Review Article

Immune Aspects of Female Infertility

Andrea Brazdova, Ph.D.[1]*, Helene Senechal, Ph.D.[1], Gabriel Peltre, Ph.D.[1], Pascal Poncet, Ph.D.[1, 2]

1. Department of Biochemistry, Allergy and Environment, Armand-Trousseau Hospital, Paris, France
2. Department of Infection and Epidemiology, Pasteur Institute, Paris, France

Abstract

Immune infertility, in terms of reproductive failure, has become a serious health issue involving approximately 1 out of 5 couples at reproductive age. Semen that is defined as a complex fluid containing sperm, cellular vesicles and other cells and components, could sensitize the female genital tract. The immune rejection of male semen in the female reproductive tract is explained as the failure of natural tolerance leading to local and/or systemic immune response. Present active immune mechanism may induce high levels of anti-seminal/sperm antibodies. It has already been proven that iso-immunization is associated with infertility. Comprehensive studies with regards to the identification of antibody-targets and the determination of specific antibody class contribute to the development of effective immuno-therapy and, on the other hand, potential immuno-contraception, and then of course to complex patient diagnosis. This review summarizes the aspects of female immune infertility.

Keywords: Immunoglobulins, Infertility, Sperm

Citation: Brazdova A, Senechal H, Peltre G, Poncet P. Immune aspects of female infertility. Int J Fertil Steril. 2016; 10(1): 1-10.

Introduction

The World Health Organization declares infertility as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility has been reported to be one of the most prevalent chronic health disorders regardless of age (1, 2). The decreased fecundity is associated with other health issues (severe avitaminosis, severe renal impairment, cancer and cachexia due to malnutrition or tumor), age, lifestyle and environment. The male partner accounts for the infertility 40% of the time, 40% from the female partner as well and 20% shared by both the man and the woman. The factors involve congenital, hormonal, morphological and immunological disorders (3). The main disorders involved in infertility include pathologic spermiogram, ovulation problems/anovulation, tubal diseases, pelvic adhesion/endometriosis, cervical factors and idiopathic reason usually qualified as the so-called unexplained infertility (UI) (4-6).

UI is diagnosed in a couple when the standard investigations including the semen analyses, test of ovulation and tubal potency do not provide specific results or do not detect any abnormality. Several reports (4, 5, 7) suggested that the diagnosis of UI is subjective and often misdiagnosed for endometriosis, tubal infertility, premature ovarian ageing and immune infertility. The prevalence of UI reaches up to 30% of infertile couples with regards to standard investigation. Severe endometriosis affects the fecundity potential. Mild endometriosis is not, however, associated with infertility in the absence of secondary organic disruption. It has been reported that approximately 20% of infertile females suffer from tubal disease, either distal or peritubal (2, 4). Follicle number is genetically dependent. Female subfertility caused by poor ovarian reserve is declared when the remaining follicle amount represents a fraction of the original value (8). In some women, the so-called poor ovarian response has been noticed when the age-

Received: 13 Sep 2014, Accepted: 31 Dec 2014
*Corresponding Address: Department of Biochemistry, Allergy and Environment, Armand-Trousseau Hospital, 26 Avenue du Dr. Arnold Netter, 75012 Paris, France
Email: andreabrazdova@centrum.cz

Royan Institute
International Journal of Fertility and Sterility
Vol 10, No 1, Apr-Jun 2016, Pages: 1-10

ing ovary produces fewer follicles, follicles grow poorly and follicular atresia occurs (5). Molecular and cellular endometrial deficiency resulting in an implantation failure can be related to UI since the natural immunosuppression does not prevent maternal immune rejection. T regulatory (Treg) cells are believed to protect the fetus from an immune attack. Treg cells function in immune tolerance exhibiting the immuno-suppressive activity. A factor of spontaneous abortion is displayed in the case of a lower number of $CD4^+CD25^+$ $Foxp3^+$ Treg cells that is, under normal conditions, elevated in the first trimester of physiological pregnancies (9, 10). UI is not necessarily linked to Treg differentiation, thus to immune suppression failure, but also to its recruitment into the implantation site. This fact is caused by the reduced expression and insufficient function of lymphocyte and chemotactic agents present in the uterus. Since Treg differentiation is regulated by transforming growth factor beta (TGFβ), idiopathic infertility may be related to a reduced availability of this factor. The lack of TGFβ results in insufficient Treg induction. Diminished $CD4^+CD25^+$ Treg population, the lower expression of Foxp3 and the failure of lymphocyte adherence and chemotaxis seem to play, however, a role in primary cause of UI (11).

Immune/immunological infertility is diagnosed when spontaneously produced antibodies bind to the antigens occurring on either the male or female gametocytes. In particular, antibodies bind to seminal proteins or structures present on the sperm or oocyte. So far, anti-sperm antibodies (ASA) have been observed more frequently than anti-oocyte antibodies (12).

