THESIS

# ASSOCIATION BETWEEN PATTERNS OF BODY CONDITION SCORE DURING EARLY LACTATION AND CONCEPTION RATE IN DAIRY COWS


Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2021



Master's Committee

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# ABSTRACT

## ASSOCIATION BETWEEN PATTERNS OF BODY CONDITION SCORE DURING EARLY LACTATION AND CONCEPTION RATE IN DAIRY COWS

The installation of precision farming technology includes the utilization of image biometrics to calculate body condition scores (BCS) in Holstein cattle. Body condition scores help dairy operations to individually estimate energy reserves for each animal, based on subcutaneous fat found throughout the body but more specifically along the spine and the pelvis. Body condition scoring was originally a visual task performed by trained personnel that required specialized training and was often subjective. With the installation of a new automated system that has been validated (DeLaval Body Condition Scoring BCS™), BCS has become more accessible and flexible as a herd management tool. The hypothesis of this study was that low BCS, or a loss in BCS, during early lactation would reduce the rate of conception at multiple artificial inseminations (AI) increasing the number of days to pregnancy. Therefore, the overall objective of this research was to evaluate the association between BCS dynamics and the probability of conception at multiple AI.

In chapter 1, a brief literature review about the challenges during the transition period, fertility, and BCS is presented. Chapter 2 is focused on the association between BCS and BCS changes (ΔBCS) at multiple time points post-parturition and conception at first AI, while chapter 3 analyzed subsequent breedings up to fourth AI. This prospective observational study was performed on a single dairy operation in Windsor, Colorado, USA with a population of 2,885 Holstein cows including 1,460 primiparous and 1,425 multiparous cows. Study cows were housed

in a free stall, cross-ventilated barn and milked three times per day. For study 1, automatic BCS was recorded using the DeLaval Body Condition Scoring BCS™. The records of BCS were gathered at 7, 21, 35, 49, and 60 d in milk (DIM) and on the d of first AI (dAI1). A 5-point scale was used to record BCS with 0.1 intervals. The categorization of BCS was defined as low (L; < mean - 1 SD), intermediate (M; mean ± 1 SD) and high (H; > mean + 1 SD). Changes in BCS were also categorized as no loss (NL; ΔBCS ≥ 0 points) and loss (Los; ΔBCS < 0 points). Multivariate logistic regression models were used to estimate the effect of explanatory variables on conception as a binary outcome. Additionally, a cox regression analysis with hazard ratios were used along with frequency analysis to further visualize the data.

The overall conception rate at first AI was 30.1% (34.6 and 25.5% in primiparous and multiparous cows, respectively). Low BCS was associated with lower conception rate to first AI, while loss of BCS resulted in greater days to conception.

The same study design was applied in chapter three; however, BCS records were gathered at 7, 30, and 60 DIM, and at dAI1, on the day of second AI (dAI2), third AI (dAI3), and fourth AI (dAI4). Low BCS during early lactation resulted in lower odds of pregnancy at multiple AI. Logistic regression analyses of ΔBCS also showed cows that lost BCS had greater odds of pregnancy at different inseminations. The likelihood that cows will conceive concurrent with a loss in BCS was greater across multiple AI compared to cows that did not lose BCS. The overall success of pregnancy was 27.8% at second AI (pAI2), 21.4% at third AI (pAI3), and 16.0% at fourth AI (pAI4).

In conclusion, low BCS were associated with lower conception rates at AI. Furthermore, a loss in BCS were associated with greater number of days from parturition to conception to first AI

However, a loss in BCS was associated with greater conception at second, third, or fourth AI. Monitoring daily automatic BCS provides potential for assessing future fertility of dairy cows.

# ACKNOWLEDGEMENTS

I would like to thank the CSU Graduate Student Council and the CSU Graduate School for initiating, commissioning, and supporting this project. I would like to thank Dr. Pablo Pinedo for his support and ensuring that I followed through with this project to completion. I would like to thank my committee members, Dr. Ahola and Dr. Salman, for making me a better writer, researcher, and scientist. I would like to thank Diego Manriquez, who helped behind the scenes ensuring all records were downloaded monthly and guiding me in the statistical analysis portion of my thesis. I would also like to thank Ana Velasquez Munoz for being a role model and a great friend. I would like to thank Wolf Creek Dairy and Larry Brasil for allowing me to continually use their systems to collect data on their herd. I would like to thank DeLaval for facilitating the Body Condition Scoring BCS™ for this research. Finally, I would like to thank my family for supporting me through these rough and challenging times. It was very difficult and would not have been able to finish without everyone's contribution on this project.

DEDICATION

*I would like to dedicate this thesis to my family and friends. If it were not for their sacrifice this project would not have come to life.*

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## NOMENCLATURE

| | |
|---|---|
| AI | Artificial insemination |
| BCS | Body condition score |
| ΔBCS | Change in body condition score |
| BHB | Beta-hydroxybutyrate |
| CNN | Convolutional neural networks |
| d | Day |
| dAI1 | Day of first artificial insemination |
| dAI2 | Day of second artificial insemination |
| dAI3 | Day of third artificial insemination |
| dAI4 | Day of fourth artificial insemination |
| DIM | Days in milk |
| DMI | Dry matter intake |
| FA | Fatty acid |
| H | BCS category "High" |
| Hig | Milking category "High" |
| L | BCS category "Low" |
| Lo | Milking category "Low" |
| Los | ΔBCS category "Loss" |
| M | BCS category "Intermediate" |
| Med | Milking category "Medium" |
| MP | Multiparous |
| NL | ΔBCS category "No loss" |
| n | Number of observations |
| pAI1 | Pregnancy to first artificial insemination |
| pAI2 | Pregnancy to second artificial insemination |
| pAI3 | Pregnancy to third artificial insemination |
| pAI4 | Pregnancy to fourth artificial insemination |
| PP | Primiparous |
| SD | Standard deviation |
| 2D | 2-dimensional |
| 3D | 3-dimensional |

# CHAPTER 1: LITERATURE REVIEW

## Introduction

Dairy production systems are vastly evolving through the years to incorporate new technological advancements that enable precision management and specialization (Mullins et al., 2019). Milk yields increase year by year as there continues to be a demand for milk products (Haile-Mariam and Pryce, 2015). Many of these dairy production systems utilize technology to continually sustain high milk yields throughout the year (Hadrich et al., 2018). Nevertheless, the objective across all these operations remains the same, optimize milk production with focus on cattle health (Roche et al., 2013), fertility (Roche et al., 2009), and feed management (McArt et al., 2013) to maintain profitability.

In order for there to be an efficient supply of milk, each cow needs to continually reach gestation and parturition every year (Goff and Horst 1997). Management of nutrition, disease and fertility has a strong association with milk efficiency (Roche et al., 2009). However, a major downfall that dairy cattle partake in is the transition period or also known as periparturient period, in which cattle are at their most vulnerable physiological state (Mather and Melancon, 1980). Furthermore, when monitoring the metabolic and nutritional status of a cow, it is crucial to adapt practices to improve upon the transition period to increase herd health (Roche et al., 2017). One management practice that producers are monitoring cattle is through the assessment of body condition scores (BCS) (Ferguson et al., 1994; Chebel et al., 2018). Body condition scoring is performed by trained personnel that visually estimate the subcutaneous fat reserves on cattle. This score is based on a scale from 1 to 5 with 0.25 increments (Ferguson et al., 1994). A change in

BCS postpartum or during the transition period dramatically affects reproductive performance and health (Fricke et al., 2020).

Evaluating body condition by trained personnel is subjective and labor intensive (Roche et al., 2009). In addition, frequent evaluations allowing for determination of trends would be more effective on detecting environmental stressors that can have impact on performance (Rojas Canadas et al., 2020). Precision livestock farming provides producers with multiple automatic monitoring systems to observe animal behavior, production, and reproduction in real-time (Berckmans, 2014). The newest installment of precision livestock farming in dairy systems, is image biometrics, which allows for automatic body condition scoring (Mullins et al., 2019).

**Transition Period**

The transition period in dairy cattle can be defined as the time three weeks prior and three weeks after parturition (Wankhade et al., 2017). In this period cattle are in their most critical physiological state and are susceptible to metabolic and infectious diseases (Goff and Horst, 1997). When a cow transitions from nonlactating to lactating, the production of milk compromises immune function leading to subclinical and clinical disorders, metabolic diseases, and temporal infertility (Thatcher, 2017). In early lactation all cattle go through a negative energy balance when nutrient requirements cannot be met due to the high demand of energy for milk production (Herdt, 2000; McArt et al., 2013).

Within the first three weeks after parturition, about one-third of dairy cows develop a clinical disease such as lameness, metritis, respiratory problem, mastitis, or a digestive issue (Stevenson et al., 2020; Ribeiro and Carvalho, 2017). To prevent the onset of diseases, it has been proven that increasing lipid consumption in the cow's diet, helps to improve the energy intake during parturition as well as improving peripartum and postpartum health and physiological function (Roche et al., 2009). The greatest risk in culling and death for a dairy cow happens within the transition period and is worsened when cows in the previous lactation had an increase in days open (Stevenson et al., 2020; Pinedo and De Vries, 2010). Nutrition, BCS, disease, and reproduction all effect the negative energy state cattle exhibit in the transition period and reduces milk yields and reproductive performance (McArt et al., 2013; Stevenson et al; 2020).

**Reproductive Performance in Dairy Cows**

Reproductive efficiency in dairy cattle can be considered one of the most important objectives for dairy operations (McArt et al., 2013). The degree and length of the postpartum negative energy state heavily influences fertility (Berry et al., 2003). The lack of energy that results in mobilization of body reserves decreases conception rate (Pryce et al., 2001). Chen et al. (2015) demonstrated that cows did exhibit a greater BCS in week 1 to 8 postpartum resumed ovarian cycles before the 21-day mark in milk. The research that has been performed thus far reinforces that to increase fertility, there needs to be an improvement in metabolized energy to further store fat in energy reserves (LeBlanc, 2010).

The correlation between BCS and fertility is continually being explored and research consistently indicates that there is a reduction in the likelihood of conception at both the first and second postpartum AI of BCS is lost (Chebel et al., 2018). Moreover, a loss in BCS during the dry period has shown a connection with reduced reproductive performance and milk production in addition to health disorders (Chebel et al., 2018). Chebel et al. (2018) also demonstrated that were less likely to lose their pregnancy when they had gained BCS during the dry period.

Parity is an additional factor affecting the success of conception. Primiparous cattle are still maturing and growing while they are in their first lactation and are more susceptible to a lower energy balance than their multiparous counterparts (Wathes et al., 2007). The nutrient and energy demand in turn affect their reproductive performance (Lucy, 2001; Walsh et al., 2011) and research has shown that multiparous cows that were left to naturally cycle had an improved time to conception when compared to primiparous cattle (Fodor et al., 2019). On the other hand, Fodor et al. (2019) showed primiparous cows had an advantage in risk of conception at first AI when estrus detection aids were used as well as a greater risk in conception when hormonal synchronization protocols where instilled.

**Monitoring the nutritional and metabolic status of the dairy cow: Use of body condition scoring**

A large provider of the body's energy can be found through non-esterified fatty acids (FA). These molecules are a small percentage of the body's fat and are released from the breakdown of triglycerides by the enzyme lipase (LeBlanc, 2010; Roche et al., 2013). Overton et al. (2017) described that during parturition a distinct increase in concentrated FAs were found within the plasma and was soon followed by the concentrations decreasing in the initial 5 to 6 weeks of lactation. The fluctuations of concentrated FA in the plasma are caused by the body's response to stressors (Kamiya et al., 2010; Roche et al., 2013). Such as periods of low energy when FA are mobilized into the blood to bring balance to a cow's physiological state (Roche et al., 2013).

