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The effect of prolonged milking interval on milk yield and quality in an automated milking system

자동 착유기에서 착유간격 연장이 산유량과 유질에 미치는 영향 연구

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농생명공학부

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Abstract

Automated milking systems allow cows to be milked voluntarily based on setting time. This typically leads to an increased milking interval, which may affect milk yield and milk quality. This study was performed to evaluate the effect of the prolonged milking interval of cows on milk yield and composition and milk quality in automated milking systems. A total of 29 lactating Holstein cows were divided into control group (N = 10) and treatment group (N = 19). The milking interval of the control group was maintained from 11-h to 12-h in automated milking systems (VMS™ with Delpro 3.0; Delaval). The milking permission time in treatment group was gradually increased from 11-h to 20-h to prolong milking interval. As a result, cows in the treatment group gradually increased milking interval from 13-h to 22-h during the 20-d experimental period. Milk yield, milk composition, and total bacterial count were measured at starting day (0-d), 7-d, and 20-d of experimental period. Somatic cell counts were measured daily during 20 experimental days. Milk yield, milk protein content, and milk lactose content were lower ($p < 0.05$) in the treatment group than in the control group at 20-d but not at 0-d and 7-d, whereas milk fat content was not different between two groups at all time points. Total bacterial count was higher ($p < 0.05$) in the treatment group than in the control group at 7-d and 20-d. Somatic cell counts was numerically higher in the treatment group than in the control group on 18-d and 19-d, but not at other days. Correlation analysis revealed a moderately positive correlation between milking interval and daily somatic cell count ($R^2 = 0.31$, $p = 0.011$).

Based on days in milk, treatment cows were sub-grouped into early to mid-lactation cow group (less 200 days in milk) and late-lactation cow group (over than 200 days in milk). The late-lactation group showed a moderately positive correlation ($R^2 = 0.36$, $p = 0.005$), whereas early to mid-lactation group did not show significant correlation ($R^2 = 0.12$, $p = 0.138$) between milking interval and somatic cell count. In conclusion, prolonged milking interval up to 22-h reduces milk yield, milk protein and milk lactose contents, and milk quality (total bacterial count), and it increases somatic cell counts especially during late lactation period.

Key words: Automated milking system, prolonged milking interval, milk quality, somatic cell count.

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List of abbreviations

AMS: Automatic milking system

AM: Automatic milking

VMS: Voluntary milking system

SCC: Somatic cell count

TPC: Total plate count

TBC: Total bacterial count

DIM: Days in milk

DMY: Daily milk yield

SPC: Standard plate count

OCC: Online cell counter

CMS: Conventional milking system

CM: Conventional milking

MY: Milk yield

ML: Milk leakage

MI: Milking interval

PMI: Prolonged milking interval

FFA: Free fatty acid

IMI: Intramammary infection

PRL: Prolactin

TJ: Tight junction

ALA: α-lactalbumin

MFG: Milk fat globule

LnSCC: Log-transformed somatic cell count

BSC: Body condition score

SCM: Subclinical mastitis

CM: Clinical mastitis

BMSCC: Bulk milk somatic cell count

ETC: Effectiveness of teat cleaning

TSTC: Technical success of teat cleaning

PMN: Polymorphonuclear leukocytes

ODM: Once daily milking

FIL: Feedback inhibitor of lactation

Units and marks

%: Percent

kg: Kilogram

L: liter

d: daily

g: Gram

mg: Milligram

ml: milliliter

CFU: colony forming unit

h : Hour

D: Day

Ⅰ. Introduction

The use of an automated milking system (AMS) reduces labor requirements but may extend or cause fluctuations in the intervals between milkings. As AMS relies, to some degree, on the voluntary movement of cows, the milking interval is influenced by each individual's behavior. For instance, some cows can be milked three times per day, whereas others are milked two times per day, it's only over 12-h intervals (Mollenhorst et al., 2011). Variance in milking frequency in conventional milking systems is known to affect somatic cell counts (SCC), and milk yield and composition. Increasing milking frequency by 2 to 3 times a day increased milk yield (Smith et al., 2002) and milk fat content (Erdman and Varner, 1995). In contrast, milking once a day with prolong milking intervals reduced milk yield by 24 to 40% (McNamara et al., 2008; Remond et al., 2009). Limited studies have conducted on the effect of milking interval on milk composition, and most of studies have been conducted in conventional milking systems. Little studies regarding milking intervals have been done in AMS.

Milking interval may also affect milk quality. The SCC was found to vary with milking interval. Furthermore, epidemiological studies have reported the deterioration of udder health among cows introduced to AMS (Rasmussen et al., 2001; Kruip et al., 2002; Mulder et al., 2004). Limited studies are available on the effect of the milking interval on milk quality in AMS.

We have hypothesized that a prolonged milking interval would affect milk yield and quality and that there would be an association between prolonged milking interval and SCC at different stages of lactation in dairy cows. The objective of this study was to evaluate the effect of the prolonged milking interval of cows on milk yield and composition, SCC, and total bacterial count (TBC) in an AMS.

Ⅱ. Literature review

1. Domestic development of automated milking system

The world is moving to a "smart farming" era, and the dairy industry is not far behind. Robotic milking systems, which were introduced in 2006, are increasingly gaining popularity. This system was introduced to Korea by three major companies, namely Lely, Delaval, and Galaxy. As of January 2016, 97 such machines were installed in 69 farms across Korea.

A robotic milking system has certain advantages over manual milking. For instance, owing to its easy management, it can increase the production rate by 5–25%. Moreover, it reduces the manual labor, as well as the time, required spent on milking. Further, it reduces the risk of mastitis in animals. Despite the major advantages, the system is not used by many since it is not free from disadvantages. For instance, the capital investment required is extremely high. Furthermore, using this system needs the user to be tech-savvy, which is not always possible to achieve, as most farmers are illiterate. Also, if rapid restoration is not supported due to high service dependence, there is concern about damages to cows such as mastitis (Lee. 2016, Feb 24).

Figure 1. Automated milking system (Lely A4)

*Available at https://roboticsandautomationnews.com/2019/01/23/festo-and-lely-unveil-new-version-of-milking-robot/20672/

Figure 2. Automated milking system (Delaval VMS)

*Available at www.delaval.com/explore-our-farm-solutions/milking/delaval-vms-series/

Figure 3. Automated milking system (Galaxy)

*Available at https://www.agriculture-xprt.com/products/galaxy-astrea-model-2020-ams-automatic-milk-system-356689

2. Transition from conventional milking to AMS

2.1. Changes identified with deviation from conventional milking to AMS

2.1.1. Milking frequency

AMS reduces the workload of the milkers, as well as the frequency of milking, thereby resulting in a reduction in the labor cost and substantial annual financial benefits (Dijkhuizen et al., 1997). Moreover, AMS allows one to determine the required milking frequency in advance, which helps with milking management and frequency adaptation depending on the lactation stage. Studies by Klei et al. (1997) and O¨ sterman and Bertilsson (2003) showed that milking a high-yield cow thrice a day can result in a 10–15% increase in the yield. Certain other studies (Wagner-Storch and Palmer, 2003; Svennersten-Sjaunja et al., 2000; Speroni et al., 2006) showed that, when compared to twice-daily conventional milking (CM), AM resulted in an average increase to 2.5 milking per day. An increase in milk production was also seen from 2% to 7% and to 8% in multiparous cows. Notably, thrice-daily CM is believed to produce a better yield than AMS. Svennersten-Sjaunja and Pettersson (2005) showed that, during the initial stages of lactation, the yield can be increased by increasing the milking frequency. It is important to note that excessive milking post-peak lactation cannot overcome the effects of a smaller milking frequency during the initial stages, as the cellular proliferation occurring in the mammary gland adapts to the frequency dynamics in the initial stages. In this line, apoptosis depends on the number of milkings per day (Stefanon et al., 2002).

2.1.2. Milking process

Milking can be termed successful when milk from both alveoli and cistern is collected. In contrast to a CM system, where the milking depends on the milker, an AMS results in consistent milking. Seabrook (1964) claimed that, among the external factors that influence the yield, an important factor is the milker's behavior. Stress can cause intermittent milk secretion, that too from just the cistern and not the alveoli (Bruckmaier and Blum, 1998). Rasmussen et al. (1990) observed increased yield following steady milking patterns. As stated before, teat stimulation activates oxytocin production, which initiates contraction, thereby

resulting in milk secretion. In AMS, the stimulation during teat cleaning (Dzidic et al., 2004) and teat cup attachment (Bruckmaier et al., 2001) result in oxytocin release. Samuelsson et al. (1993) observed that the concentrate administered in cows for increased oxytocin release (Svennersten et al., 1995) resulted in reduced milking time and enhanced milk flow and time needed for udder emptying. Moreover, the cows see the concentrate as treats, which improves their desire to get milked, thereby increasing the production (Prescott et al., 1998).

2.1.3. Milk quality

The assessment of the quality of milk is considered based on two aspects: milk composition and milk hygiene.

Milk fat quality. Increased Free fatty acids (FFA) content in milk is not a desirable condition due to its impact on deterioration of milk sensory properties. Svennersten-Sjaunja et al. (2000) conducted a comparative study of the effect of CM and AM on milk composition. The study reported increased FFA in farms used AM compared with farms used CM (Justesen and Rasmussen, 2000). Increased milking frequency (Ipema and Schuiling, 1992; Klei et al., 1997) and shorter milking intervals (Ahrne´ and Bjo¨rk, 1985) in AMS have been reported to increase the FFA levels. Moreover, the increased activation of enzymes limiting lipolysis by ensuring the membrane is intact, is also known to influence the FFA levels when the milking frequency increases. However, Wiking et al. (2006) found no evidence to support this claim, although they claimed that the size of the fat globule can be a reason for the increased FFA levels. According to them, the increase was noticed only after the milk was stored at 4°C for 24 h, which probably resulted from the impairment of the weak globule membrane. External stress in the form of pumping of milk with large globules showed the highest levels of FFA (Wiking et al., 2003). Another important finding of their study was that increased milking frequency resulted in larger milk fat globules, which were noted to be more prone to lysis. This observation was also made by several others (Svennersten-Sjaunja et al., 2004; Abeni et al., 2005). A study conducted by Hamann et al. (2004) proved that FFA content in milk collected using AM after long milking intervals was almost in the same range as for CM. Further, their results showed that milking intervals of 6 h and 12 h resulted in

FFA levels of 0.31 and 0.24 mmol/L, respectively, with an average of 0.28 mmol/L. Rasmussen et al. (2006) reported that the milk collected using AMS was more acidic than that using CMS. The possible reasons for this were stated as stirring of the milk in the milk tank (79%), pumping of the milk (67%), and chilling the milk (58%).

