Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis

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BACKGROUND: Several studies have examined the relationship between direct antisperm antibody (ASA) levels in semen and pregnancy rate after advanced assisted reproductive technologies (ARTs) but the results have been inconsistent. The aim of our study was to further evaluate the relationship between ASA and pregnancy after IVF or ICSI by systematic review and meta-analysis.

METHODS: We conducted a systematic Medline search of all relevant full papers on direct semen ASA and pregnancy after IVF or ICSI. Three investigators independently reviewed the papers, followed by group discussion to choose the included papers. Meta-analysis was performed to get an odds ratio (OR) for the effect of ASA on pregnancy using IVF or ICSI.

RESULTS: The study identified and analyzed 16 valid studies (10 IVF and 6 ICSI). The study characteristics (including the ASA cutoff values) were heterogeneous. Our meta-analysis revealed that the combined OR for failure to achieve a pregnancy using IVF or ICSI in the presence of positive semen ASA was 1.22 (95% CI: 0.84, 1.77) and 1.00 (95% CI: 0.72, 1.38), respectively. The overall (IVF + ICSI) combined OR was 1.08 (95% CI: 0.85, 1.38).

CONCLUSION: This systematic review and meta-analysis indicate that semen antisperm antibodies are not related to pregnancy rates after IVF or ICSI, suggesting that both forms of ART remain viable options for infertile couples with semen ASA. However, additional, well-designed prospective studies using appropriate ASA cutoff levels are needed to further address this issue.

Key words: sperm / antibodies / immunologic infertility / fertilization / pregnancy

Introduction

The antigenic properties of human sperm were reported as early as the end of 19th century (Metchnikoff et al., 1899). Since then, anti-sperm antibodies (ASAs) have been considered by several authors as a possible causative factor in infertility, with significant levels of ASAs detected in the semen of 5–15% of infertile men but in only 1–2% of fertile men (Ayvaliotis et al., 1985; Collins et al., 1993; Sinisi et al., 1993; Ombelet et al., 2009). Seminal ASAs generally develop as a result of an accidental or iatrogenic breach of the blood–testis barrier or from obstruction of the male reproductive tract (Bohring and Krause, 2003a,b). Typically, high levels of ASAs are found in men with a history of testicular torsion, testicular surgery, vasectomy and epididymo-orchitis (Mazumdar and Levine, 1998). Other conditions associated with ASAs include testicular carcinoma (Guazzieri et al., 1985; Hobarth et al., 1994), history of cryptorchidism (Urry et al., 1994) and HIV (Naz et al., 1990).

Sperm ASAs are believed to have an adverse impact on male fertility by (i) directly interfering with sperm motility and sperm-oocyte binding and (ii) indirectly by mediating the release of cytokines that can impair sperm function and by impeding cervical mucus penetration (Bronson et al., 1990; Steen et al., 1994; Mazumdar and Levine, 1998; Chiu and Chamley, 2004). Sperm ASAs have generally been associated with poor sperm motility, and, reduced natural pregnancy rates (Sinisi et al., 1993; Mazumdar and Levine, 1998; Lombardo et al., 2001). However, it is less clear whether ASAs have an adverse impact on ART outcomes.

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Several studies have evaluated the association between ASA and reproductive outcomes after IVF and ICSI, but the subject has not been evaluated in a systematic fashion. Studies on fertilization rate following IVF have reported contradictory results, with some studies showing a detrimental effect of ASA on fertilization rate (Junk et al., 1986; de Almeida et al., 1989; Acosta et al., 1994), and others not (Yeh et al., 1995; Ford et al., 1996; Culligan et al., 1998). A number of studies have also evaluated the association between ASA and pregnancy rate following IVF, with similarly contradictory results (Rajah et al., 1993; Vujsic et al., 2005). In contrast, the studies on ASA and pregnancy rate following ICSI have mostly shown that ASA do not influence ICSI pregnancy rates (Nagy et al., 1995; Check et al., 2000; Clarke, 2006).

As such, the objective of this study was to further examine the association between ASAs and pregnancy rates following assisted reproductive techniques (IVF and ICSI). This was done by conducting a systematic review and meta-analysis of the published studies on the topic.

