Original Article

The effect of prednisolone on sperm viability, sperm penetration rate and intra-uterine insemination rates in men with marked sperm agglutination and antisperm antibodies.

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Summary:

Background: *the immune system differentiates between the endogenous and exogenous bodies that inter the body. The break-down of blood-testis barrier results in the production of antisperm antibodies. This may occur in the case of an infection to the prostate, seminal vesicle and epididymes. Antisperm antibodies (ASA) cause sperm agglutination and affect sperm motility, viability and sperm migration in the female reproductive tract. ASA also impair fertilization process. The objective of the present work was to study the effect of prednisolone on sperm motility index (SMI), viability and sperm penetration assay (SPA) in immunologically infertile men.*

Materials and methods: *The semen and serum samples of 140 infertile men were examined by microagglutination test and slide agglutination test to detect ASA and sperm agglutination. Semen fliud analysis was performed to report sperm motility index (SMI), sperm viability and hypo-osmotic swelling test (HOST). Sperm penetration assay was done to record sperm penetration rate (SPR), sperm decondensation rate (SDR) and sperm penetration index (SPI). Men with positive ASA were treated with prednisolone and considered as treated group. Prednisolone was given orally in a dose of 5 mg three times daily for three months. The semen analysis, SMI HOST and SPA were performed before and after treatment with prednisolone. The number of semen samples in the treated group was 144 and in the control fertile group was 80. HOST-SPA positive semen was exposed to antisperm antibodies separation (ASAS) and invitro sperm activation prior to intra-uterine insemination.*

Results: *The SMI was significantly higher in the post-treated group compared to pretreated group (240 vs. 52.5, $P<0.01$). The SPI in the control group was significantly higher than the post and pre-treated groups. The HOST and viability test results were significantly increased in the post-treated group compared to pre-treated group (73.42 vs. 48.56 and 71.36 vs. 50.74 respectively, $P<0.01$). The sperm penetration rate, sperm decondensation rate and sperm penetration index were significantly increased in the post-treated vs. pre-treated groups (26.49 vs. 10.84, 10.91 vs. 3.47, $P<0.05$, 14.45 vs. 4.30, $P<0.01$ respectively). HOST-SPA positive semen was used for intra-uterine insemination (IUI). The semen was exposed to ASAS and in vitro activation prior to IUI and resulted in 42.86% pregnancy rate per cycle. The pregnancy was confirmed by the observation of gestation sac and viable fetal hear beet 5 weeks following IUI.*

Conclusion: *It was concluded from the results of the present study that treatment of immunological infertile men with prednisolone improves SMI, sperm viability and SPA results. The application of HOST-SPA positive semen for IUI resulted in successful pregnancy. The authors indicate that these viable spermatozoa with high fertilizable potential could be used for IVF and/or ICSI in immunologically infertile men.*

Introduction

The immune system is unable to recognize matured sperm during fetal development since mature sperm are found after puberty. Spermatozoa are considered as a foreign protein by man's immune system spermatozoa are protected inside the testicle from circulating immunologlobulins (Ig) by the tight junctions of the Sertoli cells so called blood-testis barrier[1]. Antisperm antibodies (ASA) of immunoglobulin class A (IgA) and IgG have been implicated in infertility by reducing the progression of human sperm through cervical mucus and/or interfere with sperm binding at the zona pellucida. ASA in the seminal plasma are tested when there is sperm agglutination or abnormal shaking sperm movement on semen analysis[2]. The most common causes of damage to blood-testis barrier which results in the formation of ASA to the sperm surface antigen include infection, varicocle repair,

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vasectomy, testicular biopsy and trauma[3]. Prostate and seminal vesicle accessory glands at the time of ejaculation secret ASA into the fluid of seminal plasma and these ASA come into contact with sperm and cause sperm agglutination and immobilization[4].

