

Improvement in sperm motility viability and normality after treatment with prednisolone, antisperm antibody separation and in vitro sperm activation in immunologically infertile men.

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Summary:

Background: Antisperm antibody (ASA) as a cause of men infertility was first reported in 1954. Different assays were developed for the detection of serum-bound antibodies.

Objective: The objective of this study is to study the effect of prednisolone, antisperm antibody separation (ASAS) and in vitro sperm activation on sperm motility, viability and morphology in immunologically infertile patients.

Patients and Methods: Semen samples of 250 immunologically infertile patients were examined by seminal fluid analysis and microagglutination test to check the presence of sperm agglutination and antisperm antibodies (ASA). The patients received 5mg prednisolone three times per day for two weeks then the dose was reduced to two tablets per day for four days and further reduced to one tablet for three days followed by one week of rest period. The treatment regimen was continued for three months and seminal fluid analysis was performed before and after the treatment.

Results: Sperm motility percent, sperm grade activity, sperm motility index, normal sperm morphology and sperm viability after the treatment were significantly improved ($P < 0.001$) while the percent of sperm agglutination, shaky head sperm movement percent of abnormal sperm morphology were significantly decreased ($P < 0.001$). The progress in the improvement of sperm quality was started at the end of 4th week after treatment and increased gradually up to the end of the 12th week.

Conclusion: Application of prednisolone therapy, ASAS technique and in vitro sperm activation found to be effective and resulted in significant improvement sperm quality. These active and viable sperm following treatment may be used for intrauterine insemination or in vitro fertilization (IVF) and embryo transfer (ET) for the treatment of immunological infertile men.

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Introduction

Antisperm antibody (ASA) as a cause of men infertility was first reported by Rumke⁽¹⁾ and Wilson⁽²⁾ in 1954 who found ASA in the serum of infertile males. Different assays were developed for the detection of serum-bound antibodies, including micro-agglutination assay⁽³⁾, mixed antiglobulin reaction⁽⁴⁾ and immunobead test⁽⁵⁾. The immunobead test (IBT) detects IgG, IgA, IgM class antibodies, while mixed antiglobulin reaction assay detects antibodies belonging to IgG class only.

The prevalence of ASA varied depending on the modality of the immunological screening. The detected ASA in the serum and seminal plasma of the infertile men was from 10% to 38 %⁽³⁾. The ASA cause agglutination of the motile sperm and resulted in the impairment of the sperm motility. It was found that a significant increase in the proportion of motile sperm involved in agglutination in the presence of sperm antibodies^(6, 7). An inverse significant correlation was observed between either an increase in the titer of the circulation ASA or the percentage of the sperm bound antibodies and the incidence of pregnancies^(8, 9, 10). Antisperm antibody rose against whole spermatozoa or defined sperm antigens can interfere with sperm functions involved in the fertilization process, thereby blocking sperm-egg interactions^(11, 12).

The objective of this research work was to study the effect of prednisolone, antisperm antibody separation (ASAS) and in vitro sperm activation on

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sperm motility, viability and morphology in immunologically infertile patients.

Materials and Methods

1. Patients

Two hundred and fifty infertile men complaining from antisperm antibodies (ASA) and sperm agglutination were involved in the present study. The mean age of the patients was 37 years with a range from 26 to 48 years.

The duration of the infertility was 4 to 10 years, with a mean of 7 years.

The patients received 5mg prednisolone three times daily for a period of two weeks and then reduced to two tablets for four days and further reduced to one tablet for three days. The patients had a rest for one week. The treatment protocols were continued for three months.

2. Semen samples

The semen was collected by masturbation in medicut culture medium containing 50% serum. Sperm agglutination percent was reported before and after treatment with prednisolone by using simple slide method. Antisperm antibodies (ASA) titers were also recoled before and after treatment with prednisolone using microagglutination (Tray agglutination) test. The details of the technique of microagglutination test are described elsewhere⁽³⁾.

