

# Correlation of ImmunoBead<sup>®</sup> and ImmunoSphere<sup>™</sup> Immunoglobulin G (IGG) tests on detecting antisperm antibody (ASA) on sperm

A. Bollendorf<sup>1</sup>, J.H. Check<sup>1,2</sup>

<sup>1</sup> Cooper Institute for Reproductive Hormonal Disorders, P.C., Mt. Laurel, NJ; <sup>2</sup> Cooper Medical School Of Rowan University,  
Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

## Summary

**Purpose:** To determine the correlation with detection of antibody on sperm by a new ImmunoSphere<sup>™</sup> Immunoglobulin test vs. ImmunoBeads<sup>®</sup>. **Materials and Methods:** A sampling of sperm tested for antisperm antibody (ASA) tested by direct ImmunoBead<sup>®</sup> assay with levels varying from zero to 100% were compared to the percentage of sperm positive for ASA by a new test using immunospheres. **Results:** The correlation was not perfect but, in general, there was good correlation. **Conclusion:** Now that the manufacturer is curtailing the manufacture of immunobeads, it is comforting to see a good correlation with immunospheres.

**Key words:** Antisperm antibodies; Direct immunobead test; Immunospheres; Post-coital test.

## Introduction

Recently a statement from the American Society for Reproductive Medicine Society stated that the post-coital test is an archaic procedure but cautiously advised that it has value when used in the hands of certain clinicians experienced with this test. The majority of infertility centers perform intrauterine insemination (IUI) each month in non-IVF cycles allegedly to improve pregnancy rates. However, at least one study found no advantage in improving pregnancy rates with IUI with normal sperm vs. sexual relations where a normal post-coital test was demonstrated [1].

Most infertility centers do not seem to determine if the sperm are laden with antisperm antibodies when they are performing the initial semen analysis. Cervical factor, where there is a primary mucus problem, only accounts for 3% of the causes of infertility. However, one study demonstrated that when >50% of sperm were coated with antisperm antibodies, only 31% had sperm progressing in the cervical mucus at least eight hours after intercourse in properly timed post-coital tests in the presence of normal follicular maturation [2]. Thus, if one did not measure antisperm antibodies with the initial semen analysis, perhaps subnormal post-coital tests could prompt repeating the semen analysis to measure antisperm antibodies.

Some would argue what would it matter if one is bypassing the mucus and performing an IUI anyhow? However, Francavilla *et al.* found no live pregnancies following 119 IUI cycles when 100% of the sperm were found to be

coated by antisperm antibodies [3]. Francavilla *et al.*'s study suggested that in addition to immobilizing antibodies (which when combined with complement in the cervical mucus impedes sperm progression) [4-7], that there may be antisperm antibodies that may impair fertility in other ways including disrupting sperm oocyte recognition and fusion [8, 9], or inhibiting sperm from binding to the zona pellucida [10-12]. Other areas inhibiting fertilization and normal pregnancies have been found including inhibiting capacitation or the acrosome reaction and even inhibiting a fertilized oocyte from cleaving [13].

Thus it seems very important in the modern era to still measure sperm for antisperm antibodies. One of the most commonly used tests has been the direct immunobead test [14]. However, the manufacturer is starting to phase out the production of ImmunoBeads<sup>®</sup>.

## Materials and Methods

In order to replace the ImmunoBeads<sup>®</sup> method, the authors chose to evaluate ImmunoSpheres<sup>™</sup> since they are a similar method. The ImmunoSpheres<sup>™</sup> have already been tested and reported to have good correlation with the ImmunoBeads<sup>®</sup> using indirect testing [15]. In this study the authors are evaluating the direct method of testing for anti-sperm antibodies on the sperm.

There are a few differences between the two manufacturers. The ImmunoBeads<sup>®</sup> tend to clump and are non-uniform in size. A phase contrast microscope needs to be used since the beads are translucent. The ImmunoSpheres<sup>™</sup> are monodispersed and uniform in size of 3.0 µm latex beads. They are also colored blue for use with either a bright field or phase contrast.