When glucose supplies are low for a cow's demanding metabolic needs, beta-hydroxybutyrate (BHB) is synthesized from fatty acids in the liver and transforms into a carrier of energy from the liver to peripheral tissue (LeBlanc, 2010; Putman et al., 2018). The ketone body BHB, is an intermediate metabolite due to the inadequate oxidation of FA (LeBlanc, 2010). The liver is unable to completely oxidize the rapid increase of FA and as a result ketone production is also increased to try and compensate for the demand in energy (LeBlanc, 2010). Near the end of lactation BHB concentrations decreased and this is attributed to the enhanced energy state and readily available carbohydrates (Putman et al., 2018).

Calcium is an essential nutrient in a cow's diet and responsible for muscle function (Aslam and Tucker, 1998). In the parturition period the production of colostrum demands all the calcium in the plasma and extracellular pools (Goff and Horst, 1993). Most cows go through this depletion of calcium known as hypocalcemia (Aslam and Tucker, 1998). Research has shown that there is a significant association between blood calcium concentrations and the time to first service (Mahen et al., 2018). The researchers further stated that calcium concentration also was associated with season, parity and BCS.

Researchers observed an increase in serum concentration of BHB and FA, accompanied by a lower concentration of glucose (Macrae et al., 2019) and a dose-response effect was determined for elevated FA or BHB with an increasing metabolic disease incidence (Ospina et al., 2013, Overton et al. 2017). BHB and FA concentrations are not related to differences in BCS at calving (Mansouryar et al., 2018), however BHB, FA and BCS changes together are indicators of negative energy balance at calving (Gärtner et al., 2019). Nutritional management in the dry period effects production after parturition and subsequent health, when overfed BHB levels increase, FA in the blood increases, while dry matter intake (DMI) decreases (McArt et al., 2013).

**Association between disease, nutritional status and fertility**

Health disorders in dairy production systems need to be strategically monitored to reduce the negative effects on wellbeing and performance (Stevenson et al., 2020). Management practices conducting early detection and treatment would prevent significant reductions in milk yield (LeBlanc et al., 2006). The risk of disease continually happens throughout a cow's lifecycle and one specific disease that has a large impact on dairy operations is metabolic diseases (Overton et al., 2017). This disorder can be defined as a cow's inability to adjust to a major physiological change such as calving (Roche et al., 2013). Cattle endure different stages of metabolic needs within their lifecycle and are exposed to various conditions that disrupt their physiological state (Overton et al., 2017).

Randall et al. (2015) expressed that within the transition period, cattle that had a BCS lower than 2.5 were at an increased risk of developing lameness. During the transition period, cattle that exhibit low BCS are at a greater risk to develop clinical endometritis (Kadivar et al., 2014). Within the dry period, cattle that lost BCS had an increase likelihood to be diagnosed with uterine diseases and indigestion (Chebel et al., 2018). Cattle that are in the parturition period and have a greater BCS, have an increased risk to have hepatic lipidosis, ketosis, and displacement of abomasum (Roche et al., 2009; Ospina et al., 2010). Moreover, cattle in parturition with low BCS had a negative impact on conception and reproductions rates (Van Straten et al., 2009) furthermore, there was also an impact on cattle health including dystocia (Gearhart et al., 1990), ketosis (Lacetera et al. 2005), and metritis (Hoedemaker et al., 2009). Some diseases that significantly affect udder health and decrease production are mastitis and low somatic cell count (SCC) (Banos et al., 2006).

The DMI of cattle was also taken into consideration when evaluating the risk of disease. One study showed that cattle had a reduction in DMI 13 days prepartum were more likely to be diagnosed with metritis postpartum (Huzzey et al., 2007). Chebel et al. (2018) reported that cattle with low BCS and disease diagnosis were more likely to receive antimicrobial, anti-inflammatory, and supportive therapies. Conception rate is decreased when cows lack the energy to mobilize body reserves (Pryce et al., 2001).

The association between disease, nutritional status and fertility can be best described in the research conducted by Roche and colleagues (2013) in which most metabolic diseases derive from an unwarranted loss in BCS, insufficient nutrition, or when metabolic conditions are less than optimal between gestational and lactating periods. Ospina et al. (2010) observed that greater than 15% of the sampled animals have FA and BHB that were above the cow level had an association with an increase incidence of metabolic disease, lower milk yield, and a decrease in reproductive performance. Overall, cows need to match the total energy consumed to the total energy output to avoid a negative energy state, a loss in BCS, and further shortfalls in disease, nutrition, or fertility (Roche et al., 2013; McArt et al., 2013; Bello et al., 2012).

**Individual and herd management of body condition scores**

Body condition score (BCS) is a visual indicator of an animal's subcutaneous fat that is recorded as a number. In the dairy industry this visual assessment is subjective and rated on a scale from 1 to 5 with 5 being overweight (Roche et al., 2013). This visual scoring system is labor intensive and takes thorough training to be considered dependable on a single operation. BCS is a factor to be considered in predicting the onset of disease, estimating reproduction, and maintaining milk production (Buckley et al., 2003; Pryce et al., 2001). Body weight is an additional tool that can help confirm BCS through visual representation of a cow's metabolic condition; however, body weight varies between cows (Roche et al., 2013). This is due to the multidimensional biological differences in age, skeletal frame, fatness and gut fill (Enevoldsen et al., 1997).

Furthermore, cattle do not consistently exhibit the same BCS due to the cycle of a cow with the fluctuations in lactation and through the dry period (Roche et al., 2009). Milk yield is heavily influenced by the variations in BCS which in turn alter the economics of the producer; this phenomenon happens not just in the lactation period but trying to maintain the appropriate metabolic status in the transition period too (Mullins et al., 2019). To aid in management, herd alarms help in detecting cattle that are at risk of poor health, reproduction, or performance specifically when they are in the transition period (Ospina et al., 2010; Overton et al., 2017). Herd alarms identify cow-level threshold that is abnormal and is associated with health, reproduction, and milk production (Ospina et al., 2010; Overton et al., 2017). BCS continuously aids in managing decisions on nutritional input (Ospina et al., 2010) improvement of fertility factors, prevention of disease (Chebel et al., 2018) and increasing milk yield (Berry et al., 2003).

**Environmental stressors and reproductive performance**

There are various environmental factors that have a large effect on dairy cattle populations which in turn can influence important outcomes (Macrae et al., 2019). To address the thermal environment, is critical to know cow's needs and climate they are housed in (Collier et al., 2006). Heat stress can have an impact on a cow's nutritional, productive, physiological, health, and behavior (Allen et al., 2015). When cattle are producing in a hot climate rations require changes due to the cow's decrease in dry matter intake (Macrae et al., 2019) when there is a decrease in DMI consumption this can impact BCS (Chebel et al., 2018). Involving proper management strategies can start with an increase in the nutrient density, altering mineral and water intake and ultimately modifying the digestive tract to combat potential negative energy balances (Collier et al., 2006). Collier et al. (2006) further states that climate can affect the expression of estrus and fertility in lactating cows. Research continues to provide evidence in reducing stress by implementing advancements in housing and cooling systems that can help cattle adapt to their thermal environments thus further improving well-being to maintain BCS.

Herd size can be overlooked when one of the primary objectives in dairy production systems is focused on financial and economic stability (Macrae et al., 2019). An increase in the transmission of disease was present in larger herds and further evidence showed an increase in asymptomatic carriers (Hume et al., 2004). Disease is influenced by stressors such as herd crowding and can be difficult to control (West, 2003). Once there is an onset of disease, BCS tends to decrease (Chebel et al., 2018; Randall et al., 2015). Considering this data, management practices need to accommodate the demand for milk yields, but also properly control the setbacks of an increased herd size.

Seasonal changes can often have negative impacts on cattle and influence milk yields (Macrae et al., 2019). When cattle were observed in the two extreme seasons, researchers found that calves born in the winter showed an increase in milk yields compared to the calves that were conceived in the summer (Pinedo and Vries, 2017). Subsequent survival was also a prominent finding that Pinedo and Vries (2017) expressed as significant with cattle conceived in the winter versus cattle conceived in the summer. Epigenetics can play a vital role in explaining this phenomenon due to the environment's influence on gene expression and phenotype when offspring are developing (Petronis, 2010).

Despite the negative effects some environmental phenomenon have on the surrounding dairy production systems, it is important to minimize its impact on nutrition, physiological, health, and production yields that can further impact BCS (Macrae et al., 2019). Strategies such as implementing proper housing and cooling systems, controlling herd size, and planning for seasonal changes can result in an improvement on the overall cattle performance.

**Use of automated Body Condition Scoring**

Precision farming in dairy production systems is on the rise as new technology emerges on the market (Britt et al., 2018). The focus of precision farming is to ensure maximum performance, financial stability, prevent disease onset with early detection, and improve overall well-being (Gargiulo et al., 2018; Mottram, 2015). As covered in research, BCS does have significance in health, fertility, and milk production, but the efficiency and time management are difficult to control (Gargiulo et al., 2018). The Elanco BCS chart depicted the lactation curve and desired BCS during different stages of lactation shown in Figure 2.1 (Modified from Ferguson et al., 1994; Elanco Animal Health, 2009). Body weight has disadvantages due to the variations of size in cows (Roche et al., 2013). Additionally, body weight records are hard to manage in a herd due to biological differences and BCS are subjective, there are new advancements to remain consistent in record keeping (Roche et al., 2013). Multiple installations in precision farming have had major impacts on the dairy industry in automating BCS (Halachmi et al., 2013; Li et al., 2017; Alvarez et al., 2018; Yukon et al., 2019; Mullins et al., 2019). Image biometrics is one solution than can make BCS objective and reduce labor management.

Thermal imaging is an older camera system that has been successful in removing the subjectivity in assessing BCS and enabled producers to improve management decisions in nutrition intake (Halachmi et al., 2013). Ultrasound has been precise in measuring the amount of backfat thickness in cattle and has had high correlations with manual assessment of BCS (Weber et al., 2013). Utilization of video cameras that continuously capture recordings of cows entering the milking parlor allowed researchers to develop Zernike models to identify shape features (Li et al., 2017). Zernike shape features successfully captures images of the tailheads of cows and had a quadratic discriminant analysis of 99.7% precision (Li et al., 2017). Convolutional Neural Networks (CNN) had accuracy within 0.5 units when assessing BCS compared to trained scorers (Alvarez et al., 2018). An additional study combined images, ultrasound and manual scores to find a linear regression coefficient of (0.976) (Yukon et al., 2019). The implementation of vision cameras, which include 2-dimensional (2D), thermal, 3-dimensional (3D), and ultrasound are a few examples of automatic BCS systems being installed in dairy operations (Song et al., 2019).

One of the more recent technological BCS systems is the DeLaval Body Condition Scoring BCS™ (BCS DeLaval International Tumba, Sweden). This device captures a 3D image of the lower back as a cow passes under the camera. The fat covering is determined by the image using its specialized DeLaval BCS software which further interprets a cows BCS. This score is then stored in the DelPro Farm Manager which can be accessed anytime with a computer.

