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# *Technical note:* Method for isolation of the bovine sweat gland and conditions for in vitro culture

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## ABSTRACT

Apocrine sweat glands in bovine skin are involved in thermoregulation. Human, horse, and sheep sweat gland epithelial cells have been isolated and grown in vitro. The present study was conducted to identify a method to isolate bovine sweat glands and culture apocrine bovine sweat gland epithelial cells in vitro. Mechanical shearing, collagenase digestion, centrifugation, and neutral red staining were used to identify and isolate the apocrine glands from skin. Bovine sweat glands in situ and after isolation comprised 2 major cell types consisting of a single layer of cuboidal epithelial cells resting on a layer of myoepithelial cells. In situ, the glands were embedded in a collagen matrix primarily comprising fibroblasts, and some of these cells were also present in the isolated material. The isolated material was transferred to complete medium (keratinocyte serum-free medium, bovine pituitary extract, and human recombinant epidermal growth factor + 2.5% fetal bovine serum) in a T 25 flask (Falcon, Franklin Lakes, NJ) with media film and then incubated at 37°C for 24 h. After sweat glands adhered to the bottom of the flask, an additional 2 mL of complete medium was added and the medium was changed every 3 d. Isolated apocrine sweat glands and bovine sweat gland epithelial cells were immunostained for cytokeratin and fibroblast specific protein, indicating fibroblast-free cultures.
**Key words:** apocrine sweat gland, bovine, isolation

## Technical Note

The mammalian sweat gland is known to be involved in secretion, excretion, and thermoregulation (Saga, 2002). Research into the role of the apocrine sweat glands of cattle in heat regulation has been undertaken by various investigators (Yamane and Ono, 1936; Brody, 1945; Findlay, 1950; Findlay and Yang, 1950). Although apocrine glands contribute little to thermal cooling in humans, they are the only effective sweat glands in hoofed animals such as the donkey, cow, horse, and camel (Bullard et al., 1970). Evaporation becomes the major route of heat loss as ambient temperature approaches core body temperature because it works on a vapor pressure gradient rather than a temperature gradient (Collier and Gebremedhin, 2015), and about 70 to 85 percent of maximal heat loss via evaporation occurs through the skin above the thermoneutral zone in cattle (Finch, 1986). Animal factors that affect the efficiency of cutaneous evaporative heat loss include hair coat density and thickness, hair length and color, skin color, and density of sweat glands (Klungland and Vage, 2003; Olson et al., 2003). Cattle with shorter hair that is greater in diameter and lighter in color are better adapted to heat stress than cattle with longer, darker hair (Bertipaglia et al., 2007). This was believed to be due to the fact that evaporation rate is greater from skin in cattle with short hair (Bertipaglia et al., 2007). This was confirmed by Dikmen et al. (2008).

The isolation and culture of sweat gland epithelial cells can provide powerful tools for studying the structure and function of sweat glands at the cellular level. However, the isolation and culture of sweat gland epithelial cells is very time consuming (Hongpaisan et al., 1996; Lei et al., 2008), and there is currently no published method for isolation of the bovine apocrine sweat gland. Because the isolation of bovine sweat gland epithelial cells has never been reported, the current study was conducted to identify a method to isolate bovine sweat glands and culture apocrine bovine sweat gland epithelial cells in vitro. Brayden and Fitzpatrick (1995) demonstrated that human eccrine sweat glands take up neutral red, which can be used as a marker to identify sweat glands during isolation. Based on the traditional method of isolation and culture of human sweat gland epithelial cells, Mörk et al. (1995)

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modified and improved later by Tao et al. (2010), we developed a new method for the isolation of bovine apocrine sweat glands. We hypothesized that we could modify techniques used to isolate eccrine sweat glands from humans and apocrine sweat glands from horse and sheep skin to isolate the sweat gland of the bovine.

