Induction of Acrosome Reaction by Calcium Ionophore A23187 in Sperm Separated by Two-layer Percoll Gradient Method

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Abstract : **The aim of this study was to compare the percentages of sperm with an acrosome reaction between those with and without calcium ionophore A23187 induction after two-layer Percoll gradient separation. Thirty normal semen samples were obtained from the male partners of infertile couples attending the Infertility Clinic at Siriraj Hospital. After the process of sperm separation by two-layer Percoll gradient technique, the final samples were divided into 2 portions. An aliquot of 10 μM of calcium ionophore A23187 was added to one portion to induce an acrosome reaction, while the other portion was used as a control. Fluorescein isothiocyanate-conjugated Pisum sativum agglutinin (FITC-PSA) staining was performed on both specimens and the acrosome reacted-sperm were evaluated. The percentage of acrosome-reacted sperm in the calcium ionophore A23187 induced group was significantly higher than those of the control group (24.8 ± 6.6 vs 15.4 ± 6.0 ; $p < 0.001$). It is concluded that calcium ionophore can significantly induce an acrosome reaction on sperm separated by two-layer Percoll gradient technique, and it may be beneficial to add calcium ionophore A23187 to sperm preparation for use in IUI or IVF.**

Key words : Acrosome reaction, calcium ionophore A23187, Percoll gradient

เรื่องย่อ : การชักนำให้เกิดอะโครโซมรีแอคชั่นโดยสารแคลเซียมไอโอโนฟอร์ เอ 23187 ในอสุจิที่แยกด้วยวิธีเปอร์คอลสองชั้น

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จุดประสงค์ของการศึกษานี้เพื่อเปรียบเทียบจำนวนร้อยละของอสุจิที่เกิดอะโครโซมรีแอคชั่นในอสุจิที่ผ่านการเตรียมด้วยวิธีเปอร์คอลสองชั้น ระหว่างอสุจิที่เติมและไม่เติมสารแคลเซียมไอโอโนฟอร์ เอ 23187 ได้นำน้ำอสุจิของคู่สมรสที่มีบุตรยากฝ่ายชายซึ่งผลการตรวจวิเคราะห์ปกติ ที่มารับการตรวจรักษาที่สาขาวิชาผู้มีบุตร

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ยาก ภาควิชาสูติศาสตร์-นรีเวชวิทยา จำนวน 30 ตัวอย่าง มาเตรียมด้วยวิธีเปอร์คอลสองชั้น แบ่งน้ำอสุจิที่ผ่านการเตรียมแล้วออกเป็น 2 ส่วน ส่วนแรกเติมสารแคลเซียมไอโอโนฟอร์ เอ 23187 จำนวน 10 ไมโครโมล และส่วนที่สองไม่เติมสารดังกล่าว หลังจากนั้นทำการตรวจนับจำนวนร้อยละของอสุจิที่เกิดอะโครโซมรีแอคชั่นโดยวิธีย้อมสารเรืองแสงและนับผ่านกล้องฟลูออเรสเซนต์ ผลปรากฏว่าร้อยละของอสุจิที่เกิดอะโครโซมรีแอคชั่นในกลุ่มที่เติมสารแคลเซียมไอโอโนฟอร์ เอ 23187 มากกว่ากลุ่มที่ไม่ได้เติมสารดังกล่าวอย่างมีนัยสำคัญทางสถิติ (24.8 ± 6.6 และ 15.4 ± 6.0 ; $p < 0.001$) สรุปได้ว่าสารแคลเซียมไอโอโนฟอร์ เอ 23187 สามารถเพิ่มการเกิดอะโครโซมรีแอคชั่นของอสุจิที่ผ่านการเตรียมด้วยวิธีเปอร์คอลสองชั้น สารดังกล่าวจึงอาจมีประโยชน์ในการนำมาใช้กับการเตรียมอสุจิที่ใช้สำหรับการฉีดอสุจิเข้าสู่โพรงมดลูกและการปฏิสนธินอกร่างกาย

INTRODUCTION

Intrauterine insemination (IUI) in conjunction with ovarian stimulation is usually offered to infertile couples with non-tubal factors, prior to using other assisted reproductive techniques. The role of standard semen parameters in predicting the probability of a successful outcome of IUI is still controversial.[1,2] There is now growing evidence that specialized sperm function testing is a better predictor of human fertilization than traditional semen parameters.[3,4] The acrosome reaction (AR) is essential for sperm penetration through the zona pellucida and for preparing fusion of sperm with the oolemma.[5] AR can be induced by chemicals such as calcium ionophore A23187 by generating an intracellular calcium signal which sets in motion a sequence of membrane-associated changes culminating in AR.[6] Therefore the AR ionophore challenge test has been used as a predictor of sperm fertilizing ability.[7]

Various sperm separation techniques have been applied to select motile sperm fractions, free from seminal plasma, for IUI and other assisted reproductive techniques.[8] Among all separation techniques, the discontinuous two-layer Percoll gradient method has been widely used in Thailand. This method has been claimed to improve fertilization rate in vitro.[9]

The aim of this study was to compare AR of Percoll separated sperm between the samples with and without calcium ionophore A23187 induction.

MATERIALS AND METHODS

Semen samples

Thirty semen samples were obtained from the male partners of infertile couples attending the Infertility Clinic, Siriraj Hospital, after 3-5 days of sexual abstinence. The ejaculates were collected by masturbation into a sterile container and allowed to liquefy. All semen parameters were normal according to the recommendation of the World Health Organization.[10]

Two-layer Percoll gradient

The stock solution of Percoll was prepared by mixing 9 volumes of Percoll with 1 volume of 10 times concentrated Ham's F-10 medium. Further dilutions were made using HTF (human tubal fluid) medium. A layer of 1.5 ml of 40% Percoll was layered over 1.5 ml of 80% Percoll in a 15 - ml conical tube. One milliliter of the semen was gently layered over this gradient and the tube was then centrifuged for 20 minutes at 600x g. The upper two layers were aspirated off until the 80% Percoll interface was reached. The remaining 80% layer at the bottom of the tube was resuspended in 2 ml of HTF medium and centrifuged for 8 minutes at 250x g. The final pellet was resuspended to a final volume of 1.0 ml of HTF. The final samples were divided into two 5-ml tubes, 0.5 ml each. Aliquots of 10 μM of calcium ionophore A23187 was added to one tube and coded. Both tubes were incubated for 30 minutes at 37°C in

5% CO_2 in air, then a sample from each tube was smeared on a glass slide.

Acrosome assessment

After air-drying, the smear was fixed in 95% ethanol for 30 minutes, washed in distilled water 2-3 times and stained for 45 minutes with 25 µg/ml Pisum sativum agglutinin labelled with fluorescein isothiocyanate (PSA;Sigma) in pH 7.4 Dulbecco phosphate buffered saline at 4°C. Finally, the slide was washed 2-3 times in distilled water and mounted with glycerine; 200 spermatozoa were counted using a fluorescence microscope and oil immersion at a magnification of x400. When more than half the head of a spermatozoon was brightly and uniformly fluoresced, the acrosome was considered as "unreacted". Spermatozoa without fluorescence or with a fluorescing band limited to the equatorial segment were considered to be acrosome-reacted. The person assessing the slides was unaware of the treatment of the sample.

Statistical analysis

The results were decoded and analyzed by SPSS for MS WINDOWS using a paired t-test. The level of significance was set at $p < 0.05$.

RESULTS

Semen characteristics of 30 samples obtained from 30 men with a mean age of 36 ± 5 years (range 26-45 years) are shown in Table 1.

The AR status of sperm separated by two-layer Percoll gradient is shown in Table 2. The samples with calcium ionophore A 23187 induction demonstrated a significantly higher percentage of acrosome reaction compared with the non-induced samples (24.8 ± 6.6 vs 15.4 ± 6.0 ; $p < 0.001$).

Table 1. Semen characteristics of the samples.

Semen parameters	Mean ± SD (range)
Volume (ml)	2.5 ± 0.3 (2.0 - 3.5)
pH	7.6 ± 0.2 (7.3 - 8.0)
Sperm concentration ($x10^6$/ml)	39.7 ± 11.1 (21.0 - 63.0)
Total sperm count ($x10^6$)	137.5 ± 13.0 (110.0 - 159.0)
Motility (%)	56.0 ± 6.0 (50.0 - 68.0)
Normal morphology (%)	42.7 ± 6.0 (34.0 - 55.0)
Viability (% live)	78.3 ± 4.9 (75.0 - 90.0)
White blood cells ($x10^6$/ml)	0.3 ± 0.3 (0 - 0.9)

Table 2. Comparison of acrosome-reacted sperm after Percoll separation between samples with and without calcium ionophore A23187 induction.

	Calcium ionophore A23187		P - value
	(+)	(-)	
Acrosome reaction (%)	24.8 ± 6.6	15.4 ± 6.0	< 0.001

Values show in mean ± standard deviation

DISCUSSION

Substantial information regarding the human sperm acrosome reaction (AR) and its relevance during the fertilization processes has accumulated during the past few years.[11,12] A low percentage of acrosome-reacted sperm after induction has been shown to be associated with a low fertilization rate in IVF (in vitro fertilization) and IUI.[13,14] Moreover, AR was significantly related to fertilization rates in vitro in teratozoospermic patients in male infertility.[15] The combined parameters of acrosome-reacted sperm, total number of motile and morphologically normal sperm had predictive value for pregnancy in infertile couples using IUI and IVF.[14,16]

In this study, it was clearly demonstrated that calcium ionophore A23187 induced a higher percentage of acrosome reaction compared with non-induced sperm after separation by two - layer Percoll gradient. AR was conducted only on sperm after preparation since sperm in raw semen are incapable of undergoing induced AR. This is consistent with other reports which showed that calcium ionophore induced-sperm significantly increased complete acrosome reaction as compared to the controls.[7,15-17] Ionophore is a known inducer substance for AR and it has no deleterious effect on the chromosome complement of the sperm.[18] Moreover, A23187 is the most effective AR inducer as compared to progesterone and follicular fluid.[19] In a recent report by Makkar et al,[20] it was shown that the ARIC (acrosome reaction ionophore challenge) score that was calculated by subtracting the spontaneous AR from the ionophore induced AR, was a better predictor of pregnancy than conventional sperm parameters and sperm velocities.

In conclusion, calcium ionophore can significantly induce acrosome reaction on sperm of normal semen after two-layer Percoll gradient separation. The addition of this compound to sperm preparations used for IUI or IVF should be considered.

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