Hypo-osmotic swelling test (HOST) is used to check the defects in sperm plasma membrane. This test is applied in *in-vitro* fertilization (IVF) centers to differentiate between viable and unviable sperm. Semen samples which have score >60% HOST positive are used for intra cytoplasmic sperm injection (ICSI) program [1].

Sperm pentration assay (SPA) was described by Yanagimachi in 1976[5]. The SPA involves incubation of sperm *in-vitro* for a period of 48 hours with hamster zona-free oocytes. The zona pellucida is removed prior to sperm to sperm-oocyte incubation. The function of zona pellucida is to maintain species specifity. The number of sperm head penetration and the nuclear decondensations reported in this assay. The SRA is used to asses *in-vitro* fertilization (IVF) ability of the oocytes and also it indirectly measures sperm capacitation and acrosome reaction [6]. Positive correlation was reported between SPA and HOST[7,8]. The technique of HOST is used in IVF centers as a predictor of assisted reproductive technology (ART) outcome. Although one research group observed a favorable correlation between HOST and ART outcome, but another group was found no predictive value of HOST [9,10]. The intra-uterine insemination (IUI) are performed when the women have cervical hostility (ASA in cervical mucous) luteal phase defect and/or the husband have oligospermia asthenospermia with sperm agglutination [11]. The aim of the present work was to study the effect of prednisolone and ASA separation on sperm viability, SPA and intra-uterine insemination outcome in immunologically infertile men with marked sperm agglutination and ASA.

Materials and Methods

1. patients

Semen and serum samples from one hundred forty men were tested by microagglutination test and slide agglutination test to detect ASA and sperm agglutination. The mean age of men was 36 years. The duration of infertility was from 4 to 8 years. The patients were received 5mg prednisolone 3 times daily for a period of 2 weeks and the dose was reduced to 2 tablet per day for another 3 days followed by one week rest period. The treatment regimen was repeated for three months. No side effects were observed during the course of prednisolone treatment. Men with positive sperm agglutination and ASA were tested by hypo-osmotic swelling test (HOST) and sperm pentration assay (SPA) and sperm motality index (SMI). SMI was calculated from multiplying sperm motility percent with sperm grade activity. The following sperm grading scale was used to grade human sperm motility:

Grade zero: Immotile sperm without motion.

Grade one: Motile sperm with circular movement without forward progressive motility.

Grade two: Slow forward progressive motility.

Grade three: Moderate forward progressive motility.

Grade four: Rapid forward progressive motility.

The technique of ASA detection, seminal fluid analysis, and antisperm antibody separation are described elsewhere [2, 11].

2. Sperm Penetration Assay (SPA)

Semen sample were collected by masturbation in a clean sterile petridish containing medicult culture medium (Medicule Culture Medium ompany, Denmark) with 50% serum. The samples were allowed to liquefy at 37°C in incubator. The semen was mixed gently with the serum and 5ml syringes with needle gauge 20 were used to separate and break the masses of sperm agglutination. Standard sperm *in-vitro* activation method was used to activate sperm motility and prepared the spermatozoa for SPA [12].

Young adult hamsters were injected with 20 international units (IU) with human menopausal gonadotropin on day one and two of the estrus cycle. The human chorionic gonadotropin (hCG) was injected intraperitoneally in a dose of IU on cycle day four.

Seventeen hour following hCG injection, the animals were sacrificed and the Fallopian tubes were removed and washed with 0.4 ml culture medium to recover the oocytes. The cells of the cumulus and corona radiate were removed ezymatically by treating the cells with hyaluronidase. The zona pellucida was removed by a trypsin solution. The zona-free oocytes were washed with culture medium and prepared for *in-vitro* insemination with human sperm. *In-vitro* activated human spermatozoa were incubated with zona-free oocytes for 24 hours in side 5% CO_2 at pH 7.4. The number of hamster zona-free oocyte in each IVF dish was 20 to 25 oocytes. The oocytes were examined for sperm penetration, and decondensation every 3, 12, and 24 hours following *in-vitro* insemination. Sperm penetration rate (SPR), sperm decondensation rate (SDR) and sperm penetration index (SPI) were calculated according to the following fomula:

SPR= No. penetration oocytes / total No. oocytes x 100

DR= No. decondensation sperm head within the oocytes / total No. oocytes x 100.