Milk hygiene. Studies conducted in the Netherlands and Denmark reported an increase in total bacteria counts in bulk milk at farms using AMS (Klungel et al., 2000; Rasmussen et al., 2002). The bacteria were thought to originate from the teat skin of an infected cow, or due to an unclean milking unit or inadequately cooled milk (Rasmussen et al., 2002). Nevertheless, after a year, the level of TBC was almost equal to that on farms with CMS (Van der Vorst et al., 2002). Furthermore, the highest coliform counts in bulk milk were found on farms with less efficient teat cleaning practices. Hovinen et al. (2005) observed that cleaning teats using a teat cup produced better results in terms of hygiene than brushing. Melin et al. (2004) tested the efficiency of teat cup cleaning techniques. The teats were first coated with sterilized manure water containing *Clostridium tyrobacterium* spores 20 min before milking. Some teats were left uncleaned, some cleaned manually, and the remaining were cleaned using an AM teat cup. Conventional manual cleaning removed 65% of the bacterial spores, whereas AM cleaning removed 98% of the spores. This showed that the AM teat cleaning was effective. Therefore, it can be concluded that, along with the teats, the milking environment must also be kept clean.

Milk somatic cell count. A few studies (Klungel et al., 2000; Rasmussen et al., 2001, 2002; Kruip et al., 2002) observed a directly proportional relationship between AMS and increased SSC. However, the mechanism behind this could not be identified, as AMS reduced the chances of infection and ensure better herd management and udder status (Zecconi et al., 2003). Bennedsgaard et al. (2006) reported that SSC remained elevated only for three months post-AMS installation, after which it came down to normal levels. However, Waller et al. (2003) reported an increase in milk leakage between milking sessions on farms using AMS and increased leakage was known to be associated with mastitis. Nevertheless, it is important to know that sometimes milk leakage can be physiological, for instance, the peak period of milk flow, teat canal protrusion (Klaas et al., 2005), or excessive cisternal deposition creating udder pressure (Rovai et al., 2007). This can also be supported by the findings of Waller et

al.'s (2003) study, in which they reported leakage in 62% of primiparous and 28% of multiparous. Notably, primiparous cows usually have smaller cisternal compartments (Pfeilsticker et al., 1996).

In addition, certain technical factors can result in elevated SSC. For instance, milking unit toppages in AMS result in higher SCC. In CMS, all cows can be milked at once over a short duration, whereas in an AM system, only one cow can be milked at a time. In a study by Pettersson et al. (2002), after prolonged stoppages of up to 4 h, the SSC of the bulk milk increased from 50,000 to 250,000 cells/mL. Repeated stoppages led to an increase in the bacterial count as well. Therefore, attempts should be made to reduce AMS downtime as much as possible. Data from certain other studies on commercial farms reported higher SCC in herds where the milking interval was not constant, especially with a standard deviation of more than 3 h. This led to the conclusion that milking intervals should be scheduled within 12-hour margins.

Overmilking increases the hardness and discoloration of milk (Hillerton et al., 2002). To avoid this problem, in farms with AMS, the teat cups are removed as soon as the milk flow reaches the predetermined level, which is called quarter milking. In a study conducted by Berglund et al. (2002) over a period of 25 weeks, SCC of cows milked using CMS and AMS were compared; it was observed that SCC in quarter strip milk was much lower in AM cows than CM cows. This study's findings were also supported by Hamann and Reinecke (2002). Berglund et al. (2002) also claimed that AM prevented teat damage as compared with CM.

AMS consists of several sensors, including ones used for mastitis detection. In addition, AMS has sensors that can assess the milk yield and composition (Linzell and Peaker, 1972) and electrical conductivity (Linzell and Peaker, 1975), which act as chemical sensors for mastitis detection (Mottram et al., 2007). de Mol and Ouweltjes (2001) suggested a model for mastitis detection, using electrical conductivity and milk yield as markers. Hamann et al. (2004) proposed using milk components such as lactose and lactate as markers of udder health. According to Berglund et al. (2007), the lactose levels, which are quite stable in general, are inversely proportional to SSC levels.

2.1.4. Cow traffic.

The success of AMS implementation depends on stable and properly functioning cow traffic. This means sufficient and uniform visits to both the feeding area and milking unit; this is to avoid the irregular pattern developed when cows are given free access to the areas. This controlled pattern is the biggest advantage of AMS. Forsberg et al. (2002) and Harms (2004) tried three patterns of movement and activities: free (Figure 4), semi-forced, and forced. They concluded that free traffic resulted in the least production and efficiency. Though the milk yield did not differ much in general, it tended to be a bit higher in free cows, which could be explained by the increased feed consumption. Another difference in behavior observed was the cows' standing time. The time spent standing in the queue in front of the milking unit increased and the resting time decreased the most for the cows in forced traffic. Forsberg et al. (2002) found that the controlling gates used for restricting traffic negatively impacted the low-ranked cows, as they ended up spending more time standing in the milking queue. Hermans et al. (2003) supported the balanced approach to traffic, that is, semi-forced; they observed that cows in this group spent more time in the feeding area, less time at the free stalls, and paid evenly distributed visits to the milking unit. The placement of the selection gate in the semi-forced traffic system also plays a significant role. Stefanowska et al. (1999a) suggested a passage with a walk-through section for cows to pass in a straight line from the lying to the feeding area and back (Figure 5).

One of the primary differences between the AMS and CMS lies in the movement pattern of the cows. While in CMS, the cows are herded in twice by the manager for milking, for AMS, the cows visit the milking unit on their own because of the almost set pattern. To enhance the output of AMS, Ketelaar et al. (2000) and Spo¨rndly and Wredle (2004) suggested combining AMS and grazing. However, they concluded that this method is not proven to be as effective as claimed to be, as the time spent walking to and from the pasture tends to decrease the visits to the milking unit. They also tried providing extra feed supplements and drinking water indoors, but neither improved the yield.

From the findings of the above-discussed studies, it can be concluded that the most efficient form of combining grazing with AMS would be allowing the cows to graze on a pasture quite close to the milking unit.

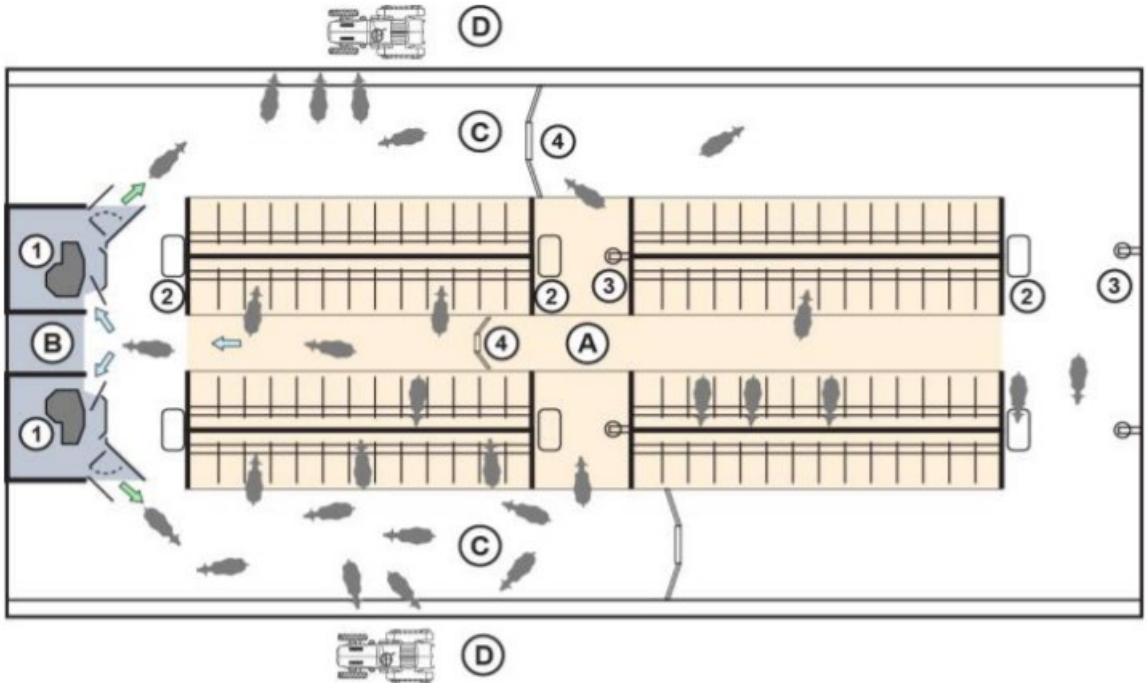


Figure 4. Layout of barn with free cow traffic: A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, 1-AMS, 2-Troughs, 3-Brushes 4- Manure scraper (Unal et al., 2017)

