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

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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 ± 7.7 and 83.0 ± 9.5 vs 70.8 ± 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

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 A23187 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
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

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

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การศึกษาเปรียบเทียบระหว่างอสุจิที่ได้รับการใส่สารเพนทอกซิฟิลลิน และ 2-ดีออกซิอะดิโนซีน
โดยใช้วิธีทดสอบการบวมน้ำของหางอสุจิ

พิทักษ์ เล่าเก่กิจยรัตน์, สมบูรณ์ คุณาธิคม, เรืองศิลป์ เสาวรัตน์, สมสิญจน์ เฟื่องยิ้ม, จปรัฐ ปรีชาพานิช

วัตถุประสงค์: เพื่อศึกษาเปรียบเทียบจำนวนร้อยละของอสุจิที่มีการบวมน้ำของหางภายใต้ภาวะไฮпоออสโมติก ในระหว่างกลุ่มที่ใส่สารเพนทอกซิฟิลลินและกลุ่ม 2-ดีออกซิอะดิโนซีน

ชนิดของการวิจัย: การวิจัยเชิงทดลอง

วัสดุและวิธีการ: นำน้ำอสุจิของคู่สมรสที่มีบุตรยากฝ่ายชายซึ่งผลการตรวจวิเคราะห์ปกติ จำนวน 30 ตัวอย่าง มาเตรียมโดยวิธีแบบสูงสองชั้น แบ่งน้ำอสุจิที่ผ่านการเตรียมแล้วออกเป็น 3 ส่วน สำนวน โดยสารเพนทอกซิฟิลลิน ส่วนที่สองใส่สาร 2-ดีออกซิอะดิโนซีน และส่วนที่สามไม่ใส่สารใด ๆ หลังจากนั้น นำน้ำอสุจิที่ 3 ส่วนผสมกับสารละลายไฮโปออสโมติก และตรวจสอบการบวมน้ำของหางอสุจิ

ผลการศึกษา: จำนวนร้อยละของอสุจิที่มีการบวมน้ำของหางในกลุ่มที่ใส่สารเพนทอกซิฟิลลินและกลุ่ม 2-ดีออกซิอะดิโนซีน มากกว่าในกลุ่มที่ไม่ใส่สาร อย่างมีนัยสำคัญทางสถิติ (82.8 ± 7.7 และ 83.0 ± 9.5 ต่อ 70.8 ± 12.7; p < 0.001) ส่วนเมื่อเปรียบเทียบระหว่างการบวมน้ำของหางในกลุ่มที่ใส่สารเพนทอกซิฟิลลินและกลุ่ม 2-ดีออกซิอะดิโนซีน ไม่พบความแตกต่างอย่างมีนัยสำคัญ (p = 0.898)

สรุป: การเติมสารเพนทอกซิฟิลลินและ 2-ดีออกซิอะดิโนซีน สามารถเพิ่มพัดกั้นของน้ำอสุจิที่ผ่านการเตรียมโดยวิธีแบบสูงสองชั้นได้มากเทียบกัน สำหรับผลดังกล่าวอาจมีประโยชน์ในการนำมาใช้กับการเตรียมอสุจิเพื่อใช้ในขั้นตอนของการช่วยให้เกิดการเจริญพันธุ์