**Materials and Methods**

**Search strategy and selection criteria**

We searched Medline database from 1980 to January 2010 using the following search terms: ‘antisperm antibodies’, ‘sperm antibodies’, ‘antibodies’, ‘ASA’, ‘semen antibodies’ in combination with ‘pregnancy’, ‘assisted reproduction’, ‘fertilization’, ‘IVF’ and ‘ICSI’. Additional studies were identified from the study reference lists. Only full articles published in English were searched. Three investigators independently reviewed the papers for eligibility and discrepancies were resolved by group discussion (Fig. 1).

**Data extraction**

We selected studies that evaluated direct sperm/semen antisperm antibodies and pregnancy in couples undergoing IVF and/or ICSI. For studies to be eligible, we had to be able to construct 2 × 2 tables from the reported data (with pregnancy rate above and below ASA cutoff). The following outcomes were prerequisites for inclusion: biochemical pregnancy (serum HCG elevation) and/or clinical pregnancy (that is, presence of a fetal heartbeat, confirmed by ultrasound). If necessary, study authors were contacted to clarify the data. We recorded the accrual type (i.e., prospective, consecutive), patient selection, female inclusion/exclusion criteria, treatment type (IVF/ICSI), sperm antisperm antibody test [mixed agglutination reaction (MAR), immunobead test (IBT)], cutoff point (definition of a positive ASA test), number of cycles or patients, fertilization rate relative to abnormal or normal test results and number of pregnancies relative to abnormal or normal test results.

From the 2 × 2 tables of test results, the following test properties were calculated for each study: sensitivity, specificity, positive predictive value, negative predictive value, proportion of abnormal tests and diagnostic odds ratio (OR). In order to perform the meta-analysis (Egger et al., 2001) and calculate a combined OR, we first tested the study homogeneity. We used the Q statistics to test homogeneity and rejected the null hypothesis at 0.10 significance level. Depending on whether homogeneity was accepted or rejected, we used the fixed or the random effect model to compute the combined OR and its 95% confidence interval (CI). Forest plots were presented to show the results of both treatment types (IVF and ICSI). The meta-analysis was conducted using the STATA 10.0 software (StataCorp LP, College Station, TX).

**Results**

A total of 16 papers (10 IVF and 6 ICSI studies) met our inclusion criteria. After group discussion, all reviewers were in agreement to include all 16 papers. A summary of the relevant IVF and ICSI data is shown in Table I. The total number of ART treatment cycles in each paper varied from 15 to 1822 and in 8 of the 16 studies more than 100 treatment cycles were reported. In total, there were 4209 ART treatment cycles (1508 IVF and 2701 ICSI cycles) and the mean clinical pregnancy rates for the group of IVF and ICSI studies were 23 and 36%, respectively. The studies varied in design (retrospective, prospective) and in the selection of ASA cutoff (1–80%). The published results regarding the presence of ASA and correlation with failure to achieve pregnancy also varied considerably.

The sensitivity of the ASA test ranged from 3 to 73% and the specificity ranged from 32 to 97% (Table II). The median percentage of couples with a positive ASA test was 18% in the ICSI studies and 31% in the IVF studies (see Table II). The OR for failing to achieve pregnancy varied from 0.53 to 6.4. Only one of the studies reported a significant association between semen ASAs and pregnancy rate (Acosta et al., 1994).

The test of heterogeneity suggested a fixed effect model and the meta-analysis revealed no significant correlation between the presence of ASA and pregnancy rates after IVF or ICSI (16 studies), with an overall combined OR for not achieving pregnancy of 1.08 (95% CI: 0.85, 1.38). Similarly, the meta-analysis revealed no significant correlation between the presence of ASA and failure to become pregnant after IVF alone (10 studies) or after ICSI alone (6 studies), with ORs of 1.22 (95% CI: 0.84, 1.77) and 1.00 (95% CI: 0.72, 1.38), respectively (Figs 2 and 3).

We have also conducted a sub-analysis of the data, including only those studies with an ASA cutoff at >50% (2 IVF and 4 ICSI studies). Limiting the analysis to these studies does not change the final conclusions (i.e. ASAs are not associated with differences in the IVF or ICSI pregnancy rates). The combined OR for not achieving pregnancy with IVF or ICSI with an ASA cutoff at >50% are 0.72 (95% CI: 0.18, 2.88) and 1.03 (95% CI: 0.72, 1.48), respectively.