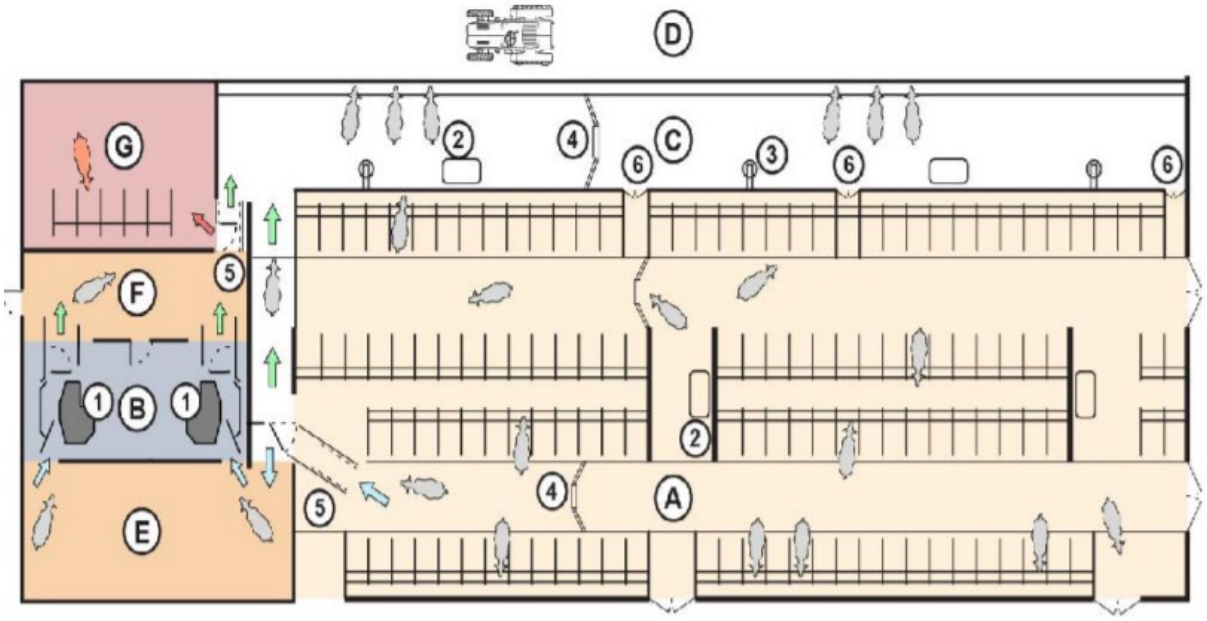


Figure 5. Layout of barn with forced cow traffic: A-Resting area, B-Milking room, C-Feeding area, D-Feeding Line, E-Waiting area, F-Exit area, G- Separation area, 1-AMS, 2-Trough, 3-Brush, 4-Manure scraper, 5-Smart gate, 6-One way gate (Unal et al., 2017)

2.1.5. Animal welfare

AMS requires cows to be forced to follow a pattern of living. Since the cows must line up for all the activities at the farm, such as eating and milking, it is important to understand how the low-ranked cows adapt to such situations. In a study by Hagen et al. (2005), it was seen that the cows subjected to such forced rules in AMS farms displayed signs of chronic stress, such as irregular heart rate. Notably, stress, as indicated by elevated cortisol levels, was not observed during milking, and this finding is in line with the findings of Gygax et al. (2006).

While most studies analyze the difference in the effect of the two milking systems on cows, Weiss et al. (2004) evaluated the physiological effects of transferring cows from CMS farms to AMS farms. The rate of adaptation varied widely across the herd. Although most cows exhibited increased heart rate during the first visit to milking unit at the AMS farm, it stabilized by the second visit. One of AMS's disadvantages is the risk of failed milking due to a detached milking cluster. The long-term effect of this might appear as a change in the cow's behavior, for instance, less time spent resting and frequent urination (Stefanowska et al., 2000).

2.2. Pros and cons in milking using AMS

The success of AMS requires active cows that frequently and regularly visit the feeding area and the milking station. When this is achieved, cows can be milked more frequently and consistently, without extra labor costs. Increased milking frequency enhances milk production. Moreover, in well-managed systems, frequent milking can improve udder health. Factors such as poor routine management, technical problems in the milking unit, or other factors inhibiting the motivation of the cows limit the success of AMS (Svennersten-Sjaunja and Pettersson, 2008).

A key factor influencing the success of AMS is well-functioning cow traffic, which can be achieved by making feed available to the cows. This necessitates investment in feeding equipment such as automatic feed wagons or feeding troughs. Lack of feed during parts of the day tends to increase asynchronized behavior among cows, resulting in queuing problems

in front of the milking unit, lower milking frequency, increased variations in milking intervals, and increased labor costs for fetching cows for milking.

AMS produces consistent milking and allows the herd manager to predict the routines, which is a prerequisite for successful milking. In AMS, crucial steps of the milking process are teat localization and teat cup attachment. These processes can be disturbed by certain problems such as a malfunctioning robotic arm, misplaced teats or abnormal udder shape, dirty teats, or a restless cow leading to an incomplete milking of one or more quarters. Highly frequent milkings ending abruptly may increase udder health problems and affect milk quality.

In AMS, many cows are milked at the same milking stall, which generally has only one milking unit. In this stall, technical equipment, including sensors, can be installed to better observe the animals' behavior in order to make an informed decision regarding their health and milking process.

One of the major disadvantages of AMS is the need for advanced technology and maintenance and initial capital investment. A highly skilled technician and a herd manager are also required to oversee the milk production and be alert for any problems that might occur during the process, such as teat cup detachment (Svennersten-Sjaunja and Pettersson, 2008).

3. Effect of milking system on udder health and SCC

Many factors such as milk productivity, the health of an animal, management, and environment affect the udder health and SSC in milk**.**

3.1. Udder health

3.1.1. Physiology of lactation

The process by which living beings, mostly mammals, produce milk is called lactation. External factors such as milking and suckling, as well as internal factors such as hormone regulation, control milk synthesis and secretion (Mepham, 1987). Post-parturition, the interaction among estrogen, progesterone, and increased prolactin (PRL) initiates lactation.

PRL is a polypeptide produced mostly by the pituitary gland, but also by mammary epithelial cells to a certain extent (Le Provost et al., 1994; Lkhider et al., 1997). PRL

maintains high concentrations of mRNA, which then enhances metabolism seen in the form of increased cell differentiation and proliferation. This enhances milk protein Another mechanism by which PRL increases the rate of milk secretion is by decreasing the permeability of tight junctions (TJ) (Linzell et al., 1975, Cowie et al., 1969; Allen, 1990). This is achieved mainly through keeping the DNA content of mammary cells constant (Flint & Gardner, 1994). As stated previously, external factors such as suckling and milking increase milk secretion; the main mechanism behind this is the increased PRL levels. A study by Gorewit et al. (1992) found that PRL increased upon teat stimulation, and the surge lasted for at least an hour post-milking. Notably, stress increases PRL levels as well, and milk secretion increases consequently (Bole-Feysot et al., 1998; Dorshkind & Horseman, 2001; do Amaral et al., 2010).

Oxytocin is a pituitary hormone synthesized in the hypothalamus (Akers, 2002). When a cow suckles her calf, the hypothalamus releases oxytocin, which helps in successful milk secretion (Mepham, 1987). When the calf suckles at its mother's teat, the stimulated receptors send signals to the brain, following which the neurohypophysis releases oxytocin into the blood circulation. The alveolar region acts as the storage unit of milk. Oxytocin pushes the milk from the alveoli into the duct system from where it reaches the udder cistern (Pfeilsticker et al., 1996; Bruckmaier et al., 1994; Bruckmaier & Hilger, 2001). Once the teat is stimulated, it takes about 40–120 s for milk secretion to begin; it is important to note that this lag time also depends on the degree to which the udder is filled (Bruckmaier et al., 1994; Bruckmaier & Hilger, 2001). A supraphysiological concentration can open the TJs, consequently arresting or limiting milk secretion (Allen, 1990; Linzel & Peaker, 1971).

In addition, lactation is regulated by corticoids (Topper & Freeman, 1980). Cortisol is a primary glucocorticoid and stress hormone. As mentioned before, stress increases PRL levels in the body, which further increases milk production. Cortisol influences alveolar cell differentiation during the last stage of lactogenesis. Moreover, it promotes casein and α-lactalbumin (ALA) gene transcription (Akers, 2002). Furthermore, cortisol ensures that the mammary epithelium remains unbroken (Zettl et al., 1992; Stelwagen et al., 1998).

3.1.2. Milk formation

Cow milk is a colloid that contains fat and proteins such as casein and whey dispersed in an aqueous solution. This solution contains lactose, vitamins, minerals, and certain other components in small quantities. The synthesis of milk is a cumulation of a chain of steps in the complex process. First, essential nutrients circulating in the blood are reabsorbed and carried into epithelial cells (Mepham, 1987). It is important to note that most of the components of milk are resynthesized in the mammary epithelial cells; on the other hand, ions and certain proteins such as immunoglobulins are transported to the synthesis unit as is.

Fat. Approximately, 3.8–4.9% of cow milk is made of fat (Akers, 2002; Blowey & Edmondson, 2010). In ruminants, acetate and b-hydroxybutyrate facilitate fatty acid synthesis (Mepham, 1987). In the epithelial cells, the smooth endoplasmic reticulum produces triglycerides in the form of small droplets containing polar lipids covered with protein (Mather & Keenan, 1998). These droplets are released into the cytoplasm, where they join each other to form a milk fat globule (MFG) that is covered with an epithelial layer that protects it from disintegration. Notably, the thickness of the epithelial membrane determines the globules capacity to resist lipolysis (Evers, 2004).

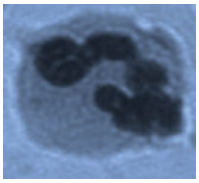
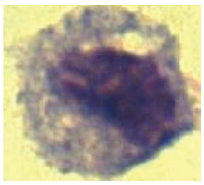
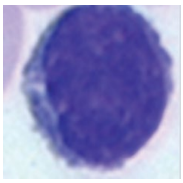
Proteins. About 3–3.6% of cow milk is made of protein (Akers, 2002; Blowey & Edmondson, 2010), which is of two types: milk synthesized and whey. These are found a ratio of 80:20. Proteins are basically made of amino acids, which are carried across the basolateral membrane for protein synthesis (Jenness, 1986; Mepham, 1987). Amino acids are joined to one another by covalent bonds. The formation of protein occurs in ribosomes and is carried out by the rough endoplasmic reticulum (Mepham, 1987). Milk contains about three major types of proteins: casein (milk proteins), ALA, and β-lactoglobulin (whey proteins). Casein is found in milk in the form of micelles, which are a combination of casein molecules, calcium, and phosphorus; this processing occurs in the Golgi apparatus. One of the most vital components for lactose production is ALA. The secretory vesicles on the Golgi carry milk proteins and lactose to the cell's apical membrane.

Lactose. Carbohydrates are energy sources of the body. About 4.6–4.8% of cow milk is made of lactose, the major carbohydrate component of milk (Akers, 2002; Blowey & Edmondson, 2010). Apart from providing energy, lactose also plays a major role in maintaining the udder's isosmotic balance (Mepham, 1987), which indicates that the volume of milk is regulated by the lactose content. Among all the components of milk, lactose is the least affected by physiological changes. However, sometimes, due to damaged TJs, the quantity of lactose gets affected; in such cases, sodium and chloride ions regulate the osmolarity of milk (Kuhn et al., 1980; Mepham, 1987). Lactose is produced in the Golgi apparatus. Two molecules of glucose are converted to galactose (Kuhn et al., 1980; Mepham, 1987), which is then converted to lactose by lactose synthase. This enzyme is made up of galactosyltransferase and ALA.

