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A PROCEDURE FOR THE PURIFICATION OF RABBIT SPERM CELLS AND THE SEPARATION OF SPERM HEADS AND TAILS

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Centrifugation of rabbit semen through a sucrose density gradient was used to remove contaminating cell debris, prostatic vesicular bodies, and accessory gland secretions from the sperm cells. Sperm heads could be detached from tails by brief sonication, and heads and tails could be separated by centrifugation through a discontinuous sucrose density gradient. The sperm cells, heads, and tails obtained in this manner were suitable for biochemical studies.

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A PROCEDURE FOR THE PURIFICATION OF RABBIT SPERM CELLS AND THE SEPARATION OF SPERM HEADS AND TAILS

1. INTRODUCTION

Sperm cells collected from mammals by electroejaculation or with an artificial vagina are contaminated by secretions and fluids from the accessory glands, cell debris, and particulate matter such as prostatic vesicular bodies.[1] Seminal plasma is commonly removed by several washes with buffer or medium and centrifugation. Sperm cells washed by this procedure are unsuitable for many biochemical studies because traces of seminal plasma remain, and particulate matter and cell debris are still present.[1,2] Accessory gland secretions and particulate matter can be effectively removed by centrifugation of sperm cells through a discontinuous sucrose density gradient. Sperm cells washed in this manner can be used for many biochemical studies.[2,3] The study of neurotransmitter receptors in rabbit sperm cells required large quantities of sperm cells and separated sperm heads and tails. A procedure, based on discontinuous sucrose density centrifugation, was developed for the large scale preparation of uncontaminated rabbit sperm cells and sperm heads and tails.

2. MATERIALS AND METHODS

2.1 Animals.

New Zealand white rabbits were individually housed in standard rabbit cages in a room maintained at 75 ± 5 °F and 50 ± 10% humidity with a 12-hr light/dark cycle. Standard laboratory rabbit chow and water were available ad libitum.

2.2 Collection of Sperm Cells.

Semen was collected with an artificial vagina.[4] Collection frequency was not more than once on alternate days.

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[1]Mann, T., and Lutwak-Mann, C., Male Reproductive Function and Semen, Chapter 3, Springer-Verlag, New York, NY, 1981.

[2]Young, R.J., "On the Integrity of Sperm DNA," Gamete Res. Vol. 2, p 223 (1979).

[3]Young, R.J., "Rabbit Sperm Chromatin is Decondensed by a Thiol-Induced Proteolytic Activity Not Endogenous to Its Nucleus," Bio. Reprod. Vol. 20, p 1001 (1979).

[4]First, N.L., "Collection and Preservation of Spermatozoa," In Methods in Mammalian Embryology, p 15, Ed. J.C. Daniel Jr., Freeman, San Francisco, CA, 1971.

A log was kept of the collection frequency and semen volume obtained from each rabbit.

2.3 Centrifugation of Sperm Cells.

2.3.1 Removal of Seminal Plasma.

Pooled semen was centrifuged at 10,000 x g at 5 °C for 3 min to remove seminal plasma. The sperm cell pellet was suspended in ST buffer (0.05M Tris-HCl, 0.15M NaCl, pH 7.4), and the yield of sperm cells was determined by counting in a Makler Counting Chamber (Sefi-Medical Instruments, Haifa, Isreal) under phase contrast optics (X100).

2.3.2 Removal of Debris.

The sperm cell suspension (up to 3 x 10^9 cells in 0.5-1 mL) was layered on top of a discontinuous sucrose density gradient (1 mL 2.2M, 5 mL 2M, 5 mL 1.8M sucrose in ST buffer) formed in a 16 by 102-mm cellulose acetate or polyallomer centrifuge tube. This suspension was centrifuged at 25,000 rpm (116,000 x g) for 1 hr at 5 °C in the 17-mL buckets of the SW 26 rotor (Beckmen Instruments, Fullerton, CA) or AH629 rotor (DuPont Instruments, Wilmington, DE).

2.4 Suspension and Sonication of Sperm Cells.

Sperm cells freed of seminal plasma and the particulate matter described above were suspended in 1 or 2 mL of ST buffer at a concentration of 2 x 10^9 cell/mL and placed in a 3.5 by 1.4-cm tube immersed in an ice bath. The cell suspension was sonicated in a sonicator (Heat Systems-Ultrasonics, Incorporated, Farmington, NY) equipped with a mircroprobe and set at 80%-pulse and 25-30% energy output until few intact sperm cells were visible under phase-contrast microscopy. A sonication time of 3-5 min was usually sufficient.

