

Immunology and immunopathology of the male genital tract

Antisperm immunity in natural and assisted reproduction

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Research conducted in the last 40 years has provided evidence that antisperm antibodies (ASA) can impair the fertilizing capacity of human spermatozoa. It is established that ASA can be present at different sites, can act against different antigens and can impair fertility in various ways. In fact, in the past it was amply demonstrated that ASA can act negatively on sperm motility and on cervical mucus penetration. In recent years, owing to the improvement and spreading of IVF techniques, it has been possible to demonstrate the effect of antibody-bound spermatozoa at the level of in-vitro gamete interaction. The literature demonstrates that the various previously used treatments for immunological infertility, i.e. medical therapy, intrauterine insemination with husband's spermatozoa (AIH) and IVF, usually had poor success. The primary choice of treatment in immunological infertility, especially in the most severe cases and when the sperm head is involved, is ICSI. ASA evaluation in all couples who undergo the various techniques of insemination or IVF is imperative.

Key words: antisperm antibodies/IVF/intracytoplasmic sperm injection/intrauterine insemination

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Introduction

Studies carried out in the late 1950s (Rumke and Hellinga, 1959) demonstrated that a significant number of infertile men show autoimmunity to spermatozoa, and suggested that antisperm antibodies (ASA) can interfere with the fertilizing ability of the spermatozoa. The ASA can act negatively both on the motility of the spermatozoa in the semen, on their ability to pass through female genital secretions, on the fusion of the gametes, which represents a key event of fertilization, and perhaps also on the first step of embryo development (Shushan and Schenker, 1992; Vazquez-Levin *et al.*, 1997; Koide *et al.*, 2000).

In the last few years, owing to the improvement and dissemination of IVF techniques, it has been possible to study

the effect of antibody-bound spermatozoa directly, at the level of in-vitro gamete interaction.

ASA and natural reproduction

As far as the effect in semen is concerned, an association between ASA and low sperm motility has been reported (Mathur *et al.*, 1984) and improvement of motility with continuous long-term, low-dose steroid therapy has been demonstrated (Hendry *et al.*, 1979). However, even though the presence of ASA is often associated with hypomotility or alterations of motility, there are a number of cases in which microscopical evaluation shows normal motility, and computerized evaluation (computer-assisted sperm analysis; CASA) confirms this for all the kinetic parameters measured by the system (Lombardo *et al.*, 1992).

ASA can affect penetration of the cervical mucus (Clarke, 1988a; Matson *et al.*, 1988; Barratt *et al.*, 1992), and ASA-coated spermatozoa may be more vulnerable to phagocytosis in the female reproductive tract (London *et al.*, 1985). Sperm-bound IgA antibodies are associated with poor cervical mucus penetration (Kremer *et al.*, 1978; Jager *et al.*, 1980; Parslow *et al.*, 1985; Wang *et al.*, 1985). It has been suggested that ASA directed against the head were of primary importance (Wang *et al.*, 1985),

Table I. Therapy for immunological infertility: protocols based on immunosuppression

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- 96 mg/day of methylprednisolone administered to the male partner from day 21 to day 28 of the partner's menstrual cycle. The success rate in terms of pregnancy is 20% (Shulman and Shulman, 1982)
 - 2 or 3 mg/day of dexamethasone for 13 or 9 weeks respectively. The reduction in antibody titre is between 0 and 50% (De Almeida and Souffir, 1977)
 - 250 mg of testosterone as an intramuscular injection to obtain a pharmacologically induced azoospermia, followed by 16 mg/day of 6-methylprednisolone by mouth for 30 days and in the following 2–4 months at 8 mg/day. The success in terms of pregnancy is ~25% (Dondero *et al.*, 1979)
 - 40 mg/day of methylprednisolone, given for a period that runs from day 1 of the partner's menstrual cycle, followed by 5 mg/day from day 11 to day 12 for three cycles. Pregnancy is achieved in ~25% of cases (Hendry *et al.*, 1986).
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while others have suggested that ASA directed against the tail are involved in this phenomenon (Clarke, 1988a). A negative correlation has been reported between IgG on the sperm tail on the one hand and forward motility and penetration of cervical mucus on the other (Barratt *et al.*, 1992).

