.

SYNTHESIS OF EMBRYOTROPHIC CYTOKINES

Cytokines synthesized by uterine and oviductal epithelial cells are secreted into the uterine luminal fluid, where they interact with the developing embryo before implantation. Several of these embryotrophic cytokines are regulated by semen exposure, providing a further nexus through which seminal fluid signaling can promote embryo development and implantation success. A principal cytokine in the postmating inflammatory response, GM-CSF, targets the preimplantation embryo to increase the number of viable blastomeres through inhibiting apoptosis and regulating glucose uptake (Robertson et al., 2001). Other cytokines targeting the developing blastocyst, including IL-6 and leukemia inhibitory factor, are induced after exposure to semen (Robertson et al., 1992). Perturbations in the growth factor environment experienced by the preimplantation embryo impair normal development of the placenta and fetus, with long-term consequences for postnatal health and metabolic programming in the progeny (Sjoblom et al., 2005).

The quality of the uterine environment is a well-recognized factor in embryonic viability and development in pigs. Treatment of gilts with seminal plasma has been shown to increase the numbers of viable embryos and to alter the embryo growth trajectory during the preimplantation period (O'Leary et al., 2004). Surprisingly, this study showed the increased number of viable blastocysts retrieved from the tract on d 9 of pregnancy was associated with a significant reduction in their average size, indicating seminal conditioning of the tract may act to support embryo survival, while constraining their pace of development. This increase in embryo survival presumably reflects indirect effects of seminal constituents on the reproductive tract environment, acting to reduce early embryo mortality, most likely through regulating epithelial cell secretion of embryotrophic factors or nutritional support. Greater developmental synchrony within the cohort of embryos, mediated through the effects of cytokines or reflecting the timing of ovulation (see below), could also contribute to improved embryo viability in a manner similar to that observed in Meishan pigs (Rivera et al., 1996).

REGULATION OF OVARIAN FUNCTION

The actions of seminal fluid can reach beyond the immediate site of deposition and exert effects in organs and systems elsewhere in the female body. In mice, macrophage populations in corpora lutea are augmented by exposure of the female tract to seminal plasma constituents (Gangnuss et al., 2004). In estrous pigs, there is a reduction in the interval between the LH surge and ovulation in response to natural mating (Signoret et al., 1972) or seminal plasma administered transcervically (Waberski et al., 1997). The effects appear to be mediated by local transport of seminal constituents or semen-induced effector molecules originating in the upper re-