SPI= Mean No. penetration sperm head / oocytes.

The technique of SPA is described in detail elsewhere [12, 13]. The number of semen sample in the treated group was 144 samples. The pre-treated semen was considered as a double control group to be compared with that of post-treated semen with prednisolone.

3. Hypo-Osmotic Swelling Test (HOST)

The HOST solution was prepared by mixing equal volume of sterile distilled water to 300-mOsmol sterile used for human intra-venous infusion (I.V. saline Sammara Drug Industry, Sammara, Iraq). This gave a final osmolarity of 150mOsmol which was used to examine human sperm plasma membrane function. The control and prednisolone treated semen sample were mixed separately with 150mOsmol hypotonic HOST solution and incubated inside incubator for a period of 60 minutes. Six hundred sperm cells were examined per sample under 1000X magnification power. Spermatozoa with swollen and coiled tails were considered viable. Semen samples with >60% swollen and coiled tails were considered normal [6, 9, 12].

4. Ovarian Stimulation and Intra-Uterine Insemination

Ovulations were induced by clomiphene citrate (50 mg twice daily from cycle day 2 to 6) and human menopausal gonadotropin (hMG 2 ampoules per day from cycle day 7 to 11). The ultrasound examination was performed on cycle day 9, 11, 12. The estradiol concentration was assayed on cycle day 10 and 12. Human chorionic gonadotropin (hCG) was injected in a dose of 10000 IU on cycle day 12 when the size of the dominant follicle was >18 mm and the estradiol concentration was between 200-300 pg/ml/follicle. Semen from post-treated men prepared for ASAS and *in-vitro* sperm activation prior to intra-uterine insemination (IUI). Motile spermatozoa were isolated from the supernatant layer of the *in-vitro* tube and examined for sperm concentration, normal sperm morphology, viability, motility and sperm agglutination. The spermatozoa concentration was adjusted to 20-30 million/ml in the transfer catheter. The IUI was performed 30-36 hours after hCG injection.

Frydman catheter was used for IUI. The catheter was loaded with 1ml od sperm suspension and introduction through the cervical canal into inside the uterine cavity. Care was taken to avoid cervical bleeding during IUI. The women were received 1500IU on cycle day 14, 17, 20 and 23. The B-hCG and progesterone concentrations were assayed 14 days after IUI. Following 5 weeks of IUI, pregnancy was documented after ultrasound examination and demonstration of viable sperm activation and IUI in infertile patients is described elsewhere [13].

Results

The effect of prednisolone , antisperm antibody separation (ASAS) and *in-vitro* activation on sperm motility index (SMI), hypo-osmotic swelling test (HOST) and sperm viability using eosin vital stain as shown in table 1. the SMI was significantly increased in post-treated group compared to pretreated group (210 versus 52.5, P<0.01). The SMI of the control fertile group was significantly higher than the post-treated group (240 versus 210, P<0.050. the HOST rate was significantly increased in the post-treated group compared to pretreated group (73.42 vs 48.56, P<0.01). the HOST rate in the control and post-treated group. The percentage of the HOST was significantly higher in the control group than that of pretreated group (77.83 vs 48.56, P <0.01). the sperm viability percentage as measured by eosin vital stain in the post-treated group significantly higher than that of pretreated group (71.36 vs 50.74 P<0.01). the eosin stain-viability rate of the control group was also significantly higher than the pretreated group (74.86 vs. 50.74, P<0.01).

Table 1: The effect of prednisolone and ASAS on hypo-osmotic swelling test, sperm viability percentage and sperm motility index in immunologically infertile males.

