Assessment of hypo-osmotic swelling test (HOST) of spermatozoa supplemented with pentoxifylline (PTX) after preparation by conventional layering technique in infertile patients undergoing intra-uterine insemination performance

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تقييم فحص كفاءة الغشاء البلازمي للنطفة البشرية تحت الضغط التنافذي الواطئ بإضافة مادة البنتوكسفلين باستخدام النظرية الطباقية البسيطة وتقنية التلقيح الاصطناعي داخل الرحم لمرضى العقم

الخلاصة:

تهدف الدراسة إلى مقارنة النسبة المئوية لفحص كفاءة الغشاء البلازمي للنطفة البشرية تحت الضغط التنافذي الواطئ بإضافة أو بدون إضافة مادة البنتوكسفلين باستخدام النظرية الطباقية البسيطة وتقنية التلقيح الاصطناعي داخل الرحم لمرضى العقم. تم اخذ ثلاثون عينة سائل منوي وكل عينة تم تقسيمها إلى قسمين وكل قسم يحتوي على (٥و٠مل) من السائل المنوي وبعد ذلك تم إضافة (٥و٠مل) من مادة البنتوكسفلين إلى مجموعة تتكون من(١٥عينة)، أما المجموعة الأخرى (١٥عينة) فبقيت كمجموعة سيطرة.تم أجراء فحص كفاءة غشاء البلازما للنطفة البشرية تحت الضغط التنافذي الواطئ قبل وبعد أجراء عملية تنشيط النطف خارج الجسم وذلك بمزج (١,٠مل) من عينة السائل المنوي مع(١,٠مل) من محلول الاختبار(Hypo-osmotic solution). أن فحوصات كفاءة النطف والتي تتضمن تركيز النطف، حركة النطف، الحركة التقدمية للنطف، والنسبة المئوية للإشكال النطف السوية تم تقييمها وفقاً إلى مقررات منظمة الصحة العالمية. أن نسبة النطف التي تعاني من انتفاخ الغشاء البلازمي للحيمن في مجموعة المعاملة والتي أضيف لها مادة البنتوكسفلين أعطت ارتفاعاً معنوياً عالياً مقارنة بمجموعة السيطرة. نستنتج من خلال الدراسة الحالية بان إضافة مادة البنتوكسفلين إلى وسط تحضير النطف أثناء أجراء عملية التنشيط وباستخدام النظرية الطباقية البسيطة يمكن أن يحسن مستوى الغشاء البلازمي للنطفة ويزيد من قابلية حصول الإخصاب بعد أجراء تقنية التلقيح الاصطناعي داخل الرحم للمرضى المصابين بعوامل مختلفة للعقم الذكري.

Abstract:

The current study was designed to compare the percentage of human sperm hypo-osmotic swelling test (HOST) with and without pentoxifylline supplemented for semen samples prepared by conventional layering technique by using 1ml of the liquefied semen was layered beneath 1ml of IVF culture medium after intra-uterine insemination (IUI) technique. Form each infertile male, Thirty normal semen samples were collected by masturbation after 3-5 days abstinence and allow liquefying at 37ºC in 5% CO2 for 30 minutes and evaluated before and after in vitro sperm activation. The final samples were divided into two tubes, each one contain 0.5 ml liquefied semen. Therefore, 0.5 ml pentoxifylline was added into one tube and another tube as a control, also hypo-osmotic swelling test (HOST) was evaluated for each semen samples. The hypo-osmotic swelling test (HOST) was performed before and after in vitro sperm preparation by mixing 0.1 ml of liquefied semen with 1.0 ml of 150 mOsm/ L NaCl as hypo-osmotic solution. The sperm function tests (SFTs) including sperm concentration, sperm motility (%), progressive sperm motility (%), and normal sperm morphology (%) were evaluated according to World Health Organization (WHO) criteria. The results of the present study indicate that the percentage of swollen spermatozoa in pentoxifylline supplemented group was significantly higher than the control group (70.80±2.32 vs. 58.75±1.58; P<0.001). It was concluded that addition of pentoxifylline to the semen samples prepared by conventional layering technique can improve functional integrity of sperm plasma membrane.

