Isolation and culture of bovine oviductal epithelial cells for use in the anatomy and physiology laboratory and undergraduate research

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Isolation and culture of bovine oviductal epithelial cells for use in the anatomy and physiology laboratory and undergraduate research. Adv Physiol Educ 30: 237–241, 2006; doi:10.1152/advan.00064.2005.—This article presents methods for the isolation and culture of epithelial cells from the bovine oviduct for use in both research and the teaching laboratory and provides examples of ways that an oviductal cell culture can be incorporated into an undergraduate research program. Cow reproductive tracts are readily available from area butchers, and the procedure for isolation of the epithelium is simple and inexpensive. The cells can be observed immediately after isolation or can be cultured for up to 72 h under simple conditions for observation over several days. For experimental use, epithelial cells are cultured in standard cell culture medium, where they continue to divide and actively secrete substances into the medium. The ease with which the tissue can be collected and isolated makes the oviductal epithelium ideal for use in both the teaching laboratory and research projects in which undergraduates serve as investigators.

A STANDARD COMPONENT of our introductory Anatomy and Physiology course is a unit on tissues, with lecture material concentrating on the structure and function of each tissue and with laboratory exercises that typically include viewing histology slides of each tissue type through a light microscope. Although useful for distinguishing differences between the tissue types, the static nature of the exercise is often viewed as boring by the laboratory participants.

To provide a more interactive learning environment, our students collected epithelial cells from their oral mucosa to be stained and viewed by simple microscopy. This provides the students with more of a hands-on role in the laboratory and is a welcomed addition to the standard histological laboratory exercise. Unfortunately, these cells can only be used during the current class time and cannot be cultured and maintained for further evaluation or investigation. The ability to successfully culture cells for an extended period of time is dependent on identifying a source material that is both easy to obtain as well as abundant in quantity. A simple, abundant, and unique source of the epithelium can be collected from female domestic animal reproductive tracts harvested shortly after animals have been slaughtered. The material is easily obtained from local butchers and can be transported back to the laboratory for preparation. Once in the laboratory, epithelial cells can be isolated by a standard procedure, and many characteristics of this tissue type can be observed over several days under simple culture conditions. One reliable source of the epithelium that is readily available and relatively easy to maintain in culture is from the bovine oviduct, or uterine tube. The ease with which this tissue can be harvested makes it ideal for use in both laboratory exercises and undergraduate research projects. Students enrolled in Introductory Physiology or Anatomy and Physiology courses can dissect the bovine reproductive tract and isolate epithelial cells as part of their laboratory experience. Additionally, students participating in upper-level undergraduate research projects have isolated the cells for prolonged culture and experimentation.

This article describes the collection, isolation, and culture of columnar epithelial cells from the oviduct of the bovine reproductive tract for both experimental and educational purposes. In addition, this protocol can easily be adapted for use with pig or sheep oviducts if those tissues are more readily available.

MATERIALS AND METHODS

Recovery of oviduct epithelial cells. Bovine oviducts, obtained from a local slaughterhouse, are transported back to the laboratory. Local butcher shops are usually willing to provide them, although it is often simpler to request the entire reproductive tract rather than just the oviducts. The oviducts can be easily separated from the reproductive tract at the laboratory. There are no special handling instructions for the tracts once they have been isolated, and they can sit in plastic bags at ambient temperature for several hours until the oviducts are removed.

Once in the laboratory, the oviducts are excised from the reproductive tract (Fig. 1), washed twice in warm (37–39°C) sterile PBS, and trimmed of excess connective tissue. PBS can be purchased or made (0.877 g NaCl and 0.142 g NaHPO4 in 100 ml distilled water, pH 7.4). If PBS is unavailable, sterile 0.9% saline prepared with distilled water can be used. After the oviducts are cut from the rest of the reproductive tract, removal of additional tissue from the oviducts prevents gross contamination of the culture with nonepithelial cells. This can be done on a clean cutting board with dissection instruments. The addition of 1% (vol/vol) penicillin-streptomycin antibiotic solution to the PBS can inhibit bacterial growth during culture if contamination with fecal material is a concern. The antibiotic stock contains 10,000 U/ml penicillin and 10,000 μg/ml streptomycin (17-602E, BioWhittaker).

Once trimmed, epithelial cells are easily extruded from the lumen of the oviduct by lightly pressing a clean microscope slide over the exterior surface of the oviduct (Fig. 2). This separates epithelial cells from the lamina propria and pushes them out of the ampulla of the oviduct. The extrusion method of collection is a widely accepted technique that effectively dislodges epithelial cells with minimal disruption of the lamina propria (17). It is easiest to move the slide toward the infundibulum as the opening here is larger than the one created where the oviduct was removed from the uterus.

