Redirecting reproductive immunology research toward pregnancy as a period of temporary immune tolerance

Norbert Gleicher^{1,2,3,4} · Vitaly A. Kushnir^{1,5} · David H Barad^{1,2}

Received: 17 November 2016 / Accepted: 16 January 2017 / Published online: 10 February 2017
© Springer Science+Business Media New York 2017

Abstract Referring to two recent publications, we here propose that clinical reproductive immunology has for decades stagnated because reproductive medicine, including assisted reproduction (AR), has failed to accept embryo implantation as an immune system-driven process, dependent on establishment of maternal tolerance toward the implanting fetal semi-allograft (and complete allograft in cases of oocyte donation). Pregnancy represents a biologically unique period of temporary (to the period of gestation restricted) tolerance, otherwise only known in association with parasitic infections. Rather than investigating the immune pathways necessary to induce this rather unique state of tolerance toward the rapidly growing parasitic antigen load of the fetus, the field, instead, concentrated on irrelevant secondary immune phenomena (i.e., “immunological noise”). It, therefore, does not surprise that interesting recent research, offering new potential insights into maternal tolerance during pregnancy, was mostly published outside of the field of reproductive medicine. This research

offers evidence for existence of inducible maternal tolerance pathways with the ability of improving maternal fecundity and, potentially, reducing such late pregnancy complications as premature labor and preeclampsia/eclampsia due to premature abatement of maternal tolerance. Increasing evidence also suggests that tolerance-inducing immune pathways are similar in successful pregnancy, successful organ transplantation and, likely also in the tolerance of “self” (i.e., prevention of autoimmunity). Identifying and isolating these pathways, therefore, may greatly benefit all three of these clinical areas, and research in reproductive immunology should be accordingly redirected.

Keywords Assisted reproduction · Immune suppression · Tolerance · Immune pathways · Implantation · Implantation failure · Pregnancy loss

Capsule We here propose that that induction of tolerance pathways is essential to establishment and maintenance of normal pregnancy.

✉ Norbert Gleicher
ngleicher@thechr.com; ngleicher@rockefeller.edu

¹ The Center for Human Reproduction, 21 East 69th Street, New York, NY 10021, USA

² Foundation for Reproductive Medicine, New York, NY 10022, USA

³ Laboratory of Stem Cell Biology and Molecular Embryology, The Rockefeller University, New York, NY 10065, USA

⁴ Department of Obstetrics and Gynecology, University of Vienna School of Medicine, 1090 Vienna, Austria

⁵ Department of Obstetrics and Gynecology, Wake Forest University, Winston Salem, NC 27109, USA

Commenting on a recently published study by Wei et al. [1] in *Fertility&Sterility* that had received considerable media attention, Zeev Blumenfeld astutely noted that one possible explanation for why women, who had lymphoid tissues (i.e., appendices and tonsils) surgically removed subsequently demonstrated improved fecundity, might be that removal of these lymphoid tissues beneficially impacted their ability to develop immunological tolerance [2]. In doing so, Blumenfeld, raised a for-decades-neglected subject in clinical reproductive medicine, which deserves renewed attention.

Treating poorly defined clinical conditions, like implantation failure and repeat pregnancy loss, with largely unproven therapies, clinical reproductive immunology has spent the last 20 years in “scientific purgatory.” Robertson et al. pointed this out recently in another timely commentary, when reviewing the use of corticosteroid therapy in assisted reproduction [3]. Considering the progress made

in most other areas of reproductive biology, one is left wondering about the disproportionate stagnation in reproductive immunology.

This is not to say that there has not been a plethora of studies in animal models trying to pierce out how maternal tolerance is established, given that maternal alloreactive lymphocytes are not depleted during pregnancies [4]. Many such studies have been published in recent years [4–6], though persistently ignored in their potential applicability to clinical practice.

