

Review

Seminal ‘priming’ for protection from pre-eclampsia—a unifying hypothesis

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Abstract

Conventional belief holds that an immune response to ejaculate antigens should interfere with fertilisation and establishment of pregnancy. However, emerging evidence now supports the opposing view—that insemination acts to activate maternal immune mechanisms exerting a positive effect on reproductive events. In a response well documented in rodents, semen triggers an influx of antigen-presenting cells into the female reproductive tract which process and present paternal ejaculate antigens to elicit activation of lymphocytes in the adaptive immune compartment. Transforming growth factor beta (TGF β), a cytokine present in abundance in seminal plasma, initiates this inflammatory response by stimulating the synthesis of pro-inflammatory cytokines and chemokines in uterine tissues. Lymphocyte activation is evident in lymph nodes draining the uterus and leads to hypo-responsiveness in T-cells reactive with paternal alloantigens. TGF β has potent immune-deviating effects and is likely to be the key agent in skewing the immune response against a Type-1 bias. Prior exposure to semen in the context of TGF β can be shown to be associated with enhanced fetal–placental development late in gestation. In this paper, we review the experimental basis for these claims and propose the hypothesis that, in women, the partner-specific protective effect of insemination in pre-eclampsia might be explained by induction of immunological hypo-responsiveness conferring tolerance to histocompatibility antigens present in the ejaculate and shared by the conceptus.

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1. Semen exposure and pregnancy outcome

Historically, seminal plasma has been viewed primarily as a transport medium for spermatozoa traversing the female reproductive tract. Indeed, seminal plasma is often alleged to play a minor role in mammalian reproduction since viable pregnancies can be initiated using epididymal or washed ejaculated sperm in artificial insemination or in vitro fertilisation/embryo transfer (Mann, 1964). However, studies from our laboratory and other groups over the past decade now clearly demonstrate that specific factors in the non-cellular fraction of semen interact with the female reproductive tract to evoke a cascade of cellular and molecular changes resembling a classical inflammatory response. Emerging information on the sequelae of this response indicate that insemination can be viewed as an immunising event acting to elicit changes in the maternal immune axis required to facilitate embryo implantation and successful pregnancy outcome.

While it is clear that seminal plasma is not mandatory for successful reproduction, studies in many animal species indicate that pregnancy, and potentially the growth and development of the fetus, are compromised if females are not exposed to seminal plasma. Experiments in which the seminal vesicle, prostate or coagulating glands are surgically removed from stud mice, rats and hamsters prior to mating each show that seminal vesicle fluid is the most vital non-sperm component of the ejaculate (Pang et al., 1979; Queen et al., 1981; Peitz and Olds Clarke, 1986; O et al., 1988). In mice, embryo transfer protocols routinely employ recipients prepared by mating to vasectomised males, but fetal loss and abnormality is considerably greater in those few reports where pseudopregnancy is achieved without exposure to seminal fluid (Watson et al., 1977). A study in rats has shown that, to some extent, this deficiency can be restored by insemination of females prior to embryo transfer (Carp et al., 1984). In pigs, artificial insemination with diluted semen reduces litter sizes but mating with a vasectomised male or administration of heat-killed semen restores litter size and improves farrowing rate (Murray et al., 1983; Mah et al., 1985).

Clinical studies in humans have shown that live birth rates in couples undergoing IVF or GIFT treatments are significantly improved when women are exposed to semen at the time of embryo or gamete transfer (Bellinge et al., 1986; Tremellen et al., 2000). Furthermore, treatment of women suffering from recurrent spontaneous abortion with seminal plasma pessaries has been reported to improve pregnancy success (Coulam and Stern, 1995). In pre-eclampsia, there is a cumulative benefit of chronic exposure to semen, with

limited sexual experience or use of barrier methods of contraception being linked with increased risk (Klonoff-Cohen et al., 1989; Robillard et al., 1995) and evidence from women where a change in male partner has occurred suggesting that the effect is partner-specific (Dekker et al., 1998). Markedly increased rates of pre-eclampsia are also evident in pregnancies initiated by donor oocytes or semen (Salha et al., 1999), when prior exposure to conceptus antigens has not occurred. Furthermore, vaginal intercourse may not be the only manner in which semen can improve reproductive outcome since it has been reported that oral sex is also associated with a diminished incidence of pre-eclampsia, provided that semen contains normal levels of soluble HLA (Koelman et al., 2000). Together, the observations of partner specificity and cumulative benefit of semen exposure imply that immunological ‘memory’ to partner’s antigens may be programmed at insemination.