3.1.3. Cells in milk

The anatomical and chemical barriers of the mammary gland are the first line of defense, followed by somatic cell count (SCC), which indicates the number of body cells, mostly desquamated mammary epithelial cells and leukocytes to a certain extent, mixed with milk. Desquamation is normal and is important for epithelial regeneration. The leukocytes present in milk serve their primary function, that is, fight diseases such as mastitis, as well as repair damaged tissues. Normal SSC is around 1 lakh cells/ml (Hillerton. 1999). If the value increases to 2 lakh CFU/ml, it is indicative of infection in at least one quarter (Vishnoi et al., 2007; Dang et al., 2008). The increase in SCC is directly proportional to the severity of the infection.

Table 1. Difference between the morphological characteristics and percentage of leukocytes in the milk of healthy cow and that of a cow with mastitis

Parameters	Neutrophils	Macrophages	Lymphocytes
Leukocyte at 100X (Olympus IX51 microscope)			
Morphological characteristics	Diameter 12-15 µm, nucleus is multilobed with bridges	Diameter 20-30 µm, the largest cell type in milk	Diameter 9-16 µm, deeply stained round nucleus with little cytoplasm
Percentage of leukocytes in healthy and mastitis milk			
Healthy cow	19	66	15
Mastitis cow	75	17	8

* Available at www.veterinaryworld.org/Vol. 11/May 2018/1.PDF

(Li et al., 2014; Alhussien et al., 2015)

Mechanism of the release of SCC in milk. In a lactating mother, milk production is a continuous process. It is secreted by epithelial cells, which absorb the necessary precursors from the blood for milk synthesis and then release it into the alveoli. If the body detects any infection in the mammary gland, the epithelial cells activate the first line of defense by releasing cellular (leukocytes) and humoral (immunoglobulin). Leukocytes consist of lymphocytes and phagocytes such as neutrophils and macrophages (Dang et al., 2008). On the other hand, humoral components include macromolecules such as antibodies, complement proteins, and antimicrobial peptides. When an infection in the mammary gland is detected, phagocytes latch onto the pathogen to destroy them, which is called phagocytosis.

3.1.4. Mastitis

Mastitis is generally caused by pathogens, including gram-positive cocci such as streptococci and staphylococci, as well as gram-negative rods such as lactose-fermenting coliforms, which enter the body through the teat canal. *Mycoplasma* spp spreads from cow to cow through aerosols. However, other pathogens such as *Staphylococcus aureus, Streptococcus agalactiae,* and *Corynebacterium bovis* generally exhibit a contagious spread upon coming in direct contact, for instance, through the milker's dirty hands or excessive shedding of bacteria from infected udders. Algae such as *Prototheca zopfii* are also known to spread contagiously. Factors such as bedding, water for udder preparation, water or mud containers, flies are common sources of infection.

Intramammary infections (IMIs) are classified into subclinical or clinical mastitis. Subclinical mastitis does not show any local inflammation or systemic signs. Apart from rare episodes of abnormal milk discharge, it is mostly asymptomatic. Although the period of infection depends on the causal agent, subclinical mastitis is mostly a chronic condition.

SSC can be used as a marker to identify the presence of infection. In general, an SCC of $\geq$ 200,000 cells/mL is mostly indicative of an infection. Similarly, herd SCCs < 200,000 cells/mL are considered desirable, and attempts must be made to lower the counts further. As SCC rises, milk production decreases. This decrease is more evident in chronic cases.

Clinical mastitis results in abnormal milk in terms of color and composition; for instance, the presence of fibrin clots during the initial stages can result in a change of color. However, if the infection persists, the udder starts showing classic signs of inflammation, such as swelling, pain, heat, and redness. Mild or moderate cases only show local signs, whereas severe cases show signs of systemic involvement, such as fever, anorexia, and shock, as well. Clinical mastitis can be either acute or chronic, mostly seen in severe and mild cases, respectively. Severe cases often present with serous secretions, which can progress to gangrenous mastitis. In most cases of clinical mastitis, only a single quarter shows signs of infection, as opposed to subclinical mastitis, in which multiple quarters get affected. Notably, Mycoplasma affects multiple quarters at once. Subclinical mastitis is typically diagnosed using milk culture.

3.2. Factors affecting SCC in milk

3.2.1. Cow factors

Productivity. A relationship exists between SCC and milk yield, although the heritability may be low. High milk-producing cows face a lot of stress; subsequently, their immunity becomes low, thereby leading to increased milk SCC (Mukherjee and Dang, 2011). High milk SCC not only negatively affects milk yield but also milk composition and quality (Cinar et al., 2015). Younger cows have lower SCC due to their low milk-producing ability (Sharma et al., 2017).

Stages of lactation. The lactation of a dairy cow can be divided into early, mid, and late lactation. Milk yield is highest during early lactation, which then decreases with the progress of lactation. The SCC was highest shortly after calving, declining rapidly to a nadir between days 25 and 45, and then rising slowly through the rest of the lactation cycle (Kennedy et al., 1982). SCC of healthy quarters increased from approximately 80×10^3 cells/ml at 35 days postpartum to 160×10^3 cells/ml at 285 days postpartum (Sheldrake et al., 1983). The mean SCC values in the milk were higher during early lactation ($1.10–1.27 \times 10^5$ cells/ml), which decreased during mid-lactation ($0.90–0.99 \times 10^5$ cells/ml), and then increased marginally during late lactation ($0.99–1.07 \times 10^5$ cells/ml) in buffaloes (Singh and Ludri, 2000). Overall, the first month of lactation showed the highest mean SCC values, followed by the second month, after which it fluctuated up to day 300 of lactation (Singh and Dang, 2002). The milk losses per unit increase of log-transformed SCC (LnSCC) in Holstein cows were estimated throughout the lactation cycle (Gonçalves et al., 2018). Milk loss was high during early lactation and reduced during mid-lactation; the highest milk losses were observed during late lactation. SCC was recorded in Belgian dairy heifers during early lactation and estimated for their impact on test-day SCC. The geometric mean of SCC (5–14 day in milk) of the 14,766 available samples was 104,000 cells/ml and decreased from 178,000 cells/ml on the 5th day to 74,000 cells/ml on the 14th day (De Vliegher et al., 2005). The stage of lactation impacts IMI; the least risk of IMI was during the 1st month of lactation and maximum during the 10th month, with an approximate difference of 6.3-fold in the probability of IMI. The prevalence

of IMI quickly reached a maximum of 79% during the 3rd month of lactation and then decreased slightly to 60% during the mid-lactation (6th month) before increasing to about 75% during late lactation in buffaloes (Moroni et al., 2006). Mammary glands of high-yielding cows have a better innate immune response during the mid-lactation stage as compared to early and late lactation and in-vitro immune response of isolated milk leukocytes (Mukherjee et al., 2013). Svensson et al. (2006), in their research in Southwest Sweden, studied the factors affected by elevated cow composite SCC (≥ 200,000 cells/ml) at first test milking after first calving in a dairy heifer. It was found that 18.1% of the animals had elevated SCC during the first test milking (21 days) after calving. The other factors associated with elevated SCC were high amounts of concentrates, moving to confined housing on the day of calving instead of earlier, and the use of restraints during milking.

Parity. Young primiparous cows produce less milk and have a lower milk SCC as compared to multiparous cows (Gonçalves et al., 2018; Saravanan et al., 2015). The mean SCC was 3.95 (51.9×10^3 cells/ml); the least-square means of SCC for bacteriological negative during first, second, and third parities were 3.80 (44.7×10^3 cells/ml), 3.93 (50.9×10^3 cells/ml), and 3.97 (53.0×10^3 cells/ml), respectively, in cows (Geneurova et al., 1993). However, the paired comparison of bacteriologically negative cows in the second lactation stage versus the first lactation was borderline significant, while the comparison between the third lactation cows versus the first lactation was not significant (Laevens et al., 1997). Another study conducted observed that the natural log of SCC from uninfected quarters of first parity cows was highest during the first stage of lactation (Schepers et al., 1997). It was also seen that the mammary gland immunity of primiparous cows was higher than in multiparous cows throughout the lactation period (Dang et al., 2014). Recently, the diurnal variation of milk SCC in Karan Fries cows across a range of parities (1, 2–4, and >4) was recorded; no difference was seen in the milk SCC up to fourth parity; however, it increased significantly ($p < 0.05$) in cows having more than four parity. Although the milk SCC of all groups fell in similar ranges as seen in the morning and evening samples, cows with more than four parities exhibited a significant diurnal variation in the DLC and were more susceptible to udder infection (Alhussien and Dang, 2017). Furthermore, the reaction of the

milk SCC to pathogens increases with age, which renders the animals more prone to new infections. There may even be long-standing infections and further tissue damage in older cows.

Body condition score (BCS) and body weight. BCS is used to evaluate fatness or thinness in cows according to a given defined scale. An increase in the BCS at calving was associated with reduced SCC in first- and second-parity cows and greater SCC in third-parity or more (Berry et al., 2007). They found that increased BCS and body weight loss during the early stage of lactation were associated with lower SCC and a lesser probability of a high test-day SCC. They further reported that the body weight was positively associated with SCC, although the effect was greater in Jersey cows than in Holstein-Friesians. They also reported a positive association between several bodyweight variables and the risk of clinical mastitis (CM).

3.2.2. Environmental factors

Season. Extreme temperatures induce stress conditions in animals, henceforth influence the feed intake. High humidity may also increase the risk of infections. The highest bulk milk somatic cell count (BMSCC) is observed in spring and summer in countries where calving patterns are non-seasonal (Morse et al., 1988). The highest BMSCC around the period of calving was observed in the winter and the lowest was observed shortly after calving (Clements et al., 2005). Higher levels of milk SCC during the hot-humid season indicate stress on the udder (Mukherjee et al., 2015). Milk contained less quantity of casein during summer and higher during winter. On the contrary, IgG and serum albumin contents were higher in summer than in winter and spring seasons (Bernabucci et al., 2015). Similarly, SCC increased in summer. Milk coagulation properties deteriorated in summer. The SCC and neutrophil:macrophage (N:M) ratio were the lowest during thermoneutral, intermediate in winter, and highest during the summer season. {please include a statement on what above stated conditions show on SCC of milk}.