The principal reason lies in the failure of clinical reproductive medicine to acknowledge the centrality of establishment of normal maternal tolerance for successful pregnancy. In other words, without establishment of proper *temporary* immune tolerance toward the implanting embryo, and subsequently expanding tolerance toward a rapidly growing volume of solidly antigenic tissue (i.e., growing fetus and placenta) for the approximately 38 weeks of normal gestational length between implantation and delivery, the reproductive process could not succeed.

Reproductive immunology as a distinct area of research within reproductive biology goes back to Medawar's and Billingham's groundbreaking transplantation studies [7, 8]. The clinical appreciation of pregnancy as an immunological paradox started entering the consciousness of clinical practitioners, however, only following establishment of the *American Society for Reproductive Immunology* in 1979, of which Billingham was a founding member. Robertson et al. recently noted [3] that it was the association of autoimmunity with pregnancy loss [9, 10], and later with implantation failure [11] that for the first time on a clinical level linked the maternal immune system with reproductive outcomes.

Exaggerations by commercial laboratory interests of the clinical relevance of reported autoimmune findings resulted in a backlash from clinical practitioners [12], and the pendulum swung to the opposite extreme, with prominent voices in clinical assisted reproduction declaring reproductive immunology inconsequential—and even demanding an end to all related diagnostic testing [13].

Within large parts of the clinical community, reproductive immunology has ever since been viewed as “scientific purgatory,” with even prominent practitioners disputing all relevance of the maternal immune system to assisted reproduction. Often rightly, clinical practitioners in this arena have been viewed as “on the fringe.” Clinical immunological knowledge in assisted reproduction, consequently, has, however, since the late 1980s largely stagnated. As Robertson et al. recently noted, like in earliest days of transplantation biology, over 60 years ago [14], suspected immunological problems in reproductive medicine are, therefore, still primarily only treated with corticosteroids [3].

Why the biology matters

Robertson et al. [3] suggested that the continuous utilization of corticosteroids in assisted reproduction is, likely, a remnant of the previously noted association of autoimmunity with various forms of reproductive failure [9–11]. Corticosteroid therapy was, however, introduced to assisted reproduction for other reasons. In the very early days of in vitro fertilization (IVF), Kemeter and Feichtinger, based on a small prospectively randomized clinical trial, suggested outcome benefits from suppression of adrenal androgens [15]. A few years later, such treatment was proposed as anti-inflammatory in association with assisted hatching of the zona pellucida [16].

Advocating corticosteroid treatments in attempts to affect autoimmune effects on reproduction, however, demonstrates how much the basic immunobiology of pregnancy is still misunderstood in the practice of IVF: Immunologically-induced reproductive failure only rarely is the consequence of failure of tolerance to “self” (i.e., autoimmunity) but, almost always the consequence of failure of tolerance toward the paternal semi-allograft (i.e., allogeneic immunity).

We here want to stress that establishment of appropriate tolerance is an essential step for normal implantation and pregnancy maintenance. It, indeed, may be more essential than even presence of endometrium since ectopic implantations and early pregnancy development occur in vivo in absence of endometrium in fallopian tubes, on ovarian surfaces, omentum and elsewhere [17]. Two recent human in vitro embryo implantation studies confirmed this, when demonstrating normal embryo development after implantation on artificial surfaces in complete absence of maternal contributions until day-14 post-fertilization, when, international conventions currently mandate termination of in vitro embryo cultures [18, 19].

To avoid being attacked by the maternal immune system, normal pregnancies, thus, must succeed in establishing timely and adequate levels of tolerance toward the fetal semi-allograft. Conversely, abnormally low levels of maternal tolerance must, therefore, result in implantation difficulties and abnormal pregnancy progression due to allogeneic (and not autoimmune) immune responses.