1. Introduction

Sperm preparation techniques and in vitro sperm treatment select the highly recovery spermatozoa with improved sperm motility, progressive sperm motility, normal sperm morphology, and supply a protective environment to maintain the functional capacity for successful fertilization potential in spermatozoa depending on the quality of the ejaculate (1). Also, the spermatozoa selected by layering technique enhanced sperm penetration results in zona free hamster egg by sperm penetration assay. The sperms prepared by conventional layering technique give greatest clinical pregnancies for intra-uterine insemination (IUI) than other sperm preparation techniques because mono-ovulation induction plus IUI do not give better clinical results compared with mono-ovulation induction plus timed vaginal intercourse and sperm preparation for IUI could improve toxic factors contained in the abnormal sperm hypo-osmotic swelling test(HOS-test) (2).

The plasma membrane integrity and fertilizing capacity of human spermatozoa can enhance by different chemical stimulators and pharmacological substances to stimulate human sperm function (3). Pentoxifylline (PTX) is a phosphodiesterase inhibitors of methylxanthine derivative, inhibits the breakdown of cyclic adenosine monophosphate (cAMP), resulting in increase intracellular levels of cAMP (4). Conversely, an increase intracellular level of cAMP through activation of cAMP protein kinase (5) involved in calcium ion movement of sperm plasma membrane (6), which itself induces sperm tail protein phosphorylation (7) with subsequent increase in sperm motility (8). In addition, Pentoxifylline improve acrosome reaction and playing a major role in improved fertilizing ability of human spermatozoa. Additionally, Pentoxifylline improves sperm egg binding ability due to increase in sperm velocity parameters, straight-line velocity (VSL), and average path velocity (VAP), and these parameters are indicators of sperm progressive motility (9). Also, PTX successfully used to increase fertilization rates in IVF (10), IUI (11), and as pre-treatment to stimulate epididymal and testicular sperm motility for intra-cytoplasmic sperm injection (ICSI) and in vitro fertilization with embryo transfer (IVF-ET) (12).

The sperm functions tests (SFTs) is an important factors in evaluation of male infertility and male reproductive potential (13, 14), including sperm concentration, sperm motility (%), normal sperm morphology (%). Sometimes, fertilization occurs despite an abnormal semen analysis, or it fails to occur when analysis values are normal. Furthermore, hypo-osmotic swelling test measure the functional integrity of sperm plasma membrane (15). However, WHO (16) considered the HOS test an optional, additional, and viability test. Conversely, it is easy to score and give additional information on the functional integrity of sperm plasma membrane (17). In addition, the functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction, and binding of spermatozoa to the egg surface (18, 19). The results of the current study certified that semen samples with sperm HOS-test supplemented with pentoxifylline give the best clinical pregnancy rate compared with those without pentoxifylline for intra-uterine insemination (IUI).

2. Subjects, Materials and Methods

2.1. Subjects

Thirty semen samples (15 samples with pentoxifylline vs. 15 without pentoxifylline) were collected from IVF Institute of Embryo Research and Infertility Treatment/Al-Nahrain University between March and May 2006. The mean of age ± S.E.M for infertile subjects 30.05 ± 4.87 years. The ejaculates were collected by masturbation after 3-5 days abstinence and allow liquefying at 37ºC in 5% CO2 for 30 minutes. The liquefied semen is then carefully mixed for few seconds, and then the specimen was examined in details by microscopic and macroscopic examinations including sperm concentration, sperm motility (%), progressive sperm motility (%), and normal sperm morphology (%) was examined. WHO criteria for normal semen values were applied.

2.2. Sperm preparation for intra-uterine insemination (IUI) technique

The sperm processing for intra-uterine insemination (IUI) prepared using conventional layering technique by mixing 1ml of the liquefied semen was layered beneath a culture medium (IVF medium, Medi-cult, Jyllinge, Denmark), after incubation for 30 minute in 5% CO2 at 37ºC.The supernatant was removed and divided into 2 tubes, 0.5 ml for each tube. One tube was mixed with 0.5 mg/ml pentoxifylline (Sigma, St, Louis, USA) and an additional tube reserved as a control tube.

2.3. Hypo-osmotic swelling test

Hypo-osmotic swelling test was performed before and after in vitro sperm preparation by mixing 0.1 ml of liquefied semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution (20). The mixture was incubated for 30 minute at 37 ºC in 5% CO2 in accordance with Jeyendran et al. (21). Spermatozoa were examined for typical tail swelling pattern under microscope at 400 x magnifications. A total of 100 spermatozoa were accounted in at least ten different fields, and sperm tail were classified into seven distinct subtype of coiling in various regions. The percentage of HOS-reactive spermatozoa (with coiled and swollen tail) and non-reactive spermatozoa(with straight or non swollen tails) were calculated; at least 50% of swollen spermatozoa were considered normal.

3. Statistical analysis

Statistical analysis was performed with the SPSS version 12.00 by the Statistical Package for Social Sciences Software. The data analysis was done using paired sample t-test to assess the statistical differences in the results of SFTs and sperm HOS-test. Mean and standard error of mean (S.E.M) obtained from crude data to compare between Pre-and Post-activation for semen parameters. P-value < 0.05 was used as a levels of statistically significant.

4. Results

The results of the present study showed that semen samples supplemented with pentoxifylline have hypo-osmotic swelling test (70.80±2.32) more than those without pentoxifylline (58.75±1.58) after in vitro sperm activation (Table 1 and 2; respectively). However, it was noticed a highly significant (P<0.001) differences in seminal fluid parameters were assessed post in vitro sperm activation for semen samples as compared to the pre-activation. Also, the

results of the present study indicated that the addition of pentoxifylline to the prepared sperm can increase sperm plasma membrane integrity and HOS test scores.
In the present study, the best results for clinical pregnancy rates (11) were observed for semen samples supplemented with pentoxifylline and HOS test more than 50% as compared with semen samples without pentoxifylline (5) after in vitro sperm preparation for intra-uterine insemination.

Table (1): Hypo-osmotic swelling test of semen samples supplemented with pentoxifylline in infertile patients* undergoing intra-uterine insemination

Parameters (N=15)	Conventional layering technique	
	Pre-activation	Post-activation
Sperm Concentration (×106 sperm/ml)	48.33±5.83	29.00±3.58 a
Sperm Motility (%)	58.66±2.60	87.13±1.16 a
Progressive sperm Motility (%)	34.66±2.52	62.40±2.28 a
Normal Sperm morphology (%)	53.66±2.57	87.33±1.07 a
Sperm HOS test score	49.93 ± 2.20	70.80 ± 2.32 a

Values are Mean ± S.E.M
a: means a highly significant (P<0.001) difference from pre-activation
***No. of infertile patients=15**

Table (2): Hypo-osmotic swelling test of semen samples without pentoxifylline supplemented in infertile patients* undergoing intra-uterine insemination.

Parameters (N=15)	Conventional layering technique	
	Pre-activation	Post-activation
Sperm Concentration (×106 sperm/ml)	40.35±6.31	22.75±3.65 a
Sperm Motility (%)	51.00±2.55	76.60±2.07 a
Progressive sperm Motility (%)	31.90±1.66	54.85±1.43 a
Normal Sperm morphology (%)	44.50±3.20	80.25±2.09 a
Sperm HOS test score	40.50 ± 1.64	58.75 ± 1.58 a

Values are Mean ± S.E.M

a: means a highly significance (P<0.001) different from pre-activation

***No. of infertile patients=15**

Discussion

The defective energy metabolism of the spermatozoa is a potential cause of male infertility, the use of motility stimulating substances to improve sperm motility, motion characteristics, and therefore fertilization rates appeared reasonable (22). However, sperm plasma membrane activity and motility significantly increase after stimulation with methylxanthine group due to augmented glycolysis and fructolysis (23). The initiation of sperm motility, hyperactivation, and capacitation are modulated by an interaction of intracellular Ca+2 levels and cAMP levels (24). In the absence of pentoxifylline, the cAMP levels remained unchanges and correlated only with hypermotility and amplitude of lateral head displacement of the spermatozoa. However, cAMP is believed to be stimulate a cAMP-dependent kinase, which itself induces sperm tail protein phosphorylation with subsequent increase in sperm motility. Furthermore, cAMP is intimately involved as a second messenger in the induction of acrosome reaction (25).

The human sperm plasma membrane is useful in assessing male infertility in addition to sperm motility. Tournaye et al. (26) assessed that concentration of ATP determinate the frequency of tail beat, and the decrease in intracellular cAMP levels due to ATP tiredness lead to decrease in sperm motility. Additionally, Gradil et al. (27) reported that pentoxifylline improves sperm egg binding ability due to an increase in sperm velocity. Therefore, it has been shown that cAMP also involved in the control of the acrosome

reaction and play a major role in improved fertilizing ability after addition of pentoxifylline to prepared semen samples for intra-uterine insemination (IUI) techniques.