All procedures used in this study were conducted under a protocol approved by the University of Arizona Animal Care and Use Committee. Sweat glands were isolated from the skin of udders taken from cattle processed at the University of Arizona abattoir. Full-thickness noncauterized skin samples were taken from fresh healthy cow udders from slaughter at the University of Arizona Food Products and Safety Lab (Tucson) to the research laboratory within 15 min while stored on ice. The skin was sprayed with 70% ethanol followed by betadine for sterilization and was shaved using an electric razor, followed by a disposable razor to remove as much hair as possible. Using a scalpel, an 8-cm × 8-cm square was cut from the udder and as much fat was removed as possible. Media and other solutions were routinely prepared by a volume/volume ratio unless otherwise stated. The tissue was added to a 50-mL conical centrifuge tube (Corning Inc., Corning, New York) that contained 10 mL of PBS with 0.1% gentamicin and 0.1% solution of penicillin (10,000 units) and streptomycin (10 mg/mL; Sigma-Aldrich, St. Louis, MO). Sections of the 8-cm × 8-cm square were additionally cut into smaller pieces in a sterile hood and were transferred into a preweighed Petri dish containing wash medium comprising 10 mL of sterile M199 (Sigma-Aldrich), 0.1% gentamicin, and 0.1% antibiotic/antimycotic (Sigma-Aldrich). The tissue weight was recorded so that 1 g of tissue was in 10 mL of medium. The skin was thinly sliced, in a serial manner, using a Stadie-Riggs hand microtome (Thomas Scientific, Swedesboro, NJ) and was immediately returned to the Petri dish.

Once all skin samples (~5 g) were thinly sliced, the tissue was placed on a sterile cutting board and wetted with medium in a sterile hood. Using standard single-edge razor blades, the tissue was minced to the consistency of applesauce for no longer than 10 min and then returned to the wash medium. This medium with tissue was added to a 250-mL fluted flask that contained the daytime digestion medium: 0.2% (wt/vol) type II collagenase (Worthington, Lakewood, NJ), an adaptation from Collier et al. (2006), medium M199 with 0.1% antibiotic/antimycotic and 5% FBS (Sigma-Aldrich), 5% (wt/vol) neutral red (Sigma-Aldrich), a modification from Brayden and Fitzpatrick (1995), and the 10 mL of wash medium with minced skin, for a total of 50 mL. The flask was placed in a 37°C shaking incubator at 175 rpm for 60 min. The digested material was transferred to a 50-mL conical centrifuge tube (Corning Inc.) and was centrifuged at 400 × *g* for 4 min. The remaining pellet was placed into a tissue culture–treated T 75 flask (Corning Inc.) containing 15 mL of M199 with 0.1% gentamicin, 0.1% antibiotic/antimycotic, 0.02% collagenase, and 1% FBS (overnight digestion media) for 20 to 24 h in an incubator at 37°C and 5% $CO_2$.

After 20 to 24 h, the digested skin was transferred to a Petri dish (Corning Inc.), which was placed on ice. A dissecting microscope sitting in a sterile hood was used to separate the sweat glands from skin and hair follicles. A micropipette was used to transfer isolated sweat glands, stained red, to a Petri dish filled with 10 mL of keratinocyte medium (Gibco, Waltham, MA) completed with 2.5% FBS, 0.1% gentamicin, and 0.1% antibiotic/antimycotic, stored on ice during collection (Figure 1). As shown in Figure 1, the sweat glands are easily identified because they selectively take up the neutral red stain. Any concentration of neutral red or collagenase greater than 5% resulted in no cell growth. The isolated sweat glands were transferred from the Petri dish to a 15-mL conical centrifuge tube (GeneMate, Kayesville, UT) and spun at 400 × *g* for 4 min, and the medium was decanted. To separate the epithelial cells from the fibroblasts, the cultures were digested with trypsin for 2 min at 37°C. Fibroblasts were then washed away and epithelial cells remained in the flask. The trypsinization of epithelial cells was then performed (Freshney, 2010). Trypsin solution was decanted, and 1 mL of keratinocyte medium was added to resuspend the glands. The medium and glands were added to the floor of the T 25flask (Falcon) that was prewetted with 1 mL of keratinocyte medium or a 24-well plate (Corning Inc.) that had been prewetted for culturing or had a collagen gel matrix as reported by Hernandez et al. (2008). Glands were dispersed as broadly as possible, and the culture dish was then placed in an incubator at 37°C and 5% $CO_2$. The glands were allowed to attach to the dish for 24 to 48 h before 2 mL of medium was added to the T 25 flask.

The 24-well plate cultures contained about 7 to 10 glands per well that had been prewetted with 50 uL of keratinocyte medium or was collagen coated. The 24-well plate was also placed in a 37°C and 5% $CO_2$ incubator. The glands were allowed to attach to the dish for 24 to 48 h before 250 uL of medium was added to each well.

Isolated sweat glands and the cells that grew from them were grown in keratinocyte medium with 2.5% FBS as reported by Tao et al. (2010). This medium consisted of keratinocyte medium (Gibco), 2.5% FBS (Sigma-Aldrich), 50 μg/mL bovine pituitary extract (**bPE**; Gibco), 5 ng/mL of human epidermal growth factor (**hEGF**; Gibco), 0.1% gentamicin, and 0.1%