Antibody formation

After an exposure to an antigenic agent, the level of immunoglobulin M (IgM) antibodies is supposed to be dominant at the early phase of a primary immune response. In response to some allergens, IgE antibodies may be prevalent in genetically predisposed individuals. The switch into IgG and IgA antibodies is induced at the late phase of primary immune response or after repeated exposure to the same antigen (13-15). When chronically exposed to the antigens, IgG_1 and IgG_4 become the predominantly produced subclasses of IgG isotype. IgG_4 is a unique antibody unable to activate the classical complement pathway and is then known as an anti-inflammatory Ig and a blocking antibody towards IgE antibodies, depending on the antigenic model. It remains unclear whether IgG_4 is a protective or pathogenic antibody (6, 16, 17). Schroeder and Cavacini speculated that IgG_1 and IgG_3 antibodies are generally induced in response to protein antigens whereas IgG_2 and IgG_4 to polysaccharide antigens (18). Other studies related to antibody distribution neither refute nor endorse this hypothesis (6, 19). We have reported IgG_1/IgG_4 predominance in anti-seminal antibodies and IgG_4/IgG_1 predominance in ASA. We have also proposed the distribution of seminal/sperm-specific antibody isotypes showing that immunoglobulins E, M, $A_{1, 2}$, G_3 are not significantly involved in pathophysiological female sensitization. Specific IgG_4 appears to be mainly produced together with specific IgG_1 (20).

Anti-sperm antibodies

The sperm antigenicity concerning the animal kingdom was first described by Landsteiner, Metchnikov and Metalnikova in 1899 as sperm toxins. In 1932, Baskin observed circulating antibodies against sperm and in 1954, Rümke observed and described the first type of ASA. They have cytotoxic, immobilizing and agglutinating functions. ASA are detectable on the systemic (blood and lymph) as well as the local level [seminal fluid (SF), cervical-vaginal mucus]. In general, the IgG isotype of ASA is mainly related to the blood circulation and IgA isotype to mucosal immunity in women. In men, IgG and IgA fractions are the most prevalent in SF, while IgG and IgM isotypes in serum (6, 21).

Semen has a very heterogeneous antigenic content. Since sperm has auto-antigenic (auto-immunization) as well as iso-antigenic (iso-immunization) potential, it is able to induce the production of sperm-reactive T-cells in men as well as in women, thus is opsonized and then targeted by the leukocytes (sperm-cytotoxic effect) (22-24). It is not a single ASA that influences fertility but more likely multiple ASA causing infertility. Furthermore, it has been postulated that antibodies against a single sperm antigen cannot cause infertility. It has also been reported that not all ASA, either produced in women or men, affect the fertility potential since the cognate antigen is not necessarily involved in the fertilization process (6, 23-26).

A highly heterogeneous sperm antigenic content could be modified during maturation and ejaculation based on antigen sequestration. Newly expressed antigens could then be in contact with any immunocompetent cells, e.g., a sperm membrane-incorporated fibronectin exhibits changes in regional antigenic expression during sperm maturation, whereas secreted fibronectin is a product of male accessory sex glands and can be attached to sperm tail during ejaculation (12, 27). Considering gastrointestinal exposure, ASA formation may be operative (21).

In men, sperm germ cells are protected in the testis from an auto-immune attack by the blood-testis barrier. When the barrier is disrupted, auto-antibodies are produced and are then detectable in blood serum, seminal plasma or directly attached to the sperm surface membrane (28). An increased risk of ASA formation may follow the congenital absence of reproductive tract components. ASA are mostly associated with genital inflammation/infection (e.g., orchitis), epididymis trauma, genital surgery, cryptorchidism and varicocele (27). The theory of auto-immune disease was supported by proving that ASA formation is related to certain human leukocyte antigen classes (29).

In women, the failure of natural tolerance may lead to sensitivity resulting in sperm elimination. ASA affect fertility potential through various pre/post-fertilization processes, such as sperm agglutination and motility, cervix mucus penetration, capacitation, acrosome reaction, zona pellucida (ZP) binding and penetration, oolemma binding, sperm-oocyte fusion and embryo implantation (30, 31). The active local immuno-regulatory mechanism is based on vaginal and cervical tissues having an active and sensitive mucosal immune system, by which the fertility potential is affected. This explains the rather high percentage of infertile women with the local reactions leading to inflammation as well as with high levels of serum anti-semen antibodies. Furthermore, ASA-coated sperm may be more vulnerable to phagocytosis in the female reproductive tract (28). Serum ASA are related to the long-term exposure of female to sperm and then to seminal deficiency in immuno-suppressive factors (32, 33).

Nevertheless, there is the evidence of ASA occurrence in fertile women and men. Some fertile individuals are positive in serum sperm agglutinins. It has been suggested that these ASA are not clinically significant. It is a physiological effect without a pathologic background as they do not inhibit the fertilization process either *in vitro* or *in vivo* (12, 23). In this case, they may be considered as the so-called natural ASA (34). They are produced by auto-reactive B cells in men that were stimulated to grow. Furthermore, natural autoantibodies may be more poly-reactive antibodies, hypothetically help remove senescent molecules and cells, and participate in immune auto-treatment of cancer (35, 36). The poly-reactive character may play a part in cytotoxic reaction at early fertilization associated with infertility.

Role of seminal fluid in female immune infertility

SF represents a part of the semen containing a range of organic/inorganic substances (e.g., neutral α-glucosidase, hyaluronidase, carnitine, glycerolphosphocholine, fructose, prostaglandins (PGs), citrate, zinc, selenium) that are necessary for the physiological metabolism of sperm. The seminal complex mixture of secretions originates in the testis, epididymis and accessory glands including the prostate, seminal vesicles and Cowper's gland. It also acts as a nutritive, transport and buffering medium of pH=7.35-7.5 that defines the main SF functions: sperm protection from the acidic environment of the vagina, metabolic support, liquefaction and clot formation. SF composition is similar to blood plasma; however, it differs in saccharide content (37-39).

