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Original Article

Establishment and characterization of female reproductive tract epithelial cell culture

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ABSTRACT

The oviductal and uterine epithelial cells have a crucial role, but are still poorly understood. Numerous studies have tried to isolate the epithelial cells from different organs in various models. The current study aimed to establish and characterize an *in vitro* monolayer culture of the oviduct and uterine horn epithelial cells by using two different techniques. Female reproductive epithelial cells from sows were cultured in follicular phase. Combined protocols to isolate the epithelial cells were performed. The viability and cell number were determined. Monolayers of epithelial cells from each group were cultured in four-well plates and were subjected to immunostaining using a Vector ABC Elite Kit. The immunohistochemical staining step was performed to evaluate the quality of the epithelial cells. Oviductal cells reached confluence faster than uterine horn cells. Cilia were seen in oviduct and uterine horn tissue culture. All the isolated cells reached confluence prior to harvesting. The number of cells was increased over the time of incubation. Monolayer culture using the trypsin/EDTA method took longer than culture with the collagenase method. Immunohistochemistry of epithelial cells showed strong staining for cytokeratin. Oviductal and uterus epithelial cells were cultured and established. Both techniques used in this experiment were useful and showed no significant differences. This cell culture model has the potential to study the secretory interactions of the female reproductive tract with spermatozoa, oocytesor embryos.

1. Introduction

In vitro cultures of cells from the female reproductive tract of different species, including humans, have been successfully established and characterized [1–5]. Numerous studies have tried to isolate epithelial cells from different organs in various models such as rats [6], cattle [5], swine [7], and humans [8,9]. Several protocols and techniques have been used to isolate the epithelial cells from different organs and organisms.

Epithelial cells are formed in the early stage of embryo development. Three distinct layers are formed, the ectoderm, mesoderm, and endoderm. The mesoderm lies between the ectoderm and endoderm. The mesoderm generates skeletal and connective tissues, while the ectoderm and endoderm generate the epithelial layers [10]. The function of epithelial cells is to transport essential nutrients, oxygen, fluids, and ions. They also secrete several components and produce certain signals. The generation of these

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signals could be due to other factors or can be generated by itself [11].

In term of physiological processes, the epithelium can be found either internally in organs or exteriorly on their surface. *In vivo*, epithelial cells are the site for malignant transformation due to their regenerative nature. Therefore, the epithelium is considered in the studies of several types of tumors [12]. The malignant tissues which composed of epithelium cells that derived for carcinogenesis tumor in prostate, lung, breast [10]. In histological sections, the stroma cell, basement membrane, is acting to separated the epithelial cell layer from other tissue. The stromal cell is also acting to regulate the different functions of the epithelial cells [13]. Squamous epithelial cells have been also studied in skin cells and determined the physiology and pathology of keratinocytes [14].

In vitro cultures are simpler and easier to establish compared with *in vivo* cultures. However, there are still several shortcomings in using *in vitro* cell culture. Specific features and functions of the oviductal epithelium are lost during *in vitro* culture [2]. Some studies showed that an increased number of oviduct culture passages resulted in decreased expression of several genes [8,15]. In addition, cell morphology alterations have also been reported with continuous cell culture [16,17].

Thibodeaux et al [5] determined a method to isolate and culture endometrial epithelium from adult bovine tissue using a trypsin–EDTA protocol. They maintained good cell viability for up to eight subcultures. In addition, a good level of post-thaw subculture was also determined. Cell separation refers to separation of epithelial cells from fibroblasts. Different components in the culture medium support epithelial cell growth and exclude fibroblasts. A simple vascular monolayer could be used for subculture and is sufficient for production of multilayers [18].

Prichard et al [19] investigated the ability to coculture oviduct and uterine cells from goats. They examined the progress of embryo development in each group with different cells. Eventually, they concluded that coculture of the embryo with oviductal or uterine cells yielded a higher rate of development. However, coculture of the embryo in medium with oviductal and uterine cells together did not improve embryo development.

In this study, we aimed to establish and characterize an *in vitro* monolayer culture of oviduct epithelial cells (OVECs) and uterine horn epithelial cells (UHECs) using two different techniques. Immunohistochemical staining was performed to confirm that the cells were epithelial cells.

2. Materials and methods

2.1. Sample collection and preparation

Reproductive tracts were obtained from sows from the local slaughterhouse (G. Wood and sons, Mansfield, UK) and transferred to the laboratory at ambient temperature. Ovaries that showed dominant follicle growth and absence of corpus luteum were selected as follicular phase. Samples in luteal phase, ovaries with pathological signs, or ambiguous samples were discarded. Samples of 15 oviducts and uterine horns were prepared at lengths of 20 cm and 15 cm, respectively. The excess tissues were trimmed away. The uterine horns and oviducts were washed three times and flushed with 37 °C phosphate-buffered saline (PBS; Gibco, Invitrogen, Paisley, UK) without magnesium and calcium, supplemented with 100 μg/mL streptomycin + 100 U/mL penicillin + 0.25 μg/mL amphotericin B mix antibiotics (Sigma, Poole, Dorset, UK). Epithelial cells were isolated from the oviducts and uterine horns.

2.2. Epithelial cell isolation from oviducts and uterine horns

Two different protocols for cell isolation were performed. The first protocol was based on an enzymatic method and the other on a mechanical or scraping technique [7]. Two separate methods were performed based on an enzymatic protocol; one with 0.25% trypsin–EDTA, as described by Thibodeaux et al [5] with some modifications. The other enzymatic method used collagenase digestion as described by Freshney [10]. Fifteen samples from each group (oviduct and uterine horn) were used. M199 medium (Invitrogen) was used as the culture medium. M199 was supplemented with 10% fetal bovine serum (Invitrogen) and 100 μg/mL streptomycin + 100 U/mL penicillin + 0.25 μg/mL amphotericin B mix (Sigma).

2.3. The 0.25% trypsin–EDTA method

Samples were kept in warm PBS (no Mg^{2+} or Ca^{2+}; Gibco) after flushing with PBS. One end of each sample was sealed tightly with cotton thread. Three to five millimeters of warm 0.25% trypsin–EDTA was injected into the sample using a 10-mL syringe, and the sample was sealed from the other side. The sealed samples were incubated at 39 °C with 5% CO_2 for 90 minutes. The cotton threads were then cut at both ends. Each sample (oviduct and uterine horn) was milked well to collect the fluid. An equal volume of the culture medium was added to the 50-mL centrifuge tube. The tubes were centrifuged at 300 *g* for 10 minutes. The pellet was resuspended in M199 medium and washed with PBS. This step was repeated two times to ensure high quality and clean pellet. Red blood cell lysis buffer was added to remove the red blood cells. The cells were washed and added to 5 mL M199 medium. The viability test was performed by 0.4% trypan blue exclusion (Sigma) to evaluate cellular integrity. Five milliliters of M199 medium was added to the pellet, which was divided into several tissue culture flasks (T-75; Nalge Nunc International, Naperville, IL, USA). The flasks were incubated in air at 39 °C and 5% CO_2. The culture medium was replaced every 12 hours.

2.4. Collagenase digestion

Collagenase Type 1A (Sigma) was prepared with Hank's Balanced Salt Solution (HBSS) without magnesium or calcium (Invitrogen). The prewarmed collagenase was filled inside the uterine horn and oviduct. The oviduct and

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