JARQ 59 (1), 17-25 (2025) https://www.jircas.go.jp

REVIEW

Culture Models to Investigate Lactation and Mastitis in Bovine Mammary Epithelial Cells

Yusaku TSUGAMI[1]*, Yuya NAGASAWA[1] and Ken KOBAYASHI[2]

[1] National Institute of Animal Health, National Agriculture and Food Research Organization, Sapporo, Japan
[2] Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

Abstract

Mammary epithelial cells (MECs) are the only cell types that produce milk components such as casein and milk fat. Synthesized milk components are secreted from the apical membranes of MECs; cell polarity is, thus, necessary for milk production in MECs. The enhanced milk production ability of MECs contributes to increased milk yield in lactating cows. MECs are confronted with mastitis-causing pathogens in mammary glands when cows afflicted with mastitis experience decreased milk production. Experiments have been conducted using lactating cows in vivo to explain the mechanisms of milk production and mastitis. However, considering costs, workers, and space constraints, setting a large sample size or treatment group for trials is difficult. In vitro culture models using bovine MECs (BMECs) have, therefore, been developed as an alternative. Previous culture models have been reported to produce several components of milk. However, limited information is available on culture models of BMECs with cell polarity. In contrast, our established culture model introduced in this study demonstrates cell polarity with milk component secretion and less-permeable tight junctions. This review introduces the establishment of a BMEC culture model and its application to investigate the mechanisms of milk production or mastitis, along with our research.

Discipline: Animal Science
Additional key words: cell signaling, milk production, tight junctions

Introduction

Mammary epithelial cells (MECs) are parenchymal cells of the mammary alveoli and ducts in the mammary glands. They are the only cells that produce milk components, such as caseins and milk fat, during lactation. To synthesize these milk components, MECs absorb nutrients such as glucose and amino acids through transporters such as glucose transporter 1 (GLUT1) and large neutral amino acid transporters in the basolateral membranes (Yu et al. 2013, Tsugami et al. 2021b, Tsugami et al. 2023). MECs, subsequently, secrete milk components from the apical membranes into the lumen of the mammary alveoli (Truchet et al. 2014, Wooding & Kinoshita 2024). The directional flow from basal to apical is crucial for milk production in MECs, and the formation of tight junctions (TJs) is required to establish this direction. TJs are composed of occludin and claudin, transmembrane proteins, and are formed at the most-apical regions in the lateral membranes (Baumgartner et al. 2017). TJs separate the apical and basolateral membranes, which contribute to establishing and maintaining cell polarity (Otani & Furuse 2020). Inducing milk component secretion and TJ formation is necessary to establish an MEC's culture model that recreates the lactation period (Fig. 1).

Milk component production and less-permeable TJ formation in the MECs of lactating mammary glands are regulated by prolactin-STAT5 and glucocorticoids-glucocorticoid receptor (GR) signaling. The activation of STAT5 promotes mRNA expression of milk components such as α-casein and α-lactalbumin (Singh et al. 2017),

*Corresponding author: tsugamiy328@affrc.go.jp
Received 4 March 2024; accepted 10 May 2024.

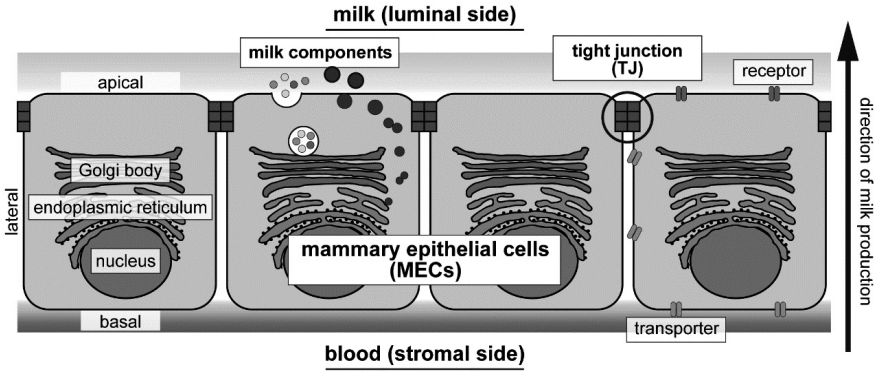

Fig. 1. Characteristics of mammary gland epithelial cells (MECs) during lactation
Schematic diagram demonstrating the characteristics of MECs during lactation. MECs take up nutrients from the blood through transporters in the basolateral membranes, synthesize milk components in the endoplasmic reticulum and Golgi body, and secrete these components into the mammary lumen from the apical membranes. Tight junctions (TJs) separate apical and basolateral membranes and regulate paracellular permeability.

and glucocorticoids especially induce TJ closure in bovine mammary glands (Wall et al. 2016). STAT5 and GR signaling regulate milk production ability and less-permeable TJs in culture models using mouse MECs (Kobayashi et al. 2017, Kobayashi et al. 2016). In contrast, milk production in mammary glands is down-regulated by activated STAT3, NFκB, and MAPK (ERK, p38, and JNK) signaling in inflammation like involution or mastitis (Singh et al. 2017, Yang et al. 2022).