The plasma membrane integrity and HOS test score is also useful in the evaluation of fertilization potential of human spermatozoa (28). Tash et al (29) reported the effect of pentoxifylline on membrane functional integrity of mouse spermatozoa and found that there was a positive correlation between percentage of tail swelling and motile spermatozoa. Also, Pentoxifylline (PTX) enhanced plasma membrane integrity and motility of fresh and cryopreserved of human spermatozoa post-thaw, not improvement was found by freezing sperm with Pentoxifylline (30). In contrast, Pentoxifylline has been used successfully to increase fertilization rates after IVF-ET and as pre-treatment to stimulate epididymal and testicular sperm motility for intracytoplasmic sperm injection (ICSI). In the present study we found that the percentage of swollen spermatozoa in semen samples supplemented with Pentoxifylline was significantly ($P<0.001$) higher than those without Pentoxifylline. Moreover, Pentoxifylline could increase the fertilizing capacity for infertile patients for human spermatozoa (31). Tournaye et al. (32) indicated that the addition of Pentoxifylline during preparation of semen samples for intra-uterine insemination could improve pregnancy rates as compared with semen samples without addition of Pentoxifylline (33).

References

1.Melis GB., Strigini F. and Mais V. (1990): Critical reappraisal of the clinical effectiveness of different methods of assisted fertilization. J. Endocrinol. Invest. 13:263-274.

2. Fakih H., Mac-Lusky N., De-Cherney A., Wallimann T. Huszar G. (1986): Enhancement of human sperm motility and velocity in vitro: Effect of calcium and creatinine phosphate. Fertil. Steril. 46:938-44.

3. Mbizvo MT., Johnston RC. and Baker GHW. (1993): The effect of the motility stimulants, caffeine, pentoxifylline, and 2- deoxyadenosine on hyper-activation of cryopreserved human sperm. Fertil. Steril. 59: 1112-17.

4. Tesarik J., Mendoza C. and Carreras A. (1992): Effects of phosphodiesterase inhibitors caffeine and pentoxifylline on spontaneous and stimulus-induced acrosome reactions in sperm. Fertil. Steril. 58: 1185-90.

5. McKinney KA., Lewis SEM. and Thompson W. (1994): Persistent effects of pentoxifylline on human sperm motility after drug removal in normozoospermic and asthenozoospermic individuals. Andrologia. 26:235-40.

6. Lambert HL., Steinleitner A. and Eiserman J. (1992): Enhanced gamete interaction in the sperm penetration assay after coincubation with pentoxifylline and human follicular fluid. Fertil. Steril. 58: 1205-8.

7. Tesarik J., Thebault A. and Testart J. (1994): Effects of pentoxifylline on sperm movement characteristics in normozoospermic and asthenozoospermic specimens. Hum. Reprod. 7:1257-1263.

8. Paul M., Sumpter JP. and Lindsay KS. (1995): Action of pentoxifylline directly on semen. Hum. Reprod. 10: 354-59.

9. Lewis SEM., Moohan JM. and Thompson W. (1993): Effects of pentoxifylline on human sperm motility in normozoospermic individuals using computer-assisted analysis. Fertil. Steril. 59: 418-23.

10. Nassar A., Morshedi M., Mahony M., Srisombut C., Lin MH. and Oehninger S. (1999): Pentoxifylline stimulates various sperm motion parameters and cervical mucus penetrability in patients with asthenozoospermia. Andrologia. 31:9-15.

11. Ponce AA., Fiol de Cuneo M. and Ruiz RD. (1999): Influence of pentoxifylline on membrane functional integrity. Arch. Androl. 43: 77-84.

12. Ombelet W., Pollet H., Bosmans E. Vereecken A. (1997): Results of a questionnaire on sperm morphology assessment. Hum. Reprod. 12: 1015-20.

13. Liu DY., Du Plessis YP., Nayudu PL., Johnston WI. and Baker HW. (1988): The use of in vitro fertilization to evaluate putative tests of human sperm function. Fertil. Steril. 49:272-7.

14. Jeyendran RS., Van Der Ven HH., Perez-Pelaez M., Crabo BG. and Zaneveld LJD. (1984): Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil. 70: 219-228.