Milking practices. Milking can be performed in three ways: either by the calf, by hand, or by machine. Apart from the cleanliness requirements for CMS, machine milking requires proper cleaning, smooth functioning, and maintenance of the machine as well. Dang et al. (2007) compared samples collected from bucket-milked and hand-milked crossbred cows. Higher ($p < 0.01$) SCC in hand-milked animals were found as compared to machine-milked cows. Moreover, post-milking teat dipping in antimicrobial solutions decreased the amount of SCC. BMSCC was compared from 24 months before installation of automatic milking system until 48 months post-installation (Castro et al., 2015). Significantly higher levels of BMSCC were observed during the 12-month post-installation period. However, these decreased over time. Therefore, it can be stated that automatic milking negatively impacts milk quality during the initial stages. An average of 188.4 days (Castro et al., 2017) later, the milk quality improved significantly, as both cows and herd managers got used to the procedure.

Pathogens. Two types of pathogens (contagious and environmental) are found in the mammary tissue of dairy animals. Contagious pathogens spread from cow to cow, and environmental pathogens spread through the herd's surroundings, such as through bedding materials, manure, and soil. *Staphylococcus aureus, Streptococcus agalactiae*, and *Streptococcus dysgalactiae* are classified as contagious pathogens, which adapt to the mammary gland conditions and spread from cow to cow through milking (Sharma et al., 2011). Pathogens such as *Streptococcus uberis*, *Enterococcus* spp., *Arcanobacterium pyogenes*, coagulase-negative *Staphylococci*, and coliforms are classified as environmental pathogens and are considered opportunistic pathogens affecting the mammary gland. These microbes, too, reach the mammary gland during milking. The degree of udder inflammation depends on the number of mastitis pathogens shedding from the infected mammary gland (Júnior et al., 2012). In a study by Souza et al. (2009), *S. aureus*-infected buffaloes had maximum milk SCC, followed by *Escherichia coli* and *S. agalactiae*; however, *S. agalactiae* pathogen was responsible for higher SCC than other pathogens in mastitis-affected dairy cows. The percentage of neutrophils in the mammary gland of buffaloes was maximum during *S. agalactiae* infection, followed by *E. coli* and *S. aureus*. Notably, the degree of mastitis did not influence the blood count, but it

affected the milk SCC of normal quarters (Dang et al., 2007). The presence of *S. aureus* and *Arcanobacterium pyogenes* increased bulk tank SCC. Significant differences were found in the presence of *S. aureus*, *S. agalactiae*, and *S. dysgalactiae* in bulk tank milk sampled from small household farms, dairy-farming communities, and large-scale dairy farms (Bi et al., 2016).

3.2.3. Milking process factors

Number of milkings and length of milking intervals. The milking frequency is more and regular in AMS than in CMS (Hogeveen et al., 2001). An appropriate milking frequency removes the bacteria from the udder, whereas long milking intervals (MI) facilitate bacterial colonization in the udder quarter once they enter the teat canal after milking (Bramley et al., 1981). This appropriate milking frequency decreases the risk of mastitis as well. Three times-a-day milking can decrease the SCC (Klei et al., 1997). Köhn et al. (2007) reported a slightly negative correlation between SCC and milking frequency in 10 AM farms and free cow traffic. In a study including more than 900 cases of clinical mastitis in AM herds (Rasmussen et al., 2007), MI was found to have increased by approximately 2 h/d just one month before the treatment for mastitis began. However, some studies claim that frequent milking increases the risk of bacterial invasion during milking, as the teat canals open up after every milking, paving the way inside for pathogens (Hillerton, 1991). A short MI does not reduce the harm to the udder either, as they leave less time for the teats to recover post-milking (Ipema and Benders, 1992). Increased SCC in AM was assumed to be mainly caused by irregular milking patterns (Kruip et al. 2002). On the one hand, long milking intervals (once-a-day milking) impair tight junctions, causing an influx of somatic cells into milk, and this influx of neutrophils into milk seems to continue even after twice-a-day milking has resumed (Stelwagen and Lacy-Hulbert, 1996). This could be due to the increased udder pressure. On the other hand, if the gap between two consecutive milking sessions is less than 3 h, even healthy quarters can have an SCC of close to 200,000 cells/mL (Olde Riekerink et al., 2007). Another study found that the geometric mean SCC remained elevated until 7 h after milking. This could be due to a hypothesized high influx of cells shortly after milking, followed by a slow dilution with the increasing milk volume for hours. Irregular milking frequency (a weekly coefficient of variation of MI > 23%) decreases the rate of milk synthesis (Bach and

Busto, 2005). In contrast, Weiss et al. (2002) found no differences in quarter SCC relative to udder filling when cows were milked at irregular intervals by AMS. AM requires milking permission depending on the expected yield or the time since the last milking, or it may be counted based on DIM and milk yield. However, the intended milking frequency may differ from the results.

Efficiency of the milking process. Kaihilahti et al. (2007) investigated 300 milking and cleaning processes on an AM farm and found that 5% of the milkings failed due to machine problems and 3% due to cow-related problems. These findings are supported by the results of several other studies (Bach & Busto, 2005; Jago et al., 2006). The problem in attaching teat cups arise from the difference in distance between teats depending on the age and parity of the cow, especially the increased distance between the fore teats in old cows and a decreased distance between the hind teats in first-parity cows (Miller et al., 1995). Incomplete emptying of the udder causes leakage (Stefanowska et al., 2000; Persson-Waller et al., 2003), discomfort for the cows due to increased udder pressure (Stefanowska et al., 2000), impaired milk ejection (Bach and Busto, 2005), and a disturbed milking routine. A study observed the development of clinical mastitis due to a rise in infrequent and incomplete milkings from 5 to 30% (Rasmussen et al., 2007). In AM, the teat must be prepared first if milking starts immediately after the attachment of the first teat cup (Dzidic et al., 2004a). If the teats were not brushed, an increase in the dead milking time (time without detectable milk flow) for the first three attached quarters was observed in AM (Jago et al., 2006). Teat preparation for AMS improves milk ejection (Bruckmaier et al., 2001; Hopster et al., 2002; Mačuhová et al., 2003; Dzidic et al., 2004a), irrespective of the teat cleaning method used, that is, brushing or cleaning with a cup of warm or cold water (Dzidic et al., 2004a,b). The time gap from the first stimulation of the udder to the attachment of the last milking cup was considerably (3 to 4 times) larger for AM than CM cows in an auto-tandem parlor (Gygax et al., 2007). Notably, prolonged attachment in AM did not negatively affect oxytocin release (Mačuhová et al., 2004).

Teat cleaning. Pathogens can enter the teat canal and get mixed with the milk. The risk of pathogenic invasion increases in cases of overmilking (Thiel et al., 1969). Poor udder and teat hygiene increase the risk of clinical mastitis (Breen et al., 2009), elevated SCC (Schreiner and Ruegg, 2003; Dohmen et al., 2010), and a high incidence of IMI (e.g., Schreiner and Ruegg, 2003). AM cows undergo cleaning using machines, which often lacks the precision and visual control of the milker as in CM. Current AMS does not have sensors to detect the results and cleaning efficiency of the machine.

Technical Success of Teat Cleaning. The research on the effectiveness of teat cleaning (ETC) and the technical success of teat cleaning (TSTC) in AM cows is limited. Kaihilahti et al. (2007) found that 8% of teat cleaning sessions per cow failed due to technical issues and 4% because of cow-related problems, including kick-offs. In a study by Jago et al. (2006), 4 teats were cleaned using a brush; the results suggested that only 67% of the cleanings were technically successful. In a field study conducted by Hovinen et al. (2005), more than one-third of the cows tested indicated unsatisfactory TSTC results. In the best-performing farm, over 95% of the teats were found to be successfully cleaned. However, the reasons for failed teat cleanings remained unclear. Although, some factors observed could be associated with improper cleanings, such as device failure, restless behavior of the cows, and abnormal udder and teat structure (Hovinen et al., 2005). When teat cup cleaning is performed, 10% of sessions failed because of cow restlessness, abrupt movement of the cow, teats slipping away from the cup, or the cow kicking the cleaning cup off the teat (Hovinen et al., 2005). Jago et al. (2006) reported an issue of 0.1 kicks/brushing. Rare physiological phenomena such as black teat pigmentation and long udder hair resulted in failed teat cup cleaning (Hovinen et al., 2005). In cases where teats were cleaned using a brush but the teat could not be localized before cleaning, 20–50% of cases failed due to restless cows or cows standing in a way so that the system could not function properly (Hovinen et al., 2005). Abnormal udder and teat structure resulted in failed cleaning attempts only in cases where the teats were cleaned using a brush (Hovinen et al., 2005).

Effectiveness of Teat Cleaning. Manual teat cleaning can ensure that no dirt remains on any part of the teat. This is where AM falls behind, as impeccable cleaning cannot be achieved in this system (Hovinen et al., 2005). Teat barrel or apex can be cleaned better than

teat orifice (Hovinen et al., 2005). Bacteria and sediment on the teat orifice can enter through the teat canal and get mixed with the raw milk. In Dohmen et al.'s (2010) study on AM cows, 8% of the cows were found to have dirty teats even after cleaning. Teat cleanliness significantly influences ETC. A study by Knappstein et al. (2004) found contradictory findings that showed that bacterial counts on the teats increased in some herds during cleaning. Moreover, in AM cows, teats cleaned with a cleaning cup were cleaner than those cleaned with a brush, especially in the case of extremely dirty teats (Hovinen et al., 2005). The complete opposite of this was shown in the study by Knappstein et al. (2004).

Maintaining the cleanliness of the animals is more important in AM farms than in CM farms. Moreover, the environment in which the animals live must also be kept clean and hygienic to ensure disease control. Thus, animal health experts must be consulted when designing AM farms.

3.3. Relation between milking frequency and milk quality

Somatic cell count. The duration of milking interval influences milk SCC. Once-daily milking (ODM) was observed to increase the SCC (Clark et al., 2006; Stelwagen and Lacy-Hulbert, 1996). AM herds tend to have a higher SCC in bulk tank milk, as well as at the cow level (Rasmussen et al., 2001, 2002). This could be due to the increased risk of mastitis' contagious spread, as well as, to some extent, due to irregular milking intervals (Bach and Busto, 2005). Further, high milking frequency was also indicative in mastitis susceptibility (Philpot and Nickerson, 2000).