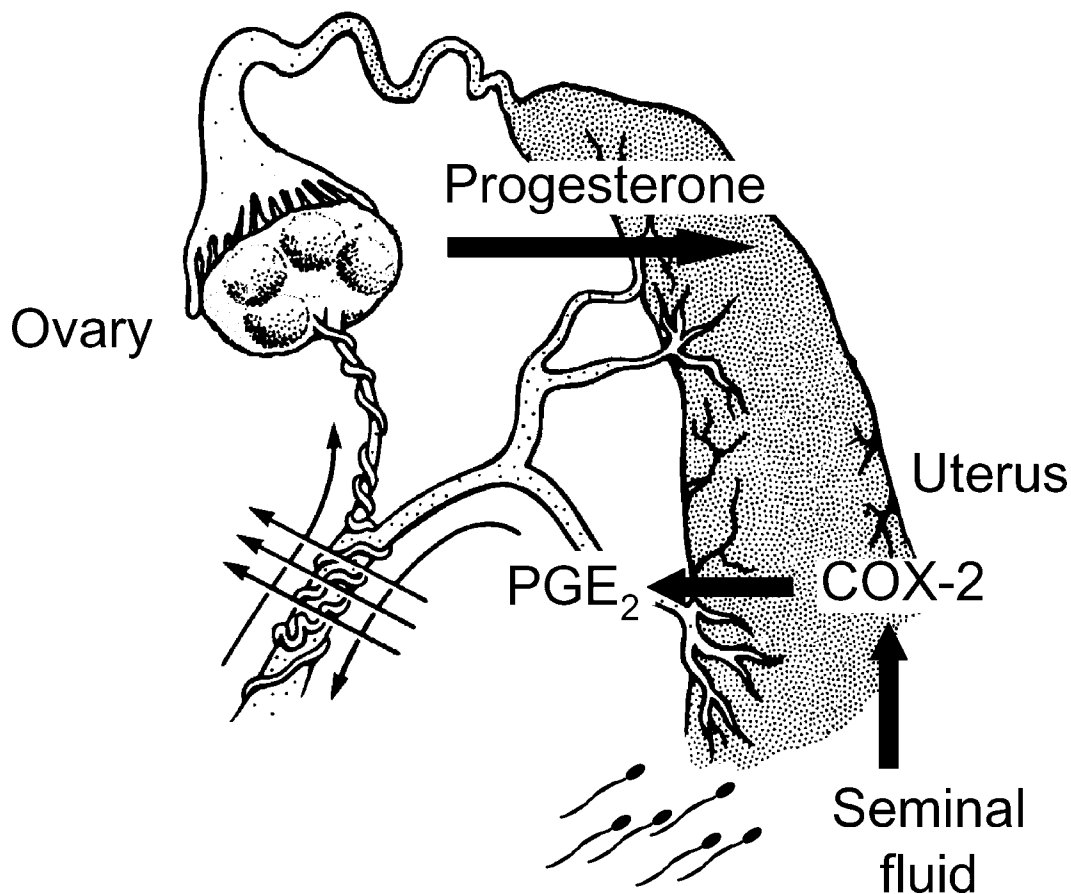


Figure 2. Schematic diagram illustrating the action of seminal fluid in the female reproductive tract to regulate corpus luteum development in the ovary. Seminal plasma acts in the endometrial tissue of gilts to increase cyclooxygenase-2 (COX-2) mRNA transcription. This is postulated to induce uterine synthesis of prostaglandins PGE and PGF_{2 α} , which access the ovary via a vascular countercurrent system to promote corpus luteum development and progesterone synthesis.

gion of the tract because the effect is seen only in ovaries ipsilateral to uterine horns receiving the seminal stimulus. In recently published work, we have shown an effect of seminal plasma on development of the corpus luteum and its steroidogenic capacity (O'Leary et al., 2006), with infiltrating macrophages implicated as the mediating cells in this process.

The mechanism and route of signal transfer from the uterus to the ovary is unknown, but an attractive possibility is the vascular counter-current mechanism linking the uterus and ovary, with uterine prostaglandins targeting the ovary to induce local inflammatory changes. Studies in which uterine horns are unilaterally ligated implicate a local communication pathway between the uterine horn and the adjacent ovary (Waberski et al., 1995). The prostaglandins PGF_{2 α} and PGE, known to be induced in the endometrium by seminal plasma estrogens (Claus, 1990) and facilitated by upregulated COX-2 expression (O'Leary et al., 2004) are likely candidates in influencing follicular development and rupture. These endometrial prostaglandins have been shown to reach the ovary by countercurrent exchange between the closely apposed uterine venous network and the ovarian

artery (Claus, 1990). In nonmated gilts, PGF_{2 α} is the major eicosanoid, but in mated gilts peak prostaglandin secretion occurs earlier and PGE₂ predominates, antagonizing the luteolytic effects of PGF_{2 α} (Christenson et al., 1994). The increase in COX-2 mRNA after exposure to seminal plasma thus raises the tantalizing possibility of a new role for seminal plasma in amplifying uterine-derived prostaglandin signals that act to precipitate ovulation, exert antiluteolytic effects, and increase progesterone synthesis (Figure 2).

SUMMARY AND CONCLUSIONS

Exposure to seminal fluid elicits inflammatory changes in the reproductive tract of all mammalian species so far examined. Studies in rodents have provided the greatest insight into the molecular and cellular basis of this response and its role in reproductive physiology, underpinning the view that seminal fluid must be considered not just a survival medium for sperm, but as a means for communication between the male and female reproductive tissues and a key factor in conditioning the female tract for pregnancy. In rodents, seminal fluid

exposure contributes to optimal pregnancy outcome, through activating inflammatory and immunological changes in the female reproductive tract that have beneficial effects for embryonic development and implantation. Many aspects of the mechanisms explaining these effects are now elucidated, with uterine cytokines and leukocytes playing central roles.