Solid organ, multi-tissue “mini” transplants, as embryos can be biologically described, can be expected to elicit similar immune responses to complex solid organ transplants. Yet, while in almost all circumstances women would violently reject any “mini”-organ transplant from a partner, they, with normally functioning immune systems, will fully tolerate such transplants in the form of implanting embryos. Already at implantation, the maternal immune system, therefore, must be “reprogrammed” from rejection to tolerance. Moreover, despite rapidly increasing allogeneic antigen load, as fetus and placenta increase in mass, adequate tolerance must be maintained for an average of approximately 38 weeks, the normal gestational period between implantation and delivery.

The normal maternal immune system in pregnancy, therefore, demonstrates an incredible level of plasticity in maintaining adequate tolerance.

As Robertson et al. correctly noted, maternal tolerance is not based on suppression of maternal immune system functions [3], as almost all principle immune functions are maintained at normal levels during pregnancy. Instead, tolerance is a consequence of inductive (i.e., stimulatory) processes. In other words, as many recent animal studies have demonstrated, it appears likely that proper maternal induction of tolerance toward implanting embryos requires proactive activation of selected immune pathways within the maternal immune system, which “reprogram” the mother’s immune responses from rejection to tolerance.

Like all physiological processes, the essential induction of tolerance pathways can, likely, malfunction. To suppress allogeneic immune responses, utilization of corticosteroids in earliest transplantation models [14], and their continuous utilization as first line anti-rejection drugs in solid organ transplantation [20], offers good evidence for their effectiveness in allogeneic immune responses. When in the past used in reproductive medicine as treatment in association with autoimmune phenomena, one, therefore, cannot conclude that their effectiveness necessarily reflects anti-autoimmunity; at least equally likely, their historical effectiveness may, have been directed against allogeneic immune responses the maternal immune system mounted against the invading fetal semi-allograft.

Hyperactive immune systems induce tolerance pathways poorly [21]. Women with autoimmunity and other immune system-activating conditions, including inflammation and allergies, therefore, likely develop tolerance-inducing pathways inadequately, and demonstrate insufficient development of tolerance toward the fetal semi-allograft in early gestation. Implanting embryos and early pregnancies, consequently, are left vulnerable to allogeneic attacks by the mothers’ immune systems.

Poorly induced tolerance pathways, therefore, can likely prevent implantation (i.e., cause implantation failure) and/or induce miscarriages (immune-induced pregnancy loss). Because autoimmunity is frequently the underlying cause for poor tolerance induction, autoimmunity has historically been associated with pregnancy loss [9, 10] and implantation failure [11]. These associations are, however, misleading because autoimmunity, itself, only unlikely causes pregnancy loss and implantation failure; the real culprit is, likely, because of autoimmunity (or other causes of immune system hyperactivity), poorly induced tolerance by eliciting reproductive failure-causing alloimmune responses.

Observed autoimmune laboratory abnormalities under such circumstances only represent “immunological noise,” and do not reflect the primary allogeneic immune defect. For decades assisted reproduction, therefore, has mistakenly concentrated on autoimmune findings or other individual test

results, often without real clinical relevance [13]. Widely tested immune markers, like anti-phospholipid and other auto-antibody abnormalities [9, 10] and NK cells [22], therefore, reflect only above noted “immunological noise,” suggesting inadequate tolerance. Themselves, these laboratory findings have little clinical meaning and practically no specificity for clinical outcomes. Previously noted lack of consensus on their meaning in association with IVF cycle outcomes, therefore, should not surprise.

It, therefore, appears timely to concentrate on how to assess tolerance, defining specific immune pathways essential for establishment of normal pregnancies and, once a diagnosis of insufficient tolerance has been reached, on how to therapeutically boost abnormally low tolerance levels. The latter point appears particularly important because normal induction of tolerance with great likelihood is an essential step in embryo implantation.

The following section describes evidence that such immune pathways exist in pregnancy, successful organ transplantation and in normal tolerance of “self.” Disturbances in these pathways result in decreased fecundity and, at times, in infertility, increased miscarriage risks and, if tolerance wears off prematurely, late pregnancy complications, like premature labor and preeclampsia/eclampsia. In allogeneic organ transplantations, the results are graft-versus-host (GVH) disease and organ rejection, and in regards to tolerance of “self,” the consequences are autoimmune diseases.

Evidence for existence of tolerance pathways

Two recently reported observations support the existence of tolerance-inducing pathways in pregnancy. The first comes from observations in a population of forager-horticulturalists in Bolivia who to significant degrees are infected by different helminth species. As parasites, those helminths—not dissimilar to fetuses—must avoid rejection. They, too, therefore, must be able to induce tolerance-producing immune pathways in the host.

Tolerance toward parasites

Blackwell et al. recently reported in *Science* that immune pathways induced by some helminths favor establishment of pregnancy. As witnessed by younger age at first birth and shorter birth intervals, women infected with roundworms (*Ascaris lumbricoides*) demonstrated strong evidence of improved fecundity in comparison to uninfected controls. Hook worm infections, in contrast, caused opposite effects, including later first births and longer birth intervals than controls [23], thus, possibly, inhibiting tolerance pathways.

Induction of pregnancy-favoring tolerance-producing immune pathways, thus, appears to be a highly specific process,

with selective environmental factors capable of influencing the female immune system in its ability to develop such pathways. Interestingly, some helminth infections have recently also been suggested to boost self-tolerance and improve clinical symptoms in, otherwise, treatment resistant autoimmune diseases, in the USA resulting in a quickly expanding treatment underground for iatrogenically induced helminth infections [24]. Further supporting these congruities, helminth infections have been reported to promote colonization resistance via type 2 immunity [25]. Normal pregnancy is also characterized by a preponderance of type 2 immunity [26].

Combined, these observations support the ability of the female immune system to respond with tolerance-inducing immune pathways in response to selected parasitic infections, which require such tolerance to survive within the human host, just as the paternal semi-allograft of pregnancy must survive within the maternal host and just like “self” must avoid autoimmune attacks. These observations further suggest that all three of these tolerance situations share overlapping tolerance pathways.

Premature termination of tolerance in pregnancy

A series of recent reports in mostly the infectious diseases (vaccination) literature potentially shed interesting additional light on essential tolerance pathways in human pregnancy. Several large-scale population studies from all around the world reported remarkably consistent effects on late pregnancy complications from influenza vaccinations during pregnancy. Though the purpose of these studies was to assess efficacy and safety of those vaccinations, they all, unexpectedly, reported an almost 50% reduction in late pregnancy complications, including premature labor [27–31].

What initiates labor, whether at term or prematurely, is still largely unknown. Increasing evidence, however, suggests that labor is an inflammatory process [32] and that, in absence of infections, its initiation may be caused by termination of the temporary tolerance of pregnancy. If that were to be the case, premature labor would reflect premature termination of temporary tolerance.

As noted earlier, immune system hyperactivity leads to diminished development of tolerance [21]. That autoimmunity is strongly associated with insufficient tolerance induction at onset of pregnancy but is also typically associated with premature labor, is, therefore, noteworthy. Premature labor is, indeed, a characteristic feature of practically all autoimmune diseases [33].

Maternal immune systems that have difficulty establishing good tolerance at initiation of gestation, thus, apparently also lose tolerance prematurely at the end of gestation. This very logical observation further supports that the establishment of temporary tolerance in pregnancy is a time-restricted event,

whether in normal pregnancy (ca. 38 weeks long) or in women with impaired tolerance (premature deliveries).

That a simple Influenza vaccination during pregnancy reduces late pregnancy complications, including premature labor, by an incredible 50% is, therefore, not only of great importance for public health but also strongly suggests that, like certain helminths, such vaccinations boost tolerance pathways in the maternal immune system. Moreover, by doing so, they extend the period of tolerance in women who, otherwise, might have experienced premature termination of tolerance and late pregnancy complications. Interestingly, helminth infections have recently also been reported to boost immune systems of offspring [34].

Extrapolating from these reported findings, increased utilization of influenza vaccinations in pregnancy should, therefore, significantly benefit current prematurity rates which, interestingly, do not greatly vary between developing and developed countries, thereby suggesting that prematurity represents an innate feature of pregnancy, independent of economic and genetic influences. Premature termination of pregnancy tolerance, of course, would represent such an innate pathophysiology, equally affecting women around the world.

The future

The evidence presented here for existence of tolerance-inducing maternal immune pathways is still largely circumstantial, and requires biological definition. Accordingly, we considered the presented evidence strong enough to initiate a prospectively randomized pilot study, investigating whether influenza vaccination in pregnancy, indeed, can affect immune tolerance pathways.

Such a study was recently approved by our center's Institutional Review Board and was registered under two protocols in autologous and donor-recipient cycles (NCT 02947217 and NCT 02946177). The hypothesis assumes that Influenza vaccination, indeed, induces immune pathways in female immune systems, which favor pregnancy through induction of tolerance. If correct, Influenza immunization, especially in women with evidence of hyperactive immune systems, should improve implantation rates and reduce spontaneous pregnancy losses (and late pregnancy complications).

We welcome potential collaborating centers to join us in this research effort, which requires larger IVF cycle numbers than a single center usually can provide. Interested centers are requested to contact the corresponding author.

Influenza immunizations in pregnancy are now strongly recommended [35]. Patients undergoing this trial will, therefore, receive a recommended treatment, with randomization only affecting timing of immunization, either shortly before or after conception.

We are currently also in the process of defining how to identify specific immune pathways activated by Influenza vaccinations. Some preliminary pathways have been reported [36], though not specifically related to infertile women. Identification of tolerance inducing pathways would not only have significance for infertility, and especially assisted reproduction treatments, but, likely, also for organ transplantation and improvements in self-tolerance (i.e., autoimmunity).

Blumenfeld pointed out the potential importance of identifying tolerance pathways [2] when discussing the interesting observation that women who had lymphoid tissues (i.e., appendices and tonsils) surgically removed demonstrated improved fecundity [1]. Assuming an effect on tolerance, removal of these lymphoid tissues would, thus, mimic above described effects of selected helminth infections [23]. It, indeed, is entirely possible that appendices and tonsils contain lymphoid cell populations with inhibitory functions on tolerance induction. Their surgical removal would then be expected to lead to improved tolerance induction and observed improvements in fecundity. Surgical removal of the spleen, the largest lymphoid organ, crucial in regulating immune homeostasis by aligning innate and adaptive immunity, and protecting against infections [37], is well documented to affect B lymphocyte responses [38] and inflammatory responses [39], has been reported to represent an independent risk factor for premature labor [40], and therefore, interestingly may be associated with early termination of tolerance.

Authors' contributions NG conceived the concept for this manuscript and wrote the first draft. VAK and DHB contributed to revisions of the manuscript. All authors approved of the final manuscript.

Compliance with ethical standards

Funding No external funds were used in support of this manuscript.

Conflict of interest NG, and DHB, are co-inventors on a number of pending and already awarded US patents claiming therapeutic benefits from androgen supplementation in women with low functional ovarian reserve (LFOR) and relating to the *FMRI* gene in a diagnostic function in female fertility. Both receive royalties from Fertility Nutraceuticals, LLC, in which NG also holds shares. NG, DHB, and VAK also are co-inventors on three pending AMH-related patent applications. They report no conflicts with the reported manuscript.

References

- Wei I, MacDonalds T, Shimi S. Association between prior appendectomy and/or tonsillectomy in women and subsequent pregnancy rate: a cohort study. *Fertil Steril*. 2016;106:1150–6.
- Blumenfeld Z. Appendectomy, tonsillectomy, and conceptions. *Fertil Steril*. 2016;106:1043–4.
- Robertson SA, Jin M, Yu D, Moldenhauer LM, Davies MJ, Hull L, et al. Corticosteroid-therapy in assisted reproduction—immune suppression is a faulty premise. *Hum Reprod* 2016; 2164–2172
- Zhang YH, Tian M, Tang MX, Liu ZZ, Liao AH. Recent insight into the role of the PD-1/PD-L1 pathway in feto-maternal tolerance and pregnancy. *Am J Reprod Immunol*. 2015;74:201–8.
- Chen T, Darrasse-Jèze G, Bergot AS, Courau T, Chriaud G, Valdivia K, et al. Self-specific memory regulatory T cells protect embryos at implantation in mice. *J Immunol*. 2013;191:2273–81.
- Liu Q, Tian FJ, Xie QZ, Zhang J, Liu L, Yang J. Fyn plays a pivotal role in fetomaternal tolerance through regulation of Th17 cells. *Am J Reprod Immunol*. 2016;75:569–79.
- Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature*. 1953;172:603–6.
- Billingham RE, Brent L, Medawar PB. Acquired tolerance of skin homografts. *Ann NY Acad Sci*. 1955;59:409–16.
- Lubbe WF, Butler WS, Palmer S, Liggins GC. Fetal survival after prednisone suppression of maternal lupus-anticoagulant. *Lancet*. 1983;19(1):1361–3.
- Gleicher N, Friberg J. IgM gammopathy and the lupus anticoagulant syndrome in habitual aborters. *JAMA*. 1985;253:3278–81.
- Geva E, Yaron Y, Lessing JB, Yovel I, Vardinon N, Burke M, et al. Circulating autoimmune antibodies may be responsible for implantation failure in vitro fertilization. *Fertil Steril*. 1994;62:802–6.
- Gleicher N, Vidali A, Karande V. The immunological “Wars of the Roses” disagreements amongst reproductive immunologists. *Hum Reprod*. 2002;17:539–42.
- Hill JA, Scott RT. Immunologic tests and IVF: “Please enough already.”. *Fertil Steril*. 2000;74:439–24.
- Billingham RE, Krohn PL, Medawar PB. Effects of cortisone on survival of skin homografts in rabbits. *Br Med J*. 1951;1:1157–63.
- Kemeter P, Feichtinger W. Prednisolone supplementation in clomid and gonadotrophin stimulation for in-vitro-fertilization—prospective randomized trial. *Hum Reprod*. 1986;1:441–4.
- Tucker MJ, Cohen J, Massey JB, Mayer MP, Wiker SR, Wright G. Partial dissection of the zona pellucida of frozen-thawed human embryos may enhance blastocyst hatching, implantation and pregnancy rates. *Am J Obstet Gynecol*. 1991;165:341–4.
- Dougal D. The clinical features of ectopic pregnancy. *Br Med J*. 1927;2:1074–7.
- Deglinclerti A, Croft GF, Pietila LN, Zernicka-Goetz M, Siggia ED, Brivanlou AH. Self-organization of the in vitro attached human embryo. *Nature*. 2016;533:251–4.
- Shahbazi MN, Jedrusik A, Vuoristo S, Recher G, Hupalowska A, Bolton V, et al. Self-organization of the human embryo in the absence of maternal tissues. *Nat Cell Biol*. 2016;18:700–8.
- Enderby C, Keller CA. An overview of immunosuppression in solid organ transplantation. *A J Manag Care*. 2015;21(1 Suppl): s12–23.
- Hobelka E, Nielsen PJ, Medgyesi D. Signaling mechanisms regulating B-lymphocyte activation and tolerance. *J Mol Med (Berl)*. 2015;93:145–58.
- Shehadri S, Sunkara SK. Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. *Hum Reprod Update*. 2014;20:429–38.
- Blackwell AD, Tamayo MA, Beheim B, Trumble BC, Stiglitz J, Hopper PL, et al. Helminth infection, fecundity, and age of first pregnancy in women. *Science*. 2015;350:970–2.
- Velasquez-Manoff M. The parasite underground. *The New York Times Magazine*, June 16, 2016.
- Ramanan D, Bowcutt R, Lee SC, Tang MS, Kurtz ZD, Ding Y, et al. Helminth infection promotes colonization resistance via type 2 immunity. *Science*. 2016;352:608–12.
- Lin H, Mosmann TR, Guilbert L, Tuntipopipat S, Wegman TG. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. *J Immunol*. 1993;151:4562–73.
- Wortman AC, Casey BM, McIntire DD, Sheffield JS. Association of influenza vaccination on decreased stillbirth rate. *Am J Perinatol*. 2015;32:571–6.