**Discussion**

In this systematic review of 16 studies involving 4209 ART treatment cycles (1508 IVF and 2701 ICSI cycles), the direct semen ASA test results were not associated with pregnancy rates (combined overall OR = 1.08, 95% CI: 0.85, 1.38). The study also indicates that ASA test results are not associated with pregnancy rates at IVF and at ICSI. The combined OR for not achieving a pregnancy using IVF or ICSI in the presence of positive semen ASA was 1.22 (95% CI: 0.84, 1.77) or 1.00 (95% CI: 0.72, 1.38), respectively. An OR of 1 indicates that an abnormal ASA test result (ASA above the cutoff point) is not associated with an increased chance of disease (i.e. failure to achieve a pregnancy). Altogether, the data suggest that both forms of ARTs remain viable options for infertile couples with semen ASAs. However, one cannot make similar conclusions regarding the influence of semen ASA levels on natural or IUI pregnancy based on these data.

The 16 studies analyzed in this systematic review were heterogeneous in terms of design and ASA test type (IBT and MAR), but
most notably in the ASA cutoff level, with the cutoff ranging form 1 to 80% (de Almeida et al., 1989; Lahteenmaki, 1993; Rajah et al., 1993; Acosta et al., 1994; Pagidas et al., 1994; Lahteenmaki et al., 1995a; Nagy et al., 1995; Sukcharoen and Keith, 1995; Clarke et al., 1997; Vazquez-Levin et al., 1997; Mercan et al., 1998; Hjort, 1999; Check et al., 2000; Vujisic et al., 2005; Clarke, 2006; Esteves et al., 2007; van Weert et al., 2008). Although the WHO recommends a 50% cutoff value (i.e. ASA test is positive if >50% of sperm are bound to IgG or IgA) (WHO, 1999), most of the analyzed studies used a different cutoff value. Only Check et al. and Esteves et al. used a 50% cutoff, while one study used 1%, three studies used 10%, five studies 20%, one study 30%, one study 70% and three studies 80% cutoff values, with no single study providing a justification for the different cutoff value. The mean pregnancy rates for the group of IVF and ICSI studies were 23 and 36%, respectively. These pregnancy rates are in keeping with the reported pregnancy rates in studies that include a wide range of maternal ages (while 4 of the 11 studies did not report female age, 7 included patients >35 years, with 3 of these studies also including patients >40) (van Loendersloot et al., 2010). The relatively larger number of ICSI (2257 or 83%) versus IVF (894 or 58%) cases in the pre-2000 era may bias the results somewhat such that one might expect overall higher IVF pregnancy rates. However, we do not believe that ASAs have a substantial impact on the IVF and ICSI pregnancy rates overall because the number of ASA positive cases in each of the studies was relatively small.

There were 10 studies which examined the relationship between ASA levels and IVF pregnancy with a combined OR = 1.22 (95% CI: 0.83, 1.77), indicating a weak and non-significant effect of ASA levels on pregnancy rates (de Almeida et al., 1989; Lahteenmaki, 1993; Rajah et al., 1993; Acosta et al., 1994; Pagidas et al., 1994; Sukcharoen and Keith, 1995; Vazquez-Levin et al., 1997; Vujisic et al., 2005; Clarke, 2006; van Weert et al., 2008). Only one of these 10 IVF studies reported a significant inverse relationship between ASA levels and IVF pregnancy rate (Acosta et al., 1994). In contrast, one
study reported that intermediate-high semen IgA levels (between 20–80% binding) were associated with a significantly higher pregnancy rate (Clarke, 2006). Out of the 10 IVF studies, 9 evaluated fertilization rates. Of these nine studies, six found a lower fertilization rate in cases with high semen ASA levels (de Almeida et al., 1989; Lahteenmaki, 1993; Rajah et al., 1993; Acosta et al., 1994; Vazquez-Levin et al., 1997; Clarke, 2006). The remaining three IVF studies found no relationship between ASA levels and fertilization rate (Pagidas et al., 1994; Sukcharoen and Keith, 1995; Vujisic et al., 2005).
Sukcharoen and Keith, 1995; Vujisic et al., 2005). A sub-analysis of the data, including only those studies with an ASA cutoff at >50% (two IVF studies) does not change the final conclusions (i.e. ASAs are not associated with IVF pregnancy rates). The combined OR for the IVF studies with an ASA cutoff at >50% was 0.72 (95% CI: 0.18, 2.88).

