

immunized syngeneic pregnant mice produced low levels of IL-2 by Con-A stimulation. The data suggested some immunogenetic factors are involved in these effects of pregnancy on CIA.

99. Correlation of Microbubble Test by New Method and Fetal Lung Maturity

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The new reliable microbubble test for the diagnosis of fetal lung maturity established instead of Pattle's test.

To produce microbubbles, 175 μ l amniotic fluid was placed on a slide, and 4 needles at regular distance of 8 mm square, vertically placed in the drop. Bubbles were made by air injected at 400 ml/h with a pump through needles. Immediately the slide was inverted. All stable microbubbles less than 15 μ m in diameter were counted in 5 fields (F) after 4 minutes. Rating of our method was carried out as follows: no stable microbubble, zero; 1 to 5 stable microbubbles/5F, Range 2; more than 11 stable microbubbles/5F, Range 3. 68 samples of amniotic fluid obtained from 15 to 42 weeks gestation were tested for their lung maturity with new method, Pattle's test, shake test, L/S ratio, disaturated phosphatidylcholin (DSPC), phosphatidylglycerole (PG). Rating of maturity was more than Range 2 in our method. In result false negative was 7.1% in our method, better than 28.6% in Pattle's test. The rate of accurate diagnosis was 97.1% in our method, 94.1% in Pattle's test, 92.6% in shake test, 92.6% in L/S ratio, 79.4% in DSPC, 83.8% in PG.

We concluded that our method was able to diagnose the fetal lung maturity in a few minutes and superior to shake test and Pattle's test in accuracy.

100. Study on the Measurement of Hyaluronidase Activity in Single Human Sperm by Substrate-slide and its Clinical Application

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Hyaluronidase is released from the sperm head during the acrosome reaction, and depolymerizes the matrix between cells of cumulus oophorus. We

developed the hyaluronic acid substrate slide in order to measure the hyaluronidase activity of human spermatozoa. In this test, hyaluronidase activity in single sperm could be detected by a halo formation on hyaluronic acid substrate slide. The purpose of this study is to refine this assay and its clinical application for evaluating the fertility potential of human spermatozoa.

A substrate slide method was described that allowed the detection of hyaluronidase activity 100% of single spermatozoa. The best incubation temperature for maximum halo size was found to be range between 35 and 42°C. The pH optima was found to be pH 5.0 and 7.4. Significant correlation was found between hyaluronidase activity and sperm concentration in male infertility. Hyaluronidase activity with higher concentration of sperm, and lowest hyaluronidase activity was observed in less than 20×10^6 /ml.

This simple method was suggested to be useful for a screening method for identifying compounds that caused developmental or genetic defects in male germ cells, or for the diagnosis of infertility due to decreased hyaluronidase activity.

101. Establishment of Human-mouse Hybridomas Producing Sperm Immobilizing Monoclonal Antibody by Semisolid Culture Medium

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A simple technique using semi-solid culture medium containing methylcellulose was applied to select hybrid cells after cell fusion of human lymphocytes and mouse myeloma cells. Human peripheral blood lymphocytes (h-PBL) from an unexplained sterile woman with strong sperm immobilizing antibody (SI-Ab) were stimulated by culturing with washed human spermatozoa and pokeweed mitogen for 6 days. These stimulated h-PBL and mouse myeloma cells (NS-1) were fused in the presence of PEG-3350. The fused cells were suspended in semi-solid medium and cultured in the dish for 14 days. Each growing colony was picked up with fine pipette and cultured in liquid medium of the microplate wells for 7 days.

The supernatants were examined for human immunoglobulin (Ig) production by ELISA and for biological activity by micro-sperm immobilization test (micro-SIT). The 246 colonies in the semi-solid me-