Comparison between Sperm treated with Pentoxifylline and 2-Deoxyadenosine using Hypo-Osmotic Swelling Test

Pitak Laokirkkiat MD*,
Somboon Kunathikom MD*, Roungsin Choavaratana MD*,
Somsin Petyim MD*, Japarath Prechapanich MD*

** Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University*

Objective: *To compare the percentage of sperm tail membrane swelling under hypo-osmotic conditions between sperm treated with pentoxifylline and 2-deoxyadenosine*
Design: *Experimental in vitro study*
Material and Method: *Thirty normal semen samples from male partners of infertile couples were collected. After sperm preparation by two-layer Percoll gradient method, each sperm sample was divided into three specimens. Pentoxifylline and 2-deoxyadenosine were separately added into two specimens, while the third specimen was used as a control. Hypo-osmotic swelling test was performed in all specimens. Percentage of swollen spermatozoa in each specimen was evaluated.*
Results: *The mean percentage of swollen spermatozoa in the semen samples supplemented with pentoxifylline and 2-deoxyadenosine were both significantly higher than those in the control (82.8 $\pm$ 7.7 and 83.0 $\pm$ 9.5 vs 70.8 $\pm$ 12.7; $p < 0.001$). There was no significant differences of swollen spermatozoa between pentoxifylline and 2-deoxyadenosine ($p = 0.898$).*
Conclusion: *Addition of pentoxifylline and 2-deoxyadenosine to the sperm prepared by the two-layer Percoll gradient method can almost equally enhance the sperm membrane integrity. Therefore, it may be beneficial to add these compounds to sperm preparation for use in assisted reproduction.*

Keywords: *Pentoxifylline, 2-Deoxyadenosine, Hypo-osmotic swelling test, Percoll gradient*

J Med Assoc Thai 2007; 90 (2): 211-5
Full text. e-Journal: *http://www.medassocthai.org/journal*

The procedures of assisted reproduction developed during recent years have revealed the need for suitable and effective techniques of sperm treatment in the laboratory. Sperm preparation by two-layer Percoll gradient method has been widely used to select spermatozoa with normal morphology and fast progressive motility. To improve the sperm quality, many pharmacological agents such as caffeine, pentoxifylline, 2-deoxyadenosine, and calcium ionophore A 23187 have been studied extensively[1-3]. Pentoxifylline is a methylxanthine derivative. It has been reported to promote hyperactivated sperm motility[4,5] and induce acrosome reaction[6]. 2-Deoxyadenosine, an analogue of adenosine with a modified ribose ring, has also been reported to enhance sperm motility and hyperactivation[1,7]. Because of these observations, the use of both pentoxifylline and 2-deoxyadenosine has been proposed as a way of enhancing fertilization in vitro when treating couples suffering from male factor infertility.

The hypo-osmotic swelling (HOS) test for investigating the functional integrity of the human sperm membrane has been introduced as a useful assay in the diagnosis of the infertile semen by Jeyendran et al[8]. The advantage of the HOS test is that it is very simple and repeatable. In fact, the HOS assay is the simplest test of all for which WHO recommended sperm optional tests[9]. There have been a number of reports that show a positive correlation between the HOS test and other sperm parameters, such as the

Correspondence to : Laokirkkiat P, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-4651, Fax: 0-2418-2662

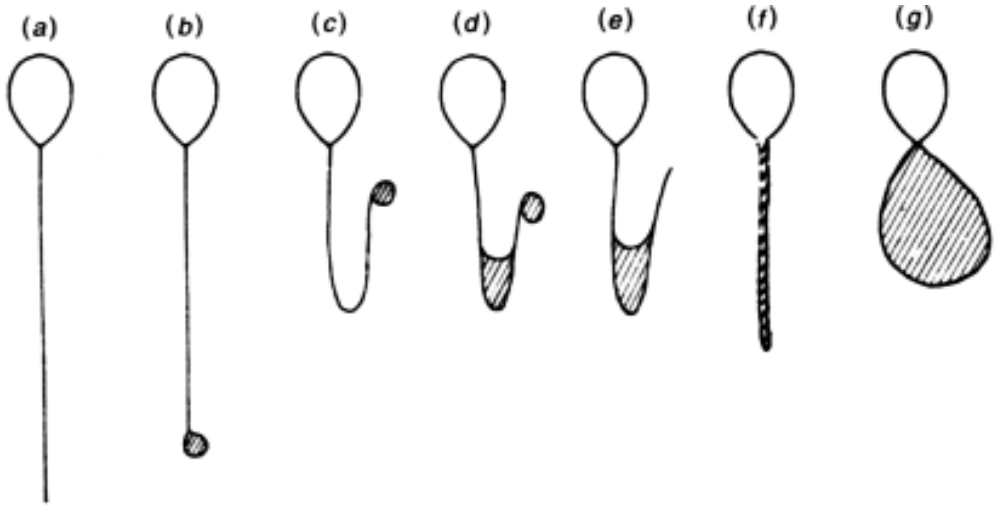

Fig. 1 Schematic representation of swelling of sperm tails: (a) = no change, (b-g) = various types of tail changes

sperm zona-free hamster ovum penetration assay and *in vitro* fertilization (IVF) outcome[10]. Most recently, the HOS test has been recommended for use in discriminating viable from non-viable spermatozoa in cases of complete astheno zoospermia[11,12].

The aim of the present study was to compare the percentage of sperm tail membrane swelling under hypo-osmotic conditions between sperm treated with pentoxifylline and 2-deoxyadenosine in sperm prepared by the two-layer Percoll gradient method.

Material and Method

Thirty semen samples were collected from male partners of infertile couples attending the Infertility Clinic at Siriraj Hospital. All samples had normal sperm concentration, motility, and morphology according to the World Health Organization criteria[9].

The 100% stock Percoll solution was prepared by diluting nine parts Percoll (Pharmacia, Uppsala, Sweden) with one part of 10% hepes buffered medium. The discontinuous Percoll gradient was prepared in two layers. The lower layer was 1.5 ml of 80% Percoll over layered by 1.5 ml of 40% Percoll in a 15-ml Falcon conical centrifuge tube (NO.2001, Becton Dickinson, USA). Then 1 ml of undiluted semen was layered carefully on top of the column, and centrifuged for 20 minutes at 500 g. After centrifugation, the semen and 40% Percoll layers were aspirated off. The remaining 80% layer was washed with 2 ml of human tubal fluid (HTF) medium by centrifugation at 200 g for 10 minutes. The final sperm pellet was resuspended in 1.2 ml HTF medium. Then, each specimen was divided into three tubes, 0.4 ml each. An aliquot in one tube was mixed with 0.4 ml of pentoxifylline (1 mg/ml) solution, the second tube mixed with 0.4 ml of 2-deoxyadenosine, and the last tube was kept as a control. All three tubes were placed in an incubator at 37 C for one hour.

A 0.1 ml aliquot of sperm sample from each tube was mixed with 1 ml hypo-osmotic solution (equal parts of 150 mOsmol fructose and 150 mOsmol sodium citrate) and then incubated at 37 C for one hour. Spermatozoa were examined with a phase-contrast microscope. Swelling of sperm is identified as changes in the shape of the tail, as shown in Fig. 1. At least 100 spermatozoa were evaluated, and the percentage of swollen tails was calculated without knowing the supplemented specimen.

Statistical analysis

Normal and standard deviation (SD) of semen characteristics were presented. One-way analysis of variance (ANOVA) was performed, and the significance of differences between the mean values was analyzed using the least significant difference test. The p-value of less than 0.05 was considered significant difference.

Results

Semen characteristics of 30 samples derived from 30 men with a mean age of 35 years (range 30-39 years) are shown in Table 1.

