Nutrient Restriction Effects on Ovulatory Follicle and Corpus Luteum Development and Progesterone Production of *Bos taurus* Cows

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Abstract

Establishment and maintenance of pregnancy is a central concern to the cattle industry, as it impacts efficiency and profitability of beef cow-calf operations strongly. The objective of this study was to determine if nutrient restriction impacts ovulatory follicle size and corpus luteum (CL) development and function of *Bos taurus* cows enrolled in estrous synchronization. A total of 26 Angus cows were housed in a facility equipped with a Calan gate system, allowing for measurement of individual animal intake. Cows were stratified by body weight (BW), and randomly assigned one of two nutritional treatments: 1) 100% of nutrient requirements (MTN; n=13) or 2) 70% of nutrient requirements (REST; n=13). Individual daily intakes were measured and adjusted weekly based on BW. All cattle underwent an acclimation period of 14 days and were then exposed to nutritional treatments for 30 days prior to estrous synchronization. Body weight was measured and recorded daily using an automated scale and a conventional livestock scale at the beginning and end of the experiment. Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4). Data were *analyzed* as repeated measures using the MIXED procedure of SAS. Initial BW tended to differ between treatments (P = 0.07; MTN 597 ± 32 kg, REST 604 ± 32 kg), but MTN had greater final BW (P < 0.001; 687 ± 24 and 556 ± 27 kg, respectively) and greater average daily gain (1.35 ± 0.18 and -0.72 ± 0.21 kg/d, respectively, $P < 0.001$) than REST. Diameter of the largest follicle was similar ($P = 0.851$) between treatments at CIDR insertion (12.6 ± 0.6 mm) and CIDR removal (12.9 ± 0.4 mm) but was greater ($P < 0.05$) for MTN than REST cows at 60 hrs after CIDR removal (14.01 ± 0.6 and 12.37 ± 0.5 mm, respectively). Volume of CL was similar ($P > 0.1$) at 5 (3211 ± 113 mm^3) and 7 (5280.3 ± 212 mm^3) days after ovulation. Concentration of P4 did not differ on days -10, -3, or 0-5. However, on days 6 and 7, P4 was greater ($P < 0.05$) for MTN than REST (2.07 ± 0.15 and 1.65 ± 0.15, and 2.27 ± 0.15 and 1.83 ± 0.15 ng/mL, respectively). In conclusion, nutrient restriction to 70% of maintenance during estrous synchronization negatively affects diameter of the ovulatory follicle and circulating P4 but did not affect CL volume in multiparous *Bos taurus* beef cows.

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General Audience Abstract

Ensuring successful pregnancy in beef cow-calf operations is crucial for the efficiency and profitability of the cattle industry. This study investigates the effects of nutrient restriction on ovulatory follicle size and corpus luteum (CL) volume in Angus cows undergoing estrous synchronization. A total of 26 cows were subjected to either a maintenance diet meeting 100% of nutrient requirements (MTN) or a diet providing 70% of nutrient requirements (REST). Intakes were updated weekly using computer software. The cows underwent a 30-day nutritional treatment before synchronization of ovulation. Results revealed that cows on the maintenance diet exhibited greater final body weight and average daily gain compared to those on the restricted diet. While estrus expression showed a numerical increase in MTN cows, the impact was not statistically significant. Analysis of ovulatory follicle size demonstrated that MTN cows had larger follicles 60 hours after synchronization compared to REST cows. Surprisingly, corpus luteum volume did not differ between the two groups at 5 and 7 days after ovulation. Additionally, circulating progesterone (P4) levels were affected by nutrient restriction, with notable differences observed on days 6 and 7. In summary, nutrient restriction during ovulation synchronization negatively influenced ovulatory follicle size and P4 levels, but did not affect corpus luteum volume in mature Angus cows. These findings contribute valuable insights for the cattle industry, emphasizing the importance of proper nutrition for optimal reproductive health in beef cows.

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I am grateful to be surrounded and lifted up by such an awesome community across the world. The formative effect that every single one of these people has had on me is incredible, and I thank God for them every day.

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Introduction

Population Growth in North America

On approximately November 15, 2022, the world population reached 8 billion people. Growth is projected to continue until 2100 when the population is estimated to peak at 10.4 billion. However, this growth rate has begun to decelerate. In 2020, the population growth rate fell to under 1 percent for the first time since 1950. (United Nations Department of Economic and Social Affairs, 2022). The escalating population underscores the increasing importance of optimizing food production, particularly as the proportion of individuals engaged in food production continues to decline. In the United States, only about 1.3% of the population is employed in farming (USDA-ERS, 2023). In 1920, 30.2% of the United States identified as living on a farm (Kalbacher and Deare, 1986).