Each one of the six studies on ASA levels and ICSI outcomes reported no measurable relationship between ASA levels and pregnancy rates and, the combined OR of these ICSI studies was 1.00 (95% CI: 0.72, 1.38). Of the six ICSI studies, four also reported on fertilization rates. Of these, three retrospective ICSI studies found no relationship between ASA levels and fertilization rates. Of these, three retrospective ICSI studies found no relationship between ASA levels and fertilization rate (Lahteenmaki et al., 1995a; Clarke et al., 1997; Mercan et al., 1998). On the other hand, one of the ICSI studies reported a higher (not lower) fertilization rate in cases with high semen ASA levels (Nagy et al., 1995). Two large prospective ICSI studies (both using a 50% ASA cutoff level) demonstrated no association between ASA levels and pregnancy rates (Check et al., 2000; Esteves et al., 2007). A sub-analysis of the data, including only those studies with an ASA cutoff at >50% (4 ICSI studies) did not change the final conclusions (i.e. ASAs are not associated with ICSI pregnancy rates). The combined OR for the ICSI studies with an ASA cutoff at >50% was 1.03 (95% CI: 0.72, 1.48).

The conclusions drawn from this meta-analysis of ASAs in IVF and ICSI studies cannot be applied to ASAs in the context of natural and IUI pregnancy. Studies have shown an association between ASAs and reduced male fertility potential (Hammitt et al., 1988; Matson et al., 1988; Adeghe, 1993; Acosta et al., 1994; Leushuis et al., 2009) and there is good evidence to show that the prevalence of ASAs in the semen of infertile men is higher that of fertile men (Collins et al., 1993; Sinisi et al., 1993). ASAs have been shown to impair sperm motility, sperm transport through the female reproductive tract, sperm survival, sperm acrosome reaction, sperm–oocyte fusion and embryo development, thus, highlighting the various mechanisms by which ASAs can impair natural fertility (Ohl and Naz, 1995). Ovarian stimulation with IUI can be used to overcome these effects and successfully treat infertile couples with semen ASAs (Check and Bollendorf, 1992; Lahteenmaki et al., 1995b; Ombelet et al., 1997;
However, studies suggest that ASAs are also associated with a reduced probability of pregnancy at IUI (Check and Bollendorf, 1992; Francavilla et al., 1992; Check et al., 2004; van Weert et al., 2005). As such, several investigators have suggested that couples with ASAs (particularly those with high levels) consider IVF and/or ICSI rather than IUI as an initial treatment (Bates, 1997; Check et al., 2004; Lombardo et al., 2004).

One of the inherent limitations of the studies included in this analysis is that they report on assays (the IBT and MAR tests) that assess antibody binding to a population of viable, motile sperm. These studies do not specifically address the potentially more severe effects of ASAs (e.g. cytotoxicity, with reduced survival rates, and agglutination, with impaired progressive motility). Moreover, the IBT and MAR tests do not examine the specificity of the antibodies (i.e. antibody binding to clinically or biologically relevant sperm antigens with associated sperm dysfunction). This is clinically relevant because only a subset of ASA-positive infertile men have cytotoxic and agglutinating ASAs (Hinting et al., 1996; Harrison et al., 1998), and it is unclear what proportion of ASAs bind to clinically relevant antigens (Ohl and Naz, 1995; Bohring and Krause, 2003b). As such, the IBT and MAR studies provide a rather crude assessment of the pathologically important ASAs and this may certainly influence how one interprets correlative studies with IVF and ICSI outcomes.

In summary, the results of the meta-analysis indicate that semen ASAs (as measured by MAR/IBT) are not related to IVF or ICSI pregnancy rates and suggest that both forms of ARTs remain viable options for couples with semen ASAs. However, the meta-analysis is weakened by the heterogeneity of the studies and the fact that few studies (particularly, the IVF studies) evaluated couples with high levels of ASAs (most studies used cutoff levels <50%). As such, it is hard to make definitive conclusions about the clinical value of ASA testing prior to IVF and/or ICSI. Additional, well-designed prospective studies using appropriate ASA cutoff levels are needed to further address this issue.

**Authors’ roles**

A.Z. was responsible for the study design, overseeing the completion of the study, editing and finalizing the manuscript. N.F., A.K. and N.A. collected the data, conducted group discussions and prepared the manuscript draft. A.C. and E.B. performed the statistical analyses.

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