Groups	HOST	Viability Percent	Sperm Motility Index
Control	77.83+10.41**	74.86 +11.03	240**
Pre-treated	48.56+7.80	50.74+9.48	52.5
Post-treated	73.42+5.78**	71.36+6.46**	210**

*P<0.05 significantly different from corresponding post-treated group.

**P<0.01 significantly different from corresponding pre-treated group.

The number of semen samples in the control group was 80 and in the treated group were 144.

The sperm penetration rate (SRP) was significantly increased in the post-treated group compared to pretreated group (26.49 +7.38 vs. 10.84+6.45 respectively, P<0.05). The SPR in the control group was significantly higher than the pretreated group (30.82 + 8.49 vs. 10.84 + 6.45, P<0.01). The sperm decondensation rate (SDR) was significantly increased in the post-treated group compared to pretreated group (10.91+3.60 vs. 3.47+ 1.87 respectively, P<0.05). The SDR was significantly not different from post-treated group but it was significantly different from pretreated group (Table 2). The sperm penetration index (SPI0 in the control and post-treated groups was significantly higher than that of pretreated group

(16.62 + 4.92 and 14.45 + 3.86 vs. 4.30 + 2.60 respectively, P< 0.01).

Table 2: The effect of prednisolone and ASAS on sperm penetration assay in immunologically infertile males.

Groups	Sperm penetration rate	sperm decondensation	Sperm penetration Index
Control	30.82+8.49**	12.80 + 3.48	16.62 + 4.92**
Pre-treated	10.84 + 6.45	3.47+1.87	4.30 + 2.60
Post-treated	26.49 + 7.38*	10.91 + 3.60*	14.45 + 3.86*

*P<0.05 significantly different from corresponding post-treated group.

**P<0.01 significantly different from corresponding pre-treated group.

The concentration of B-hCG and progesterone in the pregnant women was significantly higher than the non-pregnant women (160.75 + 9.62 vs. 3.37 + 2.05 mIU/ml and 28.73 + 10.49 vs. 4.62 + 1.38 ng/ml, respectively, P<0.001, Table 3). Intra-uterine insemination with post-treated SPA-HOST positive semen of 49 menstrual cycles resulted in 21 pregnant cycle (42.86% pregnancy rate per cycle). The pregnancy was confirmed by the presence of fetal gestation sac with viable heart beat.

Table 3: Pregnancy rate per cycle and beta-human chorionic gonadotropin (B-hCG) and progesterone concentrations in blood of pregnant and non-pregnant women following intra-uterine insemination with post-treated prednisolone SPA-HOST positive husband semen.

Groups	B-hCG mIU/ml	Progesterone ng/ml	Pregnancy rate / cycle
Pregnant	160.75 + 9.62*	28.73 + 10.49*	42.86% (21/49)
Non-pregnant	3.33 + 2.05	4.62 + 1.38	57.14 + (28/49)

*P<0.01 significantly different from corresponding non-pregnant group.

Discussion

Sperm motility index and sperm viability (measured by eosin vital stain) and HOST rates were significantly increased in the post-treated group compared to pretreated group. These improvements in spermatozoa quality are probably due to prednisolone treatment. Prednisolone has immunosuppressive action on antibody producing cells and also has anti-inflammatory. The combination of antisperm antibody separation (ASAS) and *in-vitro* sperm activation with prednisolone for the treatment of sperm agglutination resulted in significant reduction in antisperm antibodies and sperm agglutination in the present study. This was manifested by the significant improvement in sperm viability and motility index. Several investigators reported similar observation, which confirms the data of our work[14,15]. Sperm concentration, morphology and viability significantly improved in immunologically infertile men following prednisolone and antibiotic treatment for 12 weeks [16].