The DeLaval BCS system was validated on a commercial dairy farm in Greensburg, Indiana and had accurate scoring for cattle with scores of 3.0 to 3.75 with a correlation coefficient of (0.76) (Mullins et al., 2019). With the validation of the automated system and continual improvements, it is promising that producers can utilize this management tool on their operations to further their production yields, reproductive performance, and animal welfare.

**Final remarks**

Dairy production systems endure many challenges as cows go through their lifecycle. With precision farming these challenges can be assessed and provide the producer the proper tools to minimize the impact (Berckmans, 2014). Body condition scoring is one management tool that has already proved useful throughout commercial dairies; however, this visual scoring system is labor intensive and subjective (Roche et al., 2013). Precision farming advancements have greatly improved the practice of BCS, taking it to an automated level without the need to train specialized personnel (Mullins et al., 2019). Research has shown the significance that BCS have on assessing health (Roche et al., 2013), fertility, feed intake (McArt et al., 2013), and milk production (Hadrich et al., 2013). The new advancements in BCS technology have been validated with high accuracy and show objective data that can become more dependable (Mullins et al., 2019). With the automated BCS system, researchers now have the ability to improve the efficiency and explore deeper into challenges that the dairy industry faces. Furthermore, fertility continues to be one of these challenge that is of important significance to investigate. Accordingly, the objective of this thesis is to analyze consistent automated BCS and the changes of the BCS during multiple time points during a cow's lactation to determine associations with subsequent fertility.

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# CHAPTER 2: ASSOCIATION BETWEEN PATTERNS OF BODY CONDITION SCORE DURING EARLY LACTATION AND CONCEPTION RATE AT FIRST ARTIFICIAL INSEMINATION IN HOLSTEIN COWS


## Summary

Assessment of body condition score (BCS) is a management practice that uses a subjective evaluation to determine subcutaneous fat reserves in dairy cattle. The practice of assessing individual cows is performed visually by trained personnel. However, in recent years the emergence of a commercial BCS camera has allowed for automatic assessment. The aim of this study was to evaluate the association between BCS and BCS changes during early lactation and the probability of conception at first AI in Holstein cows. Cows housed in a cross-ventilated free stall barn in one dairy in Windsor, Colorado, USA were enrolled upon parturition. Automatic and validated BCS records were gathered at d 7, 21, 35, 49, and 60 d in milk (DIM), and on the d of first AI (dAI1). A total of 2,885 cows (primiparous = 1,460; multiparous = 1,425) were followed from April 2019 to April 2020. Daily milk yield was recorded up to 90 DIM. Cows were subject to the OvSynch estrous synchronization protocol starting at 60 ± 3 DIM and milked three times daily. A 5-point scale was used to record BCS with 0.1 intervals. Scores were subsequently categorized as low (L; < mean - 1 SD), intermediate (M; mean ± 1 SD) and high (H; > mean + 1 SD). Changes in BCS were also categorized as no loss (NL; ΔBCS ≥ 0 points) and loss (Los; ΔBCS < 0 points). Associations were affirmed statistically significant if *P* ≤ 0.05. Low BCS at multiple time points was associated with lower pregnancy rate to first AI. Losses in BCS for the periods 7 to 21 DIM, 7 to 35 DIM, 7 to 49 DIM, 7 to 60 DIM, and 7 to dAI1 were associated with greater pregnancy at first AI. However, loss of BCS later in lactation resulted in increased time to

conception. Monitoring daily automatic BCS provides potential for assessing future fertility of dairy cows.

## Introduction

Body condition score (BCS) is an evaluation system that assesses the level of subcutaneous fat in dairy cows on a scale from 1 (emaciated) to 5 (overweight; Roche et al., 2013). Dairy managers use BCS as a management tool to guide nutritional decisions (Ospina et al., 2010), improve fertility, prevent disease (Chebel et al., 2018), and maximize milk yield (Berry et al., 2003). However, this scoring system is a subjective measurement and a time constraining process (Mullins et al., 2019).

Proper body condition during pre-calving is crucial (Lange et al., 2016), as dairy cows go through a negative energy balance during early lactation when nutrient intake cannot meet energy requirements for milk production (Herdt, 2000; McArt et al., 2013). This condition results in loss of body condition and an increased risk of disease (Mulligan and Doherty, 2008).

It has been reported that cows with BCS $\leq 2.5$ had an increased risk of developing lameness within the transition period (Randall et al., 2015), together with a greater risk of developing clinical endometritis (Kadivar et al., 2014). Loss in BCS combined with inadequate nutrition or adverse homeostatic conditions can result in metabolic diseases (Roche et al., 2013) and milk yield was also correlated with fluctuations in BCS during the transition period (Buckley et al., 2003).

The duration and severity of a negative energy state influences the fertility of a cow (Berry et al., 2003). Conception rate is decreased due to a lack of energy to mobilize body reserves to maintain homeostatic conditions (Pryce et al., 2001) and it has been reported that both first and second post-calving artificial insemination (AI) had a reduction in the likelihood of conception when cattle exhibited a loss of BCS (Chebel et al., 2018).

Many dairies in the US have on-farm technology that collect data continually which include milk, nutrition, and health records (NAHMS, 2014). Automated BCS systems have been available in research settings that can capture an image of the topline of a cow and determine a score. Research has indicated high validation through numerous different models and precision imaging in automatic BCS (Halachmi et al., 2013; Li et al., 2017; Alvarez et al., 2018; Yukon et al., 2019). Thermal imaging has been optimized through the years to remove objectivity in manual BCS data collection and researchers found a Pearson correlation (0.94) that enabled producers to improve nutritional decision making and management (Halachmi et al., 2013). The use of an ultrasound has successfully assessed backfat thickness with a correlation of 0.76 (Weber et al., 2014) and a similar study used two video cameras to capture continuous recordings of cows entering the milking parlor and analyzing the images with Zernike moments to identify shape features (Li et al., 2017). The authors concluded that the images gathered from the tailhead had a quadratic discriminant analysis of 99.7% precision. An additional estimator of BCS has been Convolutional Neural Networks (CNN) that had a 94% probability of overall accuracy within 0.5 units (Alvarez et al., 2018). The comparison between ultrasound, manual scores, and images captured from a camera had a coefficient of 0.976 (Yukon et al., 2019).

In a more recent development, the DeLaval Body Condition Scoring BCS™ (BCS DeLaval International AB, Tumba Sweden) has become available for research and for commercial use. This system was validated indicating a correlation (coefficient = 0.76) between visual scoring and automatic BCS (Mullins et al., 2019). The DeLaval Body Condition Scoring BCS™ provides automated BCS and continuous recording of BCS data that can be utilized to monitor risk of disease, milk production, and fertility.

Establishing the association between BCS change and the probability of conception to first AI (pAI1) could inform dairy farmers on the best timing for AI. This information could also be a tool for group management and optimization of milk production and reproduction helping producers to become more financially stable. The hypothesis was that low BCS, or a loss in BCS during early lactation, would reduce the probability of pAI1, increasing the number of days to conception. Thus, the objective of this study was to estimate the association between BCS and BCS fluctuations (ΔBCS) at multiple time points during early lactation and the probability of conception at first AI.

## Materials and Methods

### *Study Population*

This was a prospective observational study. Cows were monitored during lactation starting on the day of calving and gathering data up until 90 days in milk (DIM). The observations and records were collected from a commercial dairy operation located in Windsor, Colorado, USA. Cows were categorized as primiparous or multiparous. Data were derived from a population of 2,885 Holstein cows including 1,460 primiparous and 1,425 multiparous cows. The cows were housed in a cross-ventilated, free stall barn and placed in the appropriate group pens determined by stage of lactation and amount of milk produced. These cows were monitored from April 23, 2019 to April 30, 2020.

Cows were subject to AI following a double OvSynch protocol (Nowicki et al., 2017). Cows were milked three times daily and milk yield was recorded for each session, which included data such as peak flow, average flow, milk duration, and overall yield for that session. Milk yield data were considered up to 90 DIM.

*Experimental Design and Data Collection*

Basic cow data (calving date, parity, etc.) were collected from the on-farm management software DairyCMOP305 (Valley Agricultural Software, Tulare, CA) and BCS records were collected using the DeLaval Body Condition Scoring BCS™ (BCS DeLaval International AB, Tumba Sweden). This system included two cameras located at the exit of the milking parlor and cows were scored after every milking. Scores were averaged into one daily score through a proprietary algorithm. These cameras used 3D images to calculate a BCS based on the subcutaneous fat reserves over the back and pelvis of the cow. A 0 to 5-point scale was used to record BCS with 0.1 intervals. Files were accessed using the DelPro software and BCS files were downloaded monthly. Milk files were also downloaded bi-monthly from the DelPro software.

The outcome variable was pAI1 (1 for yes and 0 for no). An explanatory variable included in the models was parity, categorized as PP (primiparous) or MP (multiparous). Season was an additional explanatory variable included and summer was considered as 1 and the remaining seasons as 0. Finally, milk yield averaged over 90 days was also included in the models. The effect of BCS was assessed at specific DIM or a as difference between two time points during early lactation. Scores of interests for the fixed time points included 7 (BCS7), 21 (BCS21), 35 (BCS35), 49 (BCS49) and 60 DIM (BCS60) and on the day of first AI (BCSAI1). Based on BCS distribution, (first, interquartile range, and upper quartile), BCS at specific time points were categorized as follows:  For 7 DIM the categories included: low (L; $< 3.1$), intermediate (M; $\geq 3.1$ and $\leq 3.5$) and high (H; $> 3.5$). For 21 DIM, 35 DIM, and 49 DIM the categories were: L $< 3.0$, M $\leq 3.4$ and 3.0 and H $> 3.4$, respectively. At 60DIM the categories were: L $< 2.9$, M $\leq 3.2$ and 2.9 and H $> 3.2$.

The BCS on day of first AI (dAI1) was categorized as: L < 2.8, M ≤ 3.3 and ≥ 2.8 and H > 3.3. Cows were also classified based on the differences between two time points to categorize the change in BCS (ΔBCS). The ΔBCS classified cows as either having no loss (NL; ΔBCS ≥ 0) or loss (Los; ΔBCS < 0 points). Milk production was evaluated up to 90 d. Mean milk production was 39.2 ± 11.04 kg/d with a median of 38.10 kg/d. Cows were categorized as low (Lo; < 25.0 kg [lower quartile]), intermediate (Med; ≥ 25.0 kg to ≤ 48.08 kg [interquartile range]), and high (Hig; > 48.1 kg [upper quartile]).

*Statistical Analysis*

Data were analyzed using SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC). To visualize the data, a test on the measures of central tendency was performed using PROC FREQ for the mean, median, minimum, and maximum values of BCS in fixed time.

A summary statistics table was used to visualize the number of cows in each category of ΔBCS during different time periods. The input of PROC FREQ gave the proportions of cows to investigate different trends between the different categories in the data and help draw conclusions. PROC FREQ was used to visualize the proportions of cows conceiving at first AI by BCS and by ΔBCS categories.

Multivariate logistic regression models (PROC LOGISTIC) were used to test binary outcomes (pAI1). This model provided odds ratios as a measure of the magnitude for the association between categorical explanatory variables and the outcome. The main explanatory variable was BCS at fixed time points (categorized as low [reference], intermediate, and high). The outcome for this multiple regression model was success in pregnancy. Other explanatory variables included parity (PP [reference variable] or MP), season of calving (summer [reference variable] vs. other season), average milk yield up to 90 DIM (low [reference], medium, or high).

