Stain for Sperm Acrosomes

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Procedure

1. After sperm preparation, adjust sperm concentration as high as possible (5-10 x 10^6/ml).
2. Prepare a smear of sperm suspention onto a slide and allow it to dry.
3. Place slides in 0.1% (w/v) naphthol yellow S in 1% (v/v) aqueous acetic acid for 30 min.
4. Blot the slides dry and then rinse them in 1% (v/v) acetic acid for 10 seconds.
5. Drain the slides and place them in a solution containing equal parts of 0.2% aqueous naphthol yellow S and 0.2% aqueous erythrosin B (solution pH 4.8), for 13 min.
6. Rinse slides with distilled water (pH 4.8) or deionized water and blot air dry.
7. Dip slides in xylene for 1-2 seconds, and then Permount. Apply cover slip with Permount.
8. In normal sperm, strong red color is observed in the acrosomal membrane, and the equatorial region is well defined. In abnormal sperm, the head appears less distintive red, and the equatorial region of the sperm's head is not defined.
9. A minimum of 100 sperm should be count to determine the percentages of acrosome membrane integrity.

Reference

R.W. Lenz, G.D. Ball, J.K. Lohse, N.L. First and R.L. Ax. Chondroitin sulfate facilitates an acrosome reaction in bovine spermatozoa as evidence by light microscopy, electron microscopy and in vitro fertilization. Biol. Reprod. 28: 683-690 (1983)

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