Prostate specific antigen (PSA), prostatic acid phosphatase (PAP) and prostate-specific protein-94 belonging to the prostate secretion are in direct contact with sperm and thus may be the first to confront the cervical tissues. PSA is a 33 kDa member of the glandular kallikrein subfamily of serine proteases participating in the liquefaction of the seminal coagulum. Its activity is strongly inhibited by zinc ions (40-42). Serum PSA is a commonly used marker of prostate cancer (43-45). PAP, a member of the histidine acid phosphatase family, is a non-specific tyrosine phosphatase that dephosphorylates macromolecules and inactivates lysophosphatidic acid in SF (46, 47). Seminal components, e.g., heparin (48) or zinc-2-glycoprotein (ZAG) (49), bind to the acrosomal sperm head region protect sperm and are then carried together

into the higher female genital tract. SF plays an important role in moving the sperm into the female reproductive tract due to its high content of TGFβ and PGE, both of which inhibit the function of natural killer (NK) cells and neutrophils that are recruited into the superficial epithelial layers of the cervical tissues. TGFβ is synthesized in the prostate and is testosterone-dependent. This glycoprotein belongs to cell-secreted molecules and occurs in 75% in the latent form in SF. It is further activated in the female reproductive tract by either the enzymes of male/female origin, acidic vaginal pH or through conformational change after an interaction with epithelial cells. The remaining proportion of TGFβ, 25%, exists in an active form (50, 51). TGFβ acting may result in the immune tolerance of seminal antigens. A divergent member of this family is growth/differentiation factor 15 (GDF 15), which is highly abundant in SF. GDF 15 has anti-tumorigenic activity, serves as a cancer marker and is likely to promote a pro-inflammatory immune response. High level of GDF 15 in female serum corresponds to spontaneous abortion as it is expressed in the placenta. It has been suggested that due to the presence of seminal antigens on a fetus, TGFβ facilitates the female immune tolerance to the fetus (52, 53).

Some seminal constituents, such as cathepsin D, are able to degrade proteins vaginally exposed that may be involved in antibody formation related to immune infertility (54). Seminal ZAG has been reported as a novel adipokine playing a significant role in fertilization, lipid mobilization, and peptide/antigen/ligand binding. ZAG may participate in the expression of female immune response since the fold is similar to major histocompatibility complex (MHC) molecules, in particular MHC I, on the antigen-presenting cells. ZAG has been proven to be an IgG-binding protein related to a pathophysiological iso-immunization. This protein belongs to immunoglobulin gene family and may have a protective role by blocking the elicited female anti-semen antibodies (6, 31, 49). SF includes a repertoire of signaling molecules interacting with the epithelium in the female reproductive tract. SF may modulate the chemotactic and phagocytic responses of the female reproductive tract. Phagocytes serve to filter out morphologically abnormal sperm. Sperm selection is based on morphological or antigenic structures. Mainly, the immune modulating properties are mediated by the PGs of the E series, complement inhibitors, cytokines and proteins capable of binding IgG antibodies (55, 56). Local reactions may lead to an inflammation. However, SF has a built-in mechanism preventing an immunological sensitization of the female against sperm as well as seminal structures. This protective system exists due to the presence of immune inhibitors originating in the male sex accessory glands (52, 57).

SF has been suggested to be the modulator of sperm-induced inflammation that leads to sperm elimination from the female genital tract. Antibody fraction interacting with seminal antigen targets most of the seminal proteins adsorbed on sperm. However, SF induces the recruitment of macrophages and dendritic cells into cervical and endometrial tissues (58). SF elicits endometrial changes by inducing pro-inflammatory cytokines and cyclooxygenase-2. Their presence leads to macrophage and dendritic cell recruitment into the female reproductive tract. Seminal components activate the income of neutrophils into the endometrial stroma (24, 52, 59). However, it has been reported that the influx of neutrophils is higher and faster when the washed sperm inseminated (60). This fact demonstrates the protective and signaling activity of SF. The immuno-suppressive activity prevents the iso-immunization of the female reproductive tract and suppresses cell-mediated cytotoxicity (61). Seminal prostaglandin D2 is known for its immuno-suppressive effect, by which ASA formation is prevented in the female genital tract. The immuno-modulating properties are mediated by PGE, complement inhibitors, cytokines and proteins capable of binding the Fc region of IgG. These IgG-binding proteins are Fcγ receptor-like soluble proteins.

In general, seminal antibody-binding proteins contribute to sperm protection against immune-mediated damage by enabling successful sperm passage in the female reproductive tract and by blocking an interaction with immune effectors such as prolactin-inducible protein, which is a secretory glycoprotein located in seminal vesicles, binds to immunoglobulin G via its Fc fragment. It may therefore be involved in immune regulation by trapping ASA and neutralizing them (62, 63). Particular deficiencies in seminal factors may lead to higher antibody production in infertile women (64).

SF has already been considered to be linked to the IgE-mediated rare reaction to semen (65). This rare phenomenon was first reported in 1945 (66). Human seminal plasma allergy (HSPA), the so-called hypersensitivity to semen, is defined by local and/or systemic symptoms after exposure to SF. The symptoms occur immediately after contact with semen or even within several hours after intercourse. The local symptoms include vulvar/vaginal itching, burning, redness and swelling. Local reaction can appear on any semen contact site and can be misdiagnosed as chronic vulvo-vaginitis caused by bacteria, yeasts, viruses and other parasites. Systemic symptoms include generalized urticaria, angioedema (face, tongue, lips, throat), dyspnea, wheezing, cough, chest tightness, rhinorrhea, nausea, vomiting, diarrhea. Generalized malaise may result in an anaphylactic shock, which is a life-threatening reaction. The symptoms can manifest after the first time intercourse in up to 50% of cases. Response mediated by IgE antibodies is then the most common mechanism. It has been suggested that female patients experiencing any allergic symptoms after/during the first time intercourse might be sensitive to other antigens/allergens that cross-react with SF. IgE cross-reactivity has already been proven among proteins from dog epithelium and PSA (67). Patients diagnosed with HSPA have difficulties conceiving but infertility has not been demonstrated, so far (65, 68, 69).

Auto-immune aspects in infertility

Auto-immune phenomena have already been associated with increased prevalence of female immune infertility. This fact concerns anti-phospholipid, anti-nuclear, anti-thyroid, anti-annexin V, anti-prothrombin, anti-laminin, anti-ZP antibody formation, the high level of NK cells as the risk factors but not as those pathognomonic (4).

ZP, as the protective layer, is composed of glycoproteins. It represents a broad antigenic content. Antibodies against ZP prevent sperm from penetrating it. Anti-ZP autoantibody concentration can be elevated if ZP shape is abnormal (deformed, thickened, thinned). These antibodies interfere with the implantation process since ZP protects a fertilized oocyte up to the 7th day after fertilization, up to embryo hatching. During this time the ZP is thickened (15-17 μm). ZP-specific antibodies are detectable in follicular and peritoneal fluid, and cervical mucus in IgG, IgA and IgM isotypes (21).

Anti-phospholipid antibodies (APA) have been associated with e.g. miscarriage, intrauterine fetal death, and placental thrombosis since the time of their discovery by Wasserman in 1906. These components of the female immune system are autoantibodies directed in particular against β2-glycoprotein, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, annexin V and cardiolipin. APA are mostly produced in IgG fractions accompanied by IgA and IgM. Phosphatidylserine-specific APA cause fetus hypotrophy as a consequence of placental vascular damage, against which the maternal immune system produces anti-coagulating factors (70). The risk of spontaneous abortion increases with the presence of anti-coagulating antibodies. Antibodies specific to annexin V and placental anti-coagulating protein are also related to reproductive failure and detectable in 5-6% of women diagnosed with pregnancy loss, 8-10% of women after unsuccessful *in vitro* fertilization, 1% of not pregnant and healthy women, and 0% of pregnant women without a pathophysiologic aspect. Complex complication is called anti-phospholipid syndrome also known as Hughes syndrome. It may cause hyper-coagulation leading to rapid organ failure (6, 21, 70).

Endometrium-specific antibodies are, inter alia, associated with polycystic ovary syndrome (PCOS) that is mainly classified as an endocrine genetic disorder. PCOS is known as Stein-Leventhal syndrome first described in 1935. It is characterized by enlarged ovaries caused by cysts, irregular ovulation, irregular or no menstruation, and increased androgen levels. With regards to androgen levels, PCOS is associated with hirsutism. On the other hand, it is associated with obesity, type 2 diabetes and high cholesterol levels. Women suffering from PCOS have usually problems with conceiving (21, 71).

Pregnancy is also complicated by endometriosis, a serious gynecological complication affecting up to 10% of women of reproductive age. Twenty-five % of women diagnosed with endometriosis are infertile. Peritoneal endometriosis is characterized by retrograde menstruation causing secondary inflammation. Factors typical for such a condition are high level of autoantibodies, presence

of T-lymphocytes in peritoneal fluid, and elevated level of NK cells (72, 73).

Mucosal immunity of the female genital tract

The mucosal immune system operates on a local level and is represented by lymphoid tissues in mucosae and external secretory glands. It limits the access of environmental antigens by which the fertility potential is significantly regulated as well. It restricts and/or prevents the penetration in the systemic compartment. The female genital tissues and secretion (vaginal washes and cervical mucus) provide the protection that differs from systemic reaction by the cell types involved and by their products, the antibodies. However, it is the initial antigen exposure to mucosae that leads to the systemic T cell hypo-responsiveness (74, 75).

Mucosal immunity in the female genital tract is influenced by the level of antibodies, cytokines and hormones. Humoral defense displayed in mucosal tissue surface provides the antibodies of the IgG, IgA and IgM isotypes. IgG, IgA and IgM levels are dependent on the menstrual cycle and are influenced by hormones. IgA and IgG reach their maximum concentrations before ovulation, which is linked to the increased level of interleukin 1 component β. In particular, estrogen causes a higher expression of secretory IgA (S-IgA), thus its selective transport is increased. This way of regulation is responsible for antibody-isotype distribution including their properties, the transport of immunoglobulin-containing cells, antigen-presenting cells, in addition to $CD4^+$ and $CD8^+$ cells in the vagina, uterus and fallopian tubes (76). In addition, it has been shown (77) that oral contraception influences IgA as well as IgG levels in the cervical mucus. It is almost one third higher than in the cervical mucus of naturally cycling women. The vaginal washes of women on oral contraception display an elevated level of IgG in comparison to IgA. Several observations showed (76, 78) that the concentration decreases in this manner: IgG>IgA>IgM. This ratio is related to the presence of IgG/A/M-producing cells. The uterine endocervix contains the highest amount of IgG- and IgA-secreting cells compared to the ectocervix, fallopian tubes and vagina (76, 79). Cervical mucus contains higher levels of IgG than IgA, both of which are locally produced. On the contrary, women on oral contraception have IgA as the predominant antibody present in cervical mucus. Among the three mentioned isotypes, IgM is the less efficiently transported antibody. The mucosal IgA antibodies are selectively transported to an external secretion based on a receptor-associated mechanism. The distribution of IgG subclasses in mucosal secretions displays a plasma proportion. IgD occurs rarely or in very low concentrations in external mucosae. The level of IgE depends on the genetic predisposition to develop allergies and then on the allergenic nature of the presented antigen (74). Despite the low IgA affinity, the avidity is high regarding the multibinding sites. Environmental antigens are usually degraded by proteolytic enzymes. IgA itself is resistant to the enzymes of proteolytic character. It has been suggested that a certain amount of not eliminated antigens circulates in the complex with IgA, which further activates the systemic immune response. It is less probable that antigens entering, at first, the mucosal tissue could circulate on itself (75, 78, 80). IgA is a multivalent antibody existing in two subclasses, IgA_1 and IgA_2. IgA has an anti-inflammatory activity proved by the inhibition of complement activation and by a diminishing effect on NK cells. These properties may avoid an early-precise diagnosis as an inflammatory marker and may not be detected. In cervical mucus as well as a vaginal wash, the IgA_1 concentration is equal to IgA_2. S-IgA is locally produced by sub-epithelial plasma cells. Most of the time, it is a polymeric molecule, which corresponds to IgA_2 since IgA_1 is rather monomeric. It has been suggested that cervical mucus contains approximately 80% of the polymeric form and the vaginal wash contains approximately 50% (74, 76). Eosinophils that cover mucosal surfaces can be degranulated by IgA antibodies. This pathology is observed when natural immune tolerance is disrupted. Further reactions may evoke an allergic reaction to the presented antigen, such as seminal and/or sperm component. Semen rejection at the level of mucosal immunity may not be reflected at the systemic level (74). The protective role depends on an antibody-dependent cell-mediated cytotoxicity, opsonization, the activation of innate humoral factors, removal and further elimination of already formed immune complexes within epithelial cells and lamina propria. IgA is able to diminish absorption of an entire antigen as well as a part of it on mucosal tissues. In comparison with IgG, which after antigen-recognition activates complement resulting in inflam-

mation, IgA acts as an inhibitor or directly avoids the adherence of antigen (81). S-IgA in a complex with an antigen is not able to efficiently activate the complement pathway. IgA antibodies have been reported to be a part of the natural antibody pool showing the characteristics of polyreactivity and hypothesized to act as the first barrier defense (82).

The uterine cervix participates in the local immune reaction by the presence of immunoglobulin-producing cells in a complex mixture known as cervical mucus/fluid/plasma. The fluid located in and around the cervix. Cervical mucus is composed mostly of water, up to 90%, depending on the menstrual cycle. Its composition is based on a glycoprotein web filled by mucus rich in immunocompetent proteins, electrolytes (calcium, sodium and potassium), simple sugars such as fructose and glucose, amino acids, C3 and C4 complement components, Th1 and Th2 cytokines, PGE, and trace elements (zinc, copper, iron, manganese, selenium). The misbalance in its content is frequently associated with immune infertility and spontaneous abortion (83-86). The value of pH is alkaline especially at ovulation in order to allow sperm survival by the elevated level of water and electrolytes. After menstruation, cervical mucus becomes rather acidic. Acidic pH is characteristic for vaginal mucus as well (87).

The basic role of cervical mucus consists in a barrier blocking uterus entrance. It is connected to "stick and thick" properties and acts as a natural lubricant because of its glycerol content. Its amount is not hormone-dependent. The mucus functions also as a transport and nourishing medium for sperm by being less concentrated, transparent with a lower amount of immuno-competent agents and high fructose level, which is essential for efficient sperm metabolism. The sugar level is progesterone dependent (21, 84, 85). On the other hand, the cervix always acts as a reservoir for sperm after sexual intercourse. Regarding the iso-immunization within the entire menstrual cycle, cervical mucus contains the antibodies directed to sperm. Their level is then crucial for sperm-cervical mucus penetration and following fertilization. ASA-positive female patients have been commonly diagnosed with immune infertility. An iso-immunization rate is observed by a local ASA level which determines the appropriate treatment (84, 85, 88). It has been shown (86) that ASA present in cervical mucus are of an agglutinating character. These locally produced ASA do not differ from those systemically produced, thus they affect sperm capacitation, acrosome reaction and may interfere with ZP penetration as well as embryo implantation. The peak of ASA in IgA and IgG fraction is reached at the luteal and follicular phases of the menstrual cycle. In contrast, their level is lowest at ovulation. The peak is related to the highest level of estradiol, usually one day before ovulation (77).

Conclusion

Infertility has been defined as reproductive failure and recognized as a disease. Idiopathic infertility correlates with certain immune aspects such as natural tolerance, in addition to the levels of immunoglobulins and specific antibodies in local and systemic secretions. Sperm displays a very heterogeneous antigenic content and has highly auto- as well as iso-antigenic potential. SF, a protective and nutritive sperm medium, is the first contact with the local immune system of the female genital tract, thus representing the potential antibody-targets. Mucosal immunity in the female genital tract is influenced by the level of natural and specific antibodies, cytokines and hormones. Seminal components also bind to the acrosomal sperm to protect it and are then carried together with it into the higher female genital tract. Iso-immunization has been associated with female immune infertility. The thorough comprehension of this pathophysiological process consists of the determination of antibody isotype mostly involved in antigen targeting; and on the other hand, consists of the characterization and identification of semen antibody-binding proteins. In particular, early determination of serum seminal/sperm-specific immunoglobulin G subclasses may make patient profiling more precise and complete the information for diagnosis. Furthermore, based on our studies, anti-seminal/sperm IgG_1 and IgG_4 could be of interest for further therapy targets. The identification of uniform auto- and iso-immunization markers would contribute to a comprehensive, detailed patient diagnosis.

