THE PRESENCE OF ANTI-SPERM ANTIBODIES IS NOT ASSOCIATED WITH SPERM DNA DAMAGE: PROSPECTIVE STUDY OF INFERTILE MEN

ARMAND ZINI[1-2], SIMON PHILLIPS[1], ABDULAZIZ BAAZEEM[2], FRANCOIS BISSONNETTE[1], ISAAC JACQUES KADOCH[1], MARIA SAN GABRIEL[2].

[1]OVO FERTILITY, MTL, QC, CND. [2]MCGILL UNIVERSITY DEPARTMENT OF UROLOGY, MTL, QC.CND.

Canadian Fertility and Andrology Society

55TH ANNUAL MEETING

19TH – 21ST NOVEMBER 2009

MONTREAL (QUEBEC)

ABSTRACT

Introduction: Anti-sperm antibodies (ASAs) have an adverse impact on male fertility by (1) directly interfering with sperm surface interactions (e.g. fertilization) and (2) indirectly by mediating the release of cytokines that can impair sperm function. However, it is not known whether ASAs can specifically impair sperm DNA integrity, whether directly or indirectly. We sought to examine the relationship between ASAs and sperm DNA integrity in semen samples from infertile men.

Methods: Semen samples were obtained from consecutive, non-azoospermic men presenting for infertility evaluation (n=75). Standard semen parameters (sperm concentration, motility and strict morphology), sperm %DFI (DNA fragmentation index: measured by flow cytometric analysis of acridine orange stained spermatozoa) and anti-sperm antibodies (direct, mixed agglutination reaction) were measured. The local ethics review board approved this study.

Results: In 64 of the 75 the samples collected, all of the sperm analyses were conducted. Eight of the men (13%) had significant levels of ASAs (>40% of sperm coated with IgG and/or IgA antibodies) and 9 (14%) had high levels of sperm DNA damage (>30% DFI). Mean (± SD) sperm concentration and progressive motility were significantly lower in ASA-positive compared to ASA-negative samples (23.4 13.1 x 106/ml and 27 15% vs. 74.6 61.2 x 106/ml and 46 18%, respectively, P<0.05) and sperm motility was inversely correlated with the percentage of IgG-bound spermatozoa (r= -0.33). In contrast, sperm %DFI and percent normal forms were not significantly different in ASA-positive compared to ASA-negative samples (17.5 17.9% and 7.5 3.0% vs. 17.4 13.5% and 6.5 2.6%, respectively).

Conclusions: Our data demonstrate that ASAs are inversely related to sperm motility and, to a lesser extent, to sperm concentration. The data also indicate that ASAs are not associated with sperm DNA damage and suggest that ASAs are unlikely to have a significant direct or indirect effect on sperm DNA integrity.

OBJECTIVE

The aim of the study was to examine the relationship between the ASAs and sperm DNA damage in semen samples from infertile men

METHODS

A prospective study was performed on consecutive semen samples of 75 men presenting for infertility evaluation.

Semen analysis was carried out according to WHO criteria[1]. Direct ASA levels (both IgG and IgA) were measured in semen by MAR test including localization of the binding (head, midpiece, tail). Furthermore an aliquot of raw semen (containing approximately 2million spermatozoa was collected and stored at -70˚C for later assessment of DNA damage. We have previously shown that testing fresh or frozen-thawed samples gives comparable results (<5% variability).[2]

Sperm DNA damage was assessed by the sperm chromatin structure assay (SCSA) and the results were expressed as sperm %DFI (DNA fragmentation index: an index of DNA damage) and %HDS (high DNA stainability: an index of chromatin compaction).[3]

At least 5000 cells were counted from two aliquots of each sample and a mean of the two sperm %DFI and %HDS values is reported. The variability of the repeat SCSA measures was <5%.

STATISTICS

Results are expressed as mean ± SD. Parametric and non-parametric tests were used to estimate differences in sperm DNA damage between ASA-positive and ASA-negative samples. Linear regression analysis was used to evaluate the relationship between dependant variables and ASA levels and conventional sperm parameters. All hypothosis testing was two-tailed with a probability value of 0.05 deemed significant.

RESULTS

Eleven of the 75 samples were excluded from the study because it was not possible to evaluate all the semen parameters either due to technical reasons or insufficient material. Eight of the men (13%) had significant ASA levels (>40% sperm with beads bound) and nine of the men (14%) had high levels of DNA damage (DFI>30%).

Mean sperm concentration and progressive motility were significantly lower in ASA-positive compared to ASA-negative samples (p<0.05) see table.

	ASA-positive	ASA-negative	*P*-value*
n	8	56	
Sperm concentration	23 ± 13 ‡	75 ± 61	0.02
Sperm progressive motility (%)	27 ± 15	46 ± 18	0.008
Sperm Stricy morphology	7.5 ±3.0	6.5 ± 2.6	NS
Sperm % DFI	17.5 ± 17.9	17.4 ± 13.5	NS
Sperm % HDS	4.0 ± 2.1	4.9 ± 3.5	NS

*Comparison between ASA-positive and ASA-negative samples by Mann-Whitney rank sum test; NS (not significant) = P>0.05
‡ Mean ± SD

Sperm progressive motility was inversely correlated with the percentage of IgG-bound (r= -0.33) and IgA-bound spermatozoa (r= -0.25). In contrast sperm %DFI and percent normal forms were not significantly different in ASA-positive compared to ASA-negative samples. Furthermore there was no significant relationships between ASA levels and DNA damage or sperm morphology.

CONCLUSIONS

In this prospective study of consecutive non-azoospermic, infertile men, we have shown that, as expected, ASAs are inversely related to sperm motility and to a lesser extent, sperm concentration.

The data indicate that ASAs are not associated with sperm DNA damage and suggest that ASAs are unlikely to have a significant early direct or indirect effect on sperm DNA integrity.

REFERENCES

1. WHO Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th Edition. Cambridge University Press. 1999.
2. Zini A et al. Fertil Steril 2001;75:674-77
3. Evenson DP, et al. Hum Reprod 1999;14:1039-49

OVO FERTILITY