

SEPARATION OF ROOSTER SPERMATOZOA IN SILICA-BASED COLLOIDAL MEDIUM BY CENTRIFUGATION

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Introduction

Moderate sperm separation can eliminate debris, immature and abnormal spermatozoa from semen ejaculation to collect functional spermatozoa for improving the efficiency of fertilization. Density gradient media have been used extensively in separating cells. However, only a few studies have attempted this technology in rooster spermatozoa. Therefore, the purpose of this study was to evaluate the effects of two silica-based colloidal media on rooster spermatozoa separation. The data obtained from these experiments will benefit the development of a feasible sperm purification protocol for the rooster.

Materials and Methods

Forty adult Taiwan Native chicken L7, L9, L11 and L12 roosters in Taiwan were used in this study. Semen was routinely collected once a week, and pooled semen was diluted 1:3 with PBS immediately after. The isotonic Percoll[®] and Percoll[®] PLUS (GE Healthcare, Uppsala, Sweden) were mixed with 1x PBS to prepare 60%, 70%, 80% and 90% Percoll and Percoll PLUS solutions, respectively, and then transferred 2 mL of these solutions each to a 15 mL conical plastic centrifuge tube. Diluted semen was gently placed on top of the solutions and centrifuged at 800 g for 20 minutes. After centrifugation, the sperm pellet was washed with PBS and resuspended in Poultry media (IMV, L'Aigle, France). Sperm viability and motility were analyzed by microcapillary flow cytometry (Guava Technologies Inc., Hayward, CA, USA; distributed by IMV Technologies) and CASA (Ceros II, IMV, L'Aigle, France).

Results and Discussion

The percentage of viable/motile/progressive sperm was significantly enhanced after Percoll 70%, 80% and 90% centrifugation by 13.1%/14.3%/26.0%, 17.3%/16.1%/27.3%, and 19.8%/16.4%/28.0%, respectively ($P < 0.01$). The similar results were also found in Percoll PLUS 70%, 80% and 90% centrifugation with 11.1%/13.3%/23.6%, 17.7%/15.7%/21.4%, and 17.4%/16.8%/19.9% increments, respectively ($P < 0.01$). Our results initially showed that either Percoll or Percoll PLUS centrifugation could be a practicable procedure for rooster spermatozoa separation. Further studies on the effects of these procedures on the fertilizing capacity of rooster spermatozoa will be determined.