Dear Editor:

Anti-sperm antibodies (ASAs) are composed of numerous antibodies interacting with multiple sperm antigens that play a role in fertility. In males, ASAs cause ‘immune infertility’ by decreasing sperm counts and normal forms, as well as reducing sperm motility and viability, markedly reducing the likelihood of natural conception[1]. The development of ASA in the male depends on the release of sequestered antigens on germ cells following the disruption of the blood-testis barrier. A surgical operation such as a vasectomy could also lead to immunogenic sperm antigens being exposed to the immune system, thus initiating an immune response to produce ASAs[2]. Vasectomy is widely regarded as a safe method of birth control, but over the years there have been many reports suggesting putative health risks associated with the procedure. ASA develops after vasectomy, which can result in autoimmune male infertility. Moreover, concerns over the possible association of vasectomy with a number of medical difficulties including cardiovascular disease, testicular cancer, prostate cancer, and a variety of immune complex-mediated disease processes have been reported[3]. There is a high correlation between vasectomy and ASA production; thus, the prevalence of ASA is higher in vasectomized men than in the general population, which causes limited success in regaining fertility, even after successful vasovasostomy[4]. The human leukocyte antigen (HLA) is the most important region in the genome with respect to autoimmunity and infection, and it is pivotal in adaptive and innate immunity. The HLA region encodes several molecules composed of the two polymorphic classes HLA-I (A, B, and C) and HLA-II (DR, DQ, and DP) that play key roles in the immune system by presenting antigens to T cells[5,6]. It is demonstrated that people with certain HLA antigens are predisposed to developing certain autoimmune diseases, but to date correlation between HLA gene and ASA production has not been fully identified[8]. We evaluated the ASA level in Iranian men one year after vasectomy and examined the association of ASA production with HLA class I and II in Iranian vasectomized men.

One hundred and ten vasectomized men (≤ 50 year) one-year post vasectomy and 130 healthy controls agreed to participate in this cross-sectional study. Written informed consent was obtained from all men and the Ethics Committee of Tehran University of Medical Sciences approved the study. Blood sample was collected from each subject to isolate peripheral blood mononuclear cells (PBMCs) and serum. For quantification of ASA in serum samples, we performed the Gelatin Agglutination Test (Kibrick) and the results were confirmed by the indirect mixed antiglobulin reaction (MAR) test. In this study, we used T cells and purified B cells as the source of HLA-I and HLA-II antigens, respectively. Firstly, PBMCs were isolated from blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, then isolated PBMCs were washed twice with PBS, placed on nylon wool columns (Biotest, Breieich, Germany) and incubated at 37°C for 30 minutes. Unabsorbed cells were removed by extensive washing with warm RPMI for HLA-A, -B and -C typing; B cells were also eluted from the column to use for HLA-DR and -DQ typing. Cell viability and cell counts were assessed by the Trypan blue exclusion method for purified cells (viability > 85%). HLA typing was carried out by a standard complement dependent microlymphocytotoxicity or Terasaki test. Microtiter plates (Biotest AG, Dreieich, Germany) containing HLA antisera with known specificity were used for determination of HLA-I or HLA-II antigens. Terasaki microtiter plates containing various HLA-I and II antibodies were seeded with 1 μL (2000 lymphocytes. After incubation at room temperature (HLA-I: 30 minutes and HLA-II: 60 minutes) and addition of rabbit complement, the results were visualized by adding 5% eosin. The assessment of lysed and non-lysed lymphocytes was carried out using an inverse phase contrast microscope. The gelatin agglutination test showed that ASA was detectable in 95% of vasectomized men, with slightly
Fig. 1 ASA titer in vasectomized and control groups. As shown, the highest ASA titers in vasectomized men are 1/32 or 1/64, whereas the maximum titer of serum ASA in normal men is 1/8.

Fig. 2 Frequency of HLA class I antigens in vasectomized and control men. HLA typing in vasectomized and normal men were carried out by a standard complement dependent microlymphocytotoxicity. There is a statistically significant correlation in the low responder group (ASA ≤ 1/8) with frequency of HLA-A2 and B5 class I antigens. (* = X² > 3.84). A: Frequency of HLA-A antigens in vasectomized and controls. B: Frequency of HLA-B antigens in vasectomized and control men. C: Frequency of HLA-C antigens in vasectomized and control men.
more than half of them (53%) having an ASA titer of 1/32 or 1/64. By contrast, serum ASA was not detectable in 86.2% of healthy controls and was found in only 13.8% of the healthy subjects with a maximum titer 1/8 (Fig. 1).

We further categorized the vasectomized subjects into the low responder group (n = 18; titer ≤ 1/8), the middle responder group (n = 92; titer between 1/16 and 1/64), and the high responder group (n = 44; titer ≥ 1/64). HLA-A, -B, -C, -DR and -DQ typing of the above three groups and control group revealed no statistically significant correlation between the high responder group and frequency of HLA antigens, whereas there was a statistically significant correlation (X² > 3.84, P < 0.05) between the low responder group and the frequency of HLA-A2 (X² = 8.85, P = 0.0029) and B5 (X² = 10.17, P = 0.0014) class I antigens (Fig. 2) as well as HLA-DRw53 (X² = 6.88, P = 0.0087) and DQw2 (X² = 5.37, P = 0.02) class II antigens (Fig. 3).

It is widely accepted that immune infertility due to ASA is one of the major causes of unexplained infertility in humans. More than 50–70% of men develop ASA after vasectomy and there is limited success in the reestablishment of fertility, even after successful surgical reanastomosis in vasovasostomy. A 2008 study by Nowroozi et al. showed that 2.5% of men had ASA at the time of vasectomy, whereas 53.5% of the study population subsequently developed ASA. The number of men with circulating ASA was increased significantly for the first three months after vasectomy, but the highest incidence of titers was one year after vasectomy. Our findings showed that ASA was produced in 95% vasectomized men one year after vasectomy. There has been lack of information on the relation between HLA antigens and ASA production in vasectomized men. Law et al. found that the HLA-A28 was strongly associated with production of ASA after vasectomy. While we found no significant association between the high responders and frequency of HLA antigens, there was a significant association in the low responders and frequency of HLA-A2, B5, -DRw53 and -DQw2. Men who had these HLA phenotypes produced a lower titer of ASA after vasectomy.

In conclusion, the predisposition to production of ASA appears to be correlated with HLA antigens. Men who have HLA-A2, -B5, -DRw53 and -DQw2 produce lower levels of ASA than other populations although a better understanding of the relation between HLA and ASA production requires further study.

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The authors reported no conflict of interests.

References

[1] Bohring C, W Krause. Immune infertility: towards a better understanding of sperm (auto)-immunity. The value of proteomic analysis. Hum Reprod 2003;18(5):915–924.

[2] Brunner-Agten S, Pavlovic R, Müller L, et al., Increased level of antibodies cross-reacting with Ves v 5 and CRISP-2 in MAR-positive patients. Int Arch Allergy Immunol 2013;160(1):47–55.

[3] Köhler TS1, Fazili AA, Brannigan RE. Putative health risks associated with vasectomy. Urol Clin North Am 2009;36(3):337–345.

[4] Heidenreich A, Bonfig R, Wilbert DM, et al. Risk factors for antisperm antibodies in infertile men. Am J Reprod Immunol 1994;31(2–3):69–76.

[5] Gough SC, Simmonds MJ. The HLA Region and Autoimmune Disease: Associations and Mechanisms of Action. Curr Genomics 2007;8(7):453–465.

[6] Chen X, PE Jensen. MHC class II antigen presentation and immunological abnormalities due to deficiency of MHC class II and its associated genes. Exp Mol Pathol 2008;85(1):40–44.