15. World Health Organization (WHO) (1999): Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 4th ed. Cambridge, Cambridge University Press UK.8-11.

16. Ved S., Montag M., Schmutzler A., Prietl G., Haidl G., Van der Ven HH. (1997): Pregnancy following intracytoplasmic sperm injection of immotile spermatozoa selected by the hypo-osmotic swelling test: a case report. Andrologia. 29: 241-242.

17. Check JH., Keifer., Check C., Wilson. and Choe JK. (2001): The relative discrepancy between viability and hypo-osmotic swelling test (HOST) scores is not related to in vitro fertilization (IVF) outcome. Proceeding of the VII international congress of andrology. Montreal, Quebec, Canada, June. 15-19.

18. Check JH., Baker A., Benfer K., Lurie D. and Katsoff D. (1996): Transfer of cryopreserved embryos improved pregnancy rates in patients with damage to the functional integrity of the sperm membrane as measured by the hypo osmotic swelling test. Fertil. Steril. 65: 1241-1244.

19. Liu J., Tasi Y., Katz E., Compton G., Garcia JE. and Baramki TA. (1997): High fertilization rates obtained after intracytoplasmic sperm injection with 100% non-motile spermatozoa selected by using a simple modified hypo-osmotic swelling test. Fertil. Steril. 66:373-5.

20. Jeyendran RS., Van der Ven HH. and Zaneveld LJD. (1992): The hypo-osmotic swelling test: an update. Arch. Androl. 29:105-106.

21. Choavaratana R., Manoch D., Treeratanaporn N. and Kunathikom S. (2003): Induction of acrosome reaction by calcium ionophore A 23187 in sperm separated by twolayer Percoll gradient method. Siriraj. Hosp. Gaz. 55: 473-77.

22. Lambert HL., Steinleitner A. and Eiserman J. (1992): Enhanced gamete interaction in the sperm penetration assay after coincubation with pentoxifylline and human follicular fluid. Fertil. Steril. 58: 1205-8.

23. De Jonge CJ., Han HL., Lawrie H., Mack SR. and Zaneveld LJD. (1992): Modulation of the human sperm acrosome reactionby effectors of the adenylate cyclase/cyclic AMP secondmessenger pathway. J. Exp. Zool. 285: 113-25.

24. Shen MR., Chiang PH. and Yang RC. (1991): Pentoxifylline stimulates human sperm motility both in vitro and after oral therapy. Br. J. Clin. Pharma. 31: 711-14.

25. Stanic P., Sonicki Z. and Suchanek E. (2002): Effect of pentoxifylline on motility and membrane integrity of cryopreserved human spermatozoa. Int. J. Androl. 25: 186-90.
26. Tournaye H., Devroey P., Camus M., Van der Linden M., Janssens R. and Van Steirteghem A. (1995): Use of pentoxifylline in assisted reproductive technology. Hum. Reprod. 10:72-79.
27. Gradil CM. and Ball BA. (2000): The use of pentoxifylline to improve motility of cryopreserved equine spermatozoa. Theriogenology. 54: 1041-1047.
28. Calogero AE., Fishel S., Hall J., Ferrara E., Vicari E., Green S., Hunter A., Burrello N., Thornton S. and D'Agata R. (1998): Correlation between intracellular cAMP content, kinematic parameters and hyperactivation of human spermatozoa after incubation with pentoxifylline. Hum. Reprod. 13:911-915.
29. Tash JS., Hidaka H. and Means AR. (1986): Axokinin phosphorylation by cAMP dependent protein kinase is sufficient for activation of sperm flagellar motility. J. Cell. Biol. 103:649-655.
30. Bracho GE., Fritch JJ. and Tash JS. (1998): Identification of flagellar proteins that initiate the activation of sperm motility in vivo. Biochem. Biophys. Res. Commun. 242:231-237.
31. Tournaye H., Janssens R., Verheyen G., Devroey P. and Van Steirteghem A. (1994): In vitro fertilization in couples with previous fertilization failure using sperm incubated with pentoxifylline and 2-deoxyadenosine. Fertil. Steril. 62:574-579.
32. Tournaye H., Janssens R., Verheyen G., Camus M., Devroey P. and Van Steirteghem A. (1994): An indiscriminate use of pentoxifylline does not improve in vitro fertilization in poor fertilizers. Hum. Reprod. 9:1289-1292.