A second multivariate logistic regression analysis was performed for ΔBCS combining multiple time periods, categorizing ΔBCS as either loss (reference) or no loss. Individual explanatory variables were examined in univariable models to build the final models for the multivariate logistic regression model. No significant interactions were determined between the explanatory variables. Therefore, the formula used for the multivariate logistic regression analysis was: Odds of pregnancy = $\beta_0 + \beta_1$BCS category (L) + $\beta_2$parity(PP) + $\beta_3$season(summer) + $\beta_1$Milk 90 category (Lo).

Cox regression analysis was used to evaluate the conception risk using PROC PHREG. This analysis uses the hazard function of $\gamma(t)$, where $\gamma(t)$ is pregnancy at first AI at time t (expressed in the number of DIM at conception), given that the cow was not bred prior to the date of conception.

Least Square Means (PROC GLM) for days to conception in cows with loss in BCS and cows with no loss in BCS were reported in DIM along with standard errors. All effects across the models were declared statistically significant if $P \leq 0.05$.

**Results**

The overall pAI1 was 30.1% (CI 95%; 28.5 to 31.8%). Pregnancy at AI1 for primiparous cows was 34.6% (CI 95%; 32.2 to 37.0%), and for multiparous cows was 25.5% (CI 95%; 23.3 to 27.8%). Descriptive statistics for BCS was calculated for the predetermined DIM and are presented in Table 2.1. The columns of the table show a decreasing trend in BCS as DIM progresses.

Logistic regression results (Odds ratios and 95% confidence intervals) are reported in Table 2.2. This table shows cows in both the high and intermediate BCS categories have greater odds of conceiving at first AI compared to cows in the low BCS category. Compared to the low BCS category, the odds of pregnancy at pAI1 were significantly greater for cows in the high BCS category at 7DIM, 21DIM, 60DIM, and dAI1. The odds of pregnancy at pAI1 were significantly greater in the intermediate BCS category at 7DIM, 21DIM, 35DIM, 49DIM, 60DIM, and dAI1.

To visualize the number of cows in each ΔBCS category, the percentage of cows was reported for pAI1 and shown in Table 2.2 for each period. The table shows a low percentage of cows initially in the no loss category and as time progresses the percentages increase. However, the inverse happened to the loss category, initially loss had greater percentages of cows and as time progressed the percentages decreased.

Percentages of cows that conceived at first AI by category of ΔBCS are presented in Table 2.4. Differences were significant for d 7 to 21, d 7 to 35, d 7 to dAI1, d 21 to 35, d 21 to 49, d 21 to 60, d 21 to dAI, d 35 to 49, d 35 to 49, d 35 to 60, d 35 to dAI1, d 49 to 60, and d 49 to dAI. The percentages for the NL category were lower initially and increased as time progressed. Inversely, the Los category had greater percentages initially and decreases as time progressed.

Results from the multivariate logistic regression analyses for ΔBCS category are listed in Table 2.5. The odds (95% confidence interval) of pAI1 were significantly lower in the NL category for d 7 to 21, d 7 to 35, and d 7 to dAI. Hazard ratios for conception were calculated, considering cows with loss in BCS as the reference (Table 2.6).

Analyzing the least square means of days to conception, some values were significantly different in specific time periods. The least square means, standard error and p-values are reported in Table 2.7. Figure 2.2 shows the differences in days to conception for cows with loss of BCS vs. cows that had NL of BCS on different time periods. For 7DIM to 60DIM cows with loss in BCS had nearly 6.5 additional days to conception compared to cows that had NL in BCS. The difference in days to conception decreased for 21DIM to 35DIM and 21DIM to 49DIM in which cows that had a loss in BCS had around 5 additional days to conception compared to cows that had NL in BCS. For 21DIM to 60DIM it was reported that cows that loss BCS had nearly 5.5 additional days to conception than cows that had NL BCS. Among cows in the period from 21DIM to dAI there were nearly 4 additional days to conception in cows that loss BCS compared to cows with NL of BCS. The lowest difference in days to conception was between 35DIM to 49DIM with less than 3.5 more days to conception. In 35DIM to 60DIM cows that loss BCS had less than 5.5 days more to conception than cows that had NL in BCS. The difference between 35DIM to dAI had nearly 4 additional days to conception in cows that loss BCS compared to cows that had NL in BCS. For 49DIM to 60DIM there were more than 5 additional days to conception in cows that had loss in BCS. The final significant difference in days of conception was 49DIM to dAI which had just over 3.5 additional days to conception in cows that loss BCS compared to cows that had NL in BCS.

**Discussion**

Results of this study established an association between BCS and fertility at pAI1. Low BCS had an association with lower pregnancy rates, however, losses in BCS early in lactation were associated with greater pregnancy at first AI. Nevertheless, loss of BCS later in lactation resulted in increased time to conception.

This study further provides evidence of automated BCS cameras effect on assessing cow's BCS. The importance of an automated BCS as a management tool in large herds can help producers in maintaining a healthy and profitable herd. The most advanced automated BCS system utilizes 3D imaging along with an algorithm to record a score as a cow passes through. The DeLaval Body Condition Scoring BCS™ has indicated high correlation coefficients of (0.76) between manual scoring and the average automatic BCS (Mullins et al., 2019). The automated BCS camera was validated for scores ranging from 3.0 to 3.75, however below 3.0 and above 3.75 the scores were inaccurate from manual scores. There were 2,637 cows (91.4%) from this study that fell into the 3.0 to 3.75 BCS range.

As expected, mean values for BCS (Table 2.1) show a decreasing trend as the cow progresses through lactation. A cow loses BCS after parturition due to the body's mobilization of energy to produce high quantities of milk and further effects reproductive efficiency (Prandi et al., 1999). It has been reported that cows continue to lose body condition 50 to 100 d post calving (Roche et al., 2009). Similarly, the number of days from the previous study matches the number of days in this study and is shown by the decreasing trend of BCS.

Cows not losing BCS are expected to exhibit better subsequent fertility. This phenomenon happens because when cows go into a negative energy balance state, the utilization of energy is needed for maintenance and growth (Williams et al., 2011). However, cows that maintain or gain in BCS can focus on fertility and reproduction (Williams et al., 2011), allowing them to be in the best condition to hold a pregnancy (Roche et al., 2013).

In our study, the results presented in Table 2.4 and 2.5 did not support the idea of no loss in BCS having an association with improved fertility. The findings exhibited cows with a loss in BCS having greater odds of conception compared to cows with no loss in BCS. A possible explanation could be in the experimental design and the categorization of ΔBCS. The category of loss in BCS was any score less than zero. This category ranged from -0.1 units to -0.9 units. Research has shown that cows still do conceive even if they lost a fraction of a BCS (Roche et al, 2009). Findings from research have shown that cows still conceived (17% conception rate) when they lost 1 BCS unit, and cows that lost less than half a BCS unit conceived (65% conception rate) (Butler and Smith 1989). Overall, the cows that had a loss in BCS had reduced conception rates, but still conceived (Domecq et al., 1997). Since nutrition was not included in the models, this factor could have played a role in conception as proven in previous studies (Ospina et al., 2010). Another potential explanation is that our analyses considered BCS changes from day 7 after calving, which excludes changes during the first week postpartum that may have a significant relevance on subsequent fertility.

The greatest number of days to conception was reported in cows that lost in BCS from 7DIM to 60DIM. Cows that did lose BCS had an additional 6.40 days to conception compared to cows that did not lose BCS. These values were significant in providing information about cows that lost BCS will have an increased number of days to conception. Similarly, Dechow and others (2002) published research stating that a greater loss in BCS was associated with an increase in days to first service.

Although Tables 2.4, 2.5, and 2.6 seem to contradict other data, this is due to several factors. The first factor is the small number of cows in the no loss category compared to the cows in the loss category. The percentages were shown in Table 2.3 and to give an example, from d 7 to 21 there was 6.2% of the total population of cows that did not lose BCS. This is compared to the 93.8% of cows that did lose BCS. The sample sizes for the ΔBCS were not evenly distributed causing the results to be skewed. An additional factor that made our data contradictory was the variables used in the model set up. For the multivariate logistic regression models and the percentages of cows conceiving, the outcome variable was pAI1. Whereas the models investigating the least square means, the outcome variable was the number of DIM. Although, the sample sizes were not proportional in ΔBCS categories the least square means model was not affected by the difference in sample sizes. Although a loss in BCS showed greater odds of conception, a loss in BCS still had greater number of days to conception compared to cows that did not lose BCS. From the data, the number of cows within different categories and the response variables made the results differ from previous studies.

Automatic BCS systems provide valuable information for assessing the probability of conception at subsequent AI. The availability of daily BCS values allows producers to evaluate and follow the changes in BCS throughout lactation. The results shown that low BCS was associated with lower pAI1. However, a loss in BCS was associated with greater pAI1 and did not support our hypothesis.

**Conclusions**

Low BCS at multiple time points was associated with lower pregnancy rate to first AI. However, a portion of the results did not support our hypothesis, as losses in BCS early in lactation were associated with greater pregnancy at first AI. Nevertheless, loss of BCS later in lactation resulted in increased time to conception. Monitoring daily automatic BCS provides potential for assessing future fertility of dairy cows.

**Table 2.1:** Measures of central tendency in dairy cow BCS for 7DIM[1], 21DIM, 35DIM, 49DIM, 60DIM, and dAI1[2] post calving (n = 2,885)[3].

| Days in milk | Measures of Central Tendency of BCS | | | | | |
|---|---|---|---|---|---|---|
| | Mean (SD)[4] | Median | Lower Quartile | Upper Quartile | Minimum | Maximum |
| 7 | 3.33 (0.20) | 3.30 | 3.20 | 3.50 | 2.30 | 4.20 |
| 21 | 3.14 (0.23) | 3.20 | 3.00 | 3.30 | 1.80 | 3.90 |
| 35 | 3.08 (0.25) | 3.10 | 2.90 | 3.20 | 1.70 | 3.90 |
| 49 | 3.04 (0.25) | 3.10 | 2.90 | 3.20 | 1.70 | 3.90 |
| 60 | 3.04 (0.26) | 3.10 | 2.90 | 3.20 | 1.60 | 3.80 |
| dAI1 | 3.04 (0.26) | 3.10 | 2.90 | 3.20 | 1.60 | 3.80 |

[1] DIM: Days in milk.
[2] dAI1: Date of first artificial insemination.
[3] n number of cows the analysis was derived from.

**Table 2.2:** Odds of pregnancy to first AI by BCS category considering low BCS as reference.

| | BCS High[1] | | | BCS Intermediate | | |
|---|---|---|---|---|---|---|
| Days in Milk | Odds Ratio | 95% CI | p-value | Odds Ratio | 95% CI | p-value |
| 7 | 1.68 | 1.20-2.36 | 0.0027 | 1.44 | 1.16-1.79 | 0.0010 |
| 21 | 2.01 | 1.37-2.94 | 0.0004 | 1.53 | 1.28-1.83 | 0.0001 |
| 35 | 1.03 | 0.61-1.74 | 0.9166 | 1.35 | 1.14-1.60 | 0.0007 |
| 49 | 0.80 | 0.46-1.40 | 0.4303 | 1.25 | 1.05-1.49 | 0.0105 |
| 60 | 1.31 | 1.01-1.69 | 0.0385 | 1.57 | 1.30-1.90 | 0.0385 |
| dAI1 | 1.45 | 1.04-2.02 | 0.0300 | 1.67 | 1.34-2.07 | 0.0001 |

[1] High (BCS > 3.5) and intermediate BCS (3.1-3.5) this was compared to low BCS (BCS < 3.1) as reference.

