

## FAILURE OF TEST YOLK BUFFER TO DECREASE ANTISPERM ANTIBODIES ON SPERM

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The objective of this study was to evaluate whether the suspension of sperm naturally bound or artificially coated with antisperm antibody (ASA) in test yolk buffer (TYB) will reduce the percentage of sperm positive for ASA. Attempts were made to repeat a study performed by Lam et al., in which they claimed a significant decrease in percentage of sperm positive for both IgA and IgG. Furthermore, the study attempted to determine if this methodology would similarly reduce the percentage of sperm naturally bound with ASA. There was no reduction in postincubation levels in percentage of sperm having attachment of IgA or IgG in either the sperm specimens naturally bound with ASA or donor sperm artificially coated with antibodies. Incubation in TYB does not appear to be an effective method to reduce the percentage of sperm bound with ASA. Possibly, it could reduce the amount of ASA bound to each sperm, but this would have to be proven by other studies.

**Keywords** immunobead test, elute, affinity, corroborate, refute

The presence of antisperm antibodies (ASA) has been associated with decreased fertility potential [2]. Since approximately 8% of the male population have ASA, it would be beneficial to have a procedure capable of eluting the antibodies from the sperm [1]. Lam et al. reported that suspending sperm coated with ASA in test yolk buffer (TYB) decreases the percentage of IgA and IgG [3]. They hypothesized that TYB may be capable of somehow altering the sperm membrane configuration, thus allowing the ASA or antigens to become detached from the sperm membrane. Another possible mechanism was that TYB proteins might absorb the antibody or antigen complexes from the sperm membrane. Another study by Zavos et al. showed a significant decrease in sperm antibody levels after treatment with TYB [4]. The suggestion was that TYB might decrease the affinity at the antibody-antigen sites; or, the test

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yolk proteins were absorbing the antibody and/or antigen complexes from the sperm membrane.

The study presented herein was designed to either corroborate or refute the findings of both Lam and Zavos, i.e., to reduce ASA on sperm membrane by incubation with TYB. Furthermore, the study attempted to determine if TYB could reduce ASA that were naturally attached to sperm from subfertile males, rather than merely artificially attached, as was done by Lam et al.

## MATERIALS AND METHODS

*Study 1: Addition of Pooled Serum Positive for ASA to Normal Donor Sperm and the Effect of TYB.* Normal sperm donors ( $n = 7$ ) with a minimum motile density (MD) of  $15 \times 10^6/\text{mL}$  and negative for ASA were used for the study. Pooled positive ASA serum was added in equal volumes to 1 mL of the sperm specimen, which was incubated at  $37^\circ\text{C}$  for 60 min; the mixture was washed with modified BWB medium and resuspended to 1 mL with modified BWB medium. A direct immunobead test (DIBT) was then performed and the percentage of IgA and IgG were recorded; 0.5 mL of the now positive sperm was incubated with equal volumes of TYB for 1 h in a  $4^\circ\text{C}$  water bath; 0.5 mL of the sperm was also incubated with an equal volume of human tubal fluid (HTF), as a control. The specimens were allowed to warm at  $37^\circ\text{C}$  for 10 min, then the DIBT was again performed and the percentages of the IgA and IgG were recorded.

*Study 2: Effect of TYB on Reducing Levels of Naturally Occurring ASA on Sperm.* Sperm from 7 men with  $>50\%$  DIBT were used. A DIBT was performed and then 0.5 mL of the sperm was incubated with equal volumes of TYB for 1 h in a  $4^\circ\text{C}$  water bath, and again 0.5 mL of the sperm was incubated with HTF, as a control. The specimens were then treated exactly as described above.

## RESULTS

The mean IgA preincubation for group 1 was 75.8%; postincubation with TYB was found to be 91.6%. For IgG, the mean preincubation DIBT was 98% and the postincubation DIBT was 99.8% (Table 1). For group 2, IgA was 76.8% preincubation and 80.7% postincubation. IgG was 95% preincubation and 98.4% after incubation (Table 1). The controls for group 1 found IgA and IgG levels after incubation with TYB at 98.6% and 99.7%, respectively. For group 2, after TYB, DIBT was 80.1% for IgA and 95.6% for IgG.

## DISCUSSION

Our findings showed a mild increase in the antibody levels, instead of the expected decrease. Unfortunately, we were unable to replicate the findings of both Lam and Zavos who

**TABLE 1** Effect of Test Yolk Buffer (TYB) in Reducing the Levels of Antibodies from Sperm Artificially and Naturally Coated with Antisperm Antibodies (ASA)

|  | Artificially Coated |         | Naturally Coated |         |
|--|---------------------|---------|------------------|---------|
|  | IgA (%)             | IgG (%) | IgA (%)          | IgG (%) |
| Mean baseline after absorption of antibodies | 75.8                | 98      | 76.8             | 95      |
| Mean binding following incubation with TYB   | 91.6                | 99.8    | 80.7             | 98.4    |
| Control                                      | 98.6                | 99.7    | 80.1             | 95.6    |

found a beneficial effect of TYB in reducing ASA levels when the antibodies were absorbed onto negative donor sperm. The study presented herein also did not demonstrate a reduction in ASA from our patients who had a positive DIBT. There did not appear to be any major differences in the techniques of this study compared to those previously reported. Possibly, the pool of serum in the present study had higher levels of ASA than used in the other experiments, which could explain the difference in conclusions.

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