2. Insemination primes the maternal immune system to paternal antigens

A mechanism by which semen might influence reproductive success or susceptibility to pre-eclampsia has not been defined, but increasing evidence of an immune aetiology in pre-eclampsia raises the tantalising possibility that exposure to semen can impact on the phenotypes or abundance of lymphocyte subsets regulating implantation and placental morphogenesis, thus potentially subsequent function of the placenta later in gestation. Studies in rodents indicate that specific populations of uterine natural killer cells and unusual populations of regulatory T-lymphocytes appear within the decidua to promote placental growth and development within the first days after implantation (Guimond et al., 1997; Arck et al., 1999) but, to date, the mechanisms regulating their ontogeny are not understood. Clues as to the functional relevance of the T-cell response to pregnancy come from the observation that successful pregnancy is associated with a systemic state of functional immune ‘tolerance’, mediated by hypo-responsiveness in T-cells reactive with paternal major histocompatibility antigens (Tafari et al., 1995). In this context, ‘tolerance’ is taken to mean active skewing of the immune response to prevent potentially injurious Type 1 immunity, rather than immunological non-responsiveness. This contemporary definition of tolerance is now commonly applied to mucosal tissues and invokes clonal anergy in T-lymphocytes, or the agency of regulatory or suppressive lymphocytes, as opposed to clonal deletion (Weiner, 2001). Intriguingly from the perspective of this paper, active immune tolerance is shown to be evident from day 4 of

pregnancy, prior to the time of implantation and when the likelihood of antigenic stimulation from the fewer than 100 cells that comprise the embryo is very low (Tafari et al., 1995).

We have formulated a working hypothesis linking the maternal immune response to ejaculate antigens with expansion of maternal regulatory or effector lymphocyte subsets implicated in implantation and placental development. This hypothesis is based on four central observations. Firstly, semen contains abundant paternal antigens shared by the conceptus. Secondly, the female reproductive tract is an immuno-competent mucosal site, primed at the time of insemination to elicit immune responses to foreign antigens. Thirdly, seminal plasma contains several powerful immunoregulatory molecules that can dampen potentially destructive Type-1 (cell-mediated) immune responses and drive immune outcomes of the quality required for functional immune tolerance. Finally, changes in T-lymphocyte status in draining lymph nodes and peripherally in the female after insemination are accompanied by evidence of a transient state of hyporesponsiveness in paternal alloantigen-reactive lymphocytes. The experimental evidence supporting our hypothesis will be briefly outlined in the following sections.

3. Semen contains paternal antigens shared by the conceptus

There is considerable relatedness between the antigenic repertoire of semen and the conceptus; indeed, semen might reasonably be said to provide the first and most frequent exposure to paternal antigens expressed by the conceptus. Semen contains abundant major and minor histocompatibility and other antigens (Thaler, 1989). MHC class I and II antigens can be detected on the surface of human sperm, albeit at a lower density than in somatic cells (Martin Villa et al., 1996). The semen of fertile men contains somatic cells such as leukocytes and desquamated genital tract epithelial cells. While somatic cells are numerically inferior to sperm, they are a more potent stimulus for maternal lymphocyte activation *in vitro*, presumably due to their higher membrane expression of MHC antigens (Rodriguez and Arnaiz, 1982). Furthermore, soluble HLA has been identified in seminal plasma (Koelman et al., 2000). Major histocompatibility antigens are expressed by placental trophoblast cells in mice and in humans, although in the latter this is restricted to the less polymorphic HLA-C and class Ib molecules HLA-E and HLA-G.

4. Insemination induces an inflammatory cascade

Shortly following deposition of semen at mating, striking changes in leukocyte populations occur within the superficial cell layers of the cervix and uterus in rodents, pigs, rabbits and women (Lovell and Getty, 1968; Phillips and Mahler, 1975; Pandya and Cohen, 1985; De et al., 1991; McMaster et al., 1992; Robertson et al., 1996). The molecular and cellular basis of this post-mating inflammatory response has been most thoroughly delineated in mice (De et al., 1991; McMaster et al., 1992; Robertson et al., 1996). The response is initiated when seminal plasma moieties interact with the estrogen-primed uterine epithelial cells to induce a surge in synthesis of pro-inflammatory cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6 and an array of chemokines (Robertson et al., 1996, 1998). Seminal vesicle-derived TGF β 1 has been identified as the principal trigger for induction of uterine epithelial GM-CSF synthesis following mating (Tremellen et al., 1998). This cytokine cascade elicits recruitment and activation of antigen-presenting cells including macrophages and dendritic cells into the endometrial stroma, where these cells accumulate subjacent to the surface epithelium, then engulf and process paternal ejaculate antigens, before trafficking to para-aortic lymph nodes draining the uterus, the mesenteric lymph nodes and spleen (Watson et al., 1983).

Activation of paternally-reactive lymphocytes in draining lymph nodes is then seen to occur during the following days. The para-aortic lymph nodes of mice transiently enlarge after allogeneic insemination (Beer and Billingham, 1974) as T-lymphocyte proliferation commences and expression of cytokines and activation antigens becomes evident (Johansson and Robertson, submitted). Amongst the responding cells are T-lymphocytes reactive with paternal MHC antigens, which are aggressively immunostimulatory in graft-versus-host assays for the first 2 days after insemination and then show evidence of suppressive regulation (Piazzon et al., 1985). Similarly, paternal-specific alloreactivity is strongly suppressed in para-aortic lymph node cells recovered on day 3 of pregnancy from rats (Kapovic and Rukavina, 1991). TGF β -producing suppressor cells have also been identified within the para-aortic lymph node from the time of implantation (Clark and McDermott, 1981).

The response to insemination in women has been less extensively characterised for obvious reasons, but the available information suggests that cellular changes comparable to those seen in the mouse may take place in the human cervix. In a recent study examining the local effects of natural insemination in peri-ovulatory women, we have identified a striking infiltration of macrophages, dendritic cells, granulocytes and lymphocytes

into the cervical epithelium and stroma shortly following intercourse (Robertson et al., 2001). The stimulus for this leukocyte influx appears to be an interaction between the cervical epithelium and semen since no inflammatory response was seen following condom-protected intercourse. TGF β has been identified as a likely candidate for the active moiety in human semen in experiments using primary human cervical keratinocyte cultures, where seminal plasma TGF β has been shown to elicit an increase in keratinocyte GM-CSF and chemokine release (Tremellen and Robertson, 1997).

5. Semen contains abundant immuno-regulatory factors

Intuitively, it seems logical that generation of an immune response to ejaculate antigens would have an adverse effect on reproduction, as is illustrated by infertility secondary to anti-sperm antibodies. Despite this, it is clear that semen does not usually elicit any hypersensitivity reaction or other form of Type 1 anti-sperm immunity. Indeed, the molecular composition of seminal plasma may ensure that the female response to seminal antigens is hypo-responsive in the Type 1 compartment. Two obvious candidates for the induction of tolerance to seminal antigens are prostaglandin E₂ (PGE₂) and TGF β , since both are present in high concentrations within mammalian semen (Nocera and Chu, 1993; Kelly and Critchley, 1997; Tremellen et al., 1998) and both have well described immune-deviating capacities in other tissues. TGF β is commonly recognised for its potent capacity to inhibit the induction of Type 1 immunity in the anterior chamber of the eye and at other mucosal surfaces (Wilbanks et al., 1992; Letterio and Roberts, 1998; Weiner, 2001). TGF β ₁ is present in extremely high concentrations (> 100 ng/ml) in murine seminal vesicle secretions (Tremellen et al., 1998), implicating this molecule as an attractive candidate for an immune-deviating agent in the ejaculate. The occurrence and likely role of TGF β in seminal plasma has been recently reviewed (Robertson et al., 2002).

6. Insemination induces immune tolerance to paternal antigens

That semen can induce functional immune tolerance to male antigens was first suggested by experiments showing that mated mice are unable to reject syngenic skin grafts of paternal origin (Lengerova and Vojtiskova, 1966). Subsequently, it was demonstrated that protection is similarly conferred to major histocompatibility antigens (Beer and Billingham, 1974), but only

when sperm is delivered in the context of seminal plasma. Washed sperm, but not whole semen, was shown to elicit transplantation immunity to paternal skin graft challenge, despite both immunisation events leading to lymph node hypertrophy. Likewise, immunisation with washed sperm but not natural insemination primed mice for generation of cytotoxic T-lymphocytes against H–Y antigen (Hancock and Faruki, 1986). The potential for a beneficial effect of this immune response on pregnancy outcome was identified in experiments showing that uterine ‘priming’ with semen can promote implantation and fetal growth in subsequent pregnancies, in a partner-specific manner (Beer et al., 1975). Consistent with an immunological mechanism, removal of lymph nodes draining the uterus after exposure to semen revoked the effect and led to a decrease in litter size and fetal and placental weight (Beer et al., 1975; Tofoski and Gill, 1977).