**Table 2.3:** Percentage and number of cows by ΔBCS category (no loss and loss) through different time periods.

| Period | No Loss[1] % (n) | Loss[2] % (n) |
|---|---|---|
| 7 to 21 | 6.2 (179) | 93.8 (2,706) |
| 7 to 35 | 6.7 (192) | 93.3 (2,693) |
| 7 to 49 | 6.8 (197) | 93.2 (2,688) |
| 7 to 60 | 8.4 (242) | 91.6 (2,643) |
| 7 to dAI1 | 11.6 (336) | 88.4 (2,549) |
| 21 to 35 | 46.5 (1,342) | 53.5 (1,543) |
| 21 to 49 | 38.3 (1,106) | 61.7 (1,779) |
| 21 to 60 | 59.0 (1,703) | 41.0 (1,182) |
| 21 to dAI1 | 42.4 (1,223) | 57.6 (1,662) |
| 35 to 49 | 60.7 (1,753) | 39.3 (1,132) |
| 35 to 60 | 48.9 (1,699) | 41.1 (1,186) |
| 35 to dAI1 | 61.3 (1,767) | 38.7 (1,118) |
| 49 to 60 | 79.2 (2,283) | 20.8 (602) |
| 49 to dAI1 | 78.4 (2,260) | 21.6 (635) |

[1] No loss is defined as $\Delta BCS \geq 0$.

[2] Loss is defined as the $\Delta BCS < 0$.

**Table 2.4:** Pregnancy to first AI (%) by time period and category of change in BCS.

| | Percentage of cows within different changes in BCS (ΔBCS) | | |
|---|---|---|---|
| Period | No Loss[1] (n) | Loss[2] (n) | p-value |
| 7 to 21 | 24.0 (43) | 30.5 (826) | 0.0124 |
| 7 to 35 | 26.6 (51) | 30.4 (818) | 0.0357 |
| 7 to 49 | 26.9 (53) | 30.4 (816) | 0.0795 |
| 7 to 60 | 28.5 (69) | 30.3 (800) | 0.0502 |
| 7 to dAI1 | 29.5 (99) | 30.2 (770) | 0.0488 |
| 21 to 35 | 31.2 (419) | 29.2 (450) | 0.0158 |
| 21 to 49 | 31.1 (344) | 29.5 (525) | 0.0220 |
| 21 to 60 | 31.8 (549) | 29.0 (320) | 0.0091 |
| 21 to dAI1 | 31.2 (381) | 29.4 (488) | 0.0191 |
| 35 to 49 | 31.5 (552) | 28.0 (317) | 0.0045 |
| 35 to 60 | 31.9 (542) | 27.6 (327) | 0.0015 |
| 35 to dAI1 | 31.6 (559) | 27.7 (310) | 0.0028 |
| 49 to 60 | 31.1 (709) | 26.6 (160) | 0.0041 |
| 49 to dAI1 | 31.1 (703) | 26.6 (166) | 0.0035 |

[1] No loss is defined as $\Delta BCS \geq 0$.
[2] Loss is defined as the $\Delta BCS < 0$.

**Table 2.5:** Odds of pregnancy to first AI by change in BCS category considering loss in BCS as reference.

| | No loss[1] | | |
|---|---|---|---|
| Period | Odds Ratio | 95% CI | p-value |
| 7 to 21 | 0.68 | 0.47-0.98 | 0.0372 |
| 7 to 35 | 0.68 | 0.48-0.97 | 0.0310 |
| 7 to 49 | 0.71 | 0.50-1.00 | 0.0522 |
| 7 to 60 | 0.79 | 0.58-1.08 | 0.1458 |
| 7 to dAI1 | 0.73 | 0.56-0.95 | 0.0198 |
| 21 to 35 | 0.93 | 0.78-1.10 | 0.3708 |
| 21 to 49 | 0.88 | 0.74-1.05 | 0.1423 |
| 21 to 60 | 0.93 | 0.78-1.10 | 0.3912 |
| 21 to dAI1 | 0.85 | 0.72-1.02 | 0.0733 |
| 35 to 49 | 1.00 | 0.84-1.18 | 0.9554 |
| 35 to 60 | 1.01 | 0.85-1.20 | 0.9159 |
| 35 to dAI1 | 0.96 | 0.81-1.15 | 0.6732 |
| 49 to 60 | 1.10 | 0.90-1.36 | 0.3555 |
| 49 to dAI1 | 1.11 | 0.90-1.36 | 0.3230 |

[1] Cows that had no loss in BCS ($\Delta BCS \geq 0$) was compared to cows that did lose BCS ($\Delta BCS < 0$) as reference.

**Table 2.6:** Hazard ratio for conception in cows with no loss in BCS relative to cows with loss in BCS.

| Period | Hazard Ratio[1] | p-value |
|---|---|---|
| 7 to 21 | 0.82 | 0.2093 |
| 7 to 35 | 0.86 | 0.3382 |
| 7 to 49 | 0.81 | 0.1697 |
| 7 to 60 | 0.74 | 0.0291 |
| 7 to dAI1 | 0.81 | 0.0656 |
| 21 to 35 | 0.85 | 0.0238 |
| 21 to 49 | 0.87 | 0.0745 |
| 21 to 60 | 0.84 | 0.0169 |
| 21 to dAI1 | 0.84 | 0.0185 |
| 35 to 49 | 0.95 | 0.4977 |
| 35 to 60 | 0.94 | 0.3883 |
| 35 to dAI1 | 0.94 | 0.3803 |
| 49 to 60 | 0.97 | 0.6928 |
| 49 to dAI1 | 0.95 | 0.6055 |

**Table 2.7:** Number of days from calving to conception comparing cows that had loss in BCS to cows with no loss in BCS at different time periods up to date of first AI.

| | No Loss[1] | | Loss[2] | | |
|---|---|---|---|---|---|
| Period | Least Squares Means (d) (n) | Standard Error | Least Squares Means (d) (n) | Standard Error | p-value[7] |
| 7 to 21 | 81.7 (43) | 3.06 | 85.1 (826) | 0.65 | 0.2777 |
| 7 to 35 | 82.2 (51) | 2.94 | 85.1 (818) | 0.65 | 0.3331 |
| 7 to 49 | 81.7 (53) | 2.96 | 85.2 (816) | 0.65 | 0.2544 |
| 7 to 60 | 79.0 (69) | 2.62 | 85.4 (800) | 0.66 | 0.0192 |
| 7 to dAI1 | 81.3 (99) | 2.15 | 85.4 (770) | 0.67 | 0.0687 |
| 21 to 35 | 82.1 (419) | 0.98 | 87.1 (450) | 0.83 | 0.0001 |
| 21 to 49 | 82.0 (344) | 1.10 | 86.5 (525) | 0.78 | 0.0008 |
| 21 to 60 | 81.5 (549) | 1.06 | 86.9 (320) | 0.79 | 0.0001 |
| 21 to dAI1 | 82.7 (381) | 1.03 | 86.4 (488) | 0.81 | 0.0040 |
| 35 to 49 | 83.7 (552) | 0.82 | 86.9 (317) | 1.01 | 0.0138 |
| 35 to 60 | 82.8 (542) | 0.83 | 88.1 (327) | 0.98 | 0.0001 |
| 35 to dAI1 | 83.5 (559) | 0.81 | 87.2 (310) | 1.02 | 0.0043 |
| 49 to 60 | 84.0 (709) | 0.71 | 89.2 (160) | 1.43 | 0.0012 |
| 49 to dAI1 | 84.3 (703) | 0.71 | 87.9 (166) | 1.45 | 0.0259 |

[1] No loss is defined as $\Delta BCS \geq 0$.
[2] Loss is defined as the $\Delta BCS < 0$.

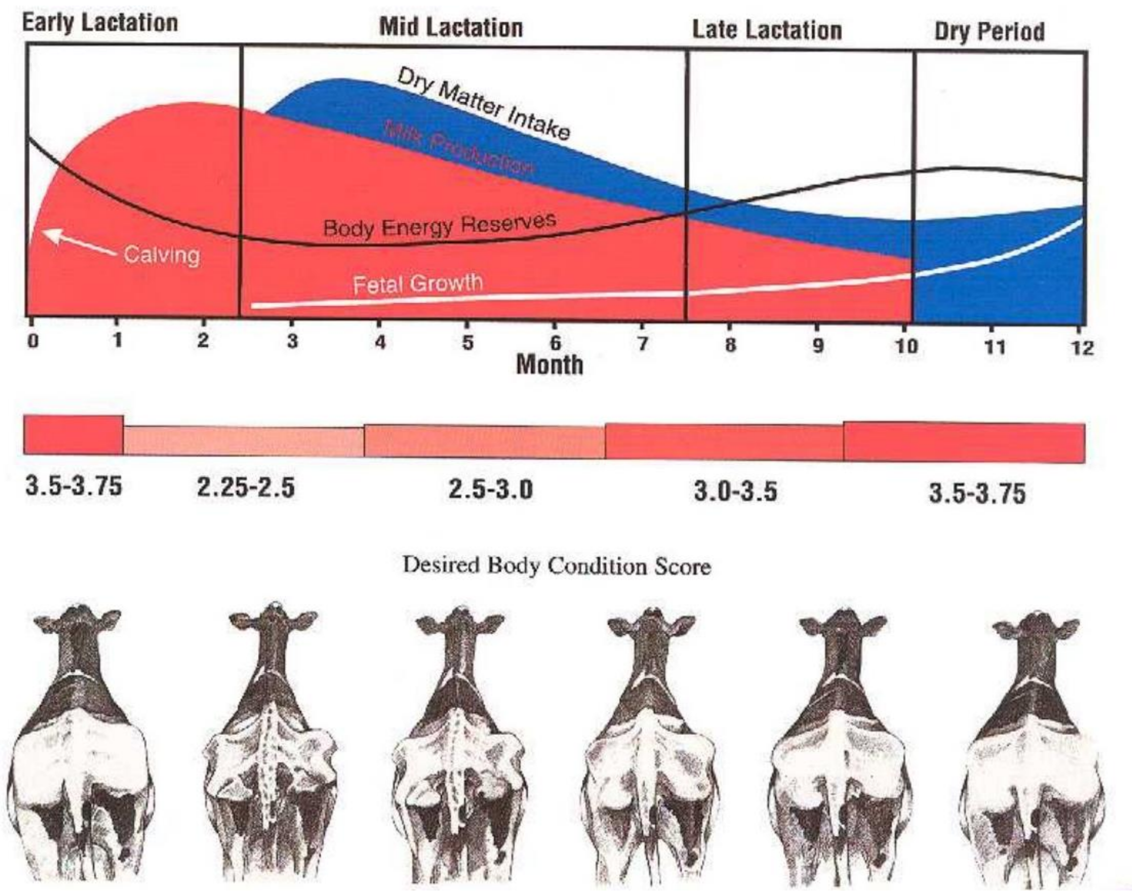


**Figure 2.1:** Elanco BCS chart depicting the lactation curve and desired body condition score during different stages of lactation (From Ferguson et al., 1994; Elanco Animal Health, 2009).