The results of the HOS test are shown in Table 2. The mean percentage of swollen spermatozoa

Table 1. Semen characteristics of 30 samples

Semen parameters	Mean ± SD
Volume (ml)	2.7 ± 0.9
pH	7.9 ± 0.1
Sperm concentration (10^6/ml)	50.9 ± 25.0
Total sperm count (10^6)	135.4 ± 80.7
Motility (%)	57.6 ± 6.0
Normal morphology (%)	37.2 ± 5.3
Viability (% live)	81.1 ± 3.8
White blood cell (10^6/ml)	0.2 ± 60.2

Table 2. Results of the HOS test in semen samples with pentoxifylline, 2-deoxyadenosine supplements, and in control (n = 30)

Semen samples	Swollen spermatozoa (%)
Control	70.8 ± 12.7
With pentoxifylline	82.8 ± 7.7
With 2-deoxyadenosine	83.0 ± 9.5

in the semen samples supplemented with pentoxifylline and 2-deoxyadenosine were both significantly higher than those in control (82.8 ± 7.7 and 83.0 ± 9.5 vs 70.8 ± 12.7; $p < 0.001$). Comparing the percentage of swollen spermatozoa between pentoxifylline and 2-deoxyadenosine supplements, there was no statistical significant differences ($p = 0.898$).

Discussion

Many compounds have been assessed in the search for an effective sperm stimulant. Both pentoxifylline and 2-deoxyadenosine are known to increase intracellular cAMP levels by stimulation of adenylate cyclase, hence may result in an adequate stimulation to restore impaired motility and increase the proportion of hyperactivated sperm cells[13]. Numerous studies have reported the use of pentoxifylline for the enhancement of sperm motility and fertilizing capacity in asthenospermia, and even for the selection of totally immotile testicular spermatozoa[13-16]. 2-Deoxyadenosine induced a 21-39% increase in the proportion of motile cells, changes in motility characteristics, particularly linear velocity but also in the frequency of sperm-head rotation[2]. In the zona-free hamster test, the percentage of penetrated oocytes was 80.8% for 2-deoxyadenosine, 92.9% for pentoxifylline, and 85.5% for pentoxifylline + 2-deoxyadenosine[2].

In addition to sperm motility, sperm membrane function is also useful in assessing male infertility. There are limited reports concerning the effects of pentoxifylline or 2-deoxyadenosine on sperm membrane integrity. Ponce et al[17] studied the effects of pentoxifylline on membrane functional integrity of mouse spermatozoa and found that there was a positive correlation between the percentage of tail swelling and motile spermatozoa. Besides sperm membrane function, the HOS test was also successfully used to differentiate between non-viable and viable immotile spermatozoa for use in intracytoplasmic sperm injection (ICSI), resulting in higher fertilization and pregnancy rate[11,12,18].

In the present study, the authors used the HOS test to evaluate two sperm stimulants, pentoxifylline and 2-deoxyadenosine, for their effects on sperm membrane functional integrity. The authors found that the percentage of swollen spermatozoa in the semen supplemented with pentoxifylline and 2-deoxy adenosine were both significantly higher than those without supplement using as a control. When comparing between pentoxifylline and 2-deoxyadenosine, there was no significant difference of the percentage of swollen spermatozoa. The results encourage the use of these two compounds to improve sperm quality in treating infertile couples by assisted reproduction. Both Yovich et al[19] and Imoedemhe et al[7] have reported respectively that pentoxifylline or 2-deoxyadenosine significantly improved fertilization *in vitro*, and with no detrimental effects to the embryos and the fetuses. However, Scott and Smith[20] have reported that both pentoxifylline and 2-deoxyadenosine have adverse effects on mouse oocytes or embryos at concentrations commonly used to activate sperm in human IVF. Hence, after the incubation, spermatozoa from the stimulants-treated samples should be washed and resuspended in culture medium before using it for insemination.