We have further investigated the possibility that exposure to semen at mating may result in immunological hypo-responsiveness or ‘tolerance’ to paternal antigens. Using inbred congenic strains of mice, we examined the effect of exposure to semen on the immune response to paternal MHC class I antigens by measuring the growth or rejection of paternal tumour cells administered to the female at the approximate time of embryo implantation. Balb/c (H-2^d) tumour cells, which are always rejected in virgin Balb/k (H-2^k) mice, were found to grow in Balb/k female mice mated with Balb/c stud males, even when the females had been sterilised by ligation of the utero–tubal junction (Robertson et al., 1997). This finding confirmed that exposure to semen is sufficient to induce specific hypo-responsiveness to paternal MHC class I antigens, irrespective of whether pregnancy ensues. The response is specific, since mating with syngeneic Balb/k or third party males did not confer any loss of resistance to the tumour, but relatively short-lived in that tumour challenges 10 or more days after insemination are invariably rejected. Whether repeated mating might act to strengthen the response or increase its duration has not been studied.

7. Immunisation with sperm and TGF β promotes subsequent pregnancy outcome

Since semen and trophoblast cells express common transplantation antigens, it is plausible that the initiation of an appropriate immune response to seminal antigens at the time of mating may later benefit the growth and survival of the semi-allogenic conceptus. In view of the central role of TGF β in this process, we examined the effect of intra-uterine immunisation of female mice with sperm in combination with rTGF β 1 on subsequent

reproductive outcome. Balb/c \times C57Bl/6 F1 females were immunised trans-cervically with CBA sperm, with or without 20 ng rTGF β ₁, 2 weeks before mating with intact stud CBA males. Prior immunisation with sperm plus rTGF β ₁ resulted in a 5% and 8% increase in mean fetal and placental weight, respectively, on day 17 of the subsequent pregnancy (Table 1). These increases were significant even when the small decrease in litter size evident in all females immunised with sperm, regardless of the presence of rTGF β ₁, was taken into account. Although a moderate increase in placental weight was observed in mice immunised with sperm alone, there was no accompanying increase in fetal weight and a decrease in the fetal:placental weight ratio, suggesting that this treatment led to a decrease in placental efficiency.

This outcome is consistent with the proposal that the immune response to pregnancy is facilitated by prior exposure to paternal antigens delivered in an immune-deviating context. Clearly, these data are preliminary and issues relating to antigenic specificity and mechanism remain to be addressed. The exciting possibility that various decidual lymphocyte lineages known to facilitate or interfere with early placental development, respectively (Guimond et al., 1997; Arck et al., 1999) might be influenced by prior exposure to semen remains to be tested. In ongoing experiments, we are examining the significance of paternal MHC haplotype, as well as the relationship between the lymphocyte populations activated at insemination and those recruited into the decidua at the time of implantation and early placental development.

Table 1

The effect on pregnancy outcome of immunisation with sperm and TGF β

	Control	Sperm + TGF β	Sperm
Number of females	12	14	10
Litter size	11.4 \pm 1.0 ^a	10.4 \pm 1.2 ^b	10.3 \pm 0.9 ^b
Number viable pups	11.2 \pm 1.3 ^a	10.1 \pm 1.5 ^b	10.1 \pm 0.9 ^b
Number resorptions	0.17 \pm 0.58 ^a	0.21 \pm 0.58 ^a	0.20 \pm 0.42 ^a
Fetal weight (mg)	645.2 \pm 61.2 ^a	677.6 \pm 56.6 ^b	646.1 \pm 49.9 ^a
Placental weight (mg)	97.7 \pm 12.1 ^a	105.2 \pm 12.4 ^b	101.8 \pm 9.8 ^b
Fetal:placental ratio	6.7 \pm 0.9 ^a	6.5 \pm 0.8 ^{ab}	6.4 \pm 0.8 ^b

Estrous adult female Balb/c \times C57Bl/6 mice were given intra-uterine immunisations, by the trans-cervical route, of 1×10^6 CBA sperm with or without 20 ng rhTGF β ₁. Control mice received PBS. Two weeks later, females were mated with CBA males. On day 17 of pregnancy, females were sacrificed and viable and resorbing fetuses were scored, and fetal and placental weights were measured. Values are mean \pm S.D. Data were analysed by one-way ANOVA, followed by Student's *t*-test. Different superscripts across rows indicate significant differences ($P < 0.05$) between groups.