Mastitis is one of the most prevalent diseases in the dairy industry. Mastitis results in significant economic losses, including increased treatment costs and decreased milk yield and quality (Ruegg 2017). There is a three-phase process in the development of mastitis: (1) the invasion of a pathogen into the mammary glands via a teat canal, (2) infection, and (3) inflammation (Murphy 1947). Over 150 mastitis-causing pathogens, including *Escherichia coli* and *Staphylococcus aureus*, have been reported. Pathogens and their cell wall components, such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), cause inflammation in the mammary glands (Kobayashi et al. 2013b, Kobayashi et al. 2022). MECs are exposed to mastitis-causing pathogens in mammary glands afflicted with mastitis.

In vivo experiments have been performed using lactating dairy cows to improve milk production and develop a mastitis treatment (Shangraw et al. 2020, Leroux et al. 2023, Choudhary et al. 2024, Hu et al. 2024, Del Valle et al. 2024). Setting a large sample size, increasing the treatment group, or increasing the number of trials is, however, difficult in vivo using lactating cows. This is because primiparous milking cows are expensive, feeding management requires a high level of expertise, and in vivo experiments using cows require many personnel. Moreover, it is impossible to investigate the changes in cell signaling in bovine MECs (BMECs) in a short period, such as minutes or hours, using whole cows. The advantages of using a culture model generally induce cost and effort saving. Several *in vitro* culture models of BMECs have, thus, been used to elucidate the mechanism of milk production in BMECs and to identify effective physiologically active substances for milk production (Wang et al. 2022, Huang et al. 2023, Yang et al. 2023, Lu et al. 2024).

In previous culture models using BMECs, BMECs have been observed to produce and secrete the milk fat (Cheng et al. 2020, Cheng et al. 2022), express the α-casein mRNA (Sakamoto et al. 2005), or secrete β-casein (Zhao et al. 2022), major milk proteins (Kumar et al. 1994). Establishing a BMEC culture model that recreates in vivo features is required to improve milk production and develop mastitis prevention and treatment methods. However, limited information is available on culture models that recreate the secretion of milk components from apical membranes with less-permeable TJs in BMECs. In contrast, our established BMEC culture model exhibits distinct cell polarity with milk component secretion and less-permeable TJs. This review introduces our established BMEC culture model and its application to investigate the mechanisms of milk production or mastitis, along with our research.

Establishment of a BMEC culture model

Milk production in BMECs is directed from the basal to the apical region, where TJ formation is vital for

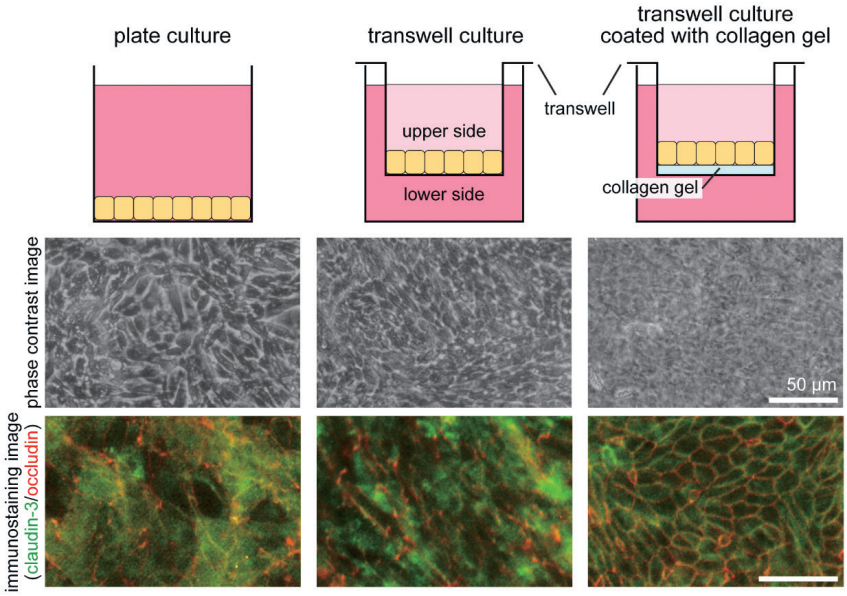

Fig. 2. Differences of culture models on tight junction (TJ) network structure
Bovine mammary epithelial cells (BMECs) were cultured on a general culture plate, transwell, or transwell coated with collagen gels. The images show phase-contrast and immunostaining images of claudin-3 (green) and occludin (red; TJ marker) in BMECs from each culture model. The scale bars represent 50 μm.

establishing cell polarity. Previous BMEC culture models have predominantly focused on milk production, leaving the formation of TJs and the establishment of cell polarity unclear. A culture model using a transwell plate recreated the formation of TJs and cell polarity similar to that in vivo in previous research using intestinal epithelial cells (Gunasekara et al. 2018, Speer et al. 2019). MECs cultured on dishes coated with type I collagen also demonstrated increased casein mRNA expression (Lee et al. 1985). Thus, the culture system and scaffold were examined to induce TJ formation and establish cell polarity.