In conclusion, the present study shows that the addition of pentoxifylline or 2-deoxyadenosine to the sperm prepared with the two-layer Percoll gradient method can almost equally enhance sperm membrane integrity. It may be beneficial to add one of these compounds to sperm preparation for use in intrauterine insemination (IUI) or IVF. However, it is clear that further studies are required to confirm its clinical importance in assisted reproduction.

References

1. Mbizvo MT, Johnston RC, Baker GH. The effect of

the motility stimulants, caffeine, pentoxifylline, and 2-deoxyadenosine on hyperactivation of cryopreserved human sperm. Fertil Steril 1993; 59: 1112-7.
2. Lanzafame F, Chapman MG, Guglielmino A, Gearon CM, Forman RG. Pharmacological stimulation of sperm motility. Hum Reprod 1994; 9: 192-9.
3. Choavaratana R, Manoch D, Treeratanaporn N, Kunathikom S. Induction of acrosome reaction by calcium ionophore A 23187 in sperm separated by two-layer percoll gradient method. Siriraj Hosp Gaz 2003; 55: 473-7.
4. Tesarik J, Thebault A, Testart J. Effect of pentoxifylline on sperm movement characteristics in normozoospermic and asthenozoospermic specimens. Hum Reprod 1992; 7: 1257-63.
5. Kay VJ, Coutts JR, Robertson L. Pentoxifylline stimulates hyperactivation in human spermatozoa. Hum Reprod 1993; 8: 727-31.
6. Tasdemir M, Tasdemir I, Kodama H, Tanaka T. Pentoxifylline-enhanced acrosome reaction correlates with fertilization in vitro. Hum Reprod 1993; 8: 2102-7.
7. Imoedemhe DA, Sigue AB, Pacpaco EL, Olazo AB. In vitro fertilization and embryonic development of oocytes fertilized by sperm treated with 2-deoxyadenosine. Int J Fertil Menopausal Stud 1993; 38: 235-40.
8. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J Reprod Fertil 1984; 70: 219-28.
9. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999: 28-31, 68-70.
10. Jeyendran RS, Van der Ven HH, Zaneveld LJ. The hypoosmotic swelling test: an update. Arch Androl 1992; 29: 105-16.
11. Casper RF, Meriano JS, Jarvi KA, Cowan L, Lucato ML. The hypo-osmotic swelling test for selection of viable sperm for intracytoplasmic sperm injection in men with complete asthenozoospermia. Fertil Steril 1996; 65: 972-6.
12. Sallam HN, Farrag A, Agameya AF, El Garem Y, Ezzeldin F. The use of the modified hypo-osmotic swelling test for the selection of immotile testicular spermatozoa in patients treated with ICSI: a randomized controlled study. Hum Reprod 2005; 20: 3435-40.
13. Tournaye H, Wieme P, Janssens R, Verheyen G, Devroey P, Van Steirteghem A. Incubation of spermatozoa from asthenozoospermic semen samples with pentoxifylline and 2-deoxyadenosine: variability in hyperactivation and acrosome reaction rates. Hum Reprod 1994; 9: 2038-43.
14. Rizk B, Fountain S, Avery S, Palmer C, Blayney M, Macnamee M, et al. Successful use of pentoxifylline in male-factor infertility and previous failure of in vitro fertilization: a prospective randomized study. J Assist Reprod Genet 1995; 12: 710-4.
15. Terriou P, Hans E, Giorgetti C, Spach JL, Salzmann J, Urrutia V, et al. Pentoxifylline initiates motility in spontaneously immotile epididymal and testicular spermatozoa and allows normal fertilization, pregnancy, and birth after intracytoplasmic sperm injection. J Assist Reprod Genet 2000; 17: 194-9.
16. Matyas S, Papp G, Kovacs P, Balogh I, Rajczy K, Kopa Z, et al. Intracytoplasmic sperm injection with motile and immotile frozen-thawed testicular spermatozoa (the Hungarian experience). Andrologia 2005; 37: 25-8.
17. Ponce AA, Fiol dC, Ruiz RD, Vincenti LM, Santilla ME, Stutz G, et al. Influence of pentoxifylline on sperm membrane functional integrity. Arch Androl 1999; 43: 77-84.
18. Peeraer K, Nijs M, Raick D, Ombelet W. Pregnancy after ICSI with ejaculated immotile spermatozoa from a patient with immotile cilia syndrome: a case report and review of the literature. Reprod Biomed Online 2004; 9: 659-63.
19. Yovich JM, Edirisinghe WR, Cummins JM, Yovich JL. Influence of pentoxifylline in severe male factor infertility. Fertil Steril 1990; 53: 715-22.
20. Scott L, Smith S. Human sperm motility-enhancing agents have detrimental effects on mouse oocytes and embryos. Fertil Steril 1995; 63: 166-75.