Antisperm antibodies combine with spermatozoa and damage the plasma membrane permeability control mechanism and this may result in unavailable sperm[14]. Al-Azzawi[17] reported positive correlation between intrauterine insemination outcome and HOST. Maladenovic et al[9] studied host technique as a quality control of sperm used for intra cytoplasmic sperm injection and embryo transfer (ICSI-ET). They reported positive correlation between HOST and outcome od assisted reproductive technology (ART) in men undergoing ICSI-ET treatment . Brito et al[18] recently studied comparison of methods to evaluate the plasmalemma of bovine sperm and their relation with *in-vitro* fertilization (IVF) rate. They reported that HOST was the only plasmalemma function evaluation method that significantly contributed to conventional sperm quality in predicting IVF rate. They also indicated that the HOST should be incorporated to routine work of seminal fluid analysis. The findings of previous investigators clearly indicate that HOST technique is predictive of IVF and ICSI outcome. It seems that prednisolone restores sperm plasma membrane function through inhibitory action on antisperm antibody and indirectly improves sperm viability and morphology[15,16]. This conclusion is supported by the results of SPA in the present study which showed significant improvement in sperm fertilization capacity in the post-treated semen compared to pretreated semen.

Prednisolone may also indirectly increase SPR and SDR. Several authors reported positive correlation between SPR and HOST[6,12] while other investigators reported positive correlation between SPA and IVF-ICSI outcome[19,20].

Post-treated semen was used for intra-uterine insemination and resulted in 42.86% pregnancy rate per cycle. The pregnancy was confirmed by the significant increase in B-hCG and progesterone concentration and later by the presence of viable gestation sac. Theses results indicate that SPA-HOST positive sperm have adequate potential to fertilize human oocytes *in vivo* and *in vitro* [20]. Clark et al.[21] reported complete inhibition of oocyte's fertilization by high titre of IgG antibodies to human sperm. Absortion of that serum with protein A produced a thousand-fold reduction in IgG antibody titre and at the same time removed the inhibitory activity upon fertilization process. They concluded that IgG antibodies could inhibit sperm fertilization. Lens[6] observed a clear correlation between the occurrence of spontaneous pregnancies, the percentage of penetrated hamster oocytes and the number of the spermatozoa, which penetrate the oocytes. The same author indicated

that the results of SPA can be important when considering whether patient couples should undertake another IVF attempt after the failure of an attempt Ridha-Barazanchi et al.[22] reported positive uterine correlation between human sperm penetration and intra-uterine insemination (IUI) outcome which confirms the result of IUI in the present work..

It was concluded from the results of the present work that the treatment of immunologically infertile men with prednisolone and application of sperm motility index test, hypo-osmotic swelling test and sperm penetration assay positive results and performance of antisperm antibody separation and intra-uterine insemination resulted in successful pregnancy.