First, primary BMECs were seeded in three ways: (1) onto a general culture plate, (2) onto a transwell plate, and (3) onto a transwell plate coated with collagen gel (Fig. 2). BMECs were isolated from deep areas within the mammary glands of parous Holstein cows using collagenase and trypsin treatment. The BMECs grew and formed tightly arranged epithelial sheets in all culture models. To examine TJ formation, cultured BMECs were immune stained for claudin-3 and occludin, the major TJ proteins in lactating mammary glands (Kobayashi & Kumura 2011, Tsugami et al. 2021b). BMECs cultured on a plate and transwell demonstrated irregular and fragmented TJ networks. In contrast, BMECs cultured on a transwell coated with collagen gel showed a continuous TJ network co-localized with claudin-3 and occludin.

The effects of STAT5 and GR signaling on milk production ability and TJ formation were subsequently examined using bovine pituitary extract (BPE), which contains prolactin, and dexamethasone (DEX), which is a substitute for glucocorticoids. BMECs secreted β-casein in the presence of BPE. DEX treatment failed to induce the secretion of β-casein, although the level of intracellular β-casein increased. In contrast, the presence of DEX enhanced the TJ barrier function. The DEX and BPE double treatment additionally resulted in higher lactose and triglyceride secretion levels with less-permeable TJs (Tsugami et al. 2020).

The localization of plasma membrane proteins was observed by immunostaining to confirm the establishment of cell polarity in BMECs cultured on a transwell plate coated with collagen gel in the presence of BPE and DEX. The claudin-3 and occludin co-localized at the most-apical region of the lateral membranes. GLUT-1 was additionally localized to the lateral membranes, and Toll-like receptor (TLR) 4 was localized to the apical membranes and cytosol near the apical membranes. The direction of milk secretion was examined, and milk components such as lactoferrin and lactose were secreted into the medium on the upper side.

These findings revealed that BMECs cultured on a transwell coated with collagen gel in the presence of BPE and DEX exhibited a TJ network, distinct cell polarity, and milk component secretion from apical membranes, similar to in vivo BMECs during lactation (Knight et al. 1998). Furthermore, approximately 2,000 samples can be

tested in a 24-well transwell from approximately 10 g of bovine mammary glands, and isolated BMECs can be cryopreserved. The following sections provide examples of the applications of our established BMEC culture model.

Application of BMEC culture model for lactation

The behavior of MECs is affected by environmental hormones and food ingredients. Isoflavones, known as phytoestrogens (Dixon 2004), are abundant in legumes that dairy cows consume, although their types and amounts vary depending on the legumes. Soybeans are rich in genistein and daidzein, whereas red clover is rich in formononetin and biochanin A (Mazur 1998, Andersen et al. 2009). Orally ingested isoflavones are often metabolized and converted into other types by the rumen, intestine, and liver. There are two pathways for the metabolic conversion of isoflavones: the biochanin A–genistein–paraethylphenol pathway and the formononetin–daidzein–equol pathway (Batterham et al. 1965, Lundh 1995, Day et al. 1998). It is, therefore, difficult to identify the effects of specific isoflavones on the mammary glands by oral ingestion using in vivo lactating cows, considering a wide variety of isoflavones exist in the body depending on metabolic conversion. In contrast, it is possible to examine the effects of specific isoflavones on milk production ability and TJs using cultured BMECs.

Using our established culture model, it was found that biochanin A and formononetin adversely affected milk production ability (Tsugami et al. 2022). Genistein demonstrated adverse effects on milk production ability at high concentrations but demonstrated positive effects at low concentrations. In contrast, daidzein and equol activated STAT5 and increased claudin-3. Paraethylphenol additionally demonstrated no significant impact on milk production ability or TJs. These findings indicated that isoflavones upstream of the metabolic pathway have adverse effects. In contrast, isoflavones downstream of the metabolic pathway have positive or no inhibitory effects on lactating BMECs (Fig. 3). This additionally suggests that isoflavones in legumes and the metabolic activity of isoflavones in dairy cows fed legumes may affect milk production ability and TJs in BMECs (Tsugami et al. 2022).

Polyphenols are particularly susceptible to metabolic conversion and conjugate formation in vivo, which affects their physiological activity (Del Rio et al. 2013, Cottart et al. 2014). It is possible to screen the effects of various ingredients, including metabolites or conjugates, and examine the dose-dependent effects of cultured BMECs, which will contribute to feed development and feed design to promote milk production by exogenous control.