New studies informed by knowledge gained in the mouse are beginning to identify cytokines and other components of seminal fluid signaling pathways in pigs. However, there is still much to learn in this species regarding (1) the nature of and interaction between active moieties present in sperm and seminal plasma, (2) the identity of the cytokine and leukocyte responses elicited in the female tissues, and (3) the downstream consequences for the immune response to male gametes, embryonic development, and endometrial receptivity. Whether seminal fluid signaling has any beneficial role in establishing pregnancy in pigs, or alternatively is concerned more with maintaining female tract homeostasis, requires further evaluation. To address this, well-designed experiments with adequate power are required to quantify the effects of exposure to seminal fluid and its constituents in early pregnancy. In addition to pregnancy and farrowing rates, characteristics including fetal development and postnatal growth trajectory, and the viability, health, and body composition of offspring all warrant investigation. These studies need to take into account the significance of collection and storage conditions on seminal fluid signaling potency and the possible importance of matching between seminal plasma and sperm donors, as well as confounding factors such as the timing of treatment relative to estrus and AI, and variation in breeding performance of different herds.

A better understanding of seminal plasma signaling in pigs could lead to improved breeding management protocols to reduce embryo mortality in early pregnancy and ensure maximal reproductive efficiency. Options to explore might include more widespread utilization of vasectomized males, less extensive dilution of seminal fluid, or utilization of proteins present in seminal fluid to improve the function of commercial semen extenders.

