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Methodology to developing bovine mammary glandular structure in immuno-deficient mice

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Abstract
Raising Indian population leads to increases the future demand of protein for consumption. Especially milk protein demand growing annually, there has been increased activity in the research field of dairy production. But other side frequent outbreaks of diseases, vaccine failures and drug resistances developed against diseases in dairy animals which are the barriers to the meet out of future protein demand. So, want to increase the research activities in the field of drug discovery and development is very important now. But there has been no increasing activity in the new drug research due to practical difficulties in use live cattle for laboratory researches and preclinical studies. It is very easy when using laboratory animals like rodents for new drug development as well as the production of milk related research, as compared with live cattle. There is more attention need to develop a bovine mammary gland structure in laboratory mouse for bovine mammary gland related studies. In India, still we were lagging to develop such a mouse model. Therefore, in this review our prime objective of discussion is how to developing bovine mammary gland in mouse model.

Keywords: Bovine, mammary gland, transplantation, Mice

Introduction
The bovine mammary gland is a complex organ, made up of various cell types that work together for milk synthesis. Cell culture is n method which was successfully used to isolate bovine mammary epithelial cells. Collagenase dissociation was used successfully during isolation and culture of bovine epithelial cells *in vitro*. Mammary epithelial cell cultures have been widely used over the years as models to understand the physiological function of mammary gland. When using cell cultures/lines, it is inherently difficult to distinguish between primary mitogens and secondary regulators of mammary gland function/development. In this article discussing the isolated cells were not immortal, thus, additional work was required to develop a bovine mammary epithelial cell line.

Bovine mammary cell transplantation
Generally, farm animals such as cattle and horses are difficult to handle for experiments. The mouse, on the other hand, is the most commonly used mammalian research model for laboratory-scale experiments. Hence, it would be beneficial to develop a mouse model of bovine mammary alveolar ducts for laboratory scale-studies. Here, we aimed to generate the bovine mammary gland ductal structure from *in vitro* bovine mammary epithelial cell culture or cell lines transplanted into nude mice dorsal tissue. The primary mammary duct invades the mammary fat pad at E17, and formation of a small, branched ductal tree begins at this time and develops its shape [1]. A previous study had established a clonal cell line from primary bovine mammary alveolar cells (MAC-T) [2]. Prolactin was used to induce the Bovine mammary epithelial cell differentiation. This differentiation, immortality, and a population doubling takes time of approximately 17 h. These cells may be very useful for dairy protein synthesis studies.

Materials and Methods
Animals and Sample Collection
All research procedures have been carried out in full accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (67/99/ CPCSEA).

All the waste materials were disposed of in a safe and sanitary manner after performing the experiments. The mice having body weight between (Ages 6–8 weeks, 25–30 gm) of either sex were used. They were maintained under standard environmental conditions (temperature 23 ± 2 ◦C, relative humidity55 ± 10%, 12 h light and 12 h dark cycle) and were fed with standard pellet diet, and water *ad libitum*. For every experiment, fresh animals were used. Mammary tissue samples used in the present study were collected from Mammary tissue from cross breed heifers (*Bos indicus*; n = 20) was collected, frozen in liquid nitrogen.

Materials

The basal growth media was DMEM/F12 containing 10% fetal bovine serum (FBS) (Invitrogen). Induction media, which could promote the synthesis of milk protein and fat, was the growth media containing 5 µg/mL bovine insulin, 5 µg/mL bovine Holo-transferrin, 5 µg/mL progesterone, 10^{-7} mol/L hydrocortisone, 10 ng/mL bovine epithelial growth factor and 5 µg/mL bovine estradiol (Sigma-Aldrich, cat. #I4434, T1283, P8783, H0888, E4127, E2758, respectively). The storage media prepared freshly was composed of 90% fetal bovine serum and 10% DMSO. A solution of 0.25% trypsin-0.02% EDTA solution (Sigma-Aldrich) used for cell digestion was prepared and stored at −20°C until use.

Tissue Isolation

Bovine mammary tissue was obtained from a three-year-old mid-lactation dairy cow. Fresh tissue was placed in sterilized tubes containing ice-cold D-Hank's (balanced salt solution) and immediately transported to the laboratory. Tissue of ca. 1 cm^3 was washed with D-Hank's solution for several times until the solution was pellucid and without milk. Tissue was then cut into 0.5~1 mm^3 cubes and washed again with D-Hank's solution until clean. These smaller pieces of tissue were transferred with sterile tips onto empty plastic cell culture dishes (Corning, U.S.A) coated with collagen. Care was taken to ensure that tissue was kept wet. Culture dishes were incubated at 38°C and 5% CO_2 and were monitored closely every 30 min. If the adjacent area surrounding the tissue was dry, several drops of basal media were added ensuring that the tissue would not float and separate from the bottom of the culture dish. After 4 h, 0.5 mL basal media were added to every culture dish and 1 mL basal media were added after 12 h. The basal media was replaced with fresh media every 48 h until cells were visibly spread across the bottom of the culture dish. Cells were detached with 0.25% trpysin-0.02% EDTA and transferred to new culture dishes, which were used to remove fibroblasts. Subsequently, the pure mammary epithelial cells were isolated after 3 passages.

Growth Characteristics of Epithelial Cells

Growth curves and doubling time were determined by seeding 5×10^4 cells/well in 12-well flat-bottom culture plates (Corning 3513, U.S.A) containing induction media. Cell number and viability were determined each day in triplicate wells between 7 to 11 d post-seeding via trypan blue exclusion. Morphology of cultured cells was routinely evaluated with an inverted microscope with phase contrast (Olympus IX71, Japan), and photomicrographs were taken.

Bovine mammary epithelial cell line

Bovine mammary epithelial (BME) cell line is cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Gaithersburg, DM, USA) containing 25 mM glucose, supplemented with 10% fetal bovine serum (FBS; Welgene, Daejeon, South Korea), 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco 15140-122). The cells were grown in a humidified 5% CO2 atmosphere at 37o C.

Bovine mammary cell transplantation to Nude Mice:

Seven-week-old female BALB/C nude mice were purchase from Jackson lab, USA and animals were housed in an environmentally controlled room with IVC System (temperature: 23±2o C, relative humidity: 50±10%, programmed ventilation, and 12:12 h light-dark cycle) prior to experiment. All of the animal experiments will be approved by the Institutional Animal Ethical Committee (IACE), IIIM, Jammu. BME cells were suspended in BD Matrigel ™ diluted 1:1 (v/v) in Hank's Balanced Salt Solution (HBSS, Gibco). Cell suspension in Matrigel was injected into the 8 weeks male BALB/C nude mice dorsal using syringe for transplantation. After 6 weeks, small portion of the transplanted tissue was dissected from BALB/C nude mice and fixed in Bouin solution for immune-staining. For analyzing of bovine mammary gland protein expression, to perform a mammalian gland biopsy from fourteen-month calf and fixed in Bouin solution.

Result and discussion:

Our result revealed significant glandular structure formation in the mice dorsal tissues of udder. In addition the histological analysis revealed the difference between before and after transplantation of the dorsal tissue of mice and bovine mammary glandular structures (Fig. 1 and Fig. 2).

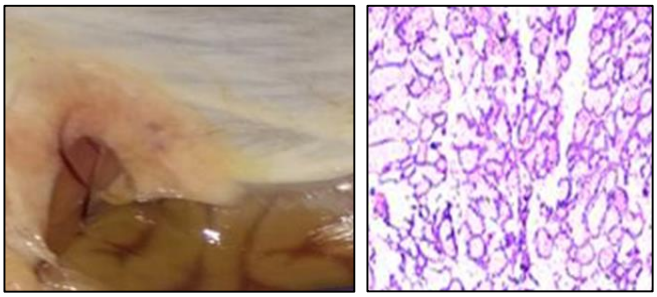

Fig 1: Before transplantation mice mammary glandular structure in control group and its hematoxylin and eosin (H&E) staining.