History of the American Beef Cattle Industry

The American beef cattle industry has long been a foundational part of the global economy and food supply since the introduction of European domesticated cattle to the Americas in the late 1400s (McTavish et al., 2013). Current-day cattle, may they be *Bos indicus* or *Bos taurus*, are thought to descend from a common ancestor, the auroch (*Bos primigeius*). The first cattle on the continent were mainly *Bos indicus* influenced, and likely looked similar to a modern-day Brahman in build and Texas Longhorn in color. In 1611, the first British, or *Bos taurus*, cattle were imported into the Jamestown Colony of Virginia (Wilson et al., 1965). The descendants of this type of cattle are widespread throughout the Northern half of the United States today. Following importation, cattle experienced somewhere between 80 to 200 generations of natural selection prior to human intervention (McTavish et al., 2013). Domestication and human intervention in the reproduction of cattle coincided with the rise of the beef and dairy cow as economically relevant species.

Current-day Beef Production Challenges

The landscape of livestock breeding and production has undergone significant evolution. Specifically, cattle systems have transitioned from free-range practices to intensively managed approaches throughout their life cycles. Technological advancements, including genetic selection, feed additives, hormonal implants, and reproductive innovations, have propelled production efficiency and increased slaughter weights. Present production strategies largely center on cow-calf producers who rear calves destined for the food chain. The ideal scenario entails each cow annually weaning a healthy calf that either re-enters production or serves as a protein source. Achieving this objective requires successfully establishing and maintaining pregnancy, calving, and nurturing the offspring to weaning. However, numerous hurdles exist between insemination and parturition, as well as from birth to weaning. Some of these early challenges encompass failure to achieve fertilization due to sperm and/or oocyte factors, aberrant development, genetic anomalies, maternal recognition of pregnancy failure, placental insufficiency, environmental stressors, or diseases. Any of these factors hold the potential to terminate the pregnancy, leading to no viable offspring. Environmental factors can include the weather, presence of other cattle, facilities, stress, and nutrition.

Although it can be difficult to quantify the pregnancy loss caused by nutritional deficit versus anything else, it is still associated with a real economic cost to producers. In 2018, it was estimated by Prevatt that the annual profit foregone by not getting cows pregnant is $200 per nonpregnant cow per year if that cow is retained in the herd. On July 1, 2023, the inventory of beef cows and heifers that had calved in the United States was approximately 29,400,000 (Service, 2023). According to those figures, a 1% increase in the weaning rate of the United States cowherd could result in up to 294,000 more calves and $58,800,000 more for producers. While it is not possible to increase the pregnancy, calving, or weaning rate to 100%, incremental increases in each can have a multiplied effect on operational income and profitability. Increased reproductive efficiency can have a profit associated over the life of a cow as well. When cows become pregnant earlier in life and earlier in the breeding season, they are more likely to have more pounds of calf weaned over their lifetime. More calves sold and more pounds of calf weaned both have the potential to lead to more profit for producers.

Review of Literature

Reproductive Management of Beef Cattle

Historically, management of reproductive success in beef and dairy cattle operations began with something as simple as grouping one or more bulls in an enclosed area with cows. During the late 19th or early 20th century, this progressed to rotation of sires every few years to limit inbreeding. Italian Spallanzani performed the first recorded successful artificial insemination (AI) of a dog in 1784 and later, in 1803, demonstrated that sperm could survive chilling (Foote, 2010; Moore and Hasler, 2017). Over 100 years after, in the late 1800s, Ivanov in Russia concentrated efforts to make AI an everyday practice in horses. His work with stallions and other species was published in 1922 in the *Journal of Animal Science* and spurred research efforts in this area around the world. The first large-scale dairy AI cooperative occurred in Denmark at the Royal Veterinary College in 1936 (Moore and Hasler, 2017). There were 1,070 cattle inseminated, of which 59% resulted in a successful pregnancy. In the United States, AI became prevalent following expansion in the 1940s, during which techniques and equipment were rapidly improved (Foote, 2010).

