

REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY

Male reproductive proteins and reproductive outcomes

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An enormous diversity of antigenically distinct proteins is found in seminal plasma. These include proteases, protease inhibitors, signal transduction molecules, protein kinases and phosphatases, transporter proteins, structural molecules, and immune response proteins.¹ Sperm, too, carry a plethora of surface proteins; these mediate species-specific binding to the zona pellucida surrounding the oocyte.² Together, semen and sperm proteins are called male reproductive proteins (MRPs).

Male reproductive proteins have roles critical to evolution and reproductive immunology. Yet their clinical and epidemiologic impact on human reproduction is largely unstudied. The purpose of this Clinical Opinion is to suggest that MRPs may have broad influences on successful reproduction, potentially with roles in the composition of vaginal microflora, the occurrence of preterm birth and preeclampsia, and complications from assisted reproduction. As such, the import of MRPs on human reproductive success is suggested as an area

Male reproductive proteins (MRPs), associated with sperm and semen, are the moieties responsible for carrying male genes into the next generation. Evolutionary biologists have focused on their capacity to control conception. Immunologists have shown that MRPs cause female genital tract inflammation as preparatory for embryo implantation and placentation. These observations argue that MRPs are critically important to reproductive success. Yet the impact of male reproductive proteins on obstetrical outcomes in women is largely unstudied. Epidemiologic and clinical observations suggest that shorter-duration exposure to MRPs prior to conception may elevate the risk for preeclampsia. A limited literature has also linked sexual behavior to bacterial vaginosis and preterm birth. We offer a clinical opinion that MRPs may have broad implications for successful reproduction, potentially involved in the composition of vaginal microflora, risks of preterm birth and preeclampsia, and success of assisted reproduction.

Key words: preeclampsia, preterm birth, reproductive immunology, semen, sexual competition

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ripe for future clinical and epidemiologic research.

Evolution of male reproductive proteins

Male reproductive proteins are among the most rapidly evolving functional genes known. This rapid evolution has been the result of strong adaptive evolution³ because MRPs are the central players in the evolutionary competition to impregnate females. Males and females are each separately incited to maximize the likelihood of transmitting their genes. They thus engage in intersexual competition characterized by mate choice (generally by the female), cryptic female choice (wherein the female disposes of male sperm without his knowledge), and mate coercion (generally by the male).⁴

Male reproductive proteins can directly influence reproductive outcomes by changing female reproductive behavior. Most intensively studied in *Drosophila*, male accessory gland proteins secreted in semen have been shown to decrease the female's propensity to remate, increase oocyte production, and even reduce female life expectancy.⁵ When male *Drosophila melanogaster*

were allowed to evolve and then mate with females who were held at a fixed phenotype, male reproductive fitness increased, but so did rates of postmating female death.⁵ Thus, MRPs have evolved to maximize male reproductive success and are the agents of sexual competition.

Reproductive proteins and inflammation

Male reproductive proteins play a central role in embryo implantation and placentation by inducing inflammation.⁶ Sexual intercourse activates cytokines and elicits cervical and uterine recruitment of epithelial and stromal macrophages, dendritic cells, lymphocytes, and natural killer cells. During the period from coitus to apposition, these cells produce high levels of interleukins (IL- α , IL-1 β , and IL-6), tumor necrosis factor (TNF- α), and colony stimulating factor-1 (CSF-1).

Seminal plasma, but not sperm, interacts with cervical and uterine epithelial cells to induce release of proinflammatory cytokines and chemokines in mice.⁷ The semen proteins dominantly inducing and modulating cervical/uterine inflammation appear to be transforming factor (TGF)- β , prostaglandin E, IL-8,

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and interferon gamma.⁸ Postcoital cervical inflammation is eliminated by condom use, implicating an etiologic role for reproductive proteins.⁸

Inflammation, mediated by seminal fluid, also plays a critical role at the trophoblast-uterine interface in placental development.⁶ Activated lymphocytes and macrophages transit into the decidua in which they produce and regulate matrix metalloproteinases and angiogenic factors, both critical for the development of the maternal-placental circulation.

Seminal plasma prevents maternal attack of paternal alloantigens expressed on trophoblast. Female mice exposed to seminal fluid in the absence of sperm show hyporesponsiveness to male major histocompatibility complex antigens and male tumor cells.⁹ Prevention of rejection during pregnancy is thought to occur, in part, via immune modulation by Th2/3 cytokines, including IL-4, IL-10, TGF- β 2, and granulocyte macrophage-colony stimulating factor. These immunotrophic or Th 2/3 molecules protect against fetal loss, whereas an overly aggressive (Th1 dominated) maternal inflammatory responsiveness has been associated with a range of perinatal morbidities including preeclampsia and preterm birth (see following text). Whether repeated stimulation by MRPs better induces immunotrophism is not clear from in vitro and animal studies but is an important area of future research.

Novel exposure to reproductive proteins and preeclampsia

No studies to date have directly examined whether and how specific MRPs have an impact on the efficacy and outcomes of human reproduction. However, indirect evidence suggests their role may be far reaching.

Preeclampsia is associated with a pathologic intravascular inflammatory reaction, involving granulocyte and monocyte subsets, proinflammatory cytokines, and the clotting and complement systems.¹⁰ Some of the indirect evidence that we believe supports a role for MRPs in the genesis of preeclampsia in-

cludes: (1) the demonstration of a paternal contribution; (2) sperm involvement in the immunologic aberrancy seen with preeclampsia; and (3) a distinct pattern of male-female sexual partnerships contributing to preeclampsia.

A paternal contribution to preeclampsia risk was demonstrated in a multigeneration study from Utah in which both men and women who were the product of a pregnancy complicated by preeclampsia were more likely than controls, in turn, to contribute to a preeclamptic pregnancy.¹¹ That the mechanism for this may be immunologic is suggested by the following case report. A woman whose partner changed between her first and second pregnancies experienced new-onset, severe preeclampsia in the second pregnancy. The mixed lymphocyte reaction between the mother's and second father's cells was 8 times stronger than the lymphocyte reaction between the mother's and the first father's cells.¹²

First pregnancies, teenage pregnancies, out-of-wedlock pregnancies, paternity change, and donor sperm insemination¹³ each represent reproductive events wherein the female is likely to be relatively naïve to her partner's MRPs, and each of these markers has been shown to increase the risk for preeclampsia. These observations must be viewed with caution because age, educational level, carriage of sexually transmitted infections (STIs), and access to health care may all confound the relationship between shorter duration partnerships and preeclampsia.

Other observations also support the idea that novel exposure to MRPs may elevate preeclampsia risk. Women undergoing intrauterine insemination with washed donor sperm have a higher risk of preeclampsia than women undergoing intrauterine insemination with washed partner (autologous) sperm, implicating exposure to unfamiliar sperm. A particularly interesting comparison of in vitro fertilization or intracytoplasmic sperm injection (ICSI) with ICSI involving surgically obtained sperm showed a 2-fold elevation in preeclampsia risk with the latter, suggesting that exposure

to sperm, in the absence of semen, might increase preeclampsia risk.¹⁴

That a particular subset of males may confer excess preeclampsia risk was suggested by the observation that men who father a child from a preeclamptic birth and then change partners are more likely to father a subsequent preeclamptic birth.¹⁵ Other studies have reported that change in sexual partner elevated the risk for preeclampsia in a second pregnancy to a level almost as high as for first pregnancies.¹⁶ However, subsequent cohort studies demonstrated that a longer interval between pregnancies might account for the apparent partner change effect.¹⁷ The interpretation that longer interval invalidates the immunoreactivity concept in preeclampsia may be overly restrictive because none of these studies specifically examined partnership length.

Women without prior preeclampsia who had a change in a partner and then gave birth within a shorter-duration interval did demonstrate an elevated risk for preeclampsia in the second pregnancy.¹⁶ This may more closely replicate short-duration sexual cohabitation prior to conception. The single study that specifically assessed shorter sexual cohabitation prior to conception showed a linear and dramatic association with preeclampsia risk. Adjusted odds ratios were 11.6 for 0-4 months of cohabitation before conception, 5.9 for 5-8 months of cohabitation, and 4.2 for 9-12 months of cohabitation, as compared with the referent group of more than 12 months of cohabitation before conception.¹⁸

Spontaneous preterm birth

Few studies have considered a possible paternal contribution to another major inflammation-mediated perinatal complication: spontaneous preterm birth. Particularly when it involves infants born at less than 32 weeks and of less than 1500 g, preterm birth is generally accompanied by evidence of infection with low virulence bacterial vaginosis (BV)-associated organisms that appear to trigger excessive inflammation (histologic chorioamnionitis, decidual inflammation, or elevations in systemic inflammatory markers).¹⁹

Could novel exposure to MRPs contribute to preterm birth? A Norwegian population-based record linkage analysis of more than 30,000 women who changed partner between the first 2 births vs 450,000 women who retained the same partner for both births found that women with a new partner had 1.8- to 2.5-fold elevations in preterm birth, low birthweight, and infant mortality, after accounting for interval between births as well as age, cohort, and education.²⁰

An increasing number of lifetime sexual partners, each representing exposure to a unique set of MRPs, prior to the index pregnancy has also been associated with spontaneous preterm birth among women at high preexisting risk.²¹ Unfortunately, few studies have examined the pattern of sexual partnerships related to preterm birth and not all studies have been supportive.²² Moreover, cigarette smoking, socioeconomic status, or infection with STIs as a result of sexual partner change might confound the links between multiple or new partnerships and preterm birth.