8. Summary and conclusion

The precise mechanisms by which exposure to semen may favour the growth and survival of the semi-allogenic fetus are not fully defined. However, on the basis of experiments in rodent models, we have generated a working model that provides a useful conceptual framework to link the immunological events of insemination with early pregnancy (Fig. 1). We have shown that exposure of the female reproductive tract to seminal TGF β initiates an influx of antigen-presenting cells that sample ejaculate antigens and subsequently activate lymphocyte populations in lymph nodes draining the uterus. In congenic mice, one consequence of this response is the induction of a state of systemic functional tolerance to paternal MHC class I antigens. TGF β is implicated as a potent immune deviating agent in the uterus. Thus, the processing of paternal transplantation antigens in a milieu containing high levels of TGF β of seminal plasma origin may result in the generation of hypo-responsiveness in paternal antigen-specific T-lymphocytes. It is reasonable to postulate that, upon re-encounter with conceptus antigens, these regulator or effector T-cells might contribute to an

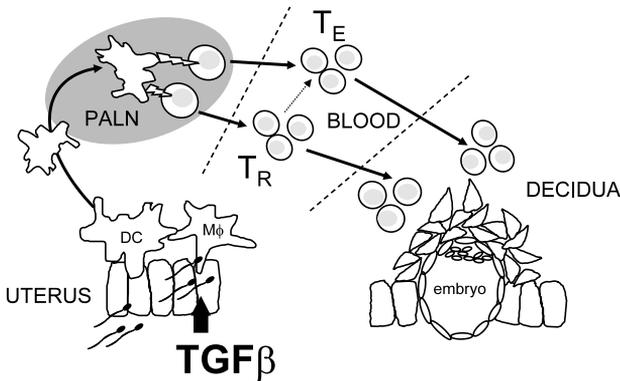


Fig. 1. Schematic illustration of a working model linking the immunological events of insemination with implantation and placental development. Seminal plasma TGF β deposited in the female tract at insemination elicits the recruitment and activation of macrophages (M ϕ) and dendritic cells (DC) which process and present seminal antigens to lymphocytes within lymph nodes draining the uterus. Activation and expansion of effector (T_E) and/or regulatory (T_R) T-lymphocytes confers a transient state of hypo-responsiveness in the paternal alloantigen reactive compartment, manifesting as a systemic immune 'tolerance' to paternal transplantation antigens. It is postulated that these lymphocyte populations facilitate implantation and placental development upon re-encounter with paternal antigens associated with the conceptus (figure modified from Robertson and Sharkey, 2001, with permission).

immunological environment favouring successful implantation and optimal placental growth.

Several challenges must be met for this hypothesis to be proven. Most importantly, it will be necessary to demonstrate identity or causal linkages between lymphocytes activated after insemination and those recruited into the decidua at embryo implantation. Experimental strategies including tracking the fate of passively transferred donor lymphocytes will assist in this endeavour. While uterine NK cells are undisputedly implicated in placental development, considerably greater research effort will be required to characterise and identify the roles of various putative regulatory T-lymphocyte populations in the placenta and decidual tissues. Encouragingly, there are phenotypic similarities between these decidual cells and unusual regulatory T-lymphocyte populations conferring tolerance in other mucosal tissues (Robertson, 2000).

While there are difficulties in justifying direct extrapolation from the rodent to the human, clearly these findings have implications for the association between semen exposure and the incidence of pathologies of human pregnancy. We speculate that the aberrant Type 1 immunity associated with 'shallow' placentation in pre-eclampsia and recurrent miscarriage (Dekker et al., 1998; Piccinni et al., 1998) can be initiated by insufficient or inappropriate immune responses to seminal antigens following intercourse, perhaps linked with seminal plasma cytokine deficiency or female incapacity to respond to seminal signals. Such couples might benefit from cumulative exposure to a specific partner's semen if each occurrence of intercourse were acting as a boost or phenotype-skewing stimulus to the woman's immune response. Achieving an appropriate quality of response to partner's semen is an additional goal potentially achievable through promoting activity of immune-deviating cytokines in semen by endogenous means or perhaps by using exogenous cytokines. A better understanding of the physiological significance of semen in pre-eclampsia requires further detailed exploration of the cellular and molecular processes occurring within the female reproductive tract at insemination, and may eventually yield novel therapies for this condition.

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