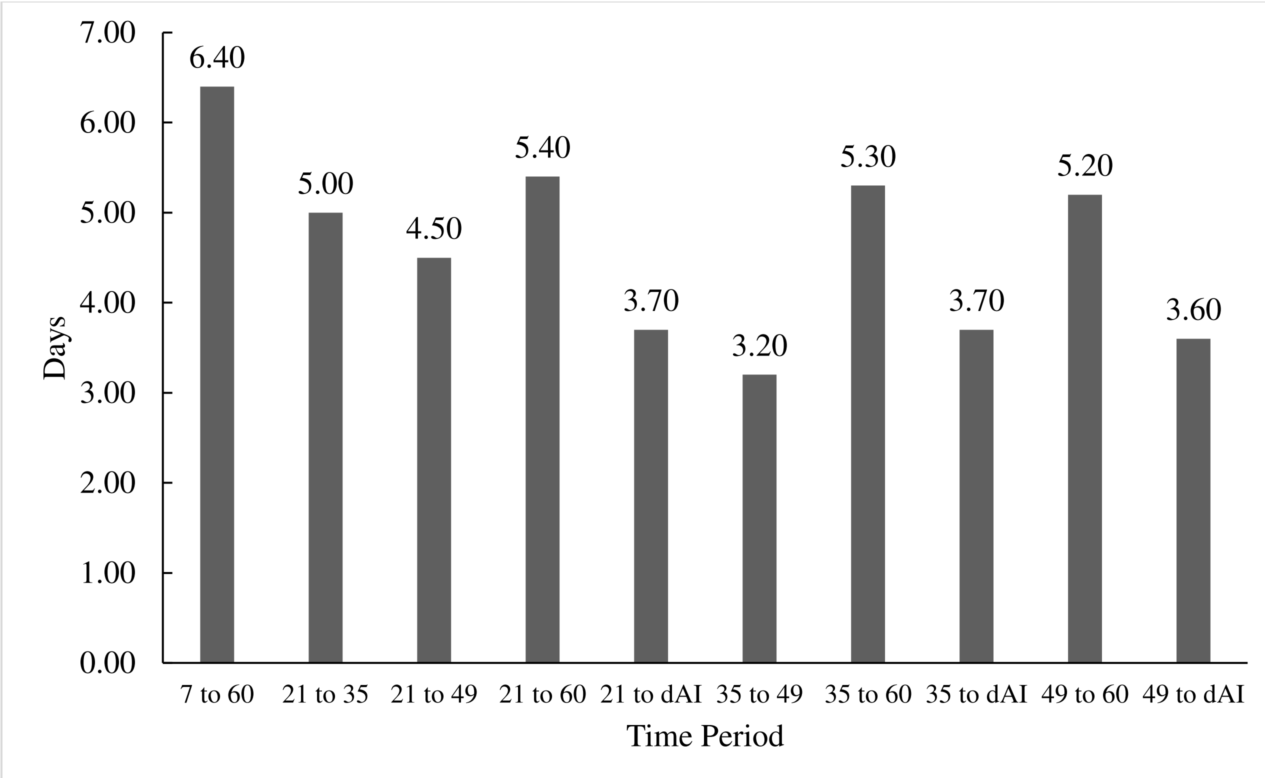


**Figure 2.2:** Differences in the number of days to conception for cows with loss of BCS vs. cows that had no loss of BCS at different time periods. These numbers were calculated by subtracting the least squares means of no loss from the least squares means of loss from Table 2.7 resulting in the additional days to conception. The number reported at the top of the bar is the additional number of days to conception in cows with loss in BCS compared to cows with no loss in BCS.

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# CHAPTER 3: ASSOCIATION BETWEEN BCS IN EARLY LACTATION AND SUBSEQUENT FERTILITY FROM SECOND TO FOURTH ARTIFICIAL INSEMINATION IN HOLSTEIN COWS

**Summary**

The objective of this study was to test the association between body condition score (BCS) and changes in BCS in early lactation and subsequent fertility from second to fourth artificial insemination (AI) in Holstein cows. The DeLaval Body Condition Scoring BCS™ was utilized on a commercial dairy operation in Windsor, Colorado. Data were collected from 2,885 lactating cows from April 2019 to April 2020. Of the total lactating cows, there were 1,460 primiparous cows and 1,425 multiparous cows. Results from this study revealed an association between BCS and fertility at multiple AI. Low BCS at multiple time points was associated with lower pregnancy rate to second, third, and fourth AI. However, losses in BCS early in lactation were generally associated with greater pregnancy at second, third, and forth AI.

**Introduction**

The transition period in dairy cows is defined as the three weeks prior after parturition (Wankhade et al., 2017). During the transition period, cows enter one of the most vulnerable physiological states of the production cycle and often fall into a negative energy balance, as high nutritional intake is required to achieve high milk yield (Herdt, 2000; McArt et al., 2013). The transition period also increases susceptibility to metabolic and infectious diseases (Goff and Horst, 1997).

Body condition scoring (BCS) is a management tool used on dairy operations to help monitor this period and has been proven to be associated with health (Lange et al., 2016, Chebel et al., 2018), nutritional decisions (Ospina et al., 2010), milk production (Berry et al., 2003) and fertility (Gargiulo et al., 2018). The visual BCS system assess subcutaneous fat and assigns a value on a scale from 1 (emaciated) to 5 (over-conditioned) (Roche et al., 2013).

Conception rate is decreased by low availability of energy (Pryce et al., 2001). At both first and second post-calving AI there is a reduction in likelihood of conception when cattle exhibited a loss in BCS (Chebel et al., 2018) and dominant follicle growth and estradiol production are decreased due to negative energy balance (Canfield and Butler, 1990). Moreover, abundant research has shown that lack of adequate body condition has a significant effect on milk production (Carvalho et al., 2014) and results in decreases in conceptions rates, loss of embryos, and anestrous (Weigel, 2006; Stevenson and Britt, 2017; Lopez et al., 2005).

Installation of automatic BCS systems have been validated on dairy production operations for research use. One specific camera system is the DeLaval Body Condition Scoring BCS™ which has been validated (Mullins et al., 2019). The authors reported a correlation of 0.76 between the scoring system and three trained manual observers. Mullins et al. (2019) indicated that the highest accuracy was at BCS 3.0 to 3.75; however, below 3.0 and above 3.75 the scores were inaccurate. Furthermore, the DeLaval BCS system continues to capture BCS regardless of a cow running, standing, or if there were multiple cows under the camera. Thus, this BCS system can minimize labor costs, subjectivity, and continuously provide herd records.

Consistent use of BCS has influenced dairy managers decisions on labor and management to improve overall animal performance and care (Stevenson and Britt, 2017). Automated BCS can provide data needed to monitor risk of disease, milk production, and fertility. The hypothesis was that low BCS or a loss in BCS during early lactation would reduce the risk of conception from second to fourth AI, increasing the number of days to conception. Thus, the objective of the study was to test the association between BCS and changes in BCS in early lactation and subsequent fertility from second to fourth AI in Holstein cows.

## Materials and Methods

### *Study Population*

This was a prospective observational study. Cows were monitored during lactation starting on the day of calving and gathering data up until 90 days in milk (DIM). The observations and records were collected from a commercial dairy operation located in Windsor, Colorado, USA. Cows were categorized as primiparous or multiparous. The initial population included 2,885 Holstein cows (1,460 primiparous and 1,425 multiparous). A total of 1,290, 528, and 200 cows received a second AI (AI2), third (AI3), and fourth AI (AI4), respectively.

Cows were subject to AI following a double OvSynch protocol (Nowicki et al., 2017). Cows were milked three times daily and milk yield was recorded including data points such as peak flow, average flow, milk duration, and overall yield per session.

### *Experimental Design and Data Collection*

Basic cow data (calving date, parity, etc.) were collected from the on-farm management software DairyCMOP305 (Valley Agricultural Software, Tulare, CA) and BCS records were collected using the DeLaval Body Condition Scoring BCS™ (BCS DeLaval International AB, Tumba Sweden). This system included two cameras located at the exit of the milking parlor and cows were scored after every milking. Scores were averaged into one daily score through a proprietary algorithm. These cameras used 3D images to calculate a BCS based on the subcutaneous fat reserves over the back and pelvis of the cow. A 0 to 5-point scale was used to record BCS with 0.1 intervals. Files were accessed using the DelPro software and BCS files were downloaded monthly. Milk files were also downloaded bi-monthly from the DelPro software.

The outcome variable was pregnancy at AI (1 for yes and 0 for no). Explanatory variables used in the models included parity, season, milk yield averaged over 90 days, BCS, and changes in BCS (ΔBCS). Parity was a binary variable and categorized as PP (primiparous) or MP (multiparous). Season was also categorized as a binary, categorical variable where summer was 1 and the remaining seasons were 0. Milk yield was averaged over 90 days and categorized into three levels as: low (Lo; < 25.0 kg [lower quartile]), intermediate (Med; ≥ 25.0 kg to ≤ 48.08 kg [interquartile range]), and high (Hig; > 48.1 kg [upper quartile]).

Based on BCS distribution, (first, interquartile range, and upper quartile), BCS at specific time points were categorized as follows:  For 7 DIM the categories included: low (L; < 3.1), intermediate (M; ≥ 3.1 and ≤ 3.5) and high (H; > 3.5). For 21 DIM, 35 DIM, and 49 DIM the categories were: L < 3.0, M ≤ 3.4 and 3.0 and H > 3.4, respectively. At 60DIM the categories were: L < 2.9, M ≤ 3.2 and 2.9 and H > 3.2. The BCS on day of first AI (dAI1) was categorized as: L < 2.8, M ≤ 3.3 and ≥ 2.8 and H > 3.3. Cows were also classified based on the differences between two time points to categorize the change in BCS (ΔBCS). The ΔBCS classified cows as either having no loss (NL; ΔBCS ≥ 0) or loss (Los; ΔBCS < 0 points). *Statistical Analysis*

The statistical software used to analyze data was SAS (9.4, SAS Institute Inc., Cary, NC). Records of BCS was continually collected and the scores of interest were 7 (BCS7), 30 (BCS30), 60 (BCS60), AI1 (BCSAI1), AI2 (BCSAI2), AI3 (BCSAI3), and AI4 (BCSAI4). To visualize the data, a test on the measures of central tendency was preformed using PROC FREQ in fixed time periods.

Multivariate logistic regression models (PROC LOGISTIC) were used to test binary outcomes (conception at AI). This model provided odds ratios as a measure of the magnitude for the association between categorical explanatory variables and the outcome. The main explanatory variable was BCS at fixed time points (categorized as low [reference], intermediate, and high). Other explanatory variables included parity (PP [reference variable] or MP), season of calving (summer [reference variable] vs. other season), average milk yield up to 90 DIM (low [reference], medium, or high). The outcome for the multiple regression model was success in pregnancy. A second multivariate logistic regression analysis was performed for ΔBCS as either loss (reference) or no loss. Individual explanatory variables were examined in univariable models to build the final models for the multivariate logistic regression model. No significant interactions were determined between the explanatory variables. Therefore, the formula used for the multivariate logistic regression analysis was: Odds of pregnancy = $\beta_0$ + $\beta_1$BCS category (L) + $\beta_2$parity(PP) + $\beta_3$season(summer) + $\beta_1$Milk 90 category (Lo).