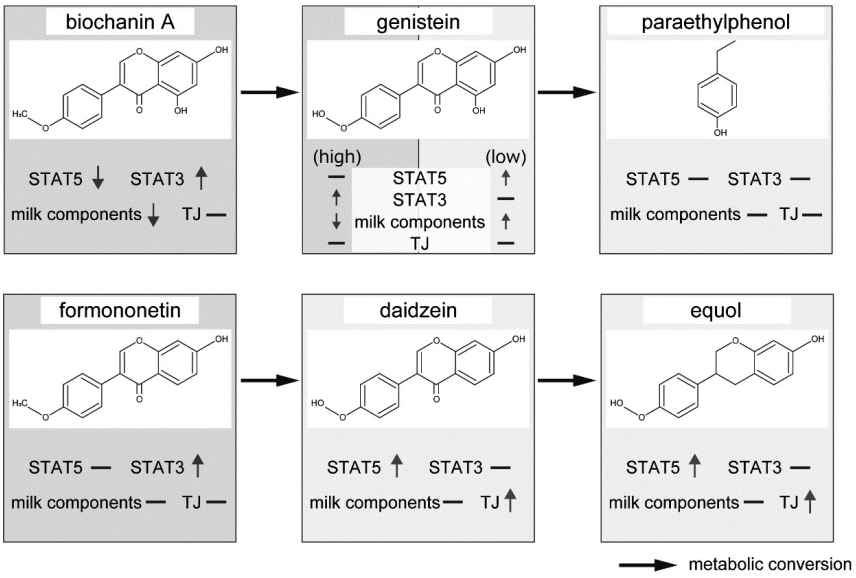

Fig. 3. Isoflavones and their metabolites influence the milk production of bovine mammary epithelial cells (BMECs) in a type-specific manner
Isoflavones (biochanin A, genistein, formononetin, and daidzein) and their metabolites (paraethylphenol and equol) influence the production of milk components, tight junctions, and regulatory pathways in BMECs in a type-specific manner. Upper arrows indicate an increase or upregulation, lower arrows indicate a decrease or downregulation, and hyphens indicate no statistical influence.

Investigating the impact of hormones such as growth hormone, estrogen, and progesterone will contribute to revealing the milk production mechanism of BMECs through endogenous control.

Application of the BMEC culture model for mastitis

Mastitis is known to reduce milk yield and quality. It is necessary to reveal the mechanisms of mastitis and understand the changes in MECs to establish methods to prevent mastitis and its treatment. Previous studies using culture models of BMECs revealed that microRNAs regulate proliferation and apoptosis induced by LPS treatment (Lu et al. 2021), and live bacteria, such as *S. aureus*, adhere to and invade BMECs (Hensen et al. 2000). LPS and LTA are cell wall components of Gram-negative and Gram-positive bacteria, respectively (Venkataranganayaka Abhilasha & Kedihithlu Marathe 2021). Our established BMEC culture model exhibited milk component secretion and less-permeable TJs, as introduced above (Tsugami et al. 2020). Therefore, the effects of LPS and LTA on milk production ability and TJs were examined.

In terms of the effects on milk component production, LPS-treated BMECs decreased the secretion of β-casein, milk fat, and lactose. In contrast, LTA-treated BMECs increased the production of lactoferrin and decreased milk fat and lactose secretion (Fig. 4). Furthermore, the effects on the size of milk fat within BMECs differed, with LPS treatment increasing the proportion of large lipid droplets and LTA treatment increasing the proportion of small lipid droplets compared to the untreated control group (Tsugami et al. 2021a). Although both LPS and LTA decreased the TJ barrier function in BMECs, the adverse effects of LPS were stronger than those of LTA.

The effects of LPS and LTA on milk production-related signaling in BMECs were examined after 48 hours of treatment. The activation of STAT5 promotes milk production (Jena et al. 2023), while the activation of STAT3 represses milk production in MECs (Matsunaga et al. 2018). Both LPS and LTA inactivated STAT5 and activated STAT3 in BMECs, but LPS inactivated STAT5 more strongly than LTA. The effects on inflammatory signaling were subsequently examined

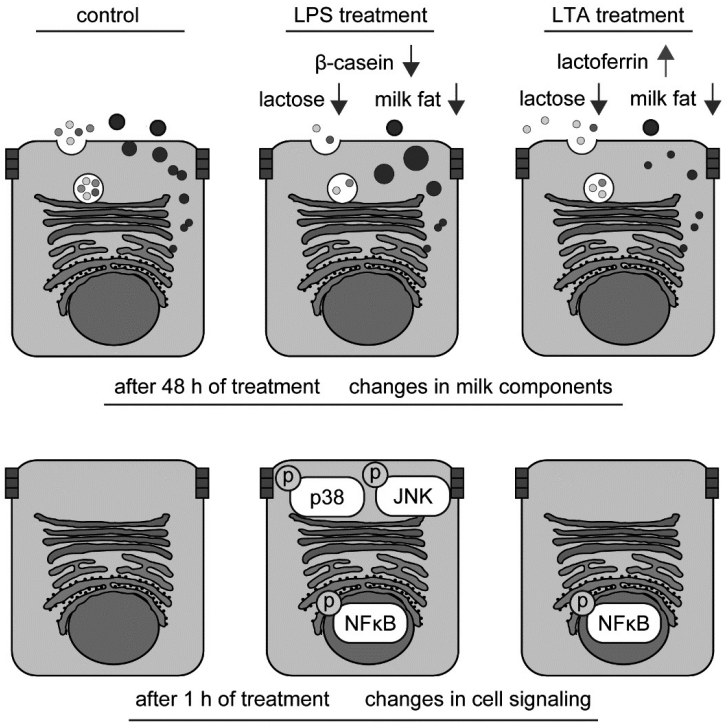

Fig. 4. Lipopolysaccharide (LPS) and lipoteichoic acid (LTA) differently influence the cell signaling and milk production of bovine mammary epithelial cells (BMECs)
LPS decreases the secretion of β-casein, lactose, and triglycerides by activating the NF-κB, p38, and JNK pathways. LTA decreased the secretion of lactose and triglycerides by activating the NF-κB pathway.