References

1. Silverberg K.M. and Turner T. "Evaluation of Sperm In: Textbook of Assisted Reproductive Technique" Garder D.K., Weissman A., Howles C.M. and Shoham Z. (eds), Blachwell Science Publishing Co. , Malden USA 2001, PP: 61-76.
2. Waheda N.E." The ffect of Antisperm Antibodies and Aerobic Bacterial Infection in Semen Parameters Infertile Males" M.Sc. Thesis, University of Baghdad, Iraq, 1993.
3. Gloumb J., Vardinon N. and Hommonnai Z. et al. "Demonstration of antispermatozoal antibodies in varicocele-related infertility with an enzymeilinked immunosorbent assay (ELISA)". *Fertil. Steril. J.* 1986, **45**: 397-405.
4. Marshburn P.B. and Kuttch W.H. "The role of antisperm antibodies in infertility" *Fertil. Steril. J.* 1994, **61**: 799-811.
5. Yanagimmachi R., Yanagimmachi H.and RogersB.J. "The use of zona-free animal ova as a free system for the assessment of their fertilizing capacity of human spermatozoa" *Biol. Reprod. J.* 1976, **15**: 471-476.
6. Lens L.W. "The Spermatozoon Practice In: IVF Lab, The Laboratory Aspects of *in Vitro* Fertilization" Brans M., Lens L.W., Piederiet M.H., Rijnders P.M., Verveld M. and Zeilmarker G.H. (eds), Organon Publishing Co. Denmark, 1996, PP: 57-68.
7. Jeyendran R.S. and Zaneveld L.J.D." Human sperm hypo-osmotic swelling test" *Fertil. Steril. J.* 1986, **46**: 151-145.
8. Chan S.Y.W., Fox E.J.and Chan M.M.C." The relationship between the human sperm hypo-osmatic swelling test routine semen analysis and the human sperm zona-free analysis" *Fertil. Steril. J.* 1985, **44**: 688-692.
9. Mladenovic I., Micic S. and Genbacev O. et al."The hypo-osmotic swelling test for quality control of sperm prepared for assisted reproduction" *Arch. Androl.J.*1995, **34**: 163-169.
10. Joshi N., Kodwany G. and Balaiah D. et al. "The importance of computer assisted semen analysis (CASA) and sperm function testing in an in vitro fertilization program" *Intrn, J. Fertil. Menopausal Stud.* 1996, **41**: 46-52.
11. Ridha-Barazanchi, M.T., Alansari S.A.M. and Khunda S.S. et al. "Human pregnancy following antisperm antibody separation (ASAS) technique and sperm intra-uterine transfer" *J. Comm. Med.* 1992, **5**: 37-43.
12. Fakhrilidin M.B." The impact of seminal fluid infections and treatment on sperm function and fertilizing capacity in infertile patients" Ph.D. Thesis, Al-Mustansiriyah University, Baghdad, Iraq, 2000.
13. Ridha-Barazanchi, M.T., Alansari S.A.M. and Alnaeib Z.T. et al."Successful life birth following human ovarian stimulation and in vitro sperm cell activation and application of sperm intra-uterine transfer technique" J. Fac. Med. Baghdad, 1991, **33**: 327.
14. Saeed H.K. "The effect of prednisolone treatment on sperm function and sperm agglutination in infertile men" High Diploma Thesis, University of Baghdad, Iraq, 1999.
15. Allow A.K., Ridha-Barazanchi, M.T. and Alansari S.A.M. "The effect of three courses of treatment with antibiotic and prednisolon on sperm agglutination and morphology in immunological infertile patients" *Kufa Med. J.* 2001, **4**: 392.
16. Allow A.K., Ridha-Barazanchi, M.T. and Alansari S.A.M. "The efficiency of antisperm antibody separation (ASAS) and intra-uterinne insemination in couples with immunological infertility" *Annals Coll. Med. Mosul*, 2002, **28**: 36-41.
17. Al-Azzawi E.A."Clinical significance of hypoosmotic swelling test in intra-uterine insemination" High Diploma Thesis, University of Baghdad IVF Institute for Embryo Research and Infertility Treatment, Baghdad, Iraq, 2003.
18. Brito L.F.C., Barth A.D. and Bilodeau-Goeseels et al. "Comparison of methods to evaluate the plasmalemma of bovine sperm and their relationship with in vitro fertilization rate" *Theriogenology* 2003, **60**: 1539-1551.
19. Ridha-Barazanchi, M.T., Alansari S.A.M. and Khunda S.S. "Successful life birth following single sperm HOST-ICSI application in TESE patients" *Kufa Med. J.* 2002, **5**: 20.
20. Alansari S.A.M. "The effect of prednisolone and luteal support therapy on embryo implantation following ICSI-ET in Immunologically Infertile patients" Ph.D. Thesis, College of Medicine, University of Baghdad, Baghdad, Iraq, 2005.
21. Clark G.N., Hyne R.V. and Du Plessis Y. et al. "Sperm antibodies and human in vitro fertilization" *Fertil. Steril. J.* 1988, **49**: 1018-1025.

22. Ridha-Barazanchi, M.T., Alansari S.A.M. and Khunda S.S. et al. "Human sperm penetration and intra-uterine insemination in infertile patients" *Middle East Fertil. J.* 2001, **6**: 36.

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