The serum samples were undergone seminal fluid analysis, antisperm antibody separation (ASAS) and *in vitro* sperm activation before and after treatments with prednisolone. The techniques of ASAS and *in vitro* sperm activation are described elsewhere^(7, 12). Seminal fluid analysis included sperm motility, viability, sperm grade activity, sperm shaky head movement, sperm motility index and normal morphology. The laboratory details of the analysis were described elsewhere^(11,13).

3. Sperm grade agglutination and motility grading:

Sperm cells were either agglutinated in the form of large masses or in the form of small masses. A scale was used to report the percent of sperm agglutination⁽¹²⁾.

The following scale was used to quantify the sperm agglutination grade:

Grade zero: No agglutination with zero percent scale.

Grade one: Mild agglutination with <10%.

Grade two: Moderate agglutination with 10 to 20%.

Grade three: Marked agglutination with 21 to 40%.

Grade four: V.marked agglutination with >40%.

The following grading system was applied for grade activity of human sperms:

Grade zero: Immotile sperm without motion.

Grade one: Motile sperm without progressive movement.

Grade two: Slow forward progressive movement.

Grade three: Moderate forward progressive movement

Grade four: Rapid forward progressive movement

Results

Sperm motility percent was significantly improved (P<0.001) following the treatment period. The sperm motility was increased starting from the end of the first four weeks period and continued up to the end of the 12th week (Fig.1).

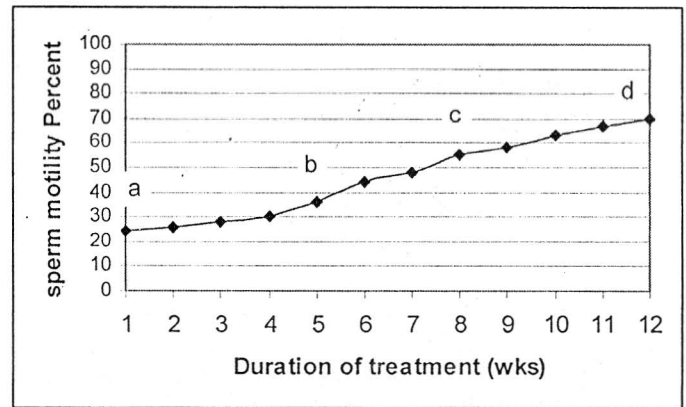


Figure e (1): The effect of Prednisolone treatment on sperm motility in immunologically infertile patients following antisperm antibody separation technique and *in vitro* sperm activation (Different letters indicates significantly different values from each other P<0.001) Number of semen samples per replicate =60

Significant improvement (P<0.001) in human sperm grade activity was reported starting at the end of 8th week period and continued to the end of duration of treatment with prednisolone (Fig. 2).

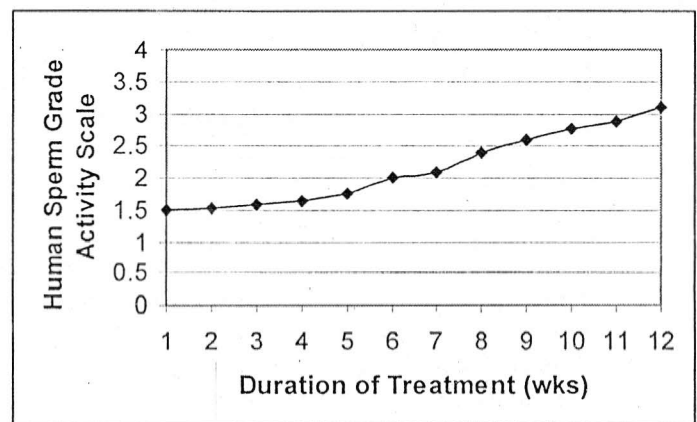


Figure (2): The effect of Prednisolone treatment on sperm grade activity in immunologically infertile patients following antisperm antibody separation technique and *in vitro* sperm activation (Different letters indicates significantly different values from each other P<0.001) Number of semen samples per replicate =60

The sperm motility index was also significantly increased following the treatment of immunologically infertile patients with prednisolone, ASAS and *in vitro* activation of

sperm ($P < 0.005$ Fig. 3). The sperm normal morphology was started to increase significantly ($P < 0.005$) after the end of four weeks treatment period and continued up to 12 weeks. This significant improvement in the recovery of morphologically normal and viable sperm was continued during the durations of treatment specially at the end of 8 weeks and 12 weeks period (Fig. 4).