History and Utilization of Artificial Insemination

Initially, all inseminations were done locally using fresh semen. This limited the influence of genetics both geographically and in number of offspring possible, however. One of the early extenders allowed for semen to be stored at 5°C and viable for up to three days. That extender was made up of an egg yolk and sodium citrate (Foote, 2010). The first successful freezing of sperm was done in the chicken in 1949. Bull semen was successfully frozen with extender in 1968, allowing for widespread use of bulls with superior or desirable traits and reduction in the transmission of venereal diseases (Foote and Kaproth, 2002). By the 1980s, AI was becoming common practice among dairy farms (Foote, 2010). According to the USDA's 2014 Dairy Health and Management Practices on U.S. Dairy Operations, 89.3% of dairy cows were exposed to AI in some fashion, while 43.7% of dairy cows were mated exclusively using AI with no live cover (USDA, 2014). Nonetheless, many beef producers are not utilizing AI in their operations. In the USDA's 2017 Beef Cow-calf Management Practices report, across all herd sizes, approximately 11.6% of beef producers in the United States are using AI, while 76.8% of beef females in 2017 were mated exclusively natural service (USDA, 2020). A few possible explanations for the poor rate of AI adoption are the location of cattle on range land in the western US, lack of labor to detect estrus, and lack of adequate facilities. Small producers utilizing beef cattle as a second income or hobby may have management goals that require minimal time and input. Approximately 81.3% of all producers and 89% of small producers (<50 head) classify their cow-calf operation as a secondary source of income (USDA, 2020).

Management of the Estrous Cycle

Implementation of AI was followed by the desire to manage the estrous cycle to maximize pregnancy results. The first step was to accurately detect estrus and optimize the time of insemination. It was found that cows serviced greater than 24 hours before or after estrus did not usually become pregnant (Bartlett and Perry, 1939; Trimberger, 1948). In research done by Trimberger in 1948, estrus was detected via cattle standing to be mounted and ovulation by palpation. Average ovulation was timed at 10.5 hours past the end of estrus. Approximately 50% of cows serviced 24 hours before their ovulation while in standing estrus became pregnant. The greatest conception rate (85%) was found in cows that were inseminated 13-18 hours before ovulation, (Trimberger, 1948). When cattle were serviced after ovulation was detected, pregnancy rates were ≤40%; thus, the “AM/PM” rule was solidified. The AM/PM rule states that cows observed in estrus in the morning should be bred that evening, and cows observed in estrus in the evening should be bred the next morning, approximately 12 hours later. This rule hinges on the timely and accurate detection of estrus.

The Bovine Estrous Cycle

The bovine estrous cycle is composed of a series of endocrine events that culminate in expression of estrus and ovulation. Hormones controlling the estrous cycle are released from a variety of tissues, including the hypothalamus, anterior pituitary, ovary (follicle & corpus luteum), and uterus. The hormone GnRH is secreted by the hypothalamus. Hormones FSH and LH originate from the anterior pituitary and are released in a pulsatile manner. Estradiol (E2) and P4 are released from the follicles and corpus luteum (CL) of the ovary, respectively. Finally, PGF2α is secreted by the uterus (Wiltbank and Casida, 1956).

One estrous cycle is commonly defined as the time from the beginning of one estrus to the beginning of the next (Frandson, 2009). On average, this is 21 days but can be as short as 17 or as long as 24 days (Lamb et al., 2010b). The estrous cycle is separated into 4 stages: proestrus, estrus, metestrus, and diestrus (Perry, 2004). These stages are each classified as follicular or luteal phase based on what structure is present on the ovary and what the primary hormone being secreted is at that point in time (Asdell, 1946). During the follicular phase, E2 is secreted from one or more follicles developing on the ovary. In the luteal phase, there is a CL actively secreting P4 on the ovary. If the follicular wave produces an ovulatory dominant follicle, a CL will form in the location where the ovum was released. In the presence of an active CL, follicles undergo atresia (RocheCrowe and Boland, 1992).

The follicular phase includes proestrus and estrus while the luteal phase is comprised of metestrus and diestrus. Estrus can also be classified as its own phase, creating a third phase of the estrous cycle (Lamb et al., 2010b). The follicular phase is the shorter of the two, accounting for about 20% of the estrous cycle while the luteal phase makes up the other 80% (Senger, 2012).