To further visualize the number of cows conceiving at first AI a PROC FREQ test was performed to see the percentage of cows that successfully conceived at first AI. The analysis used ΔBCS and looked at the percentages of cows conceiving with no loss versus the cows conceiving with loss at different DIM.

Cox regression analysis was used to evaluate conception using PROC PHREG. This analysis uses the hazard function of $\gamma(t)$, where $\gamma(t)$ is pregnancy at first AI at time t (expressed in the number of DIM at conception), given that the cow was not bred prior to the date of conception.

Least Square Means (PROC GLM) for days to conception in cows with loss in BCS and cows with no loss in BCS were reported in DIM along with standard errors. All effects across the models were declared statistically significant if $P \leq 0.05$.

To further visualize the number of cows conceiving at first AI a PROC FREQ test was used to determine the percentage of cows that successfully conceived at different AI. All effects across the models were declared statistically significant if $P \leq 0.05$.

**Results**

From the 2,885 cows analyzed through this study, pregnancy (95% Confidence Interval) at AI2, AI3, and AI4 were 27.8% (25.4% to 30.4%), 21.4% (18.0% to 24.9%), 16.0% (11.2% to 21.8%), respectively. Descriptive statistics on BCS were calculated for selected DIM and these values are listed in Table 3.1. As the days progressed, the trend in numbers in both the mean, median, and maximum columns decreased. Furthermore, the minimum column showed a different trend as it decreases until 60 DIM and then increases.

Logistic regression results are presented in Table 3.2 (a-c) and shown in Figure 3.2. The odds (95% confidence interval) of pregnancy at pAI2 were significantly higher in high BCS category compared to low BCS category at 7DIM, 60DIM, and dAI1. For pAI2 the odds of pregnancy for cows that exhibited intermediate BCS were significantly higher at 7DIM, 30DIM, 60DIM, and dAI1. The odds of pregnancy at pAI3 in the high BCS category was significantly higher only at 7DIM, but significant for intermediate BCS at 7DIM, 30DIM, and dAI1. For pAI4 the odds of pregnancy for cows that exhibited an intermediate BCS were significant in 7DIM.

Percentages of cows conceiving at different AI by change in BCS category are presented in Tables 3.3 (a-c). Pregnancy at second AI was higher in cows losing BCS (Table 3.3a) for all the study periods. However, differences in pregnancy at third AI were only significant for d 7 to dAI1, d 7 to dAI2, d 30 to 60, d 30 to dAI1, d 60 to dAI1, and d 60 to dAI3 (Table 3.3b). Differences at fourth AI were significant only for d 30 to 60, d 30 to dAI1, and d 60 to dAI1 (Table 3.3c).

Multivariate logistic regression results are presented in Table 3.4 (a-c). The odds (95% confidence interval) of pregnancy at pAI2 were significant for d 7 to 30, d 7 to 60, d 7 to dAI1, and d 7 to dAI2. The odds of pregnancy at pAI3 were significant for d 7 to dAI2 and d 7 to dAI3. The odds of pregnancy at pAI4 were not significant in any of the time periods.

Hazard ratios for conception considered loss in BCS as reference (Table 3.5a-c).

**Discussion**

Results of this study showed the association between BCS and ΔBCS with conception at different number of AI. Low BCS had an association with lower pregnancy rates. Research into the relationship between BCS and fertility efficiency has been a focus of investigation in many studies. Our findings are consistent with previous research in that cows that did lose BCS had a decrease in pregnancy at AI when compared to cows that did not lose BCS (Carvalho et al., 2014). However, the results did not support our objective of ΔBCS having an association with fertility in proximity of multiple AI. Results from Tables 3.3a to 3.3c and Tables 3.4a to 3.4c showed cows losing BCS had greater odds of conception rates compared to cows that had no loss in BCS. A possible explanation could be in the design of the experiment, more specifically the categorization of ΔBCS. The study design indicated that cows were categorized as a loss in BCS if the score was less than zero. Those category scores ranged from -0.1 units to -0.9 units. Research has been shown that cows still do conceive even if they lost a fraction of a BCS (Domecq et al., 1997; Roche et al, 2009). Findings from research has shown that cows still conceive (17% conception rate) when they lost 1 BCS unit, and cows that lost less than half a BCS unit conceived as well (65% conception rate) (Butler and Smith 1989). Since nutrition was not included in the models, this factor could have played a role in conception as proven in previous studies (Ospina et al., 2010).

The overall rate of pregnancy for second and subsequent AI was reported at 22.4% (Lopez et al., 2005) which was within the range of 15 to 39% reported by other studies (Santos et al., 2000; Sartori et al., 2004; López-Gatius et al., 2005). This falls within our findings of cows being successfully bred at AI2, AI3, and AI4, at 27.8%, 21.4%, and 16.0%, respectively.

Results from Table 3.1 showed a decrease in the average mean of BCS as the cow progresses through lactation. With the mobilization of energy, a cow loses BCS as demand for production of milk is high. This is explained through Elanco's BCS range along the lactation curve (Modified from Ferguson et al., 1994; Elanco Animal Health, 2009). The minimum BCS for all days in Table 3.1 starts high, curves, and increases. This highlights the negative energy state of cows and day 60 marks one of the lowest days before it rises.

This study has potential limitations that could be refined with further investigation on other dairy operations. The sample size is from one dairy operation in Northern Colorado that has different cofounders. Additional research from multiple facilities across different regions could provide data to better represent the association between BCS and multiple AI. Another limitation is that our analyses considered BCS changes from day 7 after calving, which excludes changes during the first week postpartum that may have a significant relevance on subsequent fertility.

An additional limitation in the study were the small number of cows in the no loss category compared to the number of cows in the loss category. Often there were zero cows in the population in the no loss category that drastically changed our results. The sample sizes for the ΔBCS were not evenly distributed causing the results to skew.

The use of continuous automated BCS has the potential to help in fertility decision making and allow producers to follow changes in BCS throughout a cow's lactation. The results have shown that low BCS was associated with lower pregnancy at multiple AI. However, a loss in BCS was associated with greater pregnancy at multiple AI and did not support our hypothesis. Adapting and utilizing this automatic system on dairy operations will help in predicting fertility.

**Conclusions**

Low BCS at multiple time points was associated with lower pregnancy rate to second to fourth AI. However, losses in BCS early in lactation were generally associated with greater pregnancy at second to forth AI. Potential explanations for this unexpected finding include our categorization of ΔBCS (no loss vs. loss) and the low numbers of cows receiving >2 AI. Monitoring daily automatic BCS provides potential for assessing future fertility of dairy cows.

**Table 3.1:** Measures of central tendency in cows BCS for 7DIM[1,2], 30DIM, 60DIM, dAI2, dAI3, and dAI4 post calving from second to fourth AI (n = 2,018).

| Days in milk | Measures of Central Tendency of BCS | | | | | |
|---|---|---|---|---|---|---|
| | Mean (SD) | Median | Lower Quartile | Upper Quartile | Minimum | Maximum |
| 7 | 3.33 (0.20) | 3.30 | 3.20 | 3.50 | 2.30 | 4.20 |
| 30 | 3.09 (0.24) | 3.10 | 3.00 | 3.20 | 1.80 | 3.80 |
| 60 | 3.04 (0.26) | 3.10 | 2.90 | 3.20 | 1.60 | 3.80 |
| dAI2 | 3.04 (0.25) | 3.00 | 2.90 | 3.20 | 2.00 | 3.60 |
| dAI3 | 3.03 (0.24) | 3.00 | 2.90 | 3.20 | 2.20 | 3.60 |
| dAI4 | 3.03 (0.26) | 3.00 | 2.90 | 3.20 | 2.50 | 3.60 |

[1] DIM: Days in milk.
[2]dAI1: Day of corresponding artificial insemination.

**Table 3.2a:** Odds of pregnancy to second AI (n = 1,290) by BCS category considering low BCS as reference.

| | BCS High[1] (n = 42)[2] | | | BCS Intermediate (n = 267) | | |
|---|---|---|---|---|---|---|
| Days in Milk | Odds Ratio | 95% CI[5] | p-value | Odds Ratio | 95% CI | p-value |
| 7 | 2.71 | 1.65-4.47 | 0.0001 | 1.98 | 1.41-2.79 | 0.0001 |
| 30 | 1.78 | 0.83-3.81 | 0.1355 | 1.66 | 1.29-2.13 | 0.0001 |
| 60 | 2.01 | 1.38-2.98 | 0.0005 | 1.50 | 1.14-1.97 | 0.0038 |
| dAI1 | 2.03 | 1.18-3.50 | 0.0108 | 1.60 | 1.20-2.14 | 0.0014 |

[1] High (BCS > 3.5) and intermediate BCS (3.1-3.5) was compared to low BCS (BCS < 3.1) as reference.

[2] Number of cows receiving AI2.

**Table 3.2b:** Odds of pregnancy to third AI (n = 528) by BCS category considering low BCS as reference.

| | BCS High[1] (n = 15)[2] | | | BCS Intermediate (n = 86) | | |
|---|---|---|---|---|---|---|
| Days in Milk | Odds Ratio | 95% CI | p-value | Odds Ratio | 95% CI | p-value |
| 7 | 2.99 | 1.23-7.24 | 0.0153 | 2.17 | 1.12-4.21 | 0.0274 |
| 30 | 1.16 | 0.24-5.59 | 0.8504 | 1.65 | 1.06-2.57 | 0.0258 |
| 60 | 1.31 | 0.59-2.89 | 0.5060 | 1.28 | 0.80-2.05 | 0.3073 |
| dAI1 | 1.71 | 0.51-5.70 | 0.3815 | 1.77 | 1.08-2.89 | 0.0228 |

[1] High (BCS > 3.5) and intermediate BCS (3.1-3.5) was compared to low BCS (BCS < 3.1) as reference.
[2] Number of cows receiving AI3.

**Table 3.2c:** Odds of pregnancy to fourth AI (n = 200) by BCS category considering low BCS as reference.

| | BCS High[1] (n = 4)[2] | | | BCS Intermediate (n = 26) | | |
|---|---|---|---|---|---|---|
| Days in Milk | Odds Ratio | 95% CI | p-value | Odds Ratio | 95% CI | p-value |
| 7 | 3.44 | 0.53-22.10 | 0.1938 | 4.55 | 1.01-20.51 | 0.0487 |
| 30 | 0 | 0 | 0.9811 | 1.96 | 0.88-4.37 | 0.0992 |
| 60 | 2.62 | 0.58-11.80 | 0.2102 | 1.51 | 0.63-3.64 | 0.3590 |
| dAI1 | 3.01 | 0.51-17.92 | 0.2267 | 0.96 | 0.43-2.17 | 0.9225 |

[1] High (BCS > 3.5) and intermediate BCS (3.1-3.5) was compared to low BCS (BCS < 3.1) as reference.
[2] Number of cows receiving AI4.

**Table 3.3a:** Pregnancy rate to second AI (%) (n = 1,290) by time period and category of change in BCS.

| | Percentage of cows within different changes in BCS (ΔBCS) | | |
|---|---|---|---|
| Period | No Loss[1] (n) | Loss[2] (n) | p-value |
| 7 to 30 | 11.8 (6) | 28.5 (353) | 0.0031 |
| 7 to 60 | 14.3 (10) | 28.6 (349) | 0.0030 |
| 7 to dAI1 | 0 (0) | 30.5 (359) | 0.0001 |
| 30 to 60 | 28.2 (182) | 27.4 (177) | 0.0472 |
| 30 to dAI1 | 0 (0) | 33.6 (359) | 0.0001 |
| 60 to dAI1 | 0 (0) | 35.7 (359) | 0.0001 |

[1] No loss is defined as $\Delta BCS \geq 0$.
[2] Loss is defined as the $\Delta BCS < 0$.

**Table 3.3b:** Pregnancy rate to third AI (%) (n = 528) by time period and category of change in BCS.

| | Change in BCS category (ΔBCS) | | |
|---|---|---|---|
| Period | No Loss[1] (n) | Loss[2] (n) | p-value |
| 7 to 30 | 15.8 (3) | 21.6 (110) | 0.5435 |
| 7 to 60 | 11.8 (2) | 21.7 (111) | 0.3247 |
| 7 to dAI1 | 0 (0) | 30.2 (113) | 0.0192 |
| 7 to dAI2 | 0 (0) | 28.1 (113) | 0.0272 |
| 30 to 60 | 17.3 (40) | 24.6 (73) | 0.0111 |
| 30 to dAI1 | 0 (0) | 31.0 (113) | 0.0001 |
| 60 to dAI1 | 0 (0) | 31.6 (113) | 0.0001 |
| 60 to dAI3 | 76.1 (102) | 2.8 (11) | 0.0001 |

[1] No loss is defined as $\Delta BCS \geq 0$.
[2] Loss is defined as the $\Delta BCS < 0$.

**Table 3.3c:** Pregnancy rate to fourth AI (%) (n = 200) by time period and category of change in BCS.

| | Change in BCS category (ΔBCS) | | |
|---|---|---|---|
| Period | No Loss[1] (n) | Loss[2] (n) | p-value |
| 7 to 30 | 0 (0) | 16.4 (32) | 0.3230 |
| 7 to 60 | 0 (0) | 16.4 (32) | 0.4142 |
| 7 to dAI1 | 0 (0) | 30.1 (32) | 0.4882 |
| 30 to 60 | 7.9 (6) | 21.0 (26) | 0.0073 |
| 30 to dAI1 | 0 (0) | 30.3 (32) | 0.0022 |
| 60 to dAI1 | 0 (0) | 30.5 (32) | 0.0001 |

[1] No loss is defined as $\Delta BCS \geq 0$.
[2] Loss is defined as the $\Delta BCS < 0$.

**Table 3.4a:** Odds of pregnancy to second AI (n = 1,290) by changes in BCS category considering no BCS loss versus BCS loss as reference.

| | No loss[1] (n = 6) | | |
|---|---|---|---|
| Period | Odds Ratio | 95% CI | p-value |
| 7 to 30 | 0.36 | 0.15-0.85 | 0.0204 |
| 7 to 60 | 0.45 | 0.22-0.90 | 0.0236 |
| 7 to dAI1 | 0.47 | 0.27-0.82 | 0.0080 |
| 7 to dAI2 | 7.77 | 3.79-15.94 | 0.0001 |
| 30 to 60 | 1.14 | 0.88-1.48 | 0.3288 |
| 30 to dAI1 | 1.10 | 0.84-1.42 | 0.4926 |
| 60 to dAI1 | 0.89 | 0.59-1.34 | 0.5760 |

[1] Cows that had no loss in BCS ($\Delta BCS \geq 0$) was compared to cows that did lose BCS ($\Delta BCS < 0$) as reference.

**Table 3.4b:** Odds of pregnancy to third AI (n = 528) by changes in BCS category considering no BCS loss versus BCS loss as reference.

| | No loss[1] (n = 3) | | |
|---|---|---|---|
| Period | Odds Ratio | 95% CI | p-value |
| 7 to 30 | 0.84 | 0.23-3.09 | 0.7934 |
| 7 to 60 | 0.76 | 0.17-3.45 | 0.7211 |
| 7 to dAI1 | 0.85 | 0.28-2.57 | 0.7664 |
| 7 to dAI2 | 17.62 | 3.53-88.01 | 0.0005 |
| 7 to dAI3 | 17.62 | 3.53-88.01 | 0.0005 |
| 30 to 60 | 0.88 | 0.55-1.41 | 0.5942 |
| 30 to dAI1 | 1.03 | 0.64-1.64 | 0.9157 |
| 60 to dAI1 | 1.38 | 0.66-2.90 | 0.3912 |

[1] Cows that had no loss in BCS ($\Delta BCS \geq 0$) was compared to cows that did lose BCS ($\Delta BCS < 0$) as reference.

**Table 3.4c:** Odds of pregnancy to fourth AI (n = 200) by changes in BCS category considering no BCS loss versus BCS loss as reference.

| | No loss[1] (n = 0) | | |
|---|---|---|---|
| Period | Odds Ratio | 95% CI | p-value |
| 7 to 30 | 0.001 | <0.001->999.99 | 0.9805 |
| 7 to 60 | 0.001 | <0.001->999.99 | 0.9805 |
| 7 to dAI1 | 0.001 | <0.001->999.99 | 0.9861 |
| 7 to dAI2 | >999.99 | <0.001->999.99 | 0.9861 |
| 7 to dAI3 | >999.99 | <0.001->999.99 | 0.9861 |
| 7 to dAI4 | >999.99 | <0.001->999.99 | 0.9861 |
| 30 to 60 | 0.52 | 0.18-1.45 | 0.2094 |
| 30 to dAI1 | 0.69 | 0.26-1.81 | 0.4492 |
| 60 to dAI1 | 3.83 | 0.48-30.51 | 0.2052 |

[1] Cows that had no loss in BCS ($\Delta BCS \geq 0$) was compared to cows that did lose BCS ($\Delta BCS < 0$) as reference.

**Table 3.5a:** Hazard ratio in cows with pregnancy to second AI (n = 1,290) with no loss relative to cows with loss.

| Period | Hazard Ratio (n = 6) | p-value |
|---|---|---|
| 7 to 30 | 1.04 | 0.9234 |
| 7 to 60 | 1.67 | 0.1271 |
| 7 to dAI1 | 1.36 | 0.2335 |
| 7 to dAI2 | 0.86 | 0.4360 |
| 30 to 60 | 1.42 | 0.0024 |
| 30 to dAI1 | 1.24 | 0.0592 |
| 60 to dAI1 | 0.84 | 0.3357 |

**Table 3.5b:** Hazard ratio in cows with pregnancy to third AI (n = 528) with no loss relative to cows with loss.

| Period | Hazard Ratio (n = 3) | p-value |
|---|---|---|
| 7 to 30 | 5.02 | 0.0092 |
| 7 to 60 | 3.32 | 0.1029 |
| 7 to dAI1 | 3.114 | 0.0341 |
| 30 to 60 | 1.54 | 0.0432 |
| 30 to dAI1 | 1.36 | 0.1459 |
| 60 to dAI1 | 0.59 | 0.1359 |

**Table 3.5c:** Hazard ratio in cows with pregnancy to fourth AI (n = 200) with no loss relative to cows with loss.

| Period | Hazard Ratio (n = 0) | p-value |
|---|---|---|
| 7 to 30 | 0 | 0 |
| 7 to 60 | 0 | 0 |
| 7 to dAI1 | 0 | 0 |
| 7 to dAI2 | 0 | 0 |
| 30 to 60 | 1.03 | 0.9634 |
| 30 to dAI1 | 1.09 | 0.8705 |
| 60 to dAI1 | 1.80 | 0.5827 |

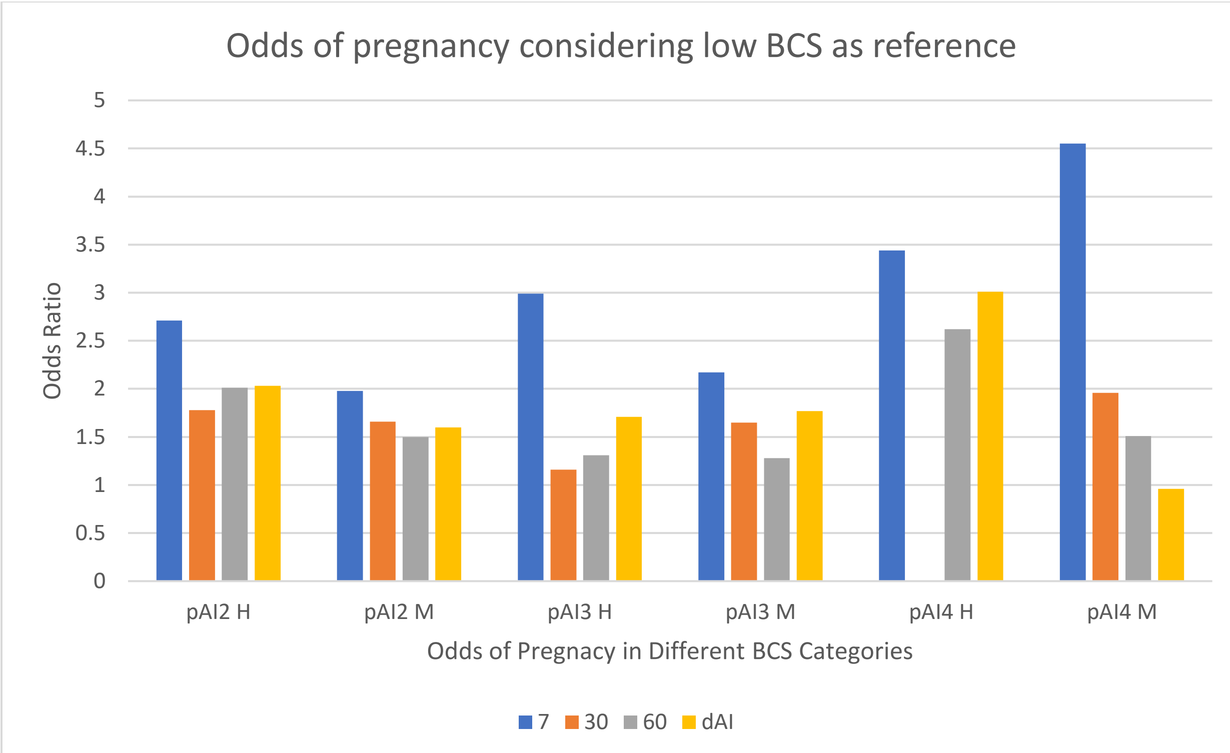


**Figure 3.1:** Logistic regression analysis showing the odds of pregnancy to multiple AI in the high and intermediate BCS category considering low BCS as reference over selected time points DIM. The blue bar depicts the odds of pregnancy in 7 DIM, the orange bar depicts 30 DIM, the grey bar is 60 DIM, and the yellow bar shows the dAI. The letter "H" stands for High BCS category and "M" stands for Intermediate BCS category.

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# CHAPTER 4: GENERAL CONCLUSIONS

Automatic BCS systems provide valuable information for assessing the probability of conception at subsequent AI. Across both studies low BCS was associated with lower success rates of pregnancy per AI. Interestingly, in general terms, cows that evidenced losses in BCS had greater odds of conception rates compared to cows that had no loss in BCS. This could be explained by several factors including the uneven distribution of cows within the no loss and loss BCS category. Another potential explanation is that our analyses considered BCS changes from day 7 after calving, which excludes changes during the first week postpartum that may have a significant relevance on subsequent fertility.

Overall, both studies displayed the beneficial use of automatic BCS have on managing fertility.