The presence of a large percentage of spermatozoa with vibratory motility is presumably due to the cross-linking of motile, antibody-coated spermatozoa to the cervical mucus gel via the Fc part of the immunoglobulins (Jager *et al.*, 1981; Clarke, 1985). Various investigations suggested that there was a relationship between sperm-bound autoantibodies of the IgA immunoglobulin class and a poor sperm cervical mucus contact test (Jager *et al.*, 1980), and between sperm antibodies and poor post-coital test results (Kremer *et al.*, 1978). It has been confirmed that, using the capillary test, sperm-bound antibodies of IgA immunoglobulin class were associated with poor in-vitro cervical mucus penetration (Clarke, 1988b). Analysis of the regional specificities indicates that antibodies at the tail endpiece do not interfere with in-vitro cervical mucus penetration, while autoantibodies at the sperm tail mainpiece are of primary importance in the interference with in-vitro cervical mucus penetration.

Such a strict correlation was not confirmed employing the slide test as the test of sperm–cervical mucus interaction (Lenzi *et al.*, 1989). In fact, above a threshold of 60% of ASA, detected using direct immunobead test (IBT), it is extremely difficult to identify clear differences — which are related to poor results of the sperm–mucus interaction test — between the immunoglobulin classes or between the binding sites. A main role for the intensity of immunization on the other immunological parameters (prevalent binding site and prevalent immunoglobulin class) has been suggested (Lenzi *et al.*, 1988, 1989).

From a therapeutic point of view, immunological pathologies can be treated by immunosuppressive steroids. Unfortunately, no double-blind, randomized study has so far been conducted, and for this reason the efficacy of this therapy has not been validated. In addition, owing to the difficulty of selecting homogeneous groups of patients, success rates are variable and inconsistent and the results are often more visible in terms of improved semen quality than in reduced autoimmune reactions. The therapeutic protocols most commonly used in humans are based on corticosteroids at various dosages and with various modes of administration (Table I). The reported results, even if they are interesting from the point of view of the percentage of pregnancies obtained, leave a doubt as to their real efficacy on autoimmune pathology, bearing in mind that these drugs are used more for their capacity to reduce inflammation than for their ability to suppress the immune response.

ASA and assisted reproduction: experimental and clinical data

As stated previously, the diffusion of the various assisted reproductive techniques increases our knowledge on the effect of ASA on the fertilizing ability of the spermatozoa. Firstly, the importance of the technique for ASA detection, the titre of antibody positivity in the indirect methods (tray agglutination test, TAT; gelatin agglutination test, GAT) or the percentage of positivity in direct methods (IBT and mixed antiglobulin reaction test, MAR/SpermMar test) (Dondero *et al.*, 1991; Gandini *et al.*, 1995) must be taken into account. Secondly, it should be noted that recent studies showed that, by treating seminal fluids strongly ASA positive using Percoll gradient separation, we obtained different sperm populations which did not show differences between them in the percentage of binding and in the regional ASA distribution versus raw semen. Moreover, immature germ cells, obtained always by Percoll separation, appear to be ASA-free (Gandini *et al.*, 1999; Lombardo *et al.*, 2000). These could be important findings with regard to the selection of the best assisted reproductive techniques in cases of immunological infertility.

The presence of ASA must be taken into account in a programme of assisted reproductive techniques in two ways; i.e. both as a possible complication (ASA production after insemination) and as an interfering factor (ASA capacity to block sperm passage through the female genital tract and interference with the fertilization process).

The first aspect refers almost exclusively to intraperitoneal insemination (IPI). With this technique, the immediate and intense effect of direct 'micro-traumatic' contact of the pool of spermatozoa with peritoneum could cause ASA production, although despite the large number of spermatozoa inseminated and even after several IPI attempts, there was no evidence of de-novo production of, or increase in, already present ASA (Livi *et al.*, 1990; Ragni *et al.*, 1993). However, due to the ASA interference with sperm progression in the female genital tract, their potential presence in the female genital secretion is sufficient reason to advise against all forms of artificial insemination (intrauterine insemination with husband's spermatozoa, AIH, IPI) and advise instead the use of IVF (Clarke *et al.*, 1984; Francavilla *et al.*, 1999).