LITERATURE CITED

- Almlid, T. 1981. Does enhanced antigenicity of semen increase the litter size in pigs? *Zeitschrift fuer tierzucht und zuchtungsbiologie*. *J. Anim. Breed. Genet.* 98:1–10.
- Aplin, J. D. 2002. Endometrial extracellular matrix. Pages 294–307 in *The Endometrium*. S. R. Glasser, J. D. Aplin, L. C. Guidice, and S. Tabibzadeh, ed. Taylor and Francis, London, UK, and New York, NY.
- Aumuller, G., and A. Riva. 1992. Morphology and functions of the human seminal vesicle. *Andrologia* 24:183–196.
- Baker, R. D., P. J. Dziuk, and H. W. Norton. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. *J. Anim. Sci.* 27:88–93.
- Beer, A. E., and R. E. Billingham. 1974. Host responses to intra-uterine tissue, cellular and fetal allografts. *J. Reprod. Fertil. Suppl.* 21:59–88.
- Bischof, R. J., M. R. Brandon, and C. S. Lee. 1995. Cellular immune responses in the pig uterus during pregnancy. *J. Reprod. Immunol.* 29:161–178.
- Bischof, R. J., C. S. Lee, M. R. Brandon, and E. Meeusen. 1994. Inflammatory response in the pig uterus induced by seminal plasma. *J. Reprod. Immunol.* 26:131–146.
- Bromfield, J. J., C. T. Roberts, and S. A. Robertson. 2004. Seminal plasma programs uterine receptivity and pregnancy outcome. *Biol. Reprod.* 37th Annual Meeting of the Society for the Study of Reproduction: 94.
- Carp, H. J., D. M. Serr, S. Mashlach, and L. Nebel. 1984. Influence of insemination on the implantation of transferred rat blastocysts. *Gynecol. Obstet. Invest.* 18:194–198.
- Chow, P. H., H. Y. Jiang, H. K. Poon, K. H. Lee, and W. S. O. 2003. Embryos sired by males without accessory sex glands induce failure of uterine support: A study of VEGF, MMP and TGF expression in the golden hamster. *Anat. Embryol. (Berl.)* 206:203–213.
- Christenson, L. K., D. B. Farley, L. H. Anderson, and S. P. Ford. 1994. Luteal maintenance during early pregnancy in the pig: Role for prostaglandin E2. *Prostaglandins* 47:61–75.
- Claus, R. 1990. Physiological role of seminal components in the reproductive tract of the female pig. *J. Reprod. Fertil. Suppl.* 40:117–131.
- De, M., R. Choudhuri, and G. W. Wood. 1991. Determination of the number and distribution of macrophages, lymphocytes, and granulocytes in the mouse uterus from mating through implantation. *J. Leukoc. Biol.* 50:252–262.
- Engelhardt, H., B. A. Croy, and G. J. King. 1997. Role of uterine immune cells in early pregnancy in pigs. *J. Reprod. Fertil. Suppl.* 52:115–131.
- Flowers, W. L., and K. L. Esbenschade. 1993. Optimizing management of natural and artificial matings in swine. *J. Reprod. Fertil. Suppl.* 48:217–228.
- Gangnuss, S., M. L. Sutton-McDowall, S. A. Robertson, and D. T. Armstrong. 2004. Seminal plasma regulates corpora lutea macrophage populations during early pregnancy in mice. *Biol. Reprod.* 71:1135–1141.
- Goetzl, E. J., M. J. Banda, and D. Leppert. 1996. Matrix metalloproteinases in immunity. *J. Immunol.* 156:1–4.
- Hunt, J. S., and S. A. Robertson. 1996. Uterine macrophages and environmental programming for pregnancy success. *J. Reprod. Immunol.* 32:1–25.
- Johansson, M., J. J. Bromfield, M. J. Jasper, and S. A. Robertson. 2004. Semen activates the female immune response during early pregnancy in mice. *Immunol.* 112:290–300.
- Kosaka, K., H. Fujiwara, K. Tatsumi, S. Yoshioka, T. Higuchi, Y. Sato, T. Nakayama, and S. Fujii. 2003. Human peripheral blood mononuclear cells enhance cell-cell interaction between human endometrial epithelial cells and BeWo-cell spheroids. *Hum. Reprod.* 18:19–25.
- Letterio, J. J., and A. B. Roberts. 1998. Regulation of immune responses by *tgf-beta*. *Annu. Rev. Immunol.* 16:137–161.
- Lovell, J. W., and R. Getty. 1968. Fate of semen in the uterus of the sow: Histologic study of endometrium during the 27 hours after natural service. *Am. J. Vet. Res.* 29:609–625.
- Maegawa, M., M. Kamada, M. Irahara, S. Yamamoto, S. Yoshikawa, Y. Kasai, Y. Ohmoto, H. Gima, C. J. Thaler, and T. Aono. 2002. A repertoire of cytokines in human seminal plasma. *J. Reprod. Immunol.* 54:33–42.
- Mah, J., J. E. Tilton, G. L. Williams, J. N. Johnson, and M. J. Marchello. 1985. The effect of repeated mating at short intervals on reproductive performance of gilts. *J. Anim. Sci.* 60:1052–1054.
- Mann, T. 1964. *The biochemistry of semen and the male reproductive tract*. John Wiley and Sons Inc., New York, NY.
- McMaster, M. T., R. C. Newton, S. K. Dey, and G. K. Andrews. 1992. Activation and distribution of inflammatory cells in the mouse uterus during the preimplantation period. *J. Immunol.* 148:1699–1705.
- Murray, F. A., P. Grifo, and C. F. Parker. 1983. Increased litter size in gilts by intrauterine infusion of seminal and sperm antigens before mating. *J. Anim. Sci.* 56:895–900.

