Human Reproduction, pp. 1–8, 2020
doi:10.1093/humrep/deaa070

human reproduction

ORIGINAL ARTICLE *Infertility*

Relationship between natural and intrauterine insemination-assisted live births and the degree of sperm autoimmunisation

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Submitted on November 20, 2019; resubmitted on March 4, 2020; editorial decision on March 13, 2020

STUDY QUESTION: What is the relationship between the degree of sperm autoimmunisation, as assessed by IgG-mixed antiglobulin reaction (MAR) test, and natural and intrauterine insemination (IUI)-assisted live births?

SUMMARY ANSWER: Compared with a lower degree of positivity (50–99%), a 100%-positive MAR test was associated with a much lower occurrence of natural live births in infertile couples, who could be successfully treated with IUI, as first-line treatment.

WHAT IS KNOWN ALREADY: The World Health Organization (WHO) has recommended screening for antisperm antibodies, through either the IgG-MAR test or an immunobead-binding test, as an integral part of semen analysis, with 50% antibody-coated motile spermatozoa considered to be the clinically relevant threshold. However, the predictive value of the degree of positivity of the MAR test above such a cut-off on the occurrence of natural pregnancies remains largely undetermined. Furthermore, the effectiveness of IUI in cases of strong sperm autoimmunisation is not yet well-established.

STUDY DESIGN, SIZE, DURATION: This was a retrospective cohort study on 108 men with a ≥50%-positive MAR test, where the couple had attended a university/hospital andrology/infertility clinic for the management of infertility from March 1994 to September 2017.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The IgG-MAR test was carried out as an integral part of semen analysis. The patients were divided into two groups: 100% and 50%–99%-positive MAR test. The post-coital test (PCT) was performed in all the couples, and IUI was offered as the first-line treatment. Laboratory and other clinical data were retrieved from a computerised database. Data on subsequent pregnancies were obtained by contacting patients over the telephone.

MAIN RESULTS AND THE ROLE OF CHANGE: A total of 84 men (77.8%) were successfully contacted by telephone, and they agreed to participate. Of these, 44 men belonged to the group with a 100%-positive MAR test, while 40 showed lower MAR test positivity. The couples with a 100%-positive MAR test showed a natural live birth rate per couple (LBR) that was considerably lower than that observed with a lower degree of positivity (4.5% vs. 30.0%; $P = 0.00001$). Among the clinical variables, a significant difference between the two groups was observed only for the PCT outcome, which was poor in the 100%-positive MAR test group. Better PCT outcomes (categorised as negative, subnormal and good) were positively associated with the occurrence of natural live births (6.3, 21.7 and 46.2%, respectively; $P = 0.0005$ for trend), for which the sole independent negative predictor was the degree of sperm autoimmunisation. IUI was performed as the first-line treatment in 38 out of 44 couples with a 100%-positive MAR test, yielding 14 live births (36.8%). In couples with lower MAR test positivity, the LBR after IUI (26.9%) was similar to the natural LBR in this group (30.0%).

LIMITATIONS, REASONS FOR CAUTION: Given the retrospective nature of the study, we cannot exclude uncontrolled variables that may have affected natural pregnancies during the follow up or a selection bias from the comparison of natural live births with those after IUI.

WIDER IMPLICATIONS OF THE FINDINGS: The routine use of the IgG-MAR test in the basic fertility workup is justified as it influences decision making. A 100%-positive IgG-MAR test can represent the sole cause of a couple's infertility, which could be successfully treated with IUI. On the other hand, a lower degree of positivity may only represent a contributing factor to a couple's infertility, and so the decision to treat or wait also depends on the evaluation of conventional prognostic factors including the PCT outcome.

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STUDY FUNDING, COMPETING INTEREST(S): This study was supported by PRIN 2017, Ministero dell'Università e della Ricerca Scientifica (MIUR), Italy. On behalf of all authors, the corresponding author states that there is no conflict of interest.