2.5 Separation of Sperm Heads and Tails.

The suspension of sonicated sperm cells was layered on top of a sucrose density gradient (1 mL 2.2M, 5 mL 2M, 5 mL 1.8M sucrose in ST buffer) formed in a 25.4 by 89-mm ultra thick polycarbonate centrifuge tube (DuPont Instruments, Wilmington, DE) and centrifuged at 25,000 rpm (112,000 x g) for 1 hr at 5 °C in the 36-mL buckets of the AH629 rotor. The sperm pellet was resuspended in 2 mL of ST buffer, layered on top of a sucrose density gradient (3 mL 2.2M, 10 mL 2M sucrose in ST buffer) formed in a 16 by 102-mm centrifuge tube and centrifuged at 25,000 rpm (116,000 x g) for 45 min at 5 °C in the 17-mL buckets of the AH629 rotor.

3. RESULTS

Cell debris, vesicles, membranes, and other pariculate matter were distributed throughout the 1.8M and 2M sucrose layers when seminal plasma-free sperm cells were centrifuged through a 1.8M, 2M, and 2.2M sucrose density gradient. The contaminants were concentrated in opalescent bands on the surface of the 1.8M sucrose layer and at the interface of the 1.8M and 2M sucrose layers, but significant amounts were also present in the clear sucrose layers. The 2.2M sucrose layer was cloudy and contained predominately intact sperm cells with some vesicles and debris. Sperm cells uncontaminated with debris, vesicles, or particulate matter were found in the pellet.

The fractions obtained by centrifugation of a sperm cell sonicate through a 1.8M, 2M discontinuous sucrose density gradient are summarized in Table 1. Opalescent bands were present on the surface of the 1.8M sucrose layer and at the interface of the 1.8M and 2M sucrose layers. The former contained tail fragments and a few heads, while these and some intact sperm cells were also present in the latter. The pellet was composed of sperm heads and some intact sperm and tail fragments. Contaminating tail fragments were removed by a second centrifugation through a 2M, 2.2M discontinuous sucrose density gradient (Table 2). Tail fragments were concentrated on the surface of the 2M sucrose layer as an opalescent band. The pellet was almost entirely sperm heads; no tail fragments and only four intact sperm cells were found in 500 heads counted over several fields. Recovery of sperm heads was 95%.

Table 1. Distribution of Sperm Cell Sonicated Over Sucrose Density Gradient.

Fraction	Description	Contents
Surface 1.8M sucrose	Opalescent	Tail fragments, membranes
1.8M sucrose layer	Clear	Tail fragments, membranes
Interface 1.8M and 2M sucrose layers	Opalescent	Tail fragments, some intact sperm cells
2M Sucrose layer	Clear	Tail fragments, occasional head and intact sperm cell
Pellet	- - - -	Heads, some intact sperm cells and tail fragments

Table 2. Distribution of Sperm Head Pellet Over Sucrose Density Gradient.

Fraction	Description	Contents
Surface 2M sucrose	Opalescent	Tail fragments
2M Sucrose layer	Clear	Tail fragments
2.2M Sucrose layer	Clear	Heads and intact sperm cells
Pellet	- - - -	Heads, occasional intact sperm cell

4. DISCUSSION

The sperm nucleus consists almost entirely of closely packed and highly condensed chromatin; therefore, the sperm cell is quite dense. Separation, by sucrose density gradient centrifugation, of sperm cells from contaminants, or of heads and tails, is based on the density difference between sperm cells and debris and vesicles, and between sperm heads and tails. Intact sperm cells form a pellet, but the contaminating debris and vesicles, being less dense, are distributed in the sucrose layers. The reason for the presence of sperm cells in the 2.2M sucrose layer is unknown. Two explanations are possible; centrifugation time was insufficient to completely pellet the sperm cells, or the sperm cells in this layer represent a separate class of less dense cells. Upon sonication, sperm tails break into fragments; but, under the chosen conditions, the head remains intact. In the first centrifugation, because of the quantity present, some tail fragments sedimented with the dense heads. The tail fragments were completely removed by a second centrifugation through a longer column of more concentrated sucrose solution.

5. CONCLUSION

The techniques of sonication and sucrose density centrifugation can be used to obtain large quantities of sperm heads free of tail fragments and sperm tails free of heads.