

## IVF-ET program.

We conclude that in oligospermic patients, (i) motile spermatozoa with forward progression can be most efficiently recovered by swim up method, when volume of layered medium is 0.5 ml and incubation time is 60 minutes and (ii) fertilization rate of preovulatory oocytes with recovered motile spermatozoa extremely low when percentage of motility is less than 20% and/or the concentration of recovered motile spermatozoa after swim up is less than  $20 \times 10^4/\text{ml}$ .

#### 24. Observation of Acrosome Reaction of Human Spermatozoa Using Combined Preparation of Triple Stain Technique and Scanning Electron Microscopy

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The purpose of this study is to investigate reliability of triple stain technique (TST). Preparation of TST was followed by scanning electron microscopy (SEM) to observe the same spermatozoa with both method. We compared the results of TST at various pH of rose Bengal solution to those of SEM.

1) Acrosome reaction was easily examined in the same spermatozoa with both TST and SEM.

2) The percentages of acrosome non-reacted (ANR) and acrosome reacted (AR) sperm were not different when spermatozoa from same semen were examined with SEM or SEM after TST (ANR 23.0%, 24.5%, AR 77.0%, 75.5% respectively).

3) When compared the results of TST to those of SEM after TST at various pH of rose Bengal solution, the results of TST at pH 5.3 were in good accordance with those of SEM (accuracy 95.9%, sensitivity 94.5%, specificity 97.6%), but results of TST at pH 4.3 and 6.3 did not agree with those of SEM (accuracy 70.5%, 61.8%, sensitivity 100%, 38.7%, specificity 21.4%, 97.5% respectively).

These data suggest that TST at pH 5.3 of rose Bengal solution is reliable and practical method for detection of acrosome reaction.

#### 25. Ultrasonic Grey Level Histogram of Ovarian Cystic Patterns in Periovarulatory Phase and Analysis of the Contents by Ultrasonically Guided

#### Aspiration

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Various types of cystic patterns in the ovary were studied with ultrasonic grey level histogram and ultrasonically guided aspiration in the periovarulatory phase.

The histographic studies were done on the cysts of ovarian follicle (n=30), lutein cyst (n=30), ovarian hyperstimulation syndrome (OHSS) (n=20) and luteinized unruptured follicle (LUF) (n=20). The diagnosis of every cystic pattern was made by daily ultrasonic observation through the cycle. The ultrasonic grey level histograms were obtained by Aloka UIP-100 computer system connected to Aloka SSD-270 ultrasonographic device.

These four cystic structures showed visual difference in the histograms and statistical studies on the histograms showed the significant difference in the echo level and histogram width (OHSS < follicle < LUF < lutein cyst).

The ultrasonic diagnosis was confirmed and further information was obtained by the examination of the contents which were aspirated from follicle (n=11), LUF (n=9), OHSS (n=2) and lutein cyst (n=4).

E<sub>2</sub> and progesterone levels of the aspirated contents were lower and testosterone levels were higher in LUF than in follicle. And in the two cases degenerated oocytes were recovered in the aspirated contents of LUF.

These results showed the utility of these two methods in the studies on preovulatory follicle, postovulatory changes and abnormal ovulatory processes.

#### 26. Studies on the Beneficial and Hazardous Factors of Ultrasonic Oocyte Collection in IVF-ET

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Ultrasonic oocyte collection has been commonly used for in vitro fertilization and embryo transfer (IVF-ET) since a few years before. A study was per-