On the other hand, when ASA are present at high titre in the serum, they are constantly associated with positivity in the follicular fluid. This means that the serum cannot be used for the culture medium and the oocyte must be washed very carefully to prevent the contamination of the medium in which the gametes will be co-incubated. The evaluation of the presence of ASA in

the serum of women involved in an IVF programme of assisted reproduction therefore helps to avoid 'unexplained' failure (Micara *et al.*, 1989).

Finally, in cases of male immune infertility, the use of various techniques for semen manipulation has been proposed (Lenzi *et al.*, 1988) in order to elute ASA bound to spermatozoa and obtain ASA-free sperm pools. The success rate of these techniques, in terms of effective recovery of spermatozoa not involved in antisperm antibody reaction, is so discouraging that in a review of therapy for immune infertility, Shulman, making a reference to the method of sperm washing and insemination, stated that 'this procedure has not had marked success, presumably because of the great difficulty of eluting the antibodies from the sperm cell surface by any method that is also sufficiently gentle to the sperm cell' (Shulman, 1986). In our experience, the preparation of semen for artificial insemination in subjects with antisperm autoantibodies has demonstrated that swim-up is not able to select spermatozoa free of antibodies bound to the sperm surface. All experiments of migration, dilution, antigenic competition and cryopreservation carried out to evaluate the theoretical possibility of a better recovery of non-'immunologically compromised' spermatozoa, have confirmed such negative results with both IBT and cytofluorimetric analysis (Lenzi *et al.*, 1988).

Another research approach takes into consideration the physiological modifications of the sperm membrane which take place during in-vitro capacitation of spermatozoa (Hinrichsen-Kohane *et al.*, 1984). This approach derives from the idea that, during in-vivo capacitation in the female genital tract and/or *in vitro* in capacitating media, there can be a loss of membrane structures which are present at the moment of ejaculation (Myles and Primakoff, 1984; Srivastava *et al.*, 1986). Some of the structures which undergo these changes are the coat molecules which are acquired by spermatozoa during epididymal maturation and ejaculation, while other structures are intrinsic to the membrane. Our hypothesis is that some of these coat and membrane molecules might be the antigens against which antibodies are directed. If this is true, by inducing in-vitro capacitation one can obtain the elimination of not only the antibody but also the entire immuno-complex without damaging the sperm membrane. In this way, one can get antibody-free spermatozoa without damaging the plasma membrane. Such spermatozoa are at an advanced stage of capacitation, but are still useful for IVF. For this purpose we have carried out direct IBT on selected post-rise populations of antibody-coated spermatozoa at various time intervals after the onset of in-vitro capacitation. In this experiment, a new scoring system was employed for the IBT microscopical evaluation, splitting sperm head positivity into the acrosomal and post-equatorial regions for the purpose of classifying the different antigenic distributions on the sperm surface. Our data indicate a significant reduction of ASA in the head acrosomal region during cell incubation. Studies of sperm function have confirmed that good motility and structural and functional integrity of the sperm membrane have been maintained. Our results demonstrate that there is a significant reduction of ASA, from both a statistical and an immunological point of view (Lenzi *et al.*, 1992). This confirms previous studies that have shown a change in the level of ASA positivity after different periods of incubation in capacitating conditions. These

researchers considered that a modification of the sperm surface was the most likely explanation (Fusi and Bronson, 1990; Monroe *et al.*, 1990). These results offer an excellent human experimental model of an oocyte fertilization by an 'immunologically compromised' spermatozoon after capacitating therapy.