TRIAL REGISTRATION NUMBER: N/A

Key words: antisperm antibodies / MAR test / male infertility / post-coital test / semen analysis

Introduction

The clinical significance of naturally occurring antisperm antibodies (ASA) in men from infertile couples, as well as their proper treatment, remains controversial despite being widely investigated (Francavilla and Barbonetti, 2017). Although a higher prevalence of ASA has been reported in some clinical conditions, such as male reproductive tract (MRT) obstructions and infections, the association between a clinical condition and ASA is only well established for acquired MRT obstructions, particularly vasectomy (Marconi and Weidner, 2009). The generation of ASA in patients with obstructions or vasectomy would involve an increased intraductal pressure of the epididymis associated with chronic absorption of sperm fragments. Acute or chronic infection or inflammation of the MRT has also been claimed as a risk factor for ASA generation due to various mechanisms, such as post-inflammatory obstruction, direct damage of the blood-testis barrier, testis immune dysregulation and cross-reactivity between antigens of microorganisms and sperm antigens; however, these associations remain controversial. Scant and inconclusive evidence provides little support for the reported associations between ASA generation and other conditions, such as varicocele, cryptorchidism, testicular trauma, surgery, torsion, tumour and sexual practices involving unprotected anal intercourse (Marconi and Weidner, 2017). In the vast majority of cases, the generation of naturally occurring ASA remains idiopathic.

The prognostic significance of ASA is also controversial. Although several studies have reported an association between a higher degree of sperm autoimmunisation and the reduced occurrence of natural pregnancies (Rumke *et al.*, 1974; Ayvaliotis *et al.*, 1985; Meinertz *et al.*, 1990; Abshagen *et al.*, 1998), the independent contribution of ASA in hindering the occurrence of natural pregnancies is yet to be proven. The latest edition of the World Health Organization's laboratory manual for the examination and processing of human semen (WHO, 2010) has reconfirmed a screening test for ASA, either the mixed antiglobulin reaction (MAR) test or the immunobead-binding test (IBT), to be an integral part of semen analysis, and has indicated that 50% antibody-coated motile spermatozoa should be the clinically relevant threshold. Nevertheless, the usefulness of the routine use of the direct IgG-MAR test in the basic fertility workup was again questioned in a prospective study, wherein a positive IgG-MAR test (≥50%) had no contribution to the prediction of natural pregnancies in subfertile couples (Leushuis *et al.*, 2009).

Similarly, the recommended treatment strategy is also contentious. On the one hand, intracytoplasmic sperm injection (ICSI) has been claimed to be the primary choice of treatment in ASA-related subfertility, especially in the case of strong sperm autoimmunisation (Lombardo *et al.*, 2001). On the other, intrauterine insemination (IUI) has been widely used as the first-line treatment, considering that the impairment of the sperm ability to penetrate through the cervical mucus is the primary mechanism whereby ASA interfere with fertility (Bronson, 1999; Francavilla *et al.*, 2007; Francavilla and Barbonetti, 2012; Francavilla and Barbonetti, 2017), as shown by *in vitro* studies (Alexander, 1984; Haas, 1986; Aitken *et al.*, 1988) and post-coital test (PCT) outcomes (Bronson *et al.*, 1984; Leushuis *et al.*, 2009; Barbonetti *et al.*, 2019). Uncertainties still remain mainly on the effectiveness of IUI in cases of strong sperm autoimmunisation (when all or nearly all spermatozoa are coated with ASA), as such an inclusion criterion (100%-positive MAR or IBT test) had only been used rarely (Francavilla *et al.*, 2009).

In a recent retrospective study on over 10 000 consecutive men who had undergone semen analysis as a part of the couple's infertility work-up, the prevalence of a positive IgG-MAR test with 50 and 100% thresholds was 3.4 and 2%, respectively (Barbonetti *et al.*, 2019). The 100%-positive MAR test, in addition to exhibiting higher consistency over time and other peculiar features such as a higher prevalence of mixed pattern of positivity (i.e. when the majority of sperm exhibits beads attached on both the head and along the tail), was also found to be significantly associated with a higher prevalence of a negative PCT outcome, compared to lower degrees of positivity. Furthermore, this finding was independent of, and without any significant contribution by, other determinants, including semen and mucus quality. In contrast, in the case of a 50–99%-positive MAR test, the semen and cervical mucus quality also contribute to the negative PCT outcome along with the degree of MAR positivity (Barbonetti *et al.*, 2019). These findings strongly suggested that a 50%-positive IgG-MAR test, proposed by WHO as the clinically relevant threshold, also includes patients with a level of autoimmunisation that could contribute to a couple's infertility in the presence of other causal factors, whereas a 100%-positive MAR test could represent the sole determinant of a couple's infertility, as it is highly predictive of a negative PCT outcome, a surrogate marker of infertility.