การศึกษาเปรียบเทียบระหว่างอสุจิที่ได้รับการใส่สารเพนทอกซิฟิลลิน และ2-ดีออกซิอะดิโนซีน โดยใช้วิธีทดสอบการบวมน้ำของหางอสุจิ

พิทักษ์ เลาห์เกริกเกียรติ, สมบูรณ์ คุณาธิคม, เรืองศิลป์ เชาวรัตน์, สมสิญจน์ เพ็ชรยิ้ม, จปรัฐ ปรีชาพานิช

วัตถุประสงค์: *เพื่อศึกษาเปรียบเทียบจำนวนร้อยละของอสุจิที่มีการบวมน้ำของหางภายใต้ภาวะไฮโปออสโมติก ระหว่างอสุจิกลุ่มที่ใส่สารเพนทอกซิฟิลลินและสาร 2-ดีออกซิอะดิโนซีน*

ชนิดของการวิจัย: *การวิจัยเชิงทดลอง*

วัสดุและวิธีการ: *นำน้ำอสุจิของคู่สมรสที่มีบุตรยากฝ่ายชายซึ่งผลการตรวจวิเคราะห์ปกติ จำนวน 30 ตัวอย่าง มาเตรียมด้วยวิธีเปอร์คอลล์สองชั้น แบ่งน้ำอสุจิที่ผ่านการเตรียมแล้วออกเป็น 3 ส่วน ส่วนแรกใส่สารเพนทอกซิฟิลลิน ส่วนที่สองใส่สาร 2-ดีออกซิอะดิโนซีน และส่วนที่สามไม่ใส่สารใด ๆ หลังจากนั้น นำน้ำอสุจิทั้ง 3 ส่วนผสมกับสารละลายไฮโปออสโมติก แล้วตรวจดูการบวมน้ำของหางอสุจิ*

ผลการศึกษา: *จำนวนร้อยละของอสุจิที่มีการบวมน้ำของหางในกลุ่มที่ใส่สารเพนทอกซิฟิลลินและ 2-ดีออกซิอะดิโนซีน มากกว่าในกลุ่มที่ไม่ได้ใส่สาร อย่างมีนัยสำคัญทางสถิติ (82.8 $\pm$ 7.7 และ 83.0 $\pm$ 9.5 ต่อ 70.8 $\pm$ 12.7; $p < 0.001$) ส่วนเมื่อเปรียบเทียบระหว่างการบวมน้ำของหางอสุจิระหว่างกลุ่มที่ใส่สารเพนทอกซิฟิลลินและ 2-ดีออกซิอะดิโนซีน ไม่พบความแตกต่างอย่างมีนัยสำคัญ ($p = 0.898$)*

สรุป: *การเติมสารเพนทอกซิฟิลลินและ 2-ดีออกซิอะดิโนซีน สามารถเพิ่มฟังก์ชั่นของผนังอสุจิที่ผ่านการเตรียมด้วยวิธีเปอร์คอลล์สองชั้นได้เท่าเทียมกัน สารทั้งสองดังกล่าวจึงอาจมีประโยชน์ในการนำมาใช้กับการเตรียมอสุจิเพื่อใช้ในขั้นตอนของการช่วยการเจริญพันธุ์*