The above data are in keeping with other findings regarding acrosomal antigens and could indicate the possible production site of such antigens. Spermatozoa, microsurgically aspirated directly from the epididymis in patients with a congenital absence of vas deferens, do not have in the acrosomal region antigens reactive with ASA deriving from sera of the same patient (Dondero *et al.*, 1993). These data which differ slightly from those of other groups (Patrizio, 1992), were obtained in men with spermatozoa positive to ASA with a high % of binding. An indirect IBT was carried out to identify such ASA and then to investigate the antigenic site. One possible explanation for the findings is that antigens acquired by spermatozoa after epididymal maturation in the cauda epididymis or after the epididymal passage are present only at the time of ejaculation and are lost during the capacitation process. These antigens could form part of the decapacitating substances which serve to stabilize the acrosome membrane until the moment of capacitation.

ASA, when present either in the man or in the woman (Clarke, 1988b), can reduce the chances of a successful assisted reproductive treatment. The experimental evidence suggests that ASA may inhibit fertilization by binding specifically to membrane antigenic structures involved in sperm-oocyte interaction (Marquant-Le Guinne and De Almeida, 1986; Munoz de Vera *et al.*, 1986). Various authors demonstrated that ASA are able to impair the fertilization process at the level of the acrosome reaction (Srivastava *et al.*, 1986), of the zona pellucida recognition and penetration (Bronson *et al.*, 1982; Saling and Lakoski, 1985; Tsukui *et al.*, 1986; Mahony *et al.*, 1991; Shibahara *et al.*, 1993), and of the sperm-vitellus interaction (O'Rand *et al.*, 1984). A number of classical studies have shown that homologous ASA can also interfere with the fertilization of zona-free hamster oocytes by human spermatozoa (Alexander, 1984; Haas *et al.*, 1985). However, even if the zona-free hamster oocyte system has been a useful model for initial studies, there is evidence to suggest that this system may be an inadequate approximation to the more complex human fertilization process. First, it has been reported that under some particular conditions spermatozoa which can penetrate zona-free hamster oocytes may not be able to penetrate human zonae (Gould *et al.*, 1983). Second, there have been conflicting reports about the degree of correlation between zona-free hamster oocyte assay and routine human IVF (Kuzan *et al.*, 1987; Zainul Rashid *et al.*, 1998). Other studies have been performed using salt-stored human zonae pellucidae (Liu *et al.*, 1989; Francavilla *et al.*, 1997). They suggest that sperm auto- and iso-antibodies of IgG or IgA immunoglobulin classes can interfere with sperm binding to, and penetration of, the human zona pellucida. It is not certain, however, whether the storage of zonae in high salt solutions may induce alterations in the sperm receptor sites so that they undergo subtle changes in their mechanism of interaction with normal or ASA-coated spermatozoa. In view of the limitations of these fertilization models, it is becoming apparent that more relevant information about the effects of the ASA on human fertilization must be derived from the study of human IVF using viable

Table II. Data of the literature on the relationship between antisperm antibodies and fertilization rate in assisted reproduction treatment

Author, year	Method	Treatment	Fertilization rate
Junk <i>et al.</i> (1986)	iIBT	IVF	reduced (IgG + IgA) ^a
Mandelbaum (1987)	IBT	IVF	reduced
Matson <i>et al.</i> (1988)	iIBT	IVF	reduced (IgG + IgA) ^a
De Almeida <i>et al.</i> (1989)	IBT	IVF	reduced (IgG + IgA) ^a
Chang <i>et al.</i> (1993)	IBT	IVF	reduced (IgG) ^b
Rajah <i>et al.</i> (1993)	IBT, MAR	IVF	reduced
Lähteenmäki (1993)	MAR, TAT	IVF	reduced (when MAR \geq 90%)
Acosta <i>et al.</i> (1994)	IBT	IVF	reduced
		GIFT	reduced PR
Sukcharoen and Keith (1995)	IBT	IVF	non-affected
Yeh <i>et al.</i> (1995)	IBT	IVF	reduced
Lähteenmäki (1995)	MAR, flow cytometry	ICSI	non-affected
Nagy <i>et al.</i> (1995)	MAR, IBT	ICSI	increased
Ford <i>et al.</i> (1996)	iIBT	IVF	reduced
Vazquez-Levin <i>et al.</i> (1997)	MAR	IVF	reduced
Clarke <i>et al.</i> (1997)	IBT	ICSI	non-affected

iIBT=indirect immunobead test; IBT=direct immunobead test; MAR=mixed antiglobulin reaction test; PR=pregnancy rates; ICSI=intracytoplasmic sperm injection; GIFT=gamete intra-Fallopian transfer.

^aReduced only when both IgG and IgA are present.

^bReduced only when IgG are present.

oocytes. Although the results of the studies in this field are suggestive of a correlation between ASA and impaired fertilizing capacity (Mandelbaum *et al.*, 1987; Clarke *et al.*, 1988; Sukcharoen and Keith, 1995; Vazquez-Levin *et al.*, 1997), it is difficult to analyse the data from an epidemiological and statistical point of view due to the fact that relatively small numbers of patients with ASA will be found in an individual programme, thus making it difficult to compare groups with a different level or type of ASA. Moreover, with small groups of patients it is also more likely that non-immunological factors may bias results in a group. For this reason, researchers try to set up an appropriate model to eliminate many of the uncertainties, employing 'spare' oocytes from consenting patients. This approach, even if it allows us to carry out various experimental programmes using whole human oocytes, carries with it a series of ethical-legal problems.

ASA and IVF: epidemiological data

A large body of evidence is present in the literature regarding the epidemiology of the interference of ASA in assisted reproductive treatments (Table II).

The immunobead binding technique has been used to identify IgA, IgG and IgM ASA in the serum, semen and follicular fluid of 40 couples undergoing IVF (Mandelbaum *et al.*, 1987). There was no correlation between ASA binding to the sperm tail tip and the fertilization rate of inseminated mature oocytes using 10% of the individual women's serum in the insemination medium. Similarly, there was no correlation between ASA binding to the sperm head in semen and male serum, and fertilization. However, the fertilization rate in couples with ASA at the sperm head of at least one isotype in the female serum was significantly less than in those without ASA at the sperm head. Also the presence of this

kind of ASA in follicular fluid correlated negatively with fertilization. The low fertilization rate reported by this group has not been reproduced by other authors.

In a study of 160 infertile couples undergoing treatment by IVF (Sukcharoen and Keith, 1995), the male partners in the study group (11 couples, 15 cycles) were positive for sperm-bound ASA determined by direct IBT. In the control group (149 couples, 152 cycles), the men had no such antibodies. There were no significant differences in the region, type and/or percentage of sperm-bound antibodies, which also had no effect on the IVF outcome, and these authors concluded that IVF is not significantly affected by autoantibody-bound spermatozoa.

The presence of both IgA and IgG in seminal plasma, using indirect IBT, was associated with a decreased incidence of good post-coital test results and a reduced rate of fertilization of human oocytes. No significant differences were found for men with IgA or IgG alone when compared to men with no detectable ASA (Matson *et al.*, 1988). On the other hand, it has been demonstrated that couples with ASA on spermatozoa, evaluated using IBT, had a lower fertilization rate and lower number of transferred embryos, with IgG as the major immunoglobulin class involved (Chang *et al.*, 1993). In this study, couples with ASA in female sera showed significant decreases in the rates of fertilization, cleavage, and number of transferred embryos only when IgM alone (i.e. without IgG or IgA) was detected. However, the presence of IgA ASA in female sera was associated with a decrease in pregnancy rate, although the number of transferred embryos was not reduced. These authors suggest that ASA can influence the results of IVF and that the specific effect is dependent upon the subtypes of ASA. In a study on 16 couples in which the male partner was positive for ASA measured by direct MAR test, direct IBT, and serum and/or seminal plasma TAT, compared with 20 couples in which the men had no such

antibodies, it was found that the ASA in the male interfered with sperm-egg fusion and subsequent fertilization, but that once fertilization has occurred the pregnancy rate remained the same (Rajah *et al.*, 1993).

In a study which involved 29 male factor patients (38 IVF cycles) with positive ASA on the spermatozoa as evaluated by IBT, and treated by IVF, and in comparison 56 similar patients (57 cycles) who were treated by GIFT (Acosta *et al.* 1994), the authors concluded that the presence of ASA on the sperm surface *per se* impairs the outcome of assisted reproduction, mainly in terms of fertilization rate of pre-ovulatory oocytes, and possibly in terms of pregnancy rate.