Epithelial cells are extruded into ~5 ml warm (37–39°C) sterile PBS in sterile 100 × 50-mm petri dishes (08-757-12, Fisher Scientific), collected with transfer pipets, and placed into conical tubes containing 5–8 ml of warm (37–39°C) PBS. Over 10 min, cells settle to the bottom of the tube, and the PBS can be removed by pipet. If cells are resuspended in fresh 37–39°C PBS and the process is complete.
cells are extruded into warm, sterile phosphate-buffered saline solution.

With this protocol, it is typical to achieve isolated cells consisting of >90% epithelial cells (17). If desired, it can be validated that they are epithelial cells by immunocytochemical detection of cytokeratin 8.13 using a commercially available antibody (Sigma Chemical). This antibody recognizes subtypes of the acidic and basic cytokeratin family that are identical to epithelial cytokeratins found in the bovine oviduct and provides a means to discriminate between cells of epithelial and stromal origin (15).

For immediate use in the teaching laboratory, washed cells can be suspended in sterile PBS, dispensed into small culture dishes or watch glasses, and observed by light microscopy. After isolation, the cells typically form spheres that move through the culture dish due to movement of the cilia. This is easily observed by simple light microscopy and does not require staining.

Culture of oviductal epithelial cells. For longer use, oviduct cells require culture at 37–39°C in PBS supplemented with 10% (vol/vol) fetal bovine serum (26140-087, GIBCO-BRL, Invitrogen Life Technologies) and 1% (vol/vol) penicillin-streptomycin. Culture in PBS in a standard incubator is not ideal, but an adequate number of cells will survive for at least 72 h to provide an opportunity for observation in the classroom laboratory over several days. Small incubators are relatively inexpensive and can be humidified by placing a beaker of distilled water inside the incubator. If the cells are cultured in PBS rather than standard culture medium, the PBS must be replaced daily.

If the washed cells are to be used for experimental purposes, they should be suspended in culture medium that has been supplemented with 5% (vol/vol) fetal bovine serum and 1% (vol/vol) penicillin-streptomycin. As these are epithelial cells with simple nutrient requirements, most standard cell culture medium will work, provided that at least 5% fetal bovine serum is added to the medium. This gives the instructor a lot of flexibility to use the resources available at their institution. Tissue Culture Medium 199 is commonly used (M-5017, Sigma Chemical), as is DMEM (11960-044, Invitrogen), both of which yield satisfactory growth.

The cells and medium are dispensed into sterile petri dishes; the size of the culture dish will depend on the nature of the experiment. Common cell culture dishes range from 60 × 15-mm circular dishes to multiwell culture plates with well diameters from 6.4 to 35 mm. These can be purchased from vendors that sell tissue culture supplies (e.g., BD Falcon tissue culture dishes, 60 × 15 mm, catalog no. 08-772F, Fisher Scientific). Cells are cultured at 39°C, 5% (vol/vol) CO₂ in air, for 72 h to allow the cells to plate or adhere to the culture dish. They divide rapidly and soon cover the bottom of the culture dish (Fig. 3). Until the culture becomes confluent, culture medium is changed every 2–3 days (17). A confluent monolayer of cells can be obtained 5–7 days after cell have been plated, and at this point they are ready for experimentation.

After cells have become confluent, they can be gently lifted from culture with a cell lifter and replated. Cell lifters or scrapers allow for the quick removal of cells from culture dishes with minimal damage and can be obtained from most laboratory supply companies (e.g., catalog no. 08-773-1, Fisher Scientific). It is not necessary to calculate the exact numbers of cells recovered during scraping to dilute and replate the cells. A simple 1:10 dilution of cells is usually adequate for replating, and this can be adjusted through trial and error as needed for different types of culture conditions and experimental purposes. The culture can continue for weeks, and cells can be lifted and replated many times. The cells will continue to grow and divide and secrete substances into the culture medium. Cell growth can be determined by lifting the cells and using a hemocytometer or other type of counting chamber (e.g., catalog no. 02-671-5, Fisher Scientific). Cell viability can be determined at the same time by staining the cells that are to be counted with a viability stain such as trypan blue (T-8154, Sigma Chemical).