28. Sheffield JS, Greer LG, Rogers VL, Roberts SW, Lytle H, McIntire DD, et al. Effect of influenza vaccination in the first trimester of pregnancy. *Obstet Gynecol.* 2012;120:532–7.
29. Bratton KN, Wardle MT, Orenstein WA, Omer SB. Maternal influenza immunization and birth outcomes of stillbirth and spontaneous abortion: a systematic review and meta-analysis. *Clin Infect Dis.* 2015;60:e11–9.
30. Olsen SJ, Mirza SA, Vonglokharn P, Khanthamaly V, Chitry B, Pholsena V, et al. The Effect of Influenza vaccination on birth outcomes in a cohort of pregnant women in Lao PDR, 2014–2015. *Clin Infect Dis.* 2016;63:487–94.
31. Regan AK, Moore HC, de Klerk N, Omer SB, Shellam G, Mak DB, et al. Seasonal trivalent influenza vaccination during pregnancy and the incidence of stillbirth: population-based retrospective cohort study. *Clin Infect Dis.* 2016;62:1221–7.
32. Ibrahim SA, Ackerman 4th WE, Summerfield TL, Lockwood CJ, Schatz F, Kniss DA. Inflammatory gene networks in term human decidual cells define a potential signature for cytokine-mediated parturition. *Am J Obstet Gynecol.* 2016;214:284. e1—284 e47.
33. Gleicher N. Does the immune system induce labor? Lessons from preterm deliveries in women with autoimmune diseases. *Clin Rev Allergy Immunol.* 2010;39:194–206.
34. Clark CE, Fay MP, Chico ME, Sandoval CA, Vaca MG, Boyd A, et al. Maternal helminth infection is associated with higher infant immunoglobulin A titers to antigen in orally administered vaccines. *J Infect Dis.* 2016;213:1996–2004.
35. ACOG. Committee opinion No 468: influenza vaccination during pregnancy. *Obstet Gynecol.* 2010;116:1006–7.
36. Samy A, El-Enbaawy M, El-Sanousi AA, Nasef SA, Hikono H, Saito T. Initiation of regulation of immune responses to immunization with whole inactivated vaccines prepared from two genetically and antigenically distinct lineages of Egyptian influenza A virus subtype H5N1. *Arch Virol.* 2016;161:2797–806.
37. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. *Lancet.* 2011;378:86–97.
38. Papatou I, Piperi C, Alexandraki K, Kattamis A, Theodoridou M, Spoulou V. Antigen-specific B-cell response to 13-valent pneumococcal conjugate vaccine in asplenic individuals with β -thalassemia previously immunized with 23-valent pneumococcal polysaccharide vaccine. *Clin Infect Dis.* 2014;59:862–5.
39. Goltz D, Huss S, Ramadori E, Büttner R, Diehl L, Meyer R. Immunomodulation by splenectomy or by FRY720 protects the heart against ischemia reperfusion injury. *Clin Exp Pharmacol Physiol.* 2015;42:1168–77.
40. Gershovitz M, Sergienko R, Friedler JM, Wiznitzer A, Zlotnik A, Sheiner E. Pregnancy outcome in women following splenectomy. *J Womens Health (Larchmt).* 2011;20:1233–7.