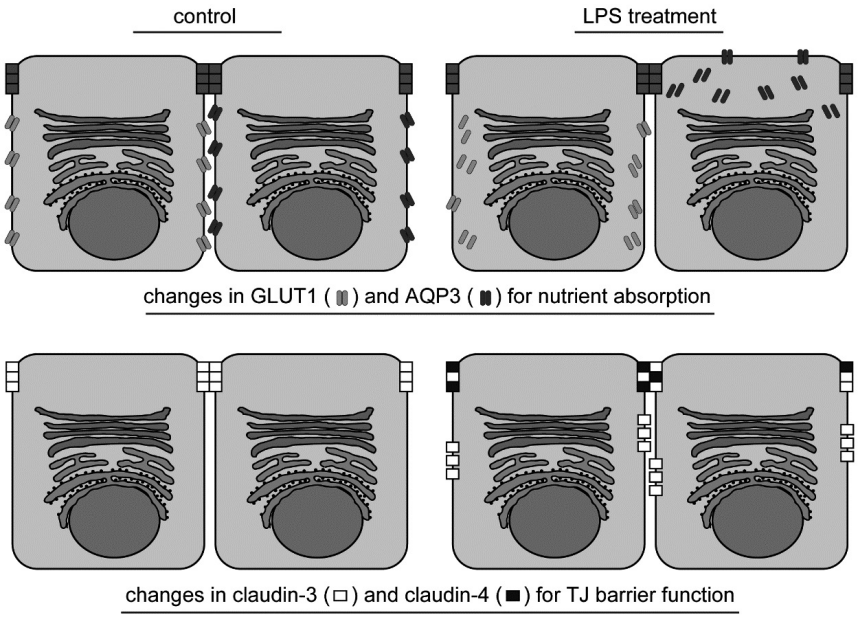

Fig. 5. Influence of lipopolysaccharide (LPS) on the localization of each membrane protein in bovine mammary epithelial cells (BMECs)
Glucose transporter 1 (GLUT1) and aquaporin 3 (AQP3) in lateral membranes are internalized by LPS. BMECs treated with LPS demonstrated localization of claudin-4 at the tight junction (TJ) region and claudin-3 in the lateral membranes, with disruption of TJ barrier function.

within six hours of treatment. NFκB and MAPK (ERK, p38, and JNK) are representative of inflammatory signaling in MECs (Yang et al. 2022). NFκB, p38, and JNK were activated in BMECs after a one-hour treatment with LPS, whereas only NFκB was activated after a one-hour treatment with LTA. Activation additionally occurred regardless of the concentration in the range of 0.1 to 10 μg/mL in both LPS and LTA treatment. Moreover, signaling inhibitors alleviated the adverse effects of LPS and LTA on milk production. These findings suggest that LPS and LTA adversely affect the milk component production of lactating BMECs in different manners, and inhibition of the p38 and NFκB pathways may help recover the decrease in milk production by *E. coli* and *S. aureus* (Tsugami et al. 2021a).

Various plasma membrane proteins are involved in milk production in lactating MECs. Our established BMEC culture model exhibited distinct cell polarity, and these plasma membrane proteins were specifically localized at the apical and basolateral membranes; the effects of LPS on these plasma membrane proteins were, therefore, examined. TLR4 was translocated to the apical membranes of BMECs after a one-hour of LPS treatment, whereas TLR2 was not significantly affected. TLR4 acts as a receptor for LPS, while TLR2 serves as a receptor for LTA (Akira et al. 2001). GLUT1 and aquaporin 3 (AQP3) are transporters and channels for glucose and water, respectively. The expression of GLUT1 and AQP3 in the basolateral membranes gradually decreased after LPS treatment. In particular, the localization of AQP3 changed to the apical membranes and the cytoplasm near the apical membranes after 48 hours of LPS treatment (Fig. 5). Additionally, claudin-3 leaked from the TJ regions into the lateral membranes. In contrast, the expression of claudin-4 increased and was localized in the TJ regions. Increased expression of claudin-4 has been observed in mammary glands with mastitis or involution (Kobayashi & Kumura 2011, Kobayashi et al. 2013a). The TJ barrier function was further weakened after LPS treatment. These findings suggest that local changes in plasma membrane proteins are related to decreases in the milk production ability of BMECs, independent of insufficient energy resulting from systemic symptoms, such as fever and anorexia, when dairy cows are afflicted with mastitis.

In the future, it is expected that the use of live bacteria will lead to the clarification of the response of BMECs against pathogens, as well as the mechanisms of pathogen adhesion and intracellular invasion. The interaction between the adhesins of pathogens and adhesin receptors expressed in cells is particularly important for pathogen adhesion to host cells. The application of our established BMEC culture model resulted in distinct cell polarity, which will contribute to

elucidating the detailed mechanism of pathogen attachment to BMECs. Experiments using our established BMEC culture model indicated that the ratio of adhesion to invasion differed among *S. aureus* strains (unpublished data). The culture model may additionally be useful for identifying physiologically active substances and developing drugs that are effective in the prevention and treatment of mastitis.