To confirm this hypothesis, this study was designed to analyse more clinically relevant endpoints in order to ascertain (i) whether a 100%-positive MAR test is associated with a low chance of natural pregnancy when compared with a lower degree of positivity (99–50%) and (ii) the effectiveness of IUI in connection with the degree of sperm autoimmunisation.

Materials and Methods

Study design and patients

Between March 1994 and September 2017, 342 men, attending the Andrology Unit of the University/Hospital of L'Aquila for semen analysis, were found to be positive for ASA, as documented by a positivity ≥50% on the IgG-MAR test, that was carried out on all ejaculates as an integral part of semen analysis. For detailed information of ASA prevalence and ASA-related seminal features see Barbonetti *et al.* (2019).

We carried out a retrospective cohort study on couples with ASA in male partners who attended our clinic for the management of couple infertility, in addition to the seminological laboratory investigation. The inclusion criteria were having undergone PCT and assessment of ovulatory function and tubal patency of the female partner. The exclusion criterion was having an untreatable cause of female infertility. There were 108 couples who met the inclusion criteria. IUI was offered as the first-line treatment for these couples. All couples were managed over time by the same physicians (F.F. and S.F.). Semen samples were analysed and processed for IUI over time by the same biologists, who also rated the PCT outcome. Semen samples were always processed using the swim-up procedure for IUI, as previously described (Francavilla *et al*., 1990).

Internal quality control was periodically carried out. The laboratory also participates in external quality assessment for semen analysis, undertaken by the UK National External Quality Assessment Service (Birmingham, UK).

The patients were divided into two groups based on the MAR test positivity: 100%-positive and 50–99%-positive. The occurrence of natural pregnancies and the effectiveness of IUI were analysed in connection with the degree of sperm autoimmunisation, also accounting for the PCT outcome. Laboratory and other clinical data were retrieved from computerised database. Data on subsequent pregnancies were obtained contacting patients by telephone.

Ethical approval

All the patients gave written informed consent for access to their clinical information and the use of their data, following the guidelines of the Italian Privacy Law. Clinical data were retrieved after the approval of the study by the Ethical Committee of the University of L'Aquila, protocol number 26652. Subsequently, patients were contacted by telephone to obtain data on subsequent pregnancies, after explaining the purpose of the interview and obtaining their informed consent.

Semen analysis

The semen samples were produced by masturbation in a private collection room located close to the laboratory. The semen samples were collected in sterile containers and analysed according to the ongoing WHO-recommended procedures as recently described (Barbonetti *et al*., 2019). The total sperm count (TSC) was calculated by multiplying the sperm concentration with the volume of the whole ejaculate; motile sperm concentration (MSC) was calculated by multiplying the sperm concentration with progressive motility (%); and the total motile sperm count (TMSC) was calculated by multiplying MSC with the volume of the ejaculate.

Direct IgG-MAR test

Immunological screening for sperm-bound antibodies was performed using the direct IgG-MAR test (SpermMAR, FertiPro N.V., Beernem, Belgium). Briefly, 10 µL of fresh semen were placed on a microscope slide and mixed with 10 µL of the IgG suspension, followed by the addition of anti-Ig incomplete antibody (as a bridging antibody). The drop was covered with a coverslip and incubated for 1 min. Then, the percentage of motile spermatozoa with attached beads and the gross prevailing regional specificity of the attachment (head, tail, tail tip) were determined using a phase-contrast microscope at ×400 magnification. When the majority of spermatozoa exhibited attached beads on the head as well as along the tail, the pattern was recorded as 'mixed'.