Milk yield and composition. The milking interval was observed to influence milk composition and yield quite significantly (Davis et al., 1999; Bernier-Dodier et al., 2010). Frequent milking had increased yield due to enhanced cell proliferation and differentiation and milk synthesis (Soberon et al., 2010). Further, the frequent milking was also casuded an increase in milk protein content (Sorensen et al., 2001; Dahl et al., 2004; Bernier-Dodier et al., 2010) by reducing plasmin activity and storage time in the udder (Sorensen et al., 2001). Frequent milking also reduced udder pressure, which maintains the stability of tight junctions, thereby preventing leakage. However, the frequent milking may negatively influence fat

content due to several factors such as increased activity of fatty acid synthetase, and higher production of short-chain fatty acids (Klei et al., 1997). This might increase the free fatty acid content in milk (Svennersten-Sjaunja et al., 2002), rendering it a sour taste. To avoid adverse effects, the milking interval was suggested to be less than 18 hours (Stelwagen et al., 1997; Bach & Busto, 2005). The decrease in yield in cows subjected to once-daily milking (ODM) could be due to a decrease in the secretory cell count due to involution. This could be because of reduced milk synthesis following increased pressure of accumulated milk (Bach and Busto, 2005).

Changes in milk composition due to longer milking intervals during ODM have been observed (Stelwagen et al., 1994; Stelwagen & Lacy-Hulbert, 1996). ODM had resulted in significantly higher SCC, protein, and fat content in the milk in addition to a decrease in milk volume. The changes in milk protein content occurred when cows were milked with prolonged milking intervals may be due to increased content of serum protein, resulting in leakage through the tight junctions (Stelwagen and Lacy-Hulbert, 1996). Increased protease activity was observed in milk from udders subjected to ODM. In general, milking frequency did not affect the quantity of casein. However, regular ODM was said to result in increased casein content (Claesson, 1965; Lacy-Hulbert et al., 1999), owing to the large micelles incapable of leaking out to the blood compartment through the tight junctions.

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Ⅲ. Materials and methods

All experimental procedures involving animals were approved by the Seoul National University Institutional Animal Use and Care Committee, Republic of Korea, and conducted in accordance with the Animal Experiment Guidelines (SNU-150603-2).

1. Animals and diets

The study was performed for three weeks from June 18 to July 8, 2015. The AMS (VMS^{TM} with Delpro 3.0; Delaval, Tumba, Sweden) was used at the farm of Seoul National University in Pyeongchang, South Korea. The cow traffic was feed-first; hence, cows that received milking permission were only able to access the AMS by sorting through a smart selection gate. A total of 29 lactating Holstein cows were allocated to either the control (N = 10) or treatment (N = 19) group. Basic milking information of the control and treatment animals is shown in Table 2. The milking interval of the control group was adjusted to approximately 12-h and was not artificially extended. In contrast, the milking interval of the treatment group was prolonged by up to 20-h in a gradual manner during the experimental period, as described below. The experimental herd was housed indoors in a sawdust barn. In the AMS, cows were supplied with 2.5 kg of concentrates per 25 kg milk production while being milked (Table 3), which was controlled by the AMS management software (Delpro3.0, DeLaval). The amounts of concentrates offered at any given milking was determined from the hours since the last milking. The forage was offered *ad libitum*. The forage part of the diets consisted of 41% timothy, 50% alfalfa and 9% oats hay.

Table 2. Descriptive statistics of milking interval, milk yield, somatic cell count (SCC), and days in lactation at starting day of experiment.

	Mean	Standard error of the mean	Minimum	Maximum
Control(n=10)				
Milking interval(h)	11.7	0.32	10.3	12.8
Milk yield (kg/day)	31.0	2.12	17.1	41.6
SCC (10^3 /ml)	73.0	13.0	27.0	164
Days in milk (days)	217	34.8	18.5	398
Treatment(n=19)				
Milking interval(h)	12.8	0.25	11.1	16.2
Milk yield (kg/day)	30.6	0.93	22.9	37.5
SCC (10^3 /ml)	74.7	12.1	14.0	234
Days in milk (days)	181	27.7	11.9	407

Table 3. Ingredients and chemical composition of the concentrate (%, otherwise stated).

Items	Concentrate
Ingredient	
Corn fine	18.9
Wheat fine	4.44
Urea	0.35
Salt	0.55
Molasses	4.50
Ammonium chloride	0.16
Palm kernel meal	9.78
Wheat flour	17.0
DDGS corn[1]-40% protein	13.0
DDGS corn	1.80
Corn gluten feed	13.6
Limestone	2.48
Protected fat	8.00
MIN PX[2]	0.15
VIT PX[3]	0.09
Soybean meal	5.16
Total	100
Chemical composition	
Dry matter (DM)	88.5
Crude protein (CP)	20.0
Ether extract (EE)	4.73
Ash	6.69
Neutral detergent fiber (aNDF)	23.9
Acid detergent fiber (ADF)	8.94
Non-fiber carbohydrates (NFC)[4]	33.7
Calcium	1.20
Phosphorus	0.49
Total digestible nutrient (TDN)[5]	75.4

[1] DDGS corn – corn Dried Distiller's Grains with Soluble

[2] MIN PX – Mineral premix contained niacin, 10,000 mg; Mn, 4,400 mg; Zn, 4,400 mg; Fe, 13,200 mg; Cu, 2,200 mg; I, 440 mg; Co, 440 mg (Grobic-DC, Bayer Health Care, Leverkusen, Germany).

[3] VIT PX – Vitamin premix contained Vit. A, 2,650,000 IU; Vit. D3, 530,000 IU; Vit. E, 1,050 IU.

[4] NFC (%) = 100 − (CP + EE + ash + aNDF)

[5] TDN (%) = NFC + CP + [(EE-1) × 2.25] + aNDF − 7 (NRC, 2016)

2. Manipulation of treatment milking interval

In the AMS, the milking permission time was based on the time elapsed since the previous milking. Cows in the treatment group gradually increased milking interval from 13-h to 22-h during the 20-d experimental period. At the beginning of the experiment, the milking permission time was changed from an 11-h milking interval to a 13-h milking interval. Henceforth, the time of permission was increased by 1 h every 4 days until 8 days had elapsed. After that, the time of permission was increased by 1 h every 2 days until a 20-h milking interval was reached. The changes in milking intervals during the experimental period in treatment group are shown in Figure 6.

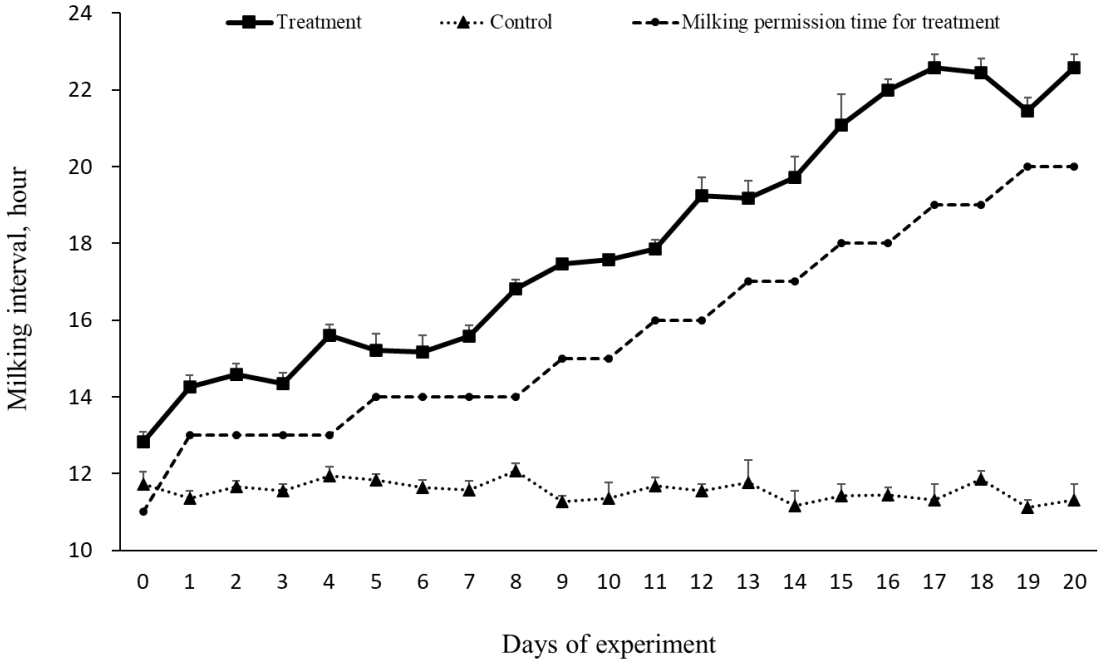

Figure 6. Milking interval of the control and treatment (prolonged milking interval) groups and the milking permission time of treatment group. Milking interval was based on the time elapsed since the previous milking. Cows in the treatment group gradually increased milking interval from 13-h to 22-h during the 20-d experimental period.

Values are mean + standard error of the mean.

3. Measurement of milk yield, composition, and quality

Milk yield was automatically recorded using built-in FloMaster units (DeLaval). Milk samples (15 ml) were collected using an automatic sampling device on 0-d, 7-d, and 20-d. Milk samples were kept at +4 ℃ until analysis. Among the collected samples, 10 ml was used for the analysis of milk composition and 5 ml for total bacterial count. Milk composition was analyzed using MilkoScan 7 RM (FOSS, Hillerød, Denmark) in the Milk Composition Analyzing Center of the Korean Animal Improvement Association (Hankyung National University, Ansung, Republic of Korea).

The SCC was measured using a Delaval Online Cell Counter. Bacterial cell counts in the raw milk were evaluated based on TBC using a 3M Petrifilm1" SM plate (3M, Minnesota, USA) at a dilution of 1:10. The Petrifilm was placed on a flat surface, and the cover film was then raised to add 1 ml of the milk sample to the center of the prescribed plating area. Then, the cover film was carefully replaced using a rolling motion, and slight pressure was applied with a plastic spreader device to distribute the sample uniformly over the prescribed counting area. The plates were then left undisturbed for 1 min to allow the gel to solidify and were incubated at 32 °C for 48 h. Irrespective of color, all colonies were counted using conventional methods and a standard colony counter. Counts were estimated either per cm^2 using the yellow grid as reference or relative to the initial volume of inoculum.

4. Statistical analysis

Statistical analyses were conducted using the software R version 4.0.4 (R Core Team, 2018). All data are expressed as mean ± standard error of the mean. The level of significance was set at $p < 0.05$. Differences between two groups were analyzed by Wilcoxon signed rank test, which is used for a non-parametric statistical hypothesis test. The simple linear regression between milking interval and somatic cell count was done.