Acknowledgements

We are immensely grateful to Mr. Brendan Fallon (Pierre and Marie Curie University) for the linguistic and grammatical editing of the manuscript.

The authors declare no conflict of interest.

References

1. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge: Cambridge University Press; 1993; 10-35.
2. Smith S, Pfeifer SM, Collins JA. Diagnosis and management of female infertility. JAMA. 2003; 290(13): 1767-1770.
3. Doherty CM, Clark MM. Infertility treatment. 1st ed. Czech Republic: Computer Press Brno; 2006; 1-121.
4. Gleicher N, Barad D. Unexplained infertility: does it really exist? Hum Reprod. 2006; 21(8): 1951-1955.
5. Siristatidis C, Bhattacharya S. Unexplained infertility: does it really exist? Does it matter? Hum Reprod. 2007; 22(8): 2084-2087.
6. Brazdova A. Study of immunological properties of sperm and seminal plasma antigens: anti-seminal and anti-sperm antibodies in female immune infertility. Characterization of targeted proteins. Presented for the Ph.D., Paris. Pierre and Marie Curie University. 2014.
7. Crosignani PC, Collins J, Cooke ID, Diczfalusy E, Rubin B. Recommendations of the ESHRE workshop on 'Unexplained Infertility'. Anacapri, August 28-29, 1992. Hum Reprod. 1993; 8(6): 977-980.
8. Nikolaou D, Templeton A. Early ovarian aging: a hypothesis. Detection and clinical relevance. Hum Reprod. 2003; 18(6): 1137-1139.
9. Agnello D, Lankford CS, Bream J, Morinobu A, Gadina M, O'Shea JJ, et al. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol. 2003; 23(3): 147-161.
10. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology. 2004; 112(1): 38-43.
11. Jasper MJ, Tremellen KP, Robertson SA. Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. Mol Hum Reprod. 2006; 12(5): 301-308.
12. Bohring C, Krause W. Immune infertility: towards a better understanding of sperm (auto)-immunity. The value of proteomic analysis. Hum Reprod. 2003; 18(5): 915-924.
13. Aalberse RC, van der Gaag R, van Leeuwen J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. J Immunol. 1983; 130(2): 722-726.
14. Nakagawa T. IgG subclass changes in response to desensitisation. Monogr Allergy. 1986; 19: 253-261.
15. Sondergaard I, Poulsen LK, Osterballe O, Weeke B. A computational approach to the description of individual immune responses. IgE and IgG-subclass allergen-specific antibodies formed during immunotherapy. Allergy. 1991; 46(1): 10-19.
16. Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. Clin Exp Allergy. 2009; 39(4): 469-477.
17. Guma M, Firestein GS. IgG4-related diseases. Best Prac Res Clin Rheumatol. 2012; 26(4): 425-438.
18. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010; 125(2 Suppl 2): S41-52.
19. Tamayo E, Fernández A, Almansa R, Carrasco E, Goncalves L, Heredia M, et al. Beneficial role of endogenous immunoglobulin subclasses and isotypes in septic shock. J Crit Care. 2012; 27(6): 616-622.
20. Brazdova A, Senechal H, Peltre G, Zidkova J, Ulcova-Gallova Z, Poncet P, et al. Immunodominant semen proteins III: IgG_1 and IgG_4 linkage in female immune infertility. Jordan J Biol Sci. 2015; 8(1): 17-21.
21. Ulcova-Gallova Z. Infertility - Attack of immunity. 1st ed. Czech Republic: Grada publishing Prague; 2006; 10-85.
22. Kurpisz M, Kamieniczna M. Immune chemistry of ASA. In: Krause W, Naz RK, editors. Immune infertility. Germany: Springer Berlin; 2009; 79-89.
23. Sedlackova T, Zidkova J, Brazdova A, Melcova M, Skop V, Cibulka J, et al. Anti-sperm antibodies. Chem Listy. 2010; 104(1): 3-6.
24. Bronson R. Biology of the male reproductive tract: its cellular and morphological considerations. Am J Reprod Immunol. 2011; 65(3): 212-219.
25. Wang M, Shi JL, Cheng GY, Hu YQ, Xu C. The antibody against a nuclear autoantigenic sperm protein can result in reproductive failure. Asian J Androl. 2009; 11(2): 183-192.
26. Haidl G. Characterization of fertility related antisperm antibodies- a step towards causal treatment of immunological infertility and immuno-contraception. Asian J Androl. 2010; 12(6): 793-794.
27. Kosanović MM, Janković MM. Molecular heterogeneity of gelatin-binding proteins from human seminal plasma. Asian J Androl. 2010; 12(3): 363-375.
28. Lu JC, Huang YF, Lu NQ. Antisperm immunity and infertility. Expert Rev Clin Immunol. 2008; 4(1): 113-126.
29. Omu AE, al-Qattan F, Ismail AA, al-Taher S, al-Busiri N. Relationship between unexplained infertility and human leukocyte antigens and expression of circulating autogeneic and allogeneic antisperm antibodies. Clin Exp Obstet Gynecol. 1999; 26(3-4): 199-202.
30. Brazdova A, Zidkova J, Peltre G, Ulcova-Gallova Z. IgG, IgA and IgE reactivities to sperm antigens in infertile women. Jordan J Biol Sci. 2012; 5(2): 85-89.
31. Brazdova A, Vermachova M, Zidkova J, Ulcova-Gallova Z, Peltre G. Immunodominant semen proteins I: new patterns of sperm proteins related to female immune infertility. Cent Eur J Biol. 2013; 8(9): 813-818.
32. Mazumdar S, Levine AS. Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment. Fertil Steril. 1998; 70(5): 799-810.
33. Bohring C, Krause E, Habermann B, Krause W. Isolation and identification of sperm membrane antigens recognized by antisperm antibodies, and their possible role in immunological infertility disease. Mol Hum Reprod. 2001; 7(2): 113-118.
34. Chamley LW, Clarke GN. Antisperm antibodies and conception. Semin Immunopathol. 2007; 29(2): 169-184.
35. Avrameas S. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol Today. 1991; 12(5): 154-159.
36. Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. N Engl J Med. 2001; 345(10): 747-755.
37. Kumar V, Hassan MI, Tomar AK, Kashav T, Nautiyal J, Singh S, et al. Proteomic analysis of heparin-binding proteins from human seminal plasma: a step towards identification of molecular markers of male fertility. J Biosci. 2009; 34(6): 899-908.
38. Rodriguez-Martinez H, Kvist U, Ernerudh J, Sanz L,