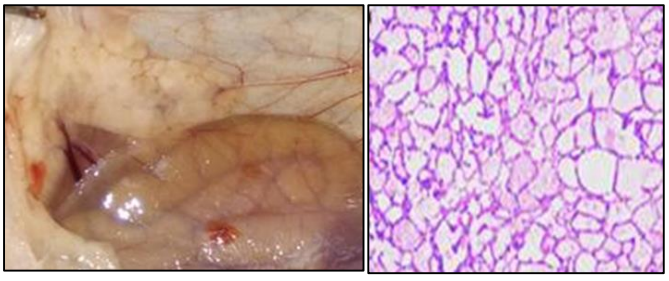

Fig 2: After transplantation Bovine mammary glandular structure in immunodeficient mice (nude mice) and its hematoxylin and eosin (H&E) staining.

Immunodeficient nude mice are defective in the natural killer cells as well as T and B cells. Which means both innate and humoral immunity defective that predominantly used for xenograft model development[6]. Our experiment suggested that these bovine mammary cells transplanted nude mice will

be useful for bovine immunity related cells against mastitis[7]. The morphogenesis of bovine mammary glandular structure is mutually regulated by mice hormones (GH), estrogen and insulin-like growth factor (IGF)-1 to generate a ductal tree[8]. During lactation, various autocrine growth factors such as of TGF*β*, IGF-1, and IGFBP-5 are implicated in the regulation of milk secretion[9].

Conclusion

It is concluded that using mammary gland transplanted mice models will be more reliable and accurate for the new drug development for mastitis as well as the production of milk related research, as compared with live cattle. There is more attention need to develop a bovine mammary gland structure in laboratory mouse for bovine mammary gland related studies. In India, still we were lagging to develop such a mouse model and further research studied required this kind of reliable transplant animal model for accurate drug targeting strategies.

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References

1. Parmar H, Cunha GR. Epithelial-stromal interactions in the mouse and human mammary gland *in vivo*. Endocr Relat Cancer 2004;11:437-458.
2. Huynh HT, Robitaille G, Turner JD. Establishment of bovine mammary epithelial cells (MAC-T): an *in vitro* model for bovine lactation. Exp Cell Res 1991;197:191-199.
3. Ryman VE, Packiriswamy N, Sordillo LM. Role of endothelial cells in bovine mammary gland health and disease. Anim Health Res Rev 2015;16:135-149.
4. Hovey RC, Trott JF, Vonderhaar BK. Establishing a framework for the functional mammary gland: from endocrinology to morphology. J Mammary Gland Biol Neoplasia 2002;7:17-38.
5. Han Hu, Jiaqi Wang, Dengpan Bu, Hongyang Wei, Linyun Zhou, Fadi Li, *et al*. *In Vitro* Culture and Characterization of a Mammary Epithelial Cell Line from Chinese Holstein Dairy Cow. PLoS One. 2009;4(11):e7636.
6. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, *et al*. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol 1995;154:1800191.
7. Líška J, Brtko J, Dubovický M, Macejová D, Kissová V, Polák Š, Ujházy E. Relationship between histology, development and tumorigenesis of mammary gland in female rat. Exp Anim 2016;65:1-9. doi: 10.1538/expanim.15-0055.
8. Howlin J, McBryan J, Martin F. Pubertal mammary gland development: insights from mouse models. J Mammary Gland Biol Neoplasia 2006;11:283-297. doi: 10.1007/s10911-006-9024-2.
9. Hovey RC, Goldhar AS, Baffi J, Vonderhaar BK. Transcriptional regulation of vascular endothelial growth factor expression in epithelial and stromal cells during mouse mammary gland development. Mol Endocrinol 2001;15:819-831. Doi:10.1210/mend.15.5.0635.