Ⅳ. Results and Discussion

1. Milking interval during experimental period

In the AMS, the milking permission time in treatment cows was increased from 11-h to 20-h to make prolonged milking interval. As a result, milking interval in treatment cows increased from 13-h at the beginning of experiment to 22-h from 17-d after the experiment time elapsed, while the milking interval in control cows maintained the range from 11-h to 12-h (Figure 6).

2. Milk yield, composition, and quality

The effect of milking interval on milk yield and milk composition is shown in Figure 7. The milk yield was lower ($p < 0.05$) in treatment group with the prolonged milking interval compared to the control. Previous studies reported a decline in milk yield with increased milking interval (Delamaire et al., 2006a; Stelwagen et al., 2008; Hanling et al., 2021). A reduction in milk yield was observed from more than 16 h of milking intervals (Rémond and Boit, 1997). The reduction of milk yield could be due to inhibition of the milk secretion caused by an autocrine effect (Bach and Busto, 2005). This autocrine effect (induced by milking interval) had identified to suppress the milk production by regulating the number of secretory cells through the activity of a protein called "feedback inhibitor of lactation" (Peaker and Wilde, 1996). Another explanation for the decline in milk yields is due to the reduction of lactose content in milk (Figure 7) with the higher milking interval (Delamaire and Guinard-Flament, 2006a). As the major osmoregulatory factor in milk, lactose content directly affects the milk yields of the cows (Osorio et al., 2016).

Milk fat percentage was higher ($p < 0.01$) in treatment cows than in control cows on day 20, but milk fat yield (kg/d) was similar between groups. The increased milk fat percentage after prolonged milking interval may be in part due to dilution effect of milk fat by numerical decrease in milk yield (Weiss et al., 2002).

The percentage of milk protein was similar between groups on 20-d, but milk protein yield was lower ($p < 0.05$) in treatment group than in the control group. Previously, prolonged milking interval up to 16 hours has no effect on milk protein percentage (Rogers and Stewart, 1982). Compared to the control, cows with a 22-h milking interval had lower ($p < 0.05$) lactose percentage and total lactose yield on 20-d. In agreement with the present study, the milk lactose yield decreased with increasing milking intervals from 8 to 24-h in dairy cows (Delamaire and Guinard-Flament, 2006b).

The treatment group showed a higher ($p < 0.05$) TBC than the control group on 7-d and 20-d. Previously, the increase in milk bacterial count was observed on farms switching from conventional systems to AMS (Rasmussen et al., 2002; de Koning et al., 2003). The increased TBC in cows with a prolonged milking interval in our study may partly be attributed to milk leakage, which could be caused by internal milk pressure overcoming during closing forces of teat canals (Persson et al., 2003). Another reason for the higher TBC with a long milking interval may be related to the increased incubation time of infected bacteria (Bramley et al., 1981).

The SCC was numerically higher in the treatment group than in the control group on 18-d and 19-d without statistical significance after adjustment of the milking interval from 13-h to 22-h (Figure 8). The milking interval has been recommended to be less than 18-h to avoid adverse effects on milk yield and milk quality (Bach and Busto, 2005; Stelwagen et al., 1997). The high SCC in the milking interval of over 20-h could be ascribed to increased udder infections and increased incubation time of infected bacteria (Hovinen et al., 2011). However, the SCC in treatment cows was sharply decreased on 20-d even under 22-h milking interval, close to the control levels, which remains unanswered.

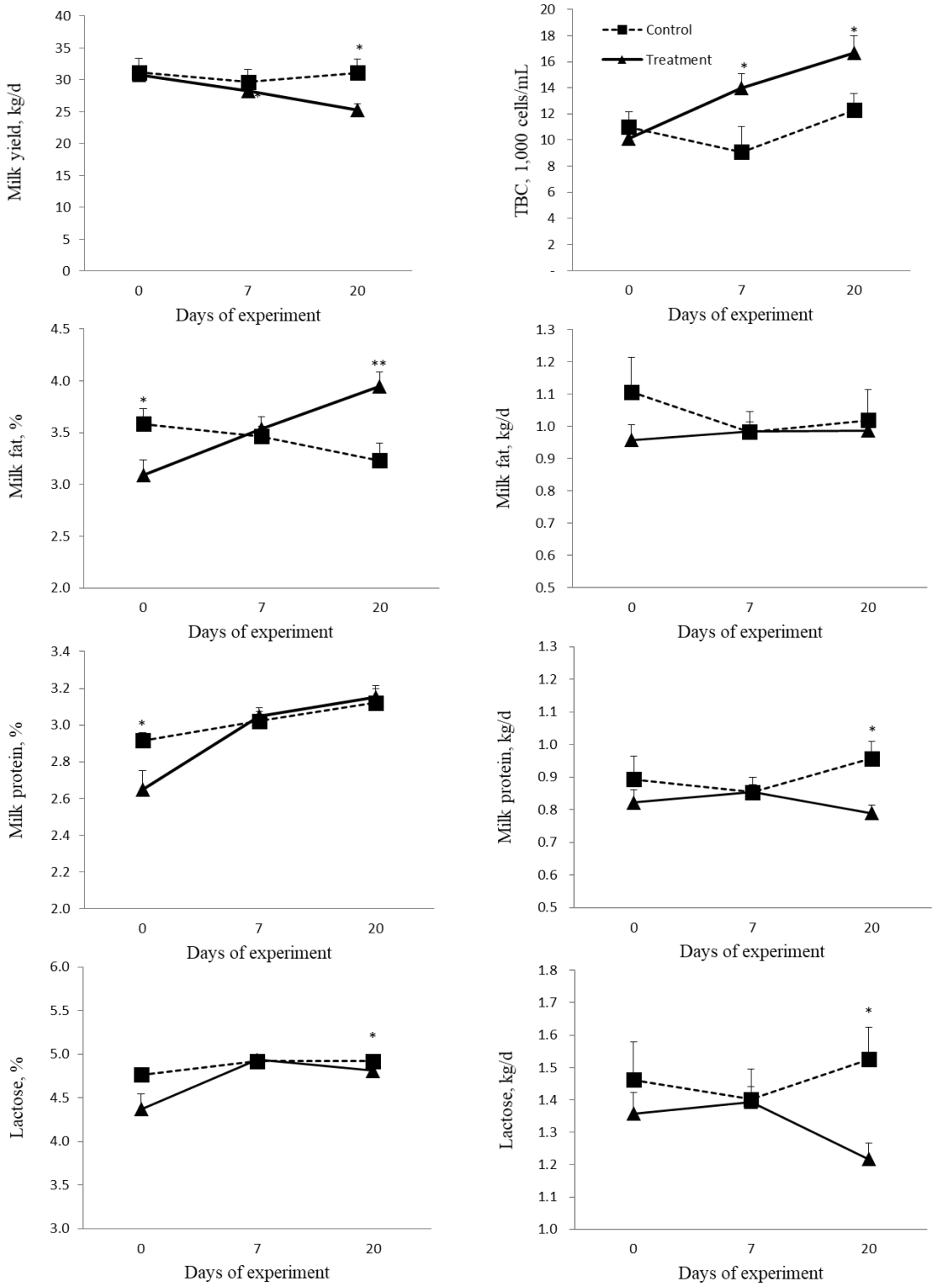

Figure 7. Milk yield and milk composition of control and treatment (prolonged milking interval) groups in Holstein cows.

Values are mean + standard error of the mean. *$p < 0.05$; **$p < 0.01$.

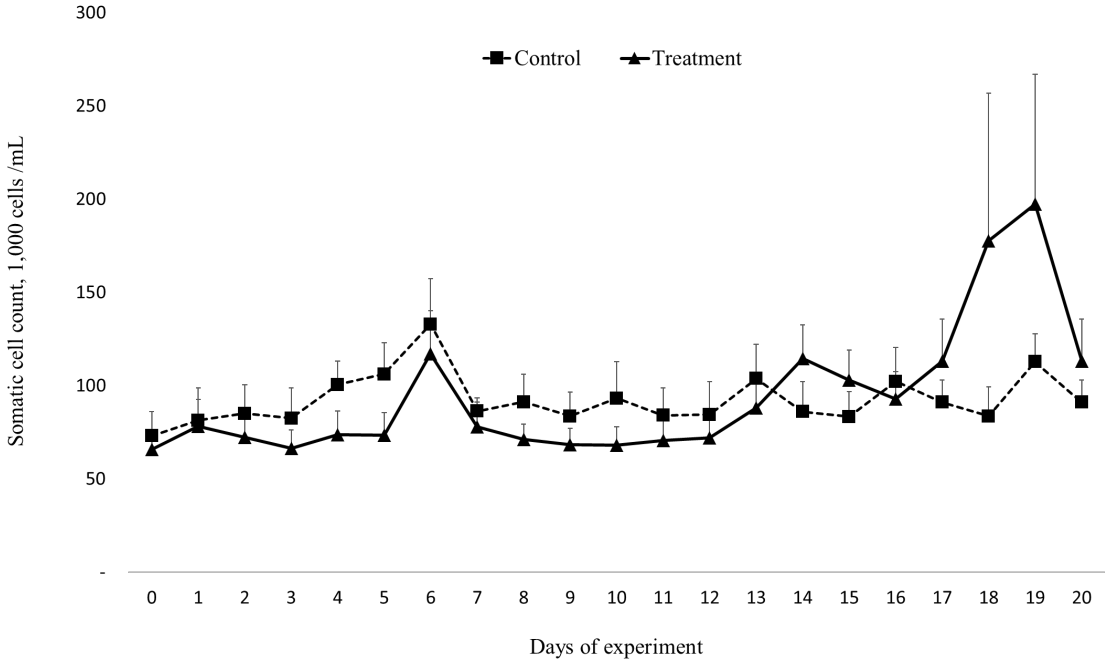

Figure 8. Somatic cell counts of control and treatment (prolonged milking interval) groups in Holstein cows during the 20-d experimental period. N = 10 for control and N =19 for treatment group.

Values are mean + standard error of the mean..