In another large study (Ford *et al.*, 1996), a series of 183 patients with positive indirect IBT on semen were studied in order to determine the correlation in semen between specific antibody types, binding sites, antibody concentration, and fertilizing ability. There was no correlation between the percentages of motile spermatozoa that bound IgA and IgG immunobeads, but the two classes of beads generally bound to the same region of the spermatozoa. A total of 63 couples went on to attempt IVF treatment, all with mature eggs recovered. The fertilization rate tended to decrease as the amount of antibody increased. The presence of antibodies to the sperm head was highly correlated with the antibody concentration but was not selected as a predictor of fertilization. The authors conclude that the nature of the antigen against which the seminal ASA is directed may be as important as the antibody concentration in affecting sperm function.

In a retrospective clinical study (Vazquez-Levin *et al.*, 1997), seven men were evaluated, treated in nine IVF cycles with surface-bound ASA, positivity ranging from 65 to 100% using direct MAR test. In these patients, the fertilization rate and early embryonic cleavage of human embryos was found to be reduced significantly. Moreover, the embryonic quality and the pregnancy rate may also be compromised.

In a study carried out in 72 couples, including 15 with ASA in the male partner's semen (Junk *et al.*, 1986), it was demonstrated that fertilization is significantly reduced only if both IgA and IgG antibodies, evaluated using indirect immunobead test (iIBT), are present in semen: there is no reduction if either class is present alone. The fertilization rate of oocytes was significantly reduced by the use of spermatozoa from oligospermic samples, and there is a further reduction in those cases with combined IgA/IgG ASA. It must be underlined that the percentage of success in cases of sperm auto-immunization are related to the degree of ASA involvement; in fact, in most series, patients with a very low or moderate sperm auto-immunization rate were also included and this might explain the high percentage of success. This bias is also present in the effects of the corticosteroid therapy, which in cases of very low immunization has been demonstrated to be very effective. If we consider only the degree of immunization there is an inverse correlation with the overall fertilization rate.

In order to confirm the importance of the level of immunization on the other immunological parameters, 20 infertile couples, undergoing IVF and with high levels (>70%) of ASA on the ejaculated spermatozoa, were evaluated using IBT (De Almeida *et al.*, 1989). Of the 15 couples who recovered oocytes, 95 mature oocytes were collected and inseminated with spermatozoa obtained by swim-up migration after rapid dilution and washing

of the ejaculates. An overall fertilization rate of 38.9% was obtained with these post-migration preparations. When >70% of the inseminated spermatozoa were covered with both IgG and IgA antibodies (five patients), six out of 43 inseminated oocytes were fertilized (14%). When >70% of the inseminated spermatozoa were covered with only one class of antibodies, either IgG or IgA (five patients), 15 out of 26 inseminated oocytes were fertilized (58%). In the five patients with <70% of spermatozoa coated with both classes of antibodies, 16 out of 26 inseminated oocytes were fertilized (62%). The IVF rate was significantly decreased when >70% of the inseminated spermatozoa were coated either with IgG ($P < 0.001$) or IgA ($P = 0.05$).

In one study, data from 33 couples suffering from male immune infertility, who underwent 47 IVF cycles, were retrospectively analysed (Lähteenmäki, 1993). The serum of all 33 male partners had elevated TAT titres ($\geq 1:16$) and positive IgG MAR test results from their semen. There was a slight correlation between these tests when conducted on semen and serum. Fertilization rates were analysed in three sperm MAR subcategories. Only the strongly positive MAR group (values $\geq 90\%$) revealed a significant reduction in fertilization rate compared to the other MAR groups. This effect was not observed with increasing serum TAT titres. Asthenozoospermic male partners showed a decreased fertilization rate (20.1%) compared to normozoospermic (34.0%) male partners. This was true also for couples not affected by immunological factors, but, when ASA were present, fertilization rates were significantly worse irrespective of whether the sperm motility was normal or decreased. Once fertilization had occurred, the pregnancy rate was not affected by the severity of immunological factors.