PCT and evaluation of cervical mucus

The timing of the PCT was scheduled for 15 to 16 days prior to the next expected menstrual period. The PCT was performed at around 10 h post-coitus following 3 to 5 days of sexual abstinence. The number of forward moving spermatozoa per high-power field (HPF) was recorded as a mean of at least 10 fields. The absence of forward-moving spermatozoa indicated a negative result, whereas the positive results were classified as subnormal in the presence of <7 forward-moving spermatozoa per HPF and good in the presence of ≥ 7 forward-moving spermatozoa per HPF. Cervical mucus quality was scored according to the Moghissi system, which was adopted by WHO in 1992 (WHO, 1992). A poor PCT outcome in the presence of unfavourable mucus was repeated later in the same cycle, if the mean diameter of the dominant follicle was <18 mm; otherwise, it was repeated, whenever possible, in a subsequent cycle following oral treatment with oestrogens. In case of ovulatory dysfunction, PCT was performed under appropriate therapy. In cases of repeated PCT, the best outcome was analysed.

IUI

In couples with male immunological infertility, IUI was performed in non-stimulated cycles, unless in the case of concurrent ovulatory dysfunction that was treated with appropriate ovulation induction. The inclusion criteria were a normal uterine cavity, tubal patency assessed by hysterosalpingography and/or laparoscopy, and the recovery of at least one million motile spermatozoa after semen processing, with 3–5 days of sexual abstinence. The timing of IUI was checked by means of a home ovulation predictor kit and, in the case of stimulated cycles, by ultrasound monitoring. Live births were assessed by reviewing the patients' charts and through phone calls to the patients.

Statistical analysis

Statistical analysis was performed using the R statistical software (version 3.0.3, 2014, The R Foundation for Statistical Computing, Vienna, Austria). Comparisons in continuous variables between pairs of groups were made using the Wilcoxon rank-sum test, while proportional variables were compared using the χ^2 test or Fisher's exact test, depending on the sample size. Multiple logistic regression models were utilised for exploring the independent contributions of the putative predictive factors such as PCT outcome, age of women, concurrent presence of female factor of infertility, duration of infertility, TMSC and percentage of MAR test positivity, to natural live births and IUI-assisted live births. The PCT outcome was categorised as positive or negative, unless stated otherwise. The statistical significance of differences in natural live births among couples with negative, subnormal and good PCT results was assessed using the χ^2 test for trend (Mantel-Haenszel test).

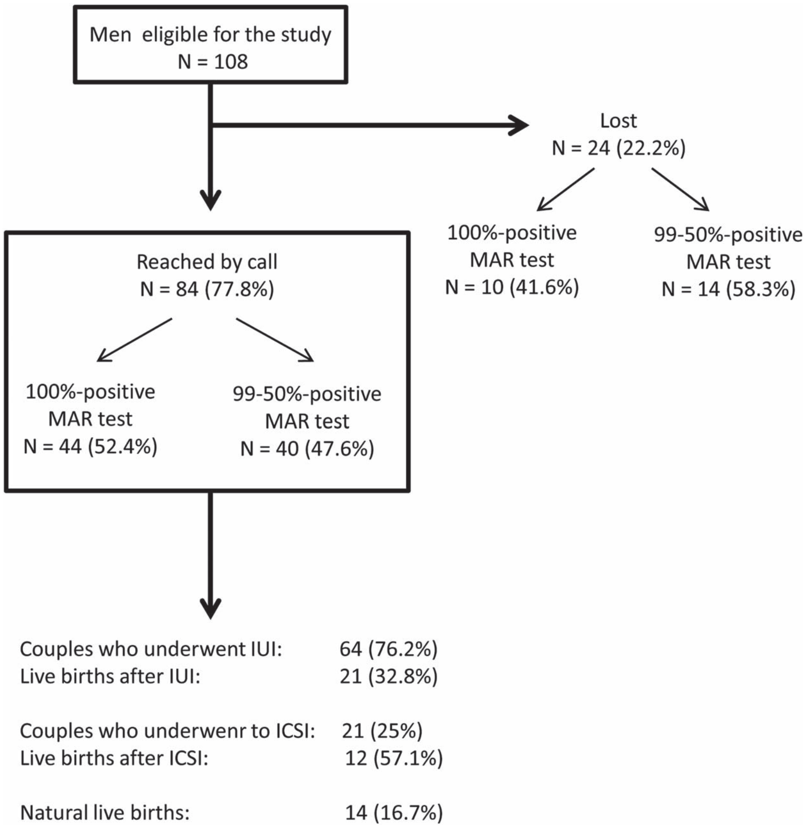

Figure 1 Study flow chart with clinical outcomes. MAR, mixed antiglobulin reaction; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination.