Revised manuscript accepted for publication July 15, 2015

The Direct ImmunoSpheres® test for IgG is performed by mixing live motile sperm with latex beads coated with antibodies that bind to human IgG antibodies. The beads are first washed with a medium containing 1-2% bovine serum albumin and can be stored up to three days at 4°C. The semen is washed three times and the sperm is diluted to give a final concentration of  $10 \times 10^6$ /ml. Five microliters of sperm suspension is mixed with five microliters of anti-IgG beads. After one to two minutes, 100 motile sperm are counted (in duplicate) and the percentage having beads attached is determined.

Procedural notes: the ImmunoBeads™ tended to have quick binding, however the bead clumping made it difficult to differentiate if the sperm were attached to the beads or trapped in the clumps of beads. The ImmunoSpheres® tended to be very sensitive with bead attachment, sometimes taking longer than the one- to two-minute incubation to form binding, as seen with the positive control. Also, controls failed often due to the weaker reactions with sperm. However, the ImmunoBeads™ consistently had normal control values.

The objective of the present study is to see if there is a reasonable correlation with a new direct antisperm antibody test from a different manufacturer but using instead of ImmunoBead™, ImmunoSphere®.

## Results

There were 29 known ASA samples that were split and the presence of ASA was measured by ImmunoBeads™ and ImmunoSpheres® tests. There were 11 ImmunoBeads™ specimens read as zero and all 11 were similarly read as zero with ImmunoSpheres®.

There were 14 specimens read as zero by ImmunoSpheres® with four slightly discordant ImmunoBeads™ tests read as 3, 2, and 7%, respectively. There were 11 ImmunoBeads™ specimens read as 100% ASA with complete agreement with ImmunoSpheres® in three; 98-99% in three, and the others showing 95% X2, 92%, and 83%, and 64%.

One ImmunoSphere® test was read as 100% and the corresponding ImmunoBeads™ was 97%. There were some larger discrepancies however. One sample was 87% by ImmunoBeads™ read as 31% ImmunoSpheres®. Other samples showed 7 vs. 0, 48 vs. 42, and 97 vs. 87.

## Discussion

There appears to be a good correlation between measuring ASA by ImmunoBeads™ vs. the ImmunoSpheres®. This confirms an older study performed over 15 years ago using different methodology than the new assay [15]. Some andrologists consider a positive test with ImmunoBeads™ ASA levels >50%, and some consider >80% as clinically important.

It is important to detect antisperm antibodies from the beginning to prevent the couple from wasting the expense, time away from work or children, and mounting frustration from performing IUI without first treating the sperm with the protein digestive enzyme chymotrypsin galactose [13,

16]. Of course in vitro fertilization with intracytoplasmic sperm injection (ICSI) is more effective (but a lot more expensive) [16].

Detection of antisperm antibodies prior to conventional insemination of normal appearing sperm can present the catastrophe of failed or poor fertilization which could have been circumvented by performing ICSI [16-20]. The present infertility center is now prepared to switch to the measurement of antisperm antibodies on sperm by ImmunoSpheres®.

## References

- [1] Check J.H., Liss J., Bollendorf A.: "Intrauterine insemination (IUI) does not improve pregnancy rates in infertile couples where semen parameters are normal and postcoital tests are adequate". *Clin. Exp. Obstet. Gynecol.*, 2013, 40, 33.
- [2] Check J.H., Bollendorf A.: "The effect of antisperm antibodies on post-coital results and the effect of intrauterine insemination on pregnancy outcome". *Arch. Androl.*, 1992, 28, 25.
- [3] Francavilla F., Romano R., Santucci R., Marrone V., Corrao G.: "Failure of intrauterine insemination in male immunological infertility in cases in which all spermatozoa are antibody-coated". *Fertil. Steril.*, 1992, 58, 587.
- [4] Steen Y., Forssman L., Lonnerstedt E., Jonasson K., Wassen A.C., Lycke E.: "Anti-sperm IgA antibodies against the equatorial segment of the human spermatozoon are associated with impaired sperm penetration and subfertility". *Int. J. Fertil.*, 1994, 39, 52.
- [5] Eggert-Kruse W., Hofstab A., Haury E., Tilgen W., Gerhard I., Runnebaum B.: "Relationship between local anti-sperm antibodies and sperm-mucus interaction in vitro and in vivo". *Hum. Reprod.*, 1991, 6, 267.
- [6] Menge A.C., Beitner O.: "Interrelationships among semen characteristics, antisperm antibodies and cervical mucus penetration assays in infertile human couples". *Fertil. Steril.*, 1989, 51, 486.
- [7] Clarke G.N.: "Immunoglobulin class and regional specificity of antispermatozoal autoantibodies blocking cervical mucus penetration by human spermatozoa". *Am. J. Reprod. Immunol. Microbiol.*, 1988, 16, 135.
- [8] Fann C.H., Lee C.Y.G.: "Monoclonal antibodies affecting sperm-zona binding and/or zona-induced acrosome reaction". *J. Reprod. Immunol.*, 1992, 21, 175.
- [9] Wolfe J.P., DeAlmeida M., Ducot B., Rodrigues D., Jouannet P.: "High levels of sperm-associated antibodies impair human sperm oolemma interaction after subzonal insemination". *Fertil. Steril.*, 1995, 63, 584.
- [10] Mahony M.C., Blackmore P.F., Bronson R.A., Alexander N.J.: "Inhibition of human sperm-zona pellucida tight binding in the presence of antisperm antibody positive polyclonal patient sera". *J. Reprod. Immunol.*, 1991, 19, 287.
- [11] Zouari R., De Almeida M.: "Effect of sperm associated antibodies on human sperm ability to bind to zona pellucida and to penetrate zona-free hamster oocytes". *J. Reprod. Immunol.*, 1993, 24, 175.
- [12] Liu D.Y., Clarke G.N., Baker H.W.G.: "Inhibition of human sperm-zona pellucida and sperm-oolemma binding by antisperm antibodies". *Fertil. Steril.*, 1991, 55, 440.
- [13] Check J.H.: "Antisperm antibodies and human reproduction". *Clin. Exp. Obstet. Gynecol.*, 2010, 37, 169.
- [14] Clark G.N., Elliott P.J., Smaila C.: "Detection of sperm antibodies in semen using the immunobead test: a survey of 813 consecutive patients". *Am. J. Reprod. Immunol.*, 1985, 7, 118.
- [15] Centola G.M., Andolina E., Deutsch A.: "Comparison of the immunobead binding test (IBT) and immunospheres (IS) assay for detecting serum antisperm antibodies". *Am. J. Reprod. Immunol.*, 1997, 37, 300.

- [16] Bollendorf A., Check J.H., Wilson C.: "A Comparison of Pregnancy Rates in Female Partners of Males with Sperm Laden with Antisperm Antibodies Following Intrauterine Insemination (IUI) with Sperm Treated with Chymotrypsin Galactose vs. In Vitro Fertilization (IVF) with Intracytoplasmic Sperm Injection (ICSI). In: International Proceedings of the 9<sup>th</sup> International Congress of Andrology, Medimond Editore, Pgs. 85-88, 2009. **Must be substituted with a published article in a Journal or Book**
- [17] Lahtenmaki A., Reima I., Hovatta O.: "Treatment of severe male immunological infertility by intracytoplasmic sperm injection". *Hum. Reprod.*, 1995, 10, 2824.
- [18] Nagy Z.P., Verheyen G., Liu J., Joris H., Janssenswillen C., Wisanto A., et al.: "Results of 55 intracytoplasmic sperm injection cycles in the treatment of male-immunological infertility". *Hum. Reprod.*, 1995, 10, 1775.
- [19] Clarke G.N., Bourne M., Baker H.W.G.: "Intracytoplasmic sperm injection for treating infertility associated with sperm autoimmunity". *Fertil. Steril.*, 1997, 68, 112.
- [20] Check M.L., Check J.H., Katsoff D., Summers-Chase D.: "ICSI as an effective therapy for male factor with antisperm antibodies". *Arch. Androl.*, 2000, 45, 125.

Address reprint requests to:  
 J.H. CHECK, M.D., Ph.D.  
 7447 Old York Road  
 Melrose Park, PA 19027 (USA)  
 e-mail: laurie@ccivf.com