- Murray, F. A., P. Grifo, and C. F. Parker. 1986. Increased litter size in gilts by presensitization with boar semen. *J. Anim. Sci.* 196(Suppl):189.
- O, W. S., H. Q. Chen, and P. H. Chow. 1988. Effects of male accessory sex gland secretions on early embryonic development in the golden hamster. *J. Reprod. Fertil.* 84:341–344.
- O'Leary, S., M. J. Jasper, S. A. Robertson, and D. T. Armstrong. 2006. Seminal plasma regulates ovarian progesterone production, leukocyte recruitment and follicular cell responses in the pig. *Reprod.* 25:1–12.
- O'Leary, S., M. J. Jasper, G. M. Warnes, D. T. Armstrong, and S. A. Robertson. 2004. Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. *Reprod.* 128:237–247.
- O'Leary, S., S. A. Robertson, and D. T. Armstrong. 2002. The influence of seminal plasma on ovarian function in pigs—A novel inflammatory mechanism? *J. Reprod. Immunol.* 57:225–238.
- Pang, S. F., P. H. Chow, and T. M. Wong. 1979. The role of the seminal vesicle, coagulating glands and prostate glands on the fertility and fecundity of mice. *J. Reprod. Fertil.* 56:129–132.
- Peitz, B., and P. Olds Clarke. 1986. Effects of seminal vesicle removal on fertility and uterine sperm motility in the house mouse. *Biol. Reprod.* 35:608–617.
- Queen, F., C. B. Dhabuwala, and C. G. Pierrepoint. 1981. The effect of removal of the various accessory sex glands on the fertility of male rats. *J. Reprod. Fertil.* 62:423–436.
- Rhodes, M., J. H. Brendemuhl, and P. J. Hansen. 2006. Litter characteristics of gilts artificially inseminated with transforming growth factor-beta. *Am. J. Reprod. Immunol.* 56:153–156.
- Rivera, R. M., C. R. Youngs, and S. P. Ford. 1996. A comparison of the number of inner cell mass and trophectoderm cells of preimplantation Meishan and Yorkshire pig embryos at similar developmental stages. *J. Reprod. Fertil.* 106:111–116.
- Robertson, S. A. 2005. Seminal plasma and male factor signalling in the female reproductive tract. *Cell Tissue Res.* 322:43–52.
- Robertson, S. A., M. Allanson, and V. J. Mau. 1998. Molecular regulation of uterine leukocyte recruitment during early pregnancy in the mouse. *Troph. Res.* 11:101–120.
- Robertson, S. A., W. V. Ingman, S. O'Leary, D. J. Sharkey, and K. P. Tremellen. 2002. Transforming growth factor beta—A mediator of immune deviation in seminal plasma. *J. Reprod. Immunol.* 57:109.
- Robertson, S. A., V. J. Mau, S. A. Hudson, and K. P. Tremellen. 1997. Cytokine-leukocyte networks and the establishment of pregnancy. *Am. J. Reprod. Immunol.* 37:438–442.
- Robertson, S. A., V. J. Mau, K. P. Tremellen, and R. F. Seamark. 1996. Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. *J. Reprod. Fertil.* 107:265–277.
- Robertson, S. A., G. Mayrhofer, and R. F. Seamark. 1992. Uterine epithelial cells synthesize granulocyte-macrophage colony-stimulating factor and interleukin-6 in pregnant and nonpregnant mice. *Biol. Reprod.* 46:1069–1079.
- Robertson, S. A., G. Mayrhofer, and R. F. Seamark. 1996. Ovarian steroid hormones regulate granulocyte-macrophage colony-stimulating factor synthesis by uterine epithelial cells in the mouse. *Biol. Reprod.* 54:183–196.
- Robertson, S. A., and D. J. Sharkey. 2001. The role of semen in induction of maternal immune tolerance to pregnancy. *Semin. Immunol.* 13:243–254.
- Robertson, S. A., C. Sjoblom, M. J. Jasper, R. J. Norman, and R. F. Seamark. 2001. Granulocyte-macrophage colony-stimulating factor promotes glucose transport and blastomere viability in murine preimplantation embryos. *Biol. Reprod.* 64:1206–1215.
- Roldan, E. R., M. Gomendio, and A. D. Vitullo. 1992. The evolution of eutherian spermatozoa and underlying selective forces: Female selection and sperm competition. *Biol. Rev. Camb. Philos. Soc.* 67:551–593.
- Rozeboom, K. J., G. Rocha-Chavez, and M. H. Tredsson. 2001a. Inhibition of neutrophil chemotaxis by pig seminal plasma in vitro: A potential method for modulating post-breeding inflammation in sows. *Reproduction* 121:567–572.