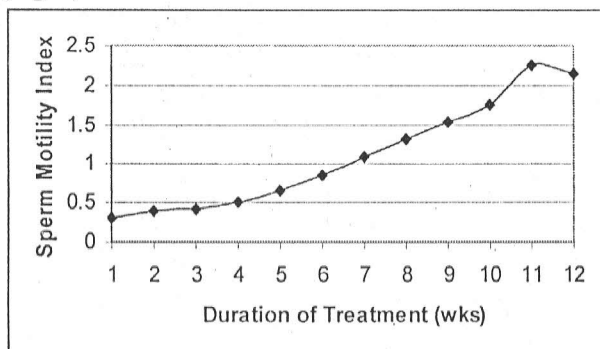


Figure (3): The effect of Prednisolone treatment on sperm motility index following antisperm antibody separation technique and in vitro sperm activation (Different letters indicates significantly different values from each other $P < 0.005$)
Number of samples per replicate =60

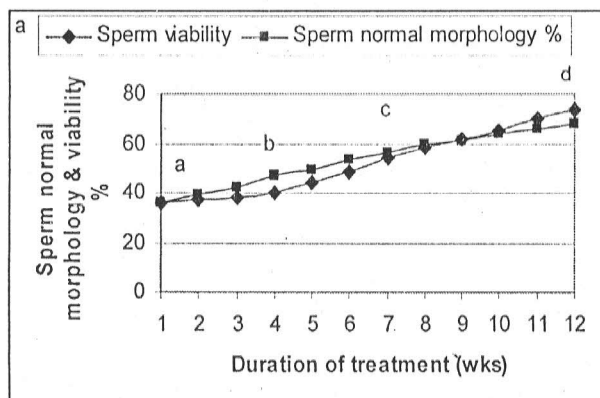


Figure (4): The effect of Prednisolone treatment on sperm normal morphology and viability percent following antisperm antibody separation technique and in vitro sperm activation (Different letters indicates significantly different values from each other $P < 0.005$)
Number of samples per replicate =60

The effect of prednisolone, ASAS and in vitro sperm activation on human sperm viability, agglutination and sperm shaky head percent in immunologically infertile patients is shown in figure 5. During the courses of prednisolone treatment significant reductions ($P < 0.001$) in sperm agglutination and sperm shaky head movement percent were observed followed at the same courses of treatment with significant increase in sperm viability. These improvements in sperm viability were very clear at the end of 8 weeks up to the 12

weeks of treatment regimen with prednisolone ($P < 0.001$).

The sperm motility percent, sperm grade activity verses sperm agglutination percent and sperm abnormal morphology percent are shown in figure 6. The sperm motility percent and the sperm grade activity showed significant ($P < 0.001$) improvement started at the end of 4 weeks treatment period and continued through the courses of treatment regimen while both sperm agglutination percent and sperm abnormal morphology significantly decreased following the same courses of treatment ($P < 0.001$).