RESULTS AND DISCUSSION

Discussion of methods for collection and culture of oviductal cells. The ease with which oviductal epithelial cells can be collected and subsequently cultured makes them ideally suited for use in Anatomy and Physiology courses in which under-
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graduates perform the isolation and culture. It is not imperative that the tissue be collected and cells isolated immediately after animals are slaughtered. Entire reproductive tracts that have remained at ambient temperature for over 7 h have provided ample live oviduct epithelial cells for the creation of cultures.

Epithelial cells can also be removed from the oviducts enzymatically, but the mechanical isolation procedure is simple, inexpensive, and has been found to be more reliable and result in the least amount of cellular damage. Additionally, mechanical isolation detaches epithelial cells from the basal lamina without damaging the lamina propria of mucosal folds (17). This helps to reduce contamination with nonepithelial cells.

Although a humidified CO2 incubator is ideal and certainly should be used for long-term experimentation, something as simple as storing tubes of cells in a 37–39°C water bath can be used to keep the cells alive for several hours, which would be long enough for observation in an introductory laboratory course. Despite the source of the material, simple aseptic techniques and antibiotic-supplemented medium are all that is needed to inhibit bacterial growth. Laminar flow culture hoods can be used, but they are not essential. All of the oviductal cell preparation described above was conducted on the laboratory bench top without any contamination of the cultures, and it is possible to change the medium regularly without the benefit of a laminar flow hood, provided that the medium and plasticware are sterile, antibiotics are used, and efforts are made to reduce exposure of the cells and medium to air. This can be done by working in a fume hood that is not turned on and by keeping the lids on the culture dishes in place until just before the medium is changed.

Using oviductal epithelial cells in the teaching laboratory. As educators in the field of physiology, we take advantage of the abundance of butcher shop “leftovers” for laboratory dissection. By obtaining living as well as preserved tissues, we can utilize these tissues and organs to enhance student understanding of physiology and to teach experimentation. The procedure described here provides an excellent opportunity for a reproductive system lesson if students are allowed to excise the epithelial cells themselves. The female bovine reproductive tract shares many similarities to the human reproductive tract. In fact, reproductive strategies in both species are quite similar, making the cow an excellent animal model for learning about human reproductive anatomy.

Reproduction is always a favorite topic of students, and an exercise where they can first observe a reproductive tract and then proceed to isolate and culture the epithelial cells provides a nice complement to the reproduction lecture. This can be accomplished by providing a brief overview of reproductive anatomy and a demonstration on how to isolate the cells before allowing the students to perform the cell isolation themselves. Students enjoy this very hands-on laboratory experience and find the cell isolation procedure to be simple. They routinely comment that the reproductive structures appeared differently than they had imagined them to look and that they have a better understanding of reproductive anatomy after participating in this laboratory exercise.

As mentioned above, this protocol can be used to isolate cells from the oviducts of many different species, including the cow, sheep, pig, and white-tailed deer. In addition, by preparing the oviducts prior to recovery of the epithelial cells, the students gain an understanding of the anatomy of the oviduct, whose layers mimic those seen in blood vessels, the renal tubule, and other tubal structures within the body (6, 7, 12). If students are harvesting the cells, the exercise will take approximately 1 h/oviduct. If combined with an anatomy lesson on the reproductive tract and some minor experimentation with the cells, the entire exercise will take approximately 3 h to complete, with a followup evaluation of the cells 72 h later.

The isolated epithelial cells can also be maintained in culture for multiweek laboratory activities. Students have found it rewarding to actively participate in the collection of cells that they can then culture and observe over several weeks, and they list this as one of their favorite components of the laboratory experience. Despite their lack of experience with aseptic techniques, it is rare that the cultures become contaminated. It is while the cells are in culture that experimentation can take place.

For example, because the cells continue to divide in culture, it is possible to incorporate this activity into a laboratory exercise on cell division. The cells can be the subject of experimentation in which variables such as temperature, pH, and culture medium are altered to evaluate their impact on cell survival and growth. Oviduct epithelial cells begin to grow in vivo as columnar epithelial cells (2), but, over time, their morphology changes, and eventually they lose their cilia and resemble squamous or cuboidal epithelium. Monitoring this change in morphology could provide students an opportunity to discuss environmental requirements needed by cells to maintain normal morphology and function and how in vitro conditions differ from the in situ environment. In all of these experiments, students observe the characteristics of live epithelial cells in culture through the use of simple light microscopy.

Use of oviductal epithelial cells for student-designed research. There is a tremendous value in involving undergraduates in scientific research, as a hands-on research experience provides opportunities for learning that cannot be duplicated in the lecture room or classroom laboratory. The oviduct cell cultures described above have provided my students with a relatively simple system for observation and experimentation.