- Rozeboom, K. J., M. H. Tredsson, and B. G. Crabo. 1998. Characterization of uterine leukocyte infiltration in gilts after artificial insemination. *J. Reprod. Fertil.* 114:195–199.
- Rozeboom, K. J., M. H. Tredsson, H. H. Hodson, G. C. Shurson, and B. G. Crabo. 2000. The importance of seminal plasma on the fertility of subsequent artificial inseminations in swine. *J. Anim. Sci.* 78:443–448.
- Rozeboom, K. J., M. H. Tredsson, T. W. Molitor, and B. G. Crabo. 1999. The effect of spermatozoa and seminal plasma on leukocyte migration into the uterus of gilts. *J. Anim. Sci.* 77:2201–2206.
- Rozeboom, K. J., M. H. Tredsson, G. R. Rocha, and B. G. Crabo. 2001b. The chemotactic properties of porcine seminal components toward neutrophils in vitro. *J. Anim. Sci.* 79:996–1002.
- Sanford, T. R., M. De, and G. W. Wood. 1992. Expression of colony-stimulating factors and inflammatory cytokines in the uterus of cd1 mice during days 1 to 3 of pregnancy. *J. Reprod. Fertil.* 94:213–220.
- Signoret, J. P., F. Du Mesnil du Buisson, and P. Mauleon. 1972. Effect of mating on the onset and duration of ovulation in the sow. *J. Reprod. Fertil.* 31:327–330.
- Sjoblom, C., C. T. Roberts, M. Wikland, and S. A. Robertson. 2005. GM-CSF alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinol.*
- Skjervold, H. 1975. Comparison of litter size by use of natural and by artificial mating. *Z. Tierzucht. Zucht. Biol.* 92:252–259.
- Sunderkotter, C., K. Steinbrink, M. Goebeler, R. Bhardwaj, and C. Sorg. 1994. Macrophages and angiogenesis. *J. Leukoc. Biol.* 55:410–422.
- Taylor, N. J. 1982. Investigation of sperm-induced cervical leucocytosis by a double mating study in rabbits. *J. Reprod. Fertil.* 66:157–160.
- Thaler, C. J. 1989. Immunological role for seminal plasma in insemination and pregnancy. *Am. J. Reprod. Immunol.* 21:147–150.
- Tomlinson, M. J., A. White, C. L. Barratt, A. E. Bolton, and I. D. Cooke. 1992. The removal of morphologically abnormal sperm forms by phagocytes: A positive role for seminal leukocytes? *Hum. Reprod.* 7:517–522.
- Tremellen, K. P., R. F. Seamark, and S. A. Robertson. 1998. Seminal transforming growth factor beta1 stimulates granulocyte-macrophage colony-stimulating factor production and inflammatory cell recruitment in the murine uterus. *Biol. Reprod.* 58:1217–1225.
- Vazquez, J. M., E. A. Martinez, J. Roca, M. A. Gil, I. Parrilla, C. Cuello, G. Carvajal, X. Lucas, and J. L. Vazquez. 2005. Improving the efficiency of sperm technologies in pigs: The value of deep intra-uterine insemination. *Theriogenology* 63:536–547.
- Waberski, D., R. Claassen, T. Hahn, P. W. Jungblut, N. Parvizi, E. Kallweit, and K. F. Weitze. 1997. LH profile and advancement of ovulation after trans-cervical infusion of seminal plasma at different stages of estrus in gilts. *J. Reprod. Fertil.* 109:29–34.
- Waberski, D., A. Dohring, F. Ardon, N. Ritter, H. Zerbe, H. J. Schuberth, M. Hewicker-Trautwein, K. F. Weitze, and R. H. Hunter. 2006. Physiological routes from intra-uterine seminal contents to advancement of ovulation. *Acta Vet. Scand.* 48:13.
- Waberski, D., H. Sudhoff, T. Hahn, P. W. Jungblut, E. Kallweit, J. J. Calvete, M. Ensslin, H. O. Hoppen, N. Wintergalen, and K. F. Weitze. 1995. Advanced ovulation in gilts by the intrauterine application of a low molecular mass pronase-sensitive fraction of boar seminal plasma. *J. Reprod. Fertil.* 105:247–252.
- Watson, J. G., J. Carroll, and S. Chaykin. 1983. Reproduction in mice: The fate of spermatozoa not involved in fertilization. *Gamete Res.* 7:75–84.
- Weiner, H. L. 2001. Oral tolerance: Immune mechanisms and the generation of Th3-type TGF-beta-secreting regulatory cells. *Microbes Infect.* 3:947–954.

References

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