Results

Study profile

Figure 1 presents the study flow chart with clinical outcomes in terms of live birth rate per couple (LBR). Out 108 men eligible for the study, 84 (77.8%) were successfully contacted by telephone and agreed to participate. Of these men, 44 (52.4%) showed a 100%-positive MAR test, while 40 (47.6%) showed lower MAR test positivity. Similar percentages were observed in the 24 patients who could not be contacted (41.6% and 58.3%, respectively; Fig. 1).

IUI had been performed as the first-line treatment in 64 couples (76.2%), yielding 21 live births (LBR = 32.8%). In 21 couples, ICSI was performed either after the failure of IUI (14 couples) or as the first-line treatment (7 couples), yielding 12 live births (LBR = 57.1%). Fourteen natural live births were recorded in the entire group during the follow-up (LBR = 16.7%).

Comparisons between 100 and 99–50%-positive MAR tests

Clinical variables.

Table I summarises the comparison of clinical variables and clinical outcomes between the two study groups. Among the clinical variables, only the PCT outcomes showed a significant difference between the two groups: the PCT outcomes were poor in the 100%-positive MAR test group in terms of both negative results and the number of motile spermatozoa per HPF.

Natural live births.

In couples with 100%-positive MAR test, the spontaneous LBR was significantly lower compared to the other couples: 2/44 (4.5%) versus 12/40 (30.0%); $P = 0.00001$ (Table I). One of the two pregnancies in the 100%-positive MAR test group was achieved after IUI failure. Three of the 12 pregnancies in the other group were achieved after IUI failure; one was after successful IUI and another was after successful ICSI. The time to pregnancy from admission was significantly longer in the two couples with a 100%-positive MAR test, compared to the other group (Table I).

As the two MAR test groups differed only in the PCT outcome, the relationship between PCT outcome and natural LBR was analysed. The LBR progressively increased in couples with negative, subnormal and good PCT outcome ($P = 0.0005$ for trend; Fig. 2). The multiple regression analysis showed that a better PCT outcome was the sole independent predictor of the occurrence of natural live births, among putative predictive factors including the age of the woman, concurrent female factor, length of infertility and TMSC (Table II, Model 1). However, even this association was no longer significant after adjustment for the percentage of MAR test positivity, as this was the sole independent predictor (Table II, Model 2).

Table I Comparison of clinical variables and clinical outcomes in the two study groups.

	100%-positive MAR test ($n = 44$)	99–50%-positive MAR test ($n = 40$)	*P* value
Clinical variables			
Secondary infertility—*n* (%)	1 (2.3%)	5 (12.5%)	0.1
Infertility length before admission (years)	2 (1–8)	2 (1–7)	0.18
Follow-up length (years)	16 (3–27)	14 (3–27)	0.26
Woman's age (years)	32.5 (23–44)	34 (24–44)	0.24
Concurrent female factor—*n* (%)	6 (13.6%)	5 (12.5%)	1
Mucus score	12 (5–15)	11 (6–15)	0.29
Negative PCT—*n* (%)	35/44 (79.5%)	13/40 (32.5%)	0.00003
Number of motile spermatozoa/HPF	0 (0.0–20.4)	2.7 (0.0–89.7)	0.00001
TMSC ($\times 10^6$)	72.5 (3.6–347.2)	95.4 (3.8–630)	0.08
Clinical outcomes			
Natural LBR—*n* (%)	2/44 (4.5%)	12/40 (30.0%)	0.00001
Time to natural pregnancy (years)	5 (4–7)	2 (1–6)	0.03
Couples receiving IUI—*n* (%)	38/44 (83.3%)	26/40 (65.0%)	0.04
Number of IUI per couple	3 (1–6)	2 (1–6)	0.01
LBR after IUI—*n* (%)	14/38 (36.8%)	7/26 (26.9%)	0.6
Time to pregnancy with IUI (years)	1 (1–2)	1 (1–6)	1
Couples receiving ICSI—*n* (%)	15/44 (34.0%)	6/40 (15.0%)	0.08
Live births after ICSI—*n* (%)	7/15 (46.7%)	5/6 (83.3%)	0.2

Data are expressed as median (min–max) for continuous variables and as percentages for categorical variables. HPF, high-power field; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; LBR, live birth rate per couple; MAR, mixed antiglobulin reaction; PCT, post-coital test; TMSC, total motile sperm count.

Table II Predictors of natural live births: multiple regression analysis.

	Occurrence of natural live births			
	Model 1		Model 2	
	β (95% CI)	*P* value	β (95% CI)	*P* value
PCT outcome*	1.02 (0.16, 1.96)	0.02	0.5 (−0.47, 1.49)	0.30
Woman's age (years)	−0.005 (−0.14, 0.12)	0.93	−0.06 (−0.22, 0.09)	0.45
Concurrent female factor	−0.02 (−2.11, 1.67)	0.98	0.05 (−2.29, 2.01)	0.96
Infertility length (years)	−0.29 (−0.94, 0.16)	0.28	−0.02 (−0.62, 0.46)	0.94
TMSC ($\times 10^6$)	0.0023 (−0.002, 0.007)	0.34	0.003 (−0.02, 0.009)	0.23
% MAR test positivity	-	-	−0.06 (−0.10, −0.02)	0.007

*Post-coital test (PCT) outcome was categorised as negative, subnormal and good. MAR, mixed antiglobulin reaction; TMSC, total motile sperm count.

Live births after IUI.

Multiple regression analysis of the entire population, using the same putative predictive factors as above, showed that only a younger female age was significantly associated with successful IUI: β coefficient: –0.17 (95% CI –0.33, −0.03), $P = 0.02$.

IUI was performed as the first-line treatment in 38 out 44 couples with 100%-positive MAR test, yielding 14 live births (LBR = 36.8%) (Table I). This rate of live births per couple was significantly higher than the natural LBR in the same group (4.5%; $P = 0.0004$). Interestingly, a negative PCT was recorded in 12 of the 14 couples with successful IUI (85.7%). The couples who had undergone IUI exhibited similar clinical variables as those who did not (Supplementary Table I).

In the 50–99%-positive MAR test group, 26/40 couples had undergone IUI (65.0%), yielding seven live births (LBR = 26.9%; $P = 1.0$ vs. natural LBR). In this group, couples who conceived (naturally or after IUI) showed a significantly shorter duration of infertility at the time of admission and tended to have a younger female age compared to those who did not conceive (Table III).

Table III Comparison of clinical variables between couples who conceived and couples who did not conceive, either naturally or after IUI, in the 50–99%-positive MAR test group.

	Couples who conceived ($n = 18$)	Couples who did not conceive ($n = 22$)	*P* value
Infertility length (years)	1.0 (1.0–3.0)	2.0 (1.0–7.0)	0.04
Woman's age (years)	32.5 (24.0–43.0)	35.0 (29.0–44.0)	0.07
Concurrent female factor—*n* (%)	4 (22.2%)	1 (4.5%)	0.1
Positive PCT outcome—*n* (%)	13 (72.2%)	14 (63.6%)	0.8
Negative PCT outcome—*n* (%)	5 (27.8%)	8 (36.4%)	
TMSC ($\times 10^6$)	94.3 (3.8–612.6)	97.2 (11.3–630.0)	0.8
MAR test % positivity	70 (50–95)	80 (50–95)	0.1

Data are expressed as median (min-max) for continuous variables and as percentages for categorical variables. MAR, mixed antiglobulin reaction; PCT, post-coital test; TMSC, total motile sperm count.

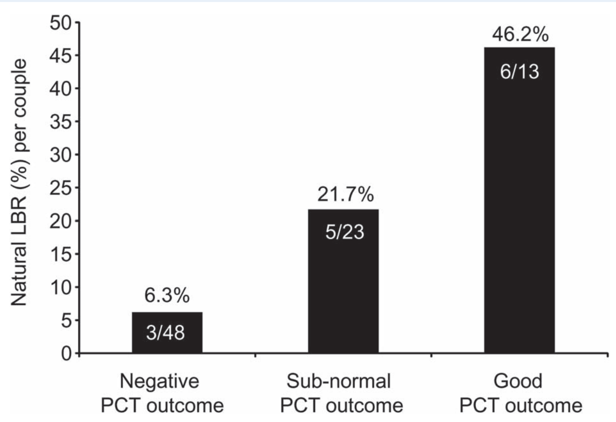

Figure 2 Natural live birth rate (LBR) per couple in couples with negative, subnormal and good post coital test (PCT) outcome. *P* for trend = 0.0005 (χ^2 Mantel–Haenszel test).