A retrospective study of 48 couples (80 IVF cycles) with males showing positive ASA on the sperm surface by IBT, who were treated by IVF (Yeh *et al.* 1995), found that IgG and IgA antibody levels had no significant correlation with total fertilization rate of preovulatory oocytes and that IgM, present in 44% of the couples, had a strong correlation with fertilization. When IgA showed very high levels of binding (>68%), and IgM binding was >40%, the fertilization rate dropped significantly. A strong correlation between the presence of antibodies and fertilization rate was seen when IgM was directed to the head or tail tip of the spermatozoa. IgA induced a statistically significant reduction of fertilization only when it was present on the head. The authors concluded that IgA had an impact only when a high level of binding was detected on the head. IgM, present in 44% of the males, was the Ig isotype which most significantly affected fertilization rates when localized at both the head and at the tail tip.

These data confirm that in IVF programmes, the degree of immunization is the most important parameter affecting fertilization, and that antisperm autoimmunization has only a relative effect on impairment of fertilization, rather than an absolute effect. In any case, the interference of ASA at the level of gamete interaction, more than at other levels (e.g. cervical mucus penetration), exhibits qualitative as well as quantitative differences among patients. The interference here is greater than that at other levels, suggesting that it depends on the relevance of the specific antigens to the fertilization process.

When ASA are present on the sperm surface they can reduce or inhibit fertilization. In this case the microinjection of the

compromised spermatozoa into the oocyte cytoplasm is likely to increase the fertilization rate. Nagy *et al.* reported the first results of ICSI treatment in couples whose infertility was due to ASA at high titre in the semen (Nagy *et al.*, 1995). They evaluated, using MAR test and IBT, 37 patients where the percentage of binding of ASA to the sperm surface was $\geq 80\%$. The fertilization rate was significantly higher than the ASA-negative control group. This can be explained by the facilitating effect of ASA on the acrosome reaction with a consequent increase in the fertilizing potential of microinjected spermatozoa (Saragüeta *et al.*, 1994). The authors conclude that fertilization, embryo development and pregnancy rate after ICSI are not influenced by the percentage of ASA-bound spermatozoa, nor by the dominant type of antibodies present, nor by the location of ASA on the spermatozoa. On the other hand, they reported a significantly poorer sperm quality in MAR-positive patients as compared to the general ICSI population.

In a study of 29 infertile couples with male ASA, detected by MAR and partly by flow cytometry, treated using ICSI (Lähteenmäki *et al.*, 1995). In a significant percentage of cases these couples had shown a poor fertilization rate in previous IVF treatments. These authors did not find differences in the fertilization and cleavage rates in ICSI between MAR-positive and MAR-negative groups. However, as in the work of Nagy, the embryo quality was lower in the MAR-positive group. Moreover, in the MAR-positive group there was a higher rate of first trimester pregnancy loss.

In order to re-examine data of the ICSI in the light of the above mentioned reports, Clarke *et al.* studied a group of 39 patients with a strong positivity at IBT (at least 80% of IgG and/or IgA) who underwent ICSI (Clarke *et al.*, 1997). The results of this study confirm that fertilization and pregnancy rate are comparable in ASA-positive and -negative patient groups; however, these authors did not confirm the decrease in embryo quality reported by Nagy *et al.* (1995). In contrast to the work of Lähteenmäki (1995), the study of Clarke did not show an increased incidence of first trimester pregnancy loss in sperm antibody-positive patients. The discrepancies in the embryo quality and pregnancy loss could be due to the small antibody-positive groups involved.

Conclusions

In conclusion, the presence of ASA may impair the fertilizing ability of the spermatozoa *in vivo* can also be a serious factor which prevents the success of the various insemination or fertilization techniques. Only ICSI seems to be able to overcome the problem, and with this technique the fertilization rate, embryo quality and pregnancy rate of ASA patients have been found to be in the same range as the general population of ICSI patients. However, due to the relative rarity of immunological infertility, the literature in this field is quite scarce and it is therefore necessary for more studies to be conducted in order to confirm that embryo quality is not impaired.

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