Other culture models using MECs

This paper introduced our established BMEC culture model and evaluated its application to lactation and mastitis based on our previous research. BMECs cultured on a transwell coated with collagen gel showed secretion of milk components and less-permeable TJs. This model used a gel primarily composed of type I collagen as a scaffold, although recent research has reported that the type of collagen around MECs changes depending on the stage of pregnancy, lactation, and involution (Tsutsui et al. 2020). Mouse MECs cultured on Matrigel, a reconstituted basement membrane from the Engelbreth-Holm-Swarm tumor, demonstrate upregulated β-casein mRNA expression (Chou et al. 1989), and extracellular matrix density regulates cell-cell adhesions of human MECs (Kumar et al. 2014). Investigating a more appropriate extracellular matrix composition or density may be the next step in enhancing milk production ability in BMECs.

Three-dimensional culture models have also been developed to investigate the structure of mammary alveoli and ducts by embedding spheroids of MECs in Matrigel or collagen gel and culturing them (Nguyen-Ngoc et al. 2015). The mechanism of the development of the mammary duct structure and the effects of heat stress or polyphenols on the structure have additionally been examined using three-dimensional culture models (Huebner et al. 2016, Kumai et al. 2020, Wakasa et al. 2022). While three-dimensional culture models are appropriate for examining structural changes, they are unsuitable for examining changes in milk production ability, considering collecting the milk components secreted into the mammary lumen is impossible. Two-dimensional culture models, therefore, remain appropriate for investigating milk secretion. It is essential to use appropriate culturing methods depending on the specific subject of investigation.

Conclusions

MECs undergo proliferation, differentiation, and regression depending on the stage of pregnancy, lactation, and involution and are the only cells that produce milk components. Our established BMEC culture model induced in this study exhibited the secretion of milk components, less-permeable TJs, and distinct cell polarity, similar to in vivo BMECs during lactation. This model was additionally used to investigate the effects of polyphenols and mastitis-causing toxins on milk component production and TJ barrier function. The effects on milk production by BMECs were partly revealed, and it was possible to identify the effects of each polyphenol metabolite and examine the changes in cell signaling in a short time. Several studies have been conducted to identify physiologically active substances that enhance milk production ability or suppress inflammation during mastitis using BMEC culture models (Ouyang et al. 2023, Yao et al. 2023, Fan et al. 2023). These results are expected to be more efficiently applied to improve milk production via feeding management and mastitis treatment at the production site using a culture model recreating milk production ability and conditions during mastitis. Moreover, the microenvironment of the mammary glands is composed of MECs as well as other cell types, such as leukocytes, fibroblasts, and adipocytes, which are partly involved in milk yield and quality or inflammatory responses (Hughes & Watson 2018, Fu et al. 2020). Investigating the interaction between MECs and other cell types based on BMEC culture models recreating in vivo conditions will contribute to revealing the unique microenvironment of the mammary glands.

Acknowledgements

We thank Naoki Isobe (Graduate School of Integrated Sciences for Life, Hiroshima University) for the helpful advice regarding our experiments and this work.

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS Kakenhi Grant Numbers JP22K14983 and JP19J10189) and the Research project for technologies to strengthen the international competitiveness of Japan's agriculture and food industry (Grant Number 21452290).

Conflict of interest

The authors declare that they have no conflicts of interest.

References

Akira, S. et al. (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol.*, **2**,

675-680.
Andersen, C. et al. (2009) Phyto-oestrogens in herbage and milk from cows grazing white clover, red clover, lucerne or chicory-rich pastures. *Animal*, **3**, 1189-1195.
Batterham, T. J. et al. (1965) Metabolism of oestrogenic isoflavones in sheep. *Nature*, **206**, 509.
Baumgartner, H. K. et al. (2017) Developmental expression of claudins in the mammary gland. *J. Mammary Gland Biol. Neoplasia*, **22**, 141-157.
Cheng, J. et al. (2022) CIDEA regulates de novo fatty acid synthesis in bovine mammary epithelial cells by targeting the AMPK/PPARγ axis and regulating SREBP1. *J. Agric. Food Chem.*, **70**, 11324-11335.
Cheng, J. et al. (2020) Sodium butyrate promotes milk fat synthesis in bovine mammary epithelial cells via GPR41 and its downstream signalling pathways. *Life Sci.*, **259**, 118375.
Chou, J. L. et al. (1989) Effects of extracellular matrix on the growth and casein gene expression of primary mouse mammary tumor cells *in vitro*. *Cancer Res.*, **49**, 5371-5376.
Choudhary, R. K. (2024) Systemic and local responses of cytokines and tissue histology following intramammary lipopolysaccharide challenge in dairy cows. *J. Dairy Sci.*, **107**, 1299-1310.
Cottart, C. H. (2014) Review of recent data on the metabolism, biological effects, and toxicity of resveratrol in humans. *Mol. Nutr. Food Res.*, **58**, 7-21.
Day, A. J. et al. (1998) Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett.*, **436**, 71-75.
Del Rio, D. et al. (2013) Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal*, **18**, 1818-1892.
Del Valle, T. A. et al. (2024) Rumen degradable protein levels on fatty acid profile of rumen bacteria and milk fat of dairy cows supplemented with calcium salts of fatty acids. *Anim. Sci. J.*, **95**, e13920.
Dixon, R. A. (2004) Phytoestrogens. *Annu. Rev. Plant Biol.*, **55**, 225-261.
Fan, Y. et al. (2023) β-sitosterol suppresses lipopolysaccharide-induced inflammation and lipogenesis disorder in bovine mammary epithelial cells. *Int. J. Mol. Sci.*, **24**, 14644.
Fu, N. Y. et al. (2020) Stem cells and the differentiation hierarchy in mammary gland development. *Physiol. Rev.*, **100**, 489-523.
Gunasekara, D. B. et al. (2018) A monolayer of primary colonic epithelium generated on a scaffold with a gradient of stiffness for drug transport studies. *Anal. Chem.*, **90**, 13331-13340.
Hensen, S. M. et al. (2000) Use of bovine primary mammary epithelial cells for the comparison of adherence and invasion ability of *Staphylococcus aureus* strains. *J. Dairy Sci.*, **83**, 418-429.
Hu, L. et al. (2024) Effects of dietary palmitic acid and oleic acid ratio on milk production, nutrient digestibility, blood metabolites and milk fatty acids profile of lactating dairy cows. *J. Dairy Sci.*, https://doi.org/10.3168/jds.2023-23801.
Huang, J. et al. (2023) Enhancement of PPARα-inhibited leucine metabolism-stimulated β-casein synthesis and fatty acid synthesis in primary bovine mammary epithelial cells. *J. Agric. Food Chem.*, **71**, 16184-16193.
Huebner, R. J. et al. (2016) Mammary epithelial tubes elongate through MAPK-dependent coordination of cell migration. *Development*, **143**, 983-993.
Hughes, K. & Watson, C. J. (2018) The mammary microenvironment in mastitis in humans, dairy ruminants, rabbits and rodents: a one health focus. *J. Mammary Gland Biol. Neoplasia*, **23**, 27-41.
Jena, M. K. et al. (2023) Molecular complexity of mammary glands development: a review of lactogenic differentiation in epithelial cells. *Artif. Cells Nanomed. Biotechnol.*, **51**, 491-508.
Knight, C. H. et al. (1998) Local control of mammary development and function. *Rev. Reprod.*, **3**, 104-112.
Kobayashi, K. & Kumura, H. (2011) Distinct behavior of claudin-3 and -4 around lactation period in mammary alveolus in mice. *Histochem. Cell Biol.*, **136**, 587-594.
Kobayashi, K. et al. (2013a) Lipopolysaccharide disrupts the milk-blood barrier by modulating claudins in mammary alveolar tight junctions. PLoS One, **8**, e62187.
Kobayashi, K. et al. (2013b) Histological analysis of mammary gland remodeling caused by lipopolysaccharide in lactating mice. Cell Tissue Res., **354**, 495-506.
Kobayashi, K. et al. (2016) Prolactin and glucocorticoid signaling induces lactation-specific tight junctions concurrent with β-casein expression in mammary epithelial cells. Biochim. Biophys. Acta Mol. Cell Res., **1863**, 2006-2016.
Kobayashi, K. et al. (2017) Distinct roles of prolactin, epidermal growth factor, and glucocorticoids in β-casein secretion pathway in lactating mammary epithelial cells. Mol. Cell. Endocrinol., **440**, 16-24.
Kobayashi, K. et al. (2022) Early effects of lipoteichoic acid from Staphylococcus aureus on milk production-related signaling pathways in mouse mammary epithelial cells. *Exp. Cell Res.*, **420**, 113352.
Kumai, A. et al. (2020) Adverse effects of coumestrol and genistein on mammary morphogenesis and future milk production ability of mammary epithelial cells. *Adv. Biosyst.*, **4**, e1900187.
Kumar, S. et al. (1994) Milk composition and lactation of beta-casein-deficient mice. *Proc. Natl. Acad. Sci. USA*, **91**, 6138-6142.
Kumar, S. et al. (2014) Extracellular matrix density promotes EMT by weakening cell-cell adhesions. *Mol. Biosyst.*, **10**, 838-850.
Lee, E. Y. et al. (1985) Interaction of mouse mammary epithelial cells with collagen substrata: regulation of casein gene expression and secretion. *Proc. Natl. Acad. Sci. USA*, **82**, 1419-1423.
Leroux, C. et al. (2023) Milk fat miRNome changes in response to LPS challenge in Holstein cows. *Vet. Res.*, **54**, 111.
Lu, H. et al. (2024) The serine protease 2 gene regulates lipid metabolism through the LEP/ampkα1/SREBP1 pathway in bovine mammary epithelial cells. *Biochem. Biophys. Res. Commun.*, **698**, 149558.
Lu, J. et al. (2021) miR-142-5p regulates lipopolysaccharide-induced bovine epithelial cell proliferation and apoptosis via targeting BAG5. *Exp. Ther. Med.*, **22**, 1425.
Lundh, T. (1995) Metabolism of estrogenic isoflavones in domestic animals. *Proc. Soc. Exp. Biol. Med.*, **208**, 33-39.

Matsunaga, K. et al. (2018) IL-1β directly inhibits milk lipid production in lactating mammary epithelial cells concurrently with enlargement of cytoplasmic lipid droplets. *Exp. Cell Res.*, **370**, 365-372.

Mazur, W. (1998) 11 Phytoestrogen content in foods. *Baillière's Clin. Endocrinol. Metab.*, **12**, 729-742.

Murphy, J. M. (1947) The genesis of bovine udder infection and mastitis; the occurrence of streptococcal infection in a cow population during a seven-year period and its relationship to age. *Am. J. Vet. Res.*, **8**, 29-42.

Nguyen-Ngoc, K. V. et al. (2015) 3D culture assays of murine mammary branching morphogenesis and epithelial invasion. *Methods Mol. Biol.*, **1189**, 135-162.

Otani, T. & Furuse, M. (2020) Tight junction structure and function revisited. *Trends Cell Biol.*, **30**, 805-817.

Ouyang, L. et al. (2023) Resveratrol inhibits LPS-induced apoptosis in bovine mammary epithelial cells: the role of PGC1α-SIRT3 axis. *In Vitro Cell. Dev. Biol. Anim.*, **59**, 264-276.

Ruegg, P. L. (2017) A 100-Year Review: Mastitis detection, management, and prevention. *J. Dairy Sci.*, **100**, 10381-10397.

Sakamoto, K. et al. (2005) Growth hormone acts on the synthesis and secretion of alpha-casein in bovine mammary epithelial cells. *J. Dairy Res.*, **72**, 264-70.

Shangraw, E. M. et al. (2020) Intramammary lipopolysaccharide infusion induces local and systemic effects on milk components in lactating bovine mammary glands. *J. Dairy Sci.*, **103**, 7487-7497.

Singh, K. et al. (2017) Temporal and spatial heterogeneity in milk and immune-related gene expression during mammary gland involution in dairy cows. *J. Dairy Sci.*, **100**, 7669-7685.

Speer, J. E. et al. (2019) Molecular transport through primary human small intestinal monolayers by culture on a collagen scaffold with a gradient of chemical cross-linking. *J. Biol. Eng.*, **13**, 36.

Truchet, S. et al. (2014) Milk secretion: The role of SNARE proteins. *J. Mammary Gland Biol. Neoplasia*, **19**, 119-130.

Tsugami, Y. et al. (2020) Establishment of an *in vitro* culture model to study milk production and the blood-milk barrier with bovine mammary epithelial cells. *Anim. Sci. J.*, **91**, e13355.

Tsugami, Y. et al. (2021a) Lipopolysaccharide and lipoteichoic acid influence milk production ability via different early responses in bovine mammary epithelial cells. *Exp. Cell Res.*, **400**, 112472.

Tsugami, Y. et al. (2021b) Adverse effects of LPS on membrane proteins in lactating bovine mammary epithelial cells. *Cell Tissue Res.*, **384**, 435-448.

Tsugami, Y. et al. (2022) Isoflavones and their metabolites influence the milk production ability of bovine mammary epithelial cells in a type-specific manner. *Anim. Sci. J.*, **93**, e13720.

Tsugami, Y. et al. (2023) Valine treatment enhances antimicrobial component production in mammary epithelial cells and the milk of lactating goats without influencing the tight junction barrier. *J. Mammary Gland Biol. Neoplasia*, **28**, 3.

Tsutsui, S. et al. (2020) Distinct expression patterns of fibrillar collagen types I, III, and V in association with mammary gland remodeling during pregnancy, lactation and weaning. *J. Mammary Gland Biol. Neoplasia*, **25**, 219-232.

Venkataranganayaka Abhilasha, K. & Kedihithlu Marathe, G. (2021) Bacterial lipoproteins in sepsis. *Immunobiology*, **226**, 152128.

Wakasa, H. et al. (2022) Adverse effects of high temperature on mammary alveolar development *in vitro*. *J. Mammary Gland Biol. Neoplasia.*, **27**, 155-170.

Wall, S. K. et al. (2016) Differential glucocorticoid-induced closure of the blood-milk barrier during lipopolysaccharide- and lipoteichoic acid-induced mastitis in dairy cows. *J. Dairy Sci.*, **99**, 7544-7553.

Wang, F. et al. (2022) MRCKα is a novel regulator of prolactin-induced lactogenesis in bovine mammary epithelial cells. *Anim. Nutr.*, **10**, 319-328.

Wooding, F. B. P. & Kinoshita, M. (2024) Milk fat globule membrane: formation and transformation. *J. Dairy Res.*, 1-9.

Yang, Y. et al. (2022) Evodiamine relieve LPS-induced mastitis by inhibiting AKT/NF-κB p65 and MAPK signaling pathways. *Inflammation*, **45**, 129-142.

Yang, Y. et al. (2023) Acetate upregulates GPR43 expression and function via PI3K-AKT-SP1 signaling in mammary epithelial cells during milk fat synthesis. *J. Agric. Food Chem.*, **71**, 16003-16015.

Yao, R. et al. (2023) Chlorogenic acid enhances PPARγ-mediated lipogenesis through preventing Lipin 1 nuclear translocation in *Staphylococcus aureus*-exposed bovine mammary epithelial cells. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids*, **1868**, 159396.

Yu, Q. et al. (2013) Functional analyse of GLUT1 and GLUT12 in glucose uptake in goat mammary gland epithelial cells. *PLoS One*, **8**, e65013.

Zhao, X. et al. (2022) Deoxynivalenol exposure inhibits biosynthesis of milk fat and protein by impairing tight junction in bovine mammary epithelial cells. *Ecotoxicol. Environ. Saf.*, **237**, 113504.