Discussion

The first finding emerging from this study is that a strong sperm autoimmunisation, defined by a 100%-positive IgG-MAR test, was associated with a very low natural LBR (4.5%) in infertile couples, whereas, the natural LBR associated with 50–99%-positive IgG-MAR test was significantly higher (30%, $P = 0.00001$). The two groups showed similar clinical characteristics, including length of infertility before admission, follow-up length, age of women, concurrent female factor and semen quality in terms of TMSC. This indicates the actual involvement of strong sperm autoimmunisation in hindering natural pregnancies. Noteworthy, the only two natural pregnancies that were registered in couples with a 100%-positive MAR test occurred after a long time (4 and 7 years) after inclusion in the study.

Prospective studies would provide the best evidence of a causal link between ASA and impairment of fertility, but obtaining such evidence is difficult especially due to the low incidence of sperm autoimmunisation. Indeed, scant information has been produced by the old prospective studies (Busacca et al., 1989; Meinertz et al., 1990; Barratt et al., 1992). In the latest prospective study, an IgG-MAR test of ≥50% positivity, detected in 3% of 1794 men from infertile couples, reduced the probability of pregnancy during a 1-year-follow-up, although the reduction was not significant (Leushuis et al., 2009). Previous retrospective cohort studies reported an inverse association between a higher degree of sperm autoimmunisation (≥50% antibody-coated spermatozoa), determined using either MAR test (Abshagen et al., 1998) or IBT (Ayvaliotis et al., 1985), and the occurrence of natural pregnancies. Accordingly, the presence of 50% antibody-coated motile spermatozoa has been indicated by the WHO as the clinically relevant threshold. However, the predictive value of the degree of MAR test positivity above such a cut-off on the occurrence of natural pregnancies remained largely undetermined. This is the first analysis that compares the prognostic value of a 100%-positive MAR test with a lower degree of positivity (99–50%).

Another novel and remarkable finding that has emerged from the present analysis is the prognostic value of PCT outcome in couples with sperm autoimmunisation, using a clinically relevant end point (LBR). This had never been demonstrated before, although the notion that ASA could impair sperm penetration through the cervical mucus is well established (Bronson, 1999; Francavilla and Barbonetti, 2017; Barbonetti et al., 2019), and an inverse correlation between the degree of sperm autoimmunisation and PCT outcome had been reported (Bronson et al., 1984; Barbonetti et al., 2019). In the present study, a significant progressive increase in the natural LBR was observed in couples with negative, subnormal and good PCT results. In addition, the multiple regression analysis indicated that a better PCT outcome was the sole independent predictor of the occurrence of natural live births, among the putative predictive factors. However, even this association was no longer significant after adjustment for the percentage of MAR test positivity, as this was the sole independent predictor. This substantiated the cause/effect relationship of the degree of sperm autoimmunisation with the impairment of sperm-cervical mucus interaction and hence with infertility. In line with this, (i) the vast majority of couples with a 100%-positive MAR test (79.5%) exhibited a negative PCT outcome, (ii) a negative PCT was recorded only in one of the two couples who conceived naturally in this group and (iii) the pregnancy occurred 4 years after the admission. On the other hand, patients in the 50–99%-positive MAR test group showed a significantly lower occurrence of negative PCT (32.5%), which corresponded with a significantly higher occurrence of live births. Noteworthily, in a previous analysis on the same series, we recently reported that, in case of a 50–99%-positive MAR test, the semen and cervical mucus quality also contributed to the negative PCT outcome besides the degree of MAR positivity, unlike in couples with a 100%-positive MAR test, which independently predicted the negative PCT outcome and did not require significant contribution from other determinants (including semen and mucus quality).

