

Spermatogenic cells isolation

Layla Simón

Abstract

After euthanasia, testicles were obtained, tunica albuginea was removed with sterile forceps, and seminiferous tubules were treated with collagenase for 10 minutes at 37°C (5 mg/ml of collagenase, SIGMA C5138). Using a trans-illumination method, cycles of spermatogenesis were detected. Sections of seminiferous tubules, developing the Golgi-acrosome complex in stages VI to VIII, were selected and cut under stereoscope. The tubules were placed on slides containing 40 µl PBS and extruded with a cover-slip in order to obtain the spermatogenic cells. Spermatogenic cells were fixed and smeared on slides or transferred to microtubes.

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Materials

✓ PBS by Contributed by users

🧪 Collagenase c5138 by Sigma

✓ Euthanyle [View](#) by Contributed by users

Protocol

Euthanasia

Step 1.

Rabbits were sacrificed applying a lethal dose of pentobarbital via pinna marginal veins

📄 AMOUNT

1 ml Additional info:
Euthanyle

Tissue collection

Step 2.

Testicles were surgically obtained and placed on Petri dishes with PBS.

Tunica albuginea was removed with sterile forceps and seminiferous tubules were treated with collagenase.

📄 CONCENTRATION

5 Mass/Volume Percent
Additional info: 5 mg/mL
collagenase in PBS

 **DURATION**

00:10:00 Additional info: at 37 °C

Seminiferous tubules selection

Step 3.

Seminiferous tubules were selected according to the spermatogenic cycle. Specifically, tubules with cells under acrosome development were isolated (stages VI to VIII). Seminiferous tubules in these stages were detected by trans-illumination method. Tubules with dark lumen (full of spermatids and sperm cells) were selected.

 **LINK:**

doi: 10.1002/jemt.1070320504

Spermatogenic cells obtaining

Step 4.

Selected seminiferous tubules were placed on slides with PBS. Tubules were extruded with a cover-slip in order to obtain spermatogenic cells. Spermatogenic cells were fixed with paraformaldehyde in PBS. Cells were smeared on slides or transferred to microtubes.

 **CONCENTRATION**

4 Mass/Volume Percent
Additional info:
paraformaldehyde