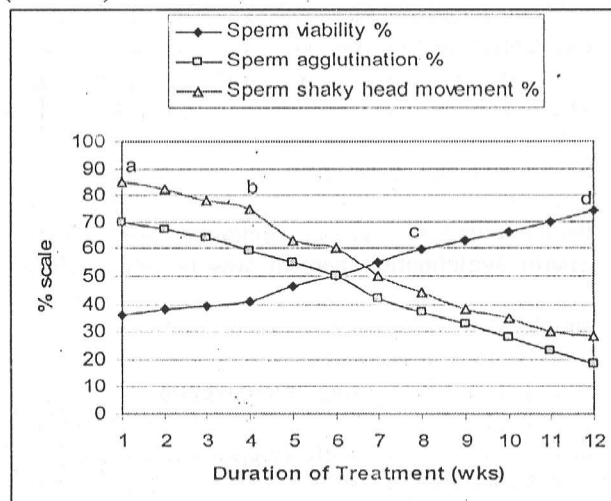


Figure (5): The effect of Prednisolone, antisperm antibody separation and in vitro sperm activation on human sperm viability, sperm shaky head movement and sperm agglutination percent in immunologically infertile patients following antisperm antibody separation technique and in vitro sperm activation (Different letters indicates significantly different values from each other $P < 0.001$)
Number of samples per replicate =60

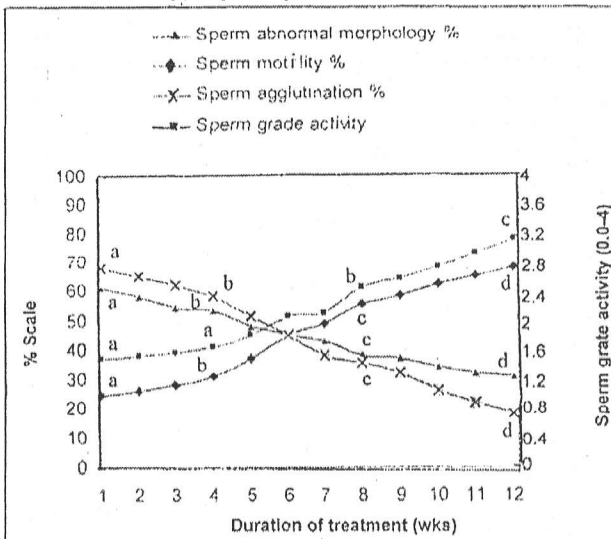


Figure (6): The effect of Prednisolone, sperm agglutination, sperm abnormal morphology, sperm

motility and sperm grade activity percent in immunologically infertile patients

(Different letters indicates significantly different values from each other $P < 0.001$)

Number of samples per replicate = 60

Discussion:

Seminal fluid parameters including sperm motility percent, sperm grade activity, sperm motility index and present of normal and viable sperm recovered following prednisolone, ASAS and sperm in vitro activation were significantly improved. These findings confirm the data of Allow et al(14) and Saeed(15) who reported that prednisolone therapy and antibiotic treatment significantly reduced sperm agglutination and improved seminal fluid parameters in patients complaining from seminal fluid infection and sperm agglutination problem.

Allow et al(11) reported 31% pregnancy rate after treatment of seminal fluid infection and sperm agglutination in infertile men. The improvements in seminal fluid parameters after prednisolone, ASAS and sperm in vitro activation were started to increase significantly at the end of 4 weeks. Best significant improvements in sperm function were observed at the end of 12 weeks treatment period. This may be due to the significant reduction in sperm agglutination which resulted from prednisolone inhibitory action on antibody production cells(16).

It was found that the presence of ASA in the seminal plasma is associated with infertility (17). Other investigators reported that ASA have been shown to reduce sperm motility, interfere with cervical mucus penetration and reduce spontaneous fertilization and pregnancy rate (18, 19). It was shown in the present study that sperm viability and activity were significantly increased when these were accompanied with significant and marked reduction in sperm agglutination, sperm shaky head movement and sperm abnormal percent. Similar data were reported by other workers (19) which confirmed our results. The former author also reported negative significant correlation between sperm agglutination versus sperm motility index. Antisperm antibodies impair sperm function by binding to the sperm membrane and result in premature acrosome reaction which makes the sperm to be unable to fertilize the oocytes (20,21). It is obvious from the data of this work that the combination of prednisolone, ASAS and in vitro sperm activation for the treatment of immunological infertile men found to be effective and adequate and resulted in significant improvements in sperm quality. These active and available sperms that obtained following prednisolone, ASAS and in vitro sperm activation may be used for intrauterine insemination and/or in

vitro fertilization and embryo transfer in order to induce pregnancy (11, 15)

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