Calvete JJ. Seminal plasma proteins: what role do they play? Am J Reprod Immunol. 2011; 66(suppl 1): 11-22.
39. Brázdová A, Zídková J, Senechal H, Peltre G, Cibulka J, Ulčová-Gallová Z. Female serum of immunoglobulins G, A, E and their immunological reaction to seminal fluid antigens. Folia Biol (Praha). 2012; 58(6): 251-255.
40. Pilch B, Mann M. Large-scale and high-confidence proteomic analysis of human seminal plasma. Genome Biol. 2006; 7(5): R40.
41. España F, Gilabert J, Estellés A, Romeu A, Aznar J, Cabo A. Functionally active protein C inhibitor/plasminogen activator inhibitor-3 (PCI/PAI-3) is secreted in seminal vesicles, occurs at high concentrations in human seminal plasma and complexes with prostate-specific antigen. Thromb Res. 1991; 64(3): 309-320.
42. Utleg AG, Yi EC, Xie T, Shannon P, White JT, Goodlett DR, et al. Proteomic analysis of human prostasomes. Prostate. 2003; 56(2): 150-161.
43. Jones WR. Allergy to coitus. Aust N Z J Obstet Gynaecol. 1991; 31(2): 137-141.
44. Ostrowski WS, Kuciel R. Human prostatic acid phosphatase: selected properties and practical applications. Clin Chim Acta. 1994; 226(2): 121-129.
45. Cao Y, Becker C, Lundwall A, Christensson A, Gadaleanu V, Lilja H, et al. Expression of protein C inhibitor (PCI) in benign and malignant prostatic tissues. Prostate. 2003; 57(3): 196-204.
46. Autiero M, Sansone G, Abrescia P. Relative ratios of lactoferrin, albumin, and acid phosphatase seminal levels as sperm quality markers in fertile and infertile men. J Androl. 1991; 12(3): 191-200.
47. Tanaka M, Kishi Y, Takanezawa Y, Kakehi Y, Aoki J, Arai H. Prostatic acid phosphatase degrades lysophosphatidic acid in seminal plasma. FEBS Lett. 2004; 571(1-3): 197-204.
48. Miller DJ, Winer MA, Ax RL. Heparin-binding proteins from seminal plasma bind to bovine spermatozoa and modulate capacitation by heparin. Biol Reprod. 1990; 42(5-6): 899-915.
49. Hassan MI, Waheed A, Yadav S, Singh TP, Ahmad F. Zinc alpha 2-glycoprotein: a multidisciplinary protein. Mol Cancer Res. 2008; 6(6): 892-906.
50. Denison FC, Grant VE, Calder AA, Kelly RW. Seminal plasma components stimulate interleukin-8 and inteleukin-10 release. Mol Hum Reprod. 1999; 5(3): 220-226.
51. Robertson SA, Ingman WV, O'Leary S, Sharkey DJ, Tremellen KP. Transforming growth factor beta--a mediator of immune deviation in seminal plasma. J Reprod Immunol. 2002; 57(1-2): 109-128.
52. Robertson SA. Seminal plasma and male factor signalling in the female reproductive tract. Cell Tissue Res. 2005; 322(1): 43-52.
53. Soucek K, Slabáková E, Ovesná P, Malenovská A, Kozubík A, Hampl A. Growth/differentiation factor-15 is an abundant cytokine in human seminal plasma. Hum Reprod. 2010; 25(12): 2962-2971.
54. Pardesi SR, Dandekar SP, Jamdar SN, Harikuma P. Identification and purification of an aspartic proteinase from human semen. Indian J Clin Biochem. 2004; 19(2): 84-90.
55. Tomlinson MJ, White A, Barratt CL, Bolton AE, Cooke ID. The removal of morphologically abnormal sperm forms by phagocytes: a positive role for seminal leukocytes? Hum Reprod. 1992; 7(4): 517-522.
56. Kelly RW, Critchley HO. Immunomodulation by human seminal plasma: a benefit for spermatozoon and pathogen? Hum Reprod. 1997; 12(10): 2200-2207.
57. Prakash C. Etiology of immune infertility. Prog Clin Biol Res. 1981; 70: 403-412.
58. Troedsson MH, Desvousges A, Alghamdi AS, Dahms B, Dow CA, Hayna J, et al. Components in seminal plasma regulating sperm transport and elimination. Anim Reprod Sci. 2005; 89(1-4): 171-186.
59. Morrell JM, Pihl J, Dalin AM, Johannisson A. Restoration of seminal plasma to stallion spermatozoa selected by colloid centrifugation increases sperm progressive motility but is detrimental to chromatin integrity. Theriogenology. 2012; 78(2): 345-352.
60. Rozeboom KJ, Troedsson MH, Molitor TW, Crabo BG. The effect of spermatozoa and seminal plasma on leukocyte migration into the uterus of gilts. J Anim Sci. 1999; 77(8): 2201-2206.
61. Lord EM, Sensabaugh GF, Stites DP. Immunosuppressive activity of human seminal plasma. I. Inhibition of in vitro lymphocyte activation. J Immunol. 1977; 118(5): 1704-1711.
62. Chiu WW, Chamley LW. Antibody-binding proteins in human seminal plasma. Am J Reprod Immunol. 2002; 48(4): 269-274.
63. Chiu WW, Chamley LW. Human seminal plasma antibody-binding protein. Am J Reprod Immunol. 2003; 50(3): 196-201.
64. Poiani A. Complexity of seminal fluid: a review. Behav Ecol Sociobiol. 2006; 60: 289-310.
65. Weidinger S, Mayerhofer A, Raemsch R, Ring J, Köhn FM. Prostate-specific antigen as allergen in human seminal plasma allergy. J Allergy Clin Immunol. 2006; 117(1): 213-215.
66. James DW. Pernicious vomiting of pregnancy due to sensitivity to semen. West J Surg. 1945; 53: 380-382.
67. Basagaña M, Bartolomé B, Pastor C, Torres F, Alonso R, Vivanco F, et al. Allergy to human seminal fluid: cross-reactivity with dog dander. J Allergy Clin Immunol. 2008; 121(1): 233-239.
68. Shah A, Panjabi C. Human seminal plasma allergy: a review of a rare phenomenon. Clin Exp Allergy. 2004; 34(6): 827-838.
69. Bernstein JA. Human seminal plasma hypersensitivity: an under-recognized women's health issue. Postgrad Med. 2011; 123(1): 120-125.
70. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Anti-phospholipid syndrome. Lancet. 2010; 376(9751): 1498-1509.
71. Mayo Foundation for Medical Education and Research -MFMER. Available from: http://www.mayoclinic.org/diseases-conditions/pcos/basics/definition/con-20028841. (11 Nov 2014).
72. Nisolle M, Paindaveine B, Bourdon A, Berlière M, Casanas-Roux F, Donnez J. Histologic study of peritoneal endometriosis in infertile women. Fertil Steril. 1990; 53(6): 984-988.
73. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. J Assist Reprod Genet. 2010; 27(8): 441-447.
74. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev. 2005; 206: 64-82.
75. Mestecky J, Russell MW, Elson CO. Perspectives on mucosal vaccines: is mucosal tolerance a barrier? J Immunol. 2007; 179(9): 5633-5638.
76. Kutteh WH, Prince SJ, Hammond KR, Kutteh CC, Mestecky J. Variations in immunoglobulins and IgA subclasses of human uterine cervical secretions around the time of ovulation. Clin Exp Immunol. 1996; 104(3): 538-542.
77. Franklin RD, Kutteh WH. Characterization of immunoglobulins and cytokines in human cervical mucus:

influence of exogenous and endogenous hormones. J Reprod Immunol. 1999; 42(2): 93-106.
78. Mestecky J, Fultz PN. Mucosal immune system of the human genital tract. J Infect Dis. 1999; 179 Suppl 3: 470-474.
79. Crowley-Nowick PA, Bell M, Edwards RP, McCallister D, Gore H, Kanbour-Shakir A, et al. Normal uterine cervix: characterization of isolated lymphocyte phenotypes and immunoglobulin secretion. Am J Reprod Immunol. 1995; 34(4): 241-247.
80. Corthésy B. Roundtrip ticket for secretory IgA: role in mucosal homeostasis? J Immunol. 2007; 178(1): 27-32.
81. Russell MW, Sibley DA, Nikolova EB, Tomana M, Mestecky J. IgA antibody as a non-inflammatory regulator of immunity. Biochem Soc Trans. 1997; 25(2): 466-470.
82. Quan CP, Berneman A, Pires R, Avrameas S, Bouvet JP. Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infection in humans. Infect Immun. 1997; 65(10): 3997-4004.
83. Moghissi KS. The function of the cervix in fertility. Fertil Steril. 1972; 23(4): 295-306.
84. Schumacher GF. Immunology of spermatozoa and cervical mucus. Hum Reprod. 1988; 3(3): 289-300.
85. Cibulka J, Ulcová-Gallová Z, Babcová K, Krauz V, Balvín M, Bibková K, et al. Electrophoretic analysis (SDS-PAGE) of ovulatory cervical mucus in patients with fertility failure and after unsuccessful IVF. Ceska Gynekol. 2005; 70(5): 331-335.
86. Ulcova-Gallova Z. Immunological and physicochemical properties of cervical ovulatory mucus. J Reprod Immunol. 2010; 86(2): 115-121.
87. Gruberová J, Biková S, Ulcová-Gallová Z, Reischig J, Rokyta Z. Ovulatory mucus and its pH, arborization and spermagglutination antibodies in women with fertility disorders. Ceska Gynekol. 2006; 71(1): 36-40.
88. Moghissi KS. The cervix in infertility. Clin Obstet Gynecol. 1979; 22(1): 27-42.