EVALUATION OF THE ACROSOME REACTION IS IMPORTANT FOR MALE INFERTILITY DIAGNOSIS. DISTURBANCE IN THE ACROSOME REACTION IS SUGGESTED AS ONE OF THE REASONS FOR DECREASED FERTILITY POTENTIAL WHICH CAN NOT BE MEASURED BY A ROUTINE SEMEN ANALYSIS. HUMAN SPERMATOZOA EXPRESSED CD46 MOLECULE ON ITS HEAD AFTER ACROSOME REACTION. ACROBEAD ASSAY IS REPORTED AS A SIMPLE, REPRODUCIBLE, COST-EFFECTIVE TEST THAT DOES NOT INVOLVE ANIMAL HANDLING, AND IS A GOOD PREDICTOR OF THE ACROSOMAL STATUS OF THE SPERM. THE TEST CAN BE PERFORMED IN A CLINICAL ANDROLOGY LABORATORY IN CONJUNCTION WITH OTHER TESTS. WE PERFORMED THE ACROBEAD TEST TO DETERMINE THE OptIMUM CAPACITATION TIME IN FRESH AND FROZEN SPECIMENS. SPERM MOTION ANALYSIS WAS DONE ON FRESH SPECIMEN. SWIM-UP METHOD WAS USED TO PROCESS ONE ALIQUOT AND THE SECOND UNPROCESSED ALIQUOT WAS FROZEN BY THE LIQUID NITROGEN VAPOR METHOD USING THE TEST-YOLK BUFFER AS THE FREEZING MEDIUM. BOTH FRESH AND FROZEN SPECIMENS WERE PREPARED BY SWIM-UP IN MBWW MEDIUM CONTAINING 0.3% HUMAN SERUM ALBUMIN (HSA) AND THEN RESUSPENDED IN MBWW CONTAINING 3.5% HSA. ONE HUNDRED μL OF THE SEMEN SAMPLES (4 X 10^6/mL) WAS ADDED IN A SERIAL DILUTION (1:1, 1:2, 1:4, AND 1:8) TO A 96-WELL TISSUE CULTURE PLATE. TEN-μL OF 1.5 X 10^6/ML IMMUNOBEADS COATED WITH ANTI-CD46 MONOCLONAL ANTIBODIES (MH-61 BEADS) WERE ADDED TO EACH WELL. ACROSOME REACTED SPERMATOZOA FORMED A SPERM-BEAD COMPLEX (AGGLOTTINATION) THAT WAS OBSERVED UNDER A PHASE-CONTRAST INVERTED MICROSCOPE. POSITIVE SPERM AGGLOTTINATION WAS IDENTIFIED BY THE ABSENCE OF ATTACHMENT TO THE MH61 BEADS. THE TEST WAS CONSIDERED NEGATIVE BY THE PRESENCE OF UNATTACHED BEADS. THE WELLS WERE SCORED AT 0, 1, 3, 6, AND 24-H INCUBATION PERIOD ON A SCALE OF 0 TO 4. AN ACROBEAD SCORE OF ≥ 2 WAS CONSIDERED NORMAL. A HIGHER ACROSOME REFLECTS A HIGH ACROSOME REACTION. IN THE FRESH SPECIMENS, 7% (1 H), 53% (3 H), 80% (6 H), AND 100% (24 H) OF THE DONORS HAD AN ACROSOME OF ≥2. THESE RESULTS INDICATE THAT A CAPACITATION PERIOD OF 6 H IS ADEQUATE FOR THE SPERMATOZOA TO UNDERGO ACROSOME REACTION. HOWEVER, SIGNIFICANTLY HIGHER NUMBER OF FROZEN SPECIMENS HAD AN ACROSOME OF ≥2 EVEN AFTER A CAPACITATION PERIOD OF 1 H (62%), AND AFTER 3 H (77%), AND 6 H (92%) INDICATING THAT IN A MAJORITY OF THESE SPECIMENS ACROSOME REACTION AND MEMBRANE DAMAGE OCCURRED DURING THE CRYOPRESERVATION PROCESS. NO CORRELATION WAS SEEN WITH SPERM COUNT, MOTILITY, AND ACROSOME IN OUR STUDY AS SEMEN SPECIMENS WERE OBTAINED FROM NORMAL HEALTHY DONORS. AN INCUBATION PERIOD OF 6 H CAN BE USED CLINICALLY TO SCREEN INDIVIDUALS WHO MAY PRESENT NORMAL SEMEN PARAMETERS BUT HAVE ACROSOME INSUFFICIENCY. ACROBEAD TEST IS NOT RELIABLE IN EVALUATING THE ACROSOMAL STATUS IN FROZEN SPECIMENS DUE TO INCREASED SPONTANEOUS ACROSOME REACTION AND MEMBRANE INDUCED DAMAGE.