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Evaluation of culture systems for attachment and proliferation of epithelial cells cultured from ovine semen

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ABSTRACT

Different culture systems were evaluated for their ability to support attachment and proliferation of the somatic cells obtained from ovine semen. Ejaculates ($n = 14$) were collected from eight rams representing three breeds, Dorper, Suffolk and Hampshire. All samples were processed immediately and somatic cells were obtained from 11 of the 14 ejaculates. These cells had classic epithelial morphology and expressed cytokeratin, indicating they were of epithelial origin. Cells from four rams with the greatest growth rates were used for subsequent studies. Cells were cultured in four different media for 5 days and total numbers of attached cells vs. total numbers of seeded cells were counted and compared each day. Four media were evaluated: (1) a supplemented medium composed of DMEM/F12, 10% fetal bovine serum (FBS), 10 ng/ml epidermal growth factor, 30 μg/ml bovine pituitary extract, 5 μg/ml insulin, 10 ng/ml cholera toxin, and 50 μg/ml gentamycin; (2) sheep fetal fibroblast (SFF)-conditioned medium; (3) swiss 3T3 fibroblast-conditioned medium; and (4) basic medium composed of DMEM/F12, 10% FBS, and 50 μg/ml gentamycin. Cell proliferation was greater in the supplemented medium, SFF-conditioned medium, and 3T3 fibroblast-conditioned medium compared to the basic medium by day 2 of culture ($p < 0.05$, $n = 24$), and greater in supplemented medium compared to the SFF-conditioned medium and 3T3 fibroblast-conditioned medium by day 4 of culture ($p < 0.05$, $n = 24$). Three different surfaces: (1) Matrigel basement membrane matrix-coated plastic; (2) collagen I-coated plastic; and (3) uncoated plastic

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were evaluated for their ability to support proliferation and attachment of the cells obtained from semen. Cell proliferation was greater when cells were cultured on the Matrigel-coated compared to the collagen I-coated and uncoated plastic by day 2 of culture ($p < 0.05$, $n = 16$). Cell attachment was greater when cells were plated on the Matrigel-coated and collagen I-coated plastic compared to the uncoated plastic ($p < 0.05$, $n = 16$). These studies describe an effective system for the culture and proliferation of epithelial cells obtained from ovine semen samples. The system may increase the likelihood of obtaining cells from frozen semen, which could be used for cloning to recover animals of genetic value in which semen is the only material that is available.

1. Introduction

There have been many cases that animals of genetic value have died and the only material available from these animals is frozen semen. Somatic cells in semen are a potential source of nuclei for cloning animals by somatic cell nuclear transfer. Culture of the cells from frozen semen, if possible, would be extremely valuable for preservation or restoration of endangered, exotic, and extinct animals when other ways of obtaining somatic cells are not available.

Extensive work has been done to identify the types of somatic cells in human semen. Cells found are epithelial cells, and various major leukocyte subpopulations including granulocytes, monocytes/macrophages, B lymphocytes, helper and suppressor/cytotoxic T lymphocytes (Phillips et al., 1978; Wolff and Anderson, 1988; Homyk et al., 1990). When human ejaculates were cultured, epithelial cells were obtained (Phillips et al., 1978).

Cells cultured from sheep and eland semen have been identified as epithelial or epithelial-like by the presence of cytokeratin (Nel-Themaat et al., 2007, 2008). In the present study, cells cultured from ram semen were assessed by more robust immunofluorescence analysis which distinguished epithelial cells (cytokeratin), mesenchymal cells (vimentin), and smooth muscle cells (α-smooth muscle actin) based on differentiation markers that each type of cell expressed.

Somatic cells have been successfully cultured from fresh and cooled ram semen, but not fast frozen semen (Nel-Themaat et al., 2007). Attempts to culture somatic cells from frozen semen in our laboratory have been unsuccessful. The culture system established herein promotes proliferation and attachment of the cells obtained from fresh ram semen samples, and may increase the likelihood of obtaining cell growth from frozen semen, which could be used for cloning to recover animals of genetic value.

2. Materials and methods

2.1. Animal care and semen collection

All procedures involving animals were approved by the Texas A&M University Institutional Animal Care and Use Committee.

Eight rams were used in the study, which included one 18-month-old Dorper, one 17-month-old Suffolk, three 18-month-old Suffolks, one 14-month-old Hampshire, and two 18-month-old Hampshires. All of the animals were in good health condition. Ejaculates ($n = 14$) were collected by electroejaculation or artificial vagina, with one to three samples being collected from each animal. Volumes of ejaculates obtained ranged from 0.5 to 2 ml.

2.2. Isolation and culture of the somatic cells in ram semen

Somatic cells were isolated using the method of Nel-Themaat et al. (2008) with minor modifications. Semen samples were extended by adding an equal volume of DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; Gibco, Grand Island, NY, USA), evenly distributed on top of two to