Male reproductive proteins might influence preterm birth through altering the composition of vaginal bacterial colonization that is common among women experiencing this adverse perinatal outcome. Bacterial vaginosis is characterized by an overgrowth of the hydrogen peroxide (H₂O₂)-producing lactobacillus that normally maintains a healthy vaginal microenvironment by endogenous facultative and anaerobic microorganisms such as *Gardnerella vaginalis*, *Mycoplasma hominis*, and anaerobic Gram-negative rods (*Bacteroides* and *Prevotella* spp.). Bacterial vaginosis is consistently associated with an increased risk for preterm delivery and premature rupture of membranes.²³ However, because antibiotic treatment of BV does not necessarily reduce the risk of these outcomes, some have suggested that BV is a risk marker rather than an etiologic factor.²⁴

A greater number of sexual partners, new sexual partners, and early age at first intercourse are associated with BV in the absence of STIs.²⁵ Whereas these risk factors raise the possibility that BV may

involve an unmeasured sexually transmitted pathogen, they may also be explained by exposure to novel MRPs. Arguing against a bacterial etiology for BV is that male partner antibiotic treatment has generally proven to be an ineffective means for preventing BV in females. However, condoms, which prevent exposure to infectious agents and MRPs, appear to reduce the risk for developing BV. In longitudinal studies assessing condom use in relation to BV, robust methods have demonstrated that condoms reduce the risk of BV by half.²⁶ Because inflammation accompanies BV,²⁷ a mechanism by which MRPs may enhance susceptibility to BV is by producing an inflammatory milieu.

Sperm donation

If novel MRPs influence reproductive outcomes, donor insemination might provide a useful framework to demonstrate this. In general, pregnancies achieved by using ovulation induction and intrauterine insemination (donor or partner) are at higher risk for perinatal complications than naturally conceived pregnancies.²⁸ It is likely that both male and female factors contribute to perinatal complications in infertile couples, and, thus, the demonstration of potential immunoreactivity from novel MRPs would require an observation of excess risk from donor sperm, in the absence of female factor infertility.

Few studies have isolated the impact of male infertility, let alone the impact of using donor (vs partner) sperm on perinatal morbidity. As reviewed in earlier text, small studies have shown an elevated risk for preeclampsia among women exposed to donor sperm.¹⁵ In 1 comparison of ovulation induction and intrauterine insemination with donor sperm, as compared with partner sperm among infertile couples, the infants born from pregnancies involving partner sperm were substantially more likely to be of low birthweight.²⁹ Inseminations with partner sperm may, however, provide an inappropriate comparison group because these sperm are not necessarily normal. The better test of the novel MRP hypothesis would be to isolate the effect of foreign sperm by comparing donor

inseminations among women without female-factor infertility to nonassisted pregnancies. This design has not been used to date.

A limitation to considering the use of donor sperm as a demonstration of the impact of novel MRPs is that donor specimens are washed of seminal proteins prior to insemination, and, thus, only sperm-related effects can be observed. That this may be a serious limitation is underscored by animal models showing that seminal fluid enhances uterine inflammation and fertilization.⁷ Finally, 1 report demonstrated that couples undergoing in vitro fertilization that were counseled to have intercourse at the time of embryo transfer achieved higher implantation rates.³⁰ In current clinical practice, the potential for in vivo fertilization of oocytes not surgically retrieved and the increased probability for multiple pregnancy limits the recommendation for intercourse around the time of oocyte retrieval/embryo transfer.

Methodologic considerations and implications

The proposed link between MRPs and adverse pregnancy outcomes in humans is speculative. Yet there is ample reason to pursue this line of research. Their physiologic role on implantation and placentation and their evolutionary role in sexual competition suggest the centrality of MRPs in reproductive success.

Previous epidemiologic and clinical study designs using indirect measures of exposure to novel MRPs have been difficult to interpret. Data on new and multiple sexual partnerships are challenged by confounding. Markers such as change in partner between pregnancies inaccurately measure length of coital duration. Finally, adequate sample size, prospective data collection, and replication will be required to establish risk. Conceptions arising from donor inseminations might be a particularly interesting setting in which to assess the impact of novel MRPs; however, an appropriate comparison group must be found and the fact that donor sperm are washed of semen eliminates the ability to study seminal proteins in this context.

Direct measures relating specific sperm and semen proteins to conception and birth outcomes will be needed to test the import of MRPs. Direct tests of adverse pregnancy outcomes by length and exclusivity of sexual partnerships are needed. In vitro and animal studies comparing single vs repeated exposure to MRPs will be informative. Genetic similarity between the proteins expressed on egg and sperm should be studied in relation to pregnancy outcomes.

The clinical and public health relevance of research on MRPs are many. If exposure to semen (in addition to sperm) is important to perinatal health, then intrauterine insemination protocols may be improved by a 2-stage approach involving priming intracervical inseminations with unwashed specimens followed by intrauterine inseminations targeting conception. In natural conceptions, it may be preferable to extend the length of sexual cohabitation prior to attempted conception. These are but a few examples. In general, a more complete understanding of the basic biology underlying the interaction between male and female reproductive proteins may translate into a wide range of prediction, prevention, and treatment tools. ■

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