

females (17.8%) ] attending Institute of Embryo Research and Infertility Treatment at AL-Nahrain University were included in this study during the period from March 2007 to May 2008.

Ages of males ranged from twenty-four to fifty-seven years. Ages of females ranged from twenty-one to thirty-five years. Information concerning smoking, drinking alcohol, varicocele and varicocelectomy were collected from them. To detect antisperm antibodies in the sera of these patients indirect immunofluorescent test kit (Euroimmune, Germany) was used.

This kit contained BIOCHIP slides and each slide contained ten BIOCHIPS coated with smears of human spermatozoa. As a counterstainer, Evans Blue pigment was used. Once the blood sample was collected from each patient, it was centrifuged till the serum was obtained. This serum was collected into an eppendorf tube and was kept at 0 C until performing the test. To perform the test, four serial dilutions for each sample were prepared (1/10, 1/100, 1/1000, 1/10000).

To prepare 1/10 dilution, 11.1 microleters of the serum were added to 100 microleters of phosphate buffer saline-Tween (PBS-Tween). For the other dilutions, 11.1 microleters of each previous dilution were added to 100 microleters of PBS-Tween. Then 25 microleters of each dilution of each sample were applied to each reaction field of the reagent tray. The reaction started by fitting the BIOCHIP slide into the corresponding recesses of the reagent tray. Each sample contacted with its BIOCHIP. Then the slide was incubated for 30 minutes at room temperature.

After incubation, the BIOCHIP slide was rinsed with a flush of PBS-Tween and was immediately immersed in a dish containing PBS-Tween. After

5 minutes, 20 microleters of fluorescein-labeled anti-human globulin were added to each reaction field of a clean reagent tray and within 5 seconds, the BIOCHIP slide was removed from the dish and the slide was immediately put into the recesses of the reagent tray. The slide was protected from the direct sunlight and was incubated for 30 minutes at room temperature.

After 30 minutes, the BIOCHIP slide was rinsed with a flush of PBS-Tween and was put into a dish containing 150 millileters of phosphate buffer added to it 10 drops Evans Blue pigment as a counterstainer and was left for 5 minutes. Then, 10 microleters of glycerol per each reaction field was added onto a coverglass and within 5 seconds the BIOCHIP slide was removed. The BIOCHIP slide with the BIOCHIPS facing downwards was put onto the prepared coverglass and it was now ready for checking by using fluorescent microscope at power 40X.

Under this power, any portion of spermatozoa with green colour indicated positive reaction and any portion of spermatozoa with red colour indicated negative reaction .

Of 60 males, seminal fluids were collected by masturbation after three days of abstinence and seminal fluid analyses were performed within two hours. It was estimated according to WHO guideline in year 1999. The following parameters were concerned in this study: sperm agglutination, sperm motility and presence of pus cells. Of 13 females included in this study, vaginal swabs were done and subjected to direct microscopic examination.

### **Results**

In this research only the first dilution (1/10) showed positive reaction and the other dilutions (1/100, 1/1000, 1/10000) exhibited no positive reactions