A third noteworthy finding emerging from this study is that IUI can represent an effective first-line treatment, even in case of strong sperm autoimmunisation (100%-positive MAR test). In the debate regarding the effectiveness of IUI in the presence of ASA in males, favourable results were reported when cases of low/moderate sperm-autoimmunisation were included (Lahteenmaki *et al.*, 1995; Robinson *et al.*, 1995; Ombelet *et al.*, 1997; van Weert *et al.*, 2005). In these cases, a well-timed intrauterine deposition of the proportion of antibody-free spermatozoa in the semen (functional oligozoospermia) would explain the effectiveness of IUI. In case of strong autoimmunisation (when all the spermatozoa are coated with ASA), the effectiveness of IUI had not yet been well-established, as such an inclusion criterion had never been used until our previous report (Francavilla *et al.*, 2009). In that prospective cross-over trial, 7 out of 10 couples with strong sperm autoimmunisation conceived with IUI, either without (LBR per cycle: 19%) or with ovarian stimulation (LBR per cycle: 18%), whereas none conceived in cycles with natural intercourse. In the present larger series, in case of strong sperm autoimmunisation, the IUI-assisted LBR was significantly higher than the natural LBR and similar to the IUI-assisted LBR recorded in couples with lower sperm autoimmunisation (99–50%-positive MAR test). These data suggest that although ASA could affect sperm functions involved in the gamete interaction to some extent (Francavilla *et al.*, 2007, for review), the clinical interference of ASA with fertility could be overcome by bypassing the cervical mucus barrier. Obviously, the success rate of IUI also depends on other established clinical predictors such as the age of females (independent predictor in the present study) as well as different inclusion criteria; in the aforementioned crossover study (Francavilla *et al.*, 2009), the ASA-positive samples were otherwise normal, instead, the recovery of at least one million progressive motile spermatozoa was the inclusion criterion in the present series. Therefore, IUI can represent a proper first-line treatment also in the case of strong sperm autoimmunisation and especially when semen samples are otherwise normal.

The present analysis has limitations that arise from the uncontrolled nature of historical cohort studies. Particularly, although couples were carefully evaluated in their infertility work-up and the two groups were similar with respect to the other relevant clinical variables, we cannot exclude uncontrolled variables that may have affected natural pregnancies during the follow-up. Similarly, due to the lack of randomisation, a selection bias cannot be excluded from the comparison of natural live births with those after IUI, even though the clinical variables were not different in couples who had or had not undergone IUI (Supplementary Table I). Finally, the degree of sperm autoimmunisation during the follow-up has not been analysed, as it was unknown in most cases. Nevertheless, we recently reported that a 100%-positive MAR test exhibited a considerably higher consistency over time compared to a lower degree of positivity (Barbonetti *et al.*, 2019).

Conclusion

This study, using clinically relevant endpoints such as natural and IUI-assisted live births, indicates that the impairment of sperm penetration through the cervical mucus represents a pre-eminent mechanism whereby ASA interfere with fertility, given (i) the correlation between better PCT outcome and natural live births, the sole independent negative predictor of which was the degree of sperm autoimmunisation, and (ii) the effectiveness of IUI in the treatment of strong sperm autoimmunisation.

The findings of this study have clinically relevant implications. In the context of the debated prognostic significance of a MAR test positivity $\geq$50%, suggested by WHO as the clinically-relevant threshold, a 100%-positive MAR test has a highly negative prognostic value and can represent the sole cause of a couple's infertility, which could be successfully treated with IUI, as the first-line treatment. On the other hand, a lower degree of sperm autoimmunisation (50–99%-positive MAR test) might only represent a contributing factor to a couple's infertility, for which the indication to treat or wait also depends on the evaluation of conventional prognostic factors, including the PCT outcome. Therefore, the routine use of IgG-MAR test in the basic fertility workup is justified, as it influences decision making.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

A.B., F.F.: study concept and design; C.C., S.D., E.M., M.T.: acquisition of data; A.B., S.D.: data analysis; A.B., F.F., S.D., S.F.: interpretation of data; A.B., F.F.: drafting the article; S.F.: support with important intellectual content; all authors: final approval.

Funding

PRIN 2017, Ministero dell'Università e della Ricerca Scientifica (MIUR), Italy.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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