In the experiments described below, each student formulated his or her own hypothesis. Students were responsible for the care and maintenance of all cell cultures in addition to conducting the experiments. Proper utilization of aseptic techniques is critical to successful cell culture, and students had an extensive opportunity to learn the value and importance of proper aseptic techniques. The majority of the undergraduates at our campus plan to enter a healthcare field, so this learning opportunity was especially beneficial to them. All of the projects to date have involved extensive use of microscopy. More cellular detail can be observed with sophisticated microscopes, but simple light microscopy was adequate for most projects.

Students who indicated interest in research were invited to participate in an independent research project after the completion of the Anatomy and Physiology course. The majority of the students were second-semester sophomores or juniors, and they conducted research over the course of an academic semester. They developed a research proposal after consultation with the faculty sponsor and conducted the research after approval by a faculty committee.
One student in my laboratory sought to determine how treating cultured oviduct epithelial cells with norepinephrine affected their ability to synthesize and secrete proteins. Norepinephrine has been identified in bovine oviductal fluid (18). The mRNA for β₂-adrenergic receptors has been found on bovine oviduct epithelial cells (5), and studies have indicated that the formation of oviductal fluid in rabbits can be stimulated by β-adrenergic agonists (4). For this project, oviduct cells were treated after they had been plated with concentrations of norepinephrine that represented physiological concentrations of norepinephrine found in bovine oviductal fluid (18). After treatment, the medium conditioned by the oviduct epithelial cells was collected and evaluated for differences in total protein concentration (1) as well as differences in the banding pattern of these proteins when the medium was evaluated by protein gel electrophoresis (11). These additional techniques provided students an opportunity to learn more about the characteristics of proteins and about experimental methods used to investigate proteins.

Subsequent students examined the medium conditioned by the oviductal epithelial cells for proteins previously identified in oviductal fluid (3) using antibodies specific to those proteins and Western blot analysis techniques.

Another student was interested in the sperm reservoir formed by the oviduct. In cattle (9) and many other domestic species (8, 10), the isthmus region of the oviduct forms a reservoir for sperm, which attach to the epithelium and are released at the time of ovulation. In this study, bovine sperm were added to confluent monolayers of oviduct epithelial cells to evaluate sperm binding to the epithelium.

These are just a few examples of research projects that could be generated from a simple culture of oviduct epithelial cells. The original student project in our laboratory initiated a research program that was perpetuated by subsequent students. The research material collected by the first student was used by additional students, and, as is expected in scientific research, the first student’s results helped to steer the direction of future studies. The students were involved in every aspect of the project from sample collection to data interpretation. Their research was conducted in the teaching laboratory, which allowed other students to observe the projects as they progressed and stimulated significant student discussion and interest. This ancillary benefit cannot be overlooked, as it resulted in additional students participating in research projects. All of the students went on to express how positive the research experience was for them, which encouraged additional students to participate.

Any student at our university who participates in research as an independent study project is required to submit a written report, with interpretation of the results and justification for this interpretation. In addition, students involved in projects with this investigator were required to present orally to peers in the Anatomy and Physiology class required of our nursing and allied health majors. Students who participated described the experience as beneficial to their understanding of the physiological related to their project and as increasing their interest in scientific discovery.

The literature describing both qualitative and quantitative analysis of the undergraduate research experience is very positive. Undergraduates who participate in research projects describe gains in skills, personal and professional development, and clarification in their career or future educational goals (13, 16). The students surveyed valued research advisors who were knowledgeable, available, patient, and enthusiastic. In fact, these traits were more important to the students than whether an advisor was well known in his or her field, externally funded, or working at the cutting edge of research in that discipline (14). These student perceptions emphasize that a lot can be accomplished even in a small facility, given that the research advisor makes a commitment to the program. Of the skills obtained from their research experience, students listed technical skills, problem-solving skills, and development of a healthy professional self-confidence as the most valuable. This speaks to the universal benefits of hands-on research experiences as many of the skills described are life skills with wide-ranging applications.

In conclusion, isolation and culture of oviduct epithelial cells from the bovine reproductive tract provides a simple, abundant source of cells for observation, culture, and experimentation. Although the culture methods for experimentation are more narrowly defined, a simple modified version can be used for observation in the teaching laboratory. Student research projects can be generated from this type of cell isolation and culture, providing the undergraduate student with the opportunity to conduct scientific research as part of their academic experience.

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