3. Correlation between somatic cell count and milking interval

Scatterplot of the daily mean milking interval against the daily mean somatic cell count of 19 treatment cows was drawn for 20 experimental days. A positive correlation was found between milking interval and SCC in treatment cows (R^2 = 0.31, p = 0.011; Figure 9). Similarly, the SCC increased after prolonged milking interval in Holstein cows (Kohler et al., 2016; Lakic et al., 2011). The increased SCC associated with longer milking intervals may be due to leaky tight junctions between mammary epithelial cells (McKusick et al., 2002). The increased SCC in a 24-h milking interval was mainly due to an influx of neutrophils into the milk (Stelwagen and Lacy-Hulbert, 1996). In addition, cows may have more udder pressure with a prolonged milking interval, which would increase infection of pathogens and affect mammary gland permeability and non-cellular inflammatory indicators (Stelwagen and Knight, 1997), contributing to the increased SCC.

Based on days in milk, treatment cows were sub-grouped into early to mid-lactation cow group (over 200 days in milk) and late-lactation cow group (less than 200 days in milk) (Table 4). Late-lactation cow group showed a positive correlation (R^2 = 0.36, p = 0.005) between milking interval and SCC, whereas the early to mid-lactation cow group did not show significant correlation (R^2 = 0.12, p = 0.138) (Figure 10). Our study demonstrates that late-lactation cows group increased SCC with increasing milking intervals, suggesting that prolonged milking intervals may reduce milk quality especially during late lactation period. The SCC was increased in late lactation (at 285 days in milk) compared with early lactation (35 days in milk) (Sheldrake et al., 1983).

Table 4. Sorting of treatment animals into sub-group by days in milk (DIM) at starting day of experiment.

Variables	Sub-group	N	Milk yield (kg/day)	Days in milk (days)	Somatic cell count (x1,000cells/mL)
Days in milk (days)	Early to Mid-lactation (DIM < 200)	N=10	31.5±1.92	70.3±14.7	59.3±11.0
	Late Lactation (DIM > 200)	N=9	31.9±1.58	285±21.8	101±27.2

Values are mean ± standard error of the mean.

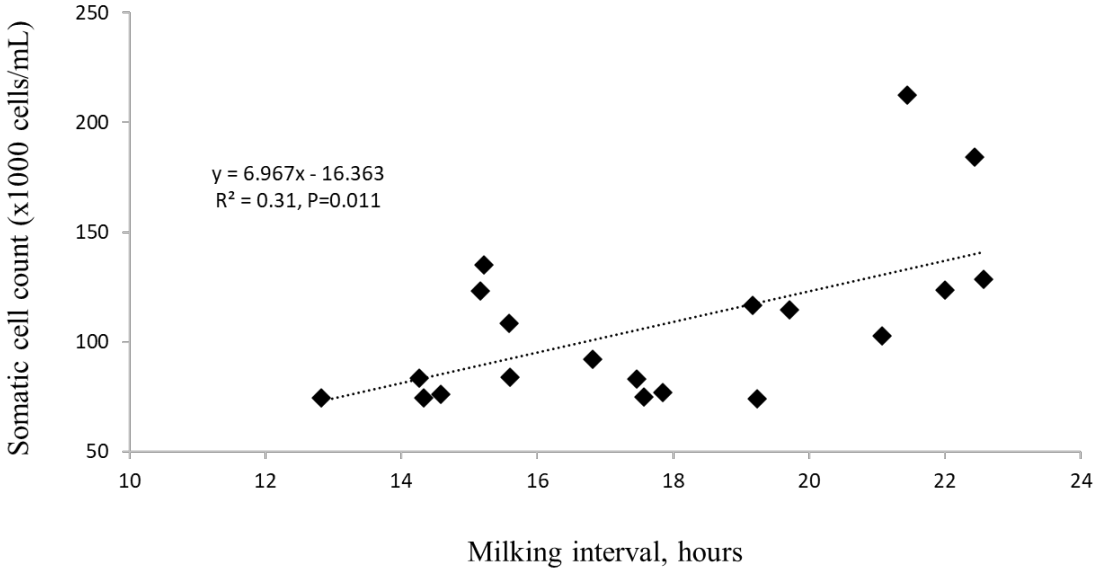

Figure 9. Scatterplot of the daily mean milking interval against the daily mean somatic cell count of 19 treatment (prolonged milking interval) Holstein cows for 20 experimental days. The dotted line is the estimated simple linear line.

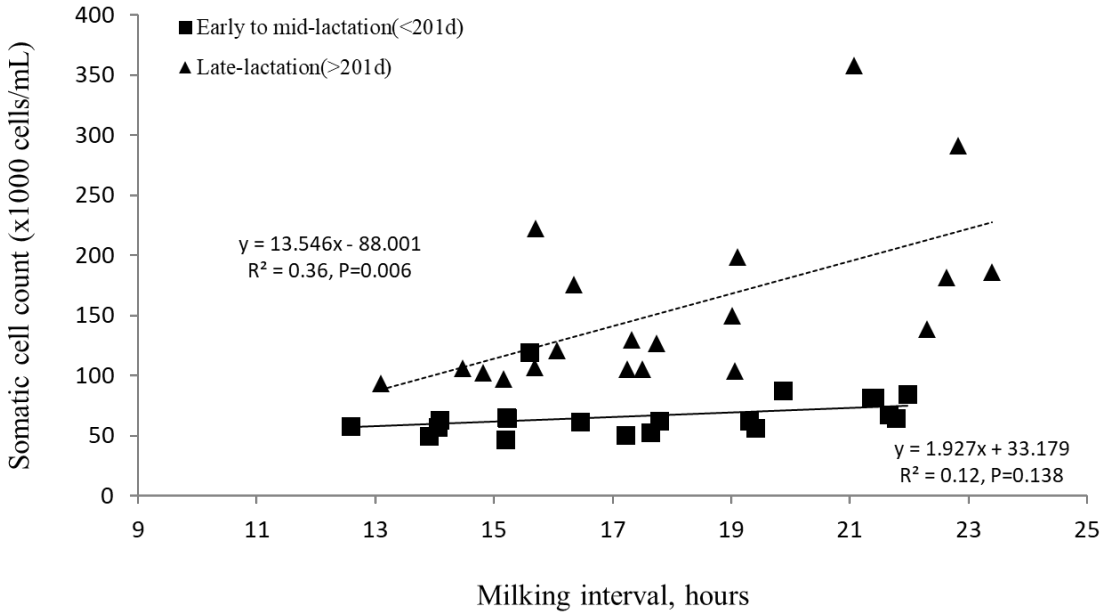

Figure 10. Scatterplot of the mean of daily milking interval against the mean of daily somatic cell count of sub-group sorted by days in milk in treatment (prolonged milking interval) Holstein cows for 20 experimental days. The solid and dotted lines are the estimated simple linear lines for early to mid-lactation cows (N=10) and late lactation cows (N=9), respectively. Square indicates the mean of daily somatic cell count associated with mean of daily milking interval for 20 experimental days in early to mid-lactation sub-group cows (N =10). Triangle indicates the mean of daily somatic cell count associated with mean of daily milking interval for 20 experimental days in late-lactation sub-group cows (N = 9).

V. Conclusion

The prolonged milking interval up to 22-h reduced milk yield and milk protein and milk lactose contents of dairy cows, but it increased total bacterial count in automatic milking systems. The prolonged milking interval increased somatic cell counts especially during late lactation period. Our study demonstrates that the prolonged milking interval adversely affects milk yield and composition as well as milk quality. It is recommended that farmers monitor the proper milking interval of individual cow when using an AMS in order to prevent reduction of the productivity of dairy cows.

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Ⅶ. Summary in Korean

자동 착유 시스템에서 젖소는 착유 허용 시간 설정에 따라 자발적으로 착유할 수 있다. 이는 일반적으로 착유 간격을 증가를 야기시켜 우유 생산량과 우유 품질에 영향을 미칠 수 있다. 이 연구는 자동 착유 시스템에서 젖소의 장기간 착유 간격 연장이 산유량과 우유 성분 및 우유 품질에 미치는 영향을 평가하기 위해 수행되었다. 총 29 마리의 착유중인 홀스타인 젖소를 대조군(N=10)과 처리군(N=19)으로 나누었다. 대조군의 착유 간격은 자동 착유 시스템(VMS™ with Delpro 3.0, Delaval)에서 11 시간에서 12 시간으로 유지되었으며 처리군의 착유 허가 시간은 11 시간에서 20 시간으로 점차 증가시켰다. 그 결과, 처리군의 젖소는 실험초기 13 시간에서 17 일에는 22 시간으로 점차적으로 착유간격이 증가하였고, 실험일 20 일까지 22 시간의 착유간격을 유지하였다. 유성분은 실험 시작일(0 일), 7 일 및 20 일에 측정하였고, 체세포 수는 실험 기간 20 일 동안 매일 측정한 결과 처리군은 실험 20 일째 산유량, 우유 단백질 함량 및 유당 함량이 대조군에 비해 낮았다 ($p < 0.05$). 유지방 함량은 모든 시점에서 두 군 간에 차이가 없었으며 총 세균 수는 실험일 7 일, 20 일에 처리군이 대조군보다 높았다 ($p < 0.05$). 측정된 체세포 수는 18 일과 19 일에 대조군보다 처리군에서 수치적으로 더 높았지만 다른 날에는 그렇지 않았다. 20 일의 실험기간 동안 처리구 젖소 19 마리의 일일 평균 체세포 수에 대한 일일 평균 착유 간격의 산점도는 단순 선형 회귀 분석에 의해 그려졌다. 착유 간격과 체세포 수 사이에는 중간 정도의 양의 상관관계가 있는 것으로 나타났다($R^2 = 0.31$, $p = 0.011$). 착유일수를 기준으로 실험군 젖소를 초기에서 중간 착유일수 그룹(우유에서 200 일 미만)과 후기 착유일수 그룹(착유일수 200 일 이상)으로 하위 그룹화 하였다. 착유초기-중기 젖소 그룹은 유의한 상관관계를 나타내지 않은 반면($R^2 = 0.12$, $p = 0.138$), 착유후기 젖소 그룹은 착유 간격과 체세포 수 사이에 중간 정도의 양의 상관관계($R^2 = 0.36$, $p = 0.005$)를 보였다. 착유 후기 젖소 그룹이 착유 간격이 증가함에 따라 체세포 수가 증가하였다. 결론적으로, 착유 간격을 22 시간까지 연장하면 우유 생산량, 우유 단백질 및 우유 유당 함량, 우유 품질(총 세균 수)이 감소하고 특히 착유 후기에 체세포 수가 증가한다.