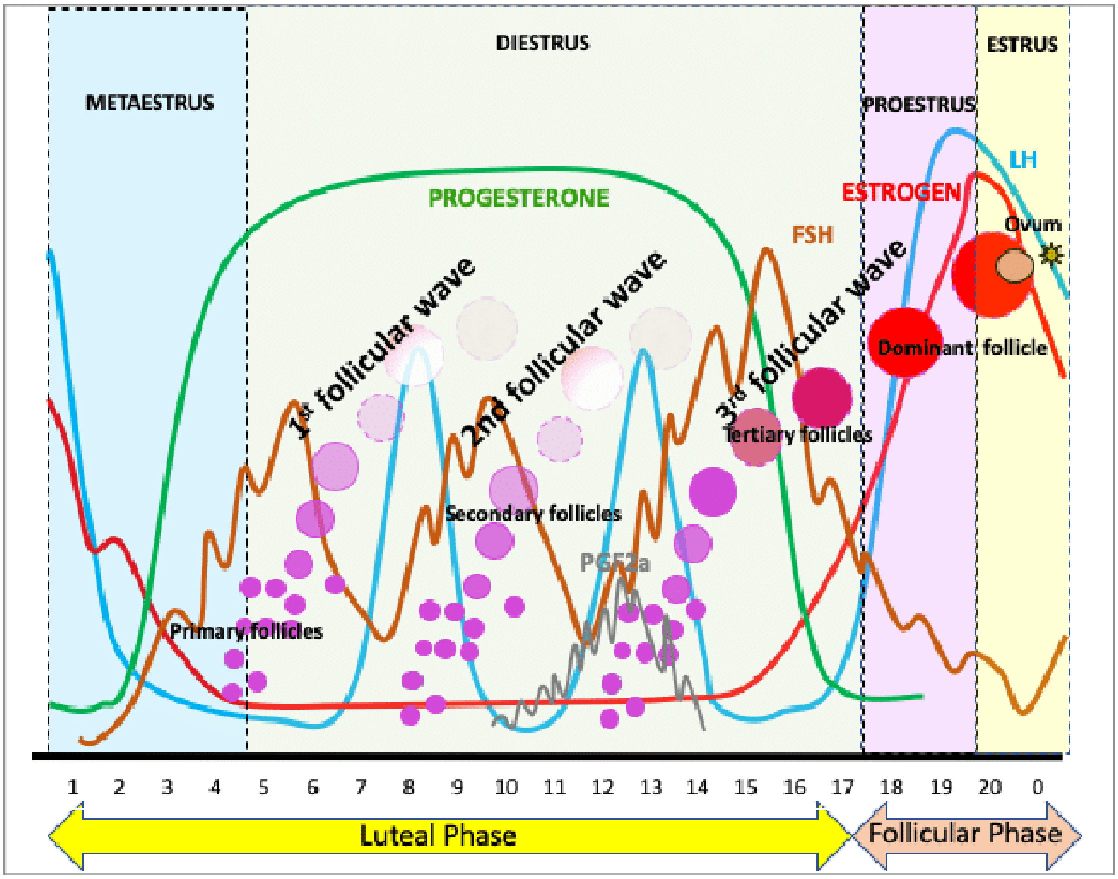

Figure 1. *Hormonal Dynamics of the Bovine Estrous Cycle (Garcia Buitrago, 2021).*

Follicular phase

Proestrus

The initiation of the follicular phase, proestrus, spans from 2-5 days prior to estrus and is marked by regression of the CL. Also termed luteolysis, this results in a marked decrease in circulating P4 (Perry, 2004). Concurrently, LH pulse frequency increases as well as follicular estradiol secretion. Follicular E2 synthesis and release is stimulated by the action of LH and FSH on the follicle. As E2 concentrations continue to increase, they eventually lead to the preovulatory surge of GnRH thus the preovulatory surge of LH. The LH surge serves to do several things. It acts on the follicle to encourage oocyte maturation and granulosa cells to trigger ovulation and start the process of luteinization (Webb et al., 2003; Frandson, 2009). The LH surge also stimulates granulosa cells to begin the synthesis of prostaglandins and causes an inflammatory-like response to weaken the follicle wall and allow for ovulation (Frandson, 2009). This LH surge generally coincides with the onset of estrus.

Estrus

Estrus is defined by increased circulating concentrations of E2 produced by the dominant follicle, LH surge, and ovulation. Cattle ovulate within 12-20 hours after the end of standing estrus (Lamb et al., 2010b). Follicular E2 is responsible for internal changes as well as the outward signs of estrus and generally peaks around the time of peak behavioral estrus expression (Lyimo et al., 2000). Internally, blood flow to the reproductive organs increases, more mucus is secreted, and there is elevated myometrial tone. The primary sign of estrus is standing to be mounted. There are several secondary signs, including vaginal discharge, mounting other animals, restlessness, vocalization, decreased feed intake, an increased body temperature, and swelling of the vulva (Lyimo et al., 2000).

Luteal Phase

Metestrus

Metestrus is the stage of estrous between ovulation and the formation of a functional CL. It is characterized by a marked decrease in E2 and a comparatively decreased circulating concentration of E2 and P4. The follicle that has just ovulated rapidly changes to form a CL: the process of luteinization (Fitz et al., 1984). At the same time, a new wave of follicles is also being recruited (Webb et al., 2004). It is likely that these follicles will not ever progress to ovulation. Instead, they will regress in the presence of increased P4 during diestrus. However, this follicular wave does cause a slight rise then a decrease in E2 and FSH levels. Towards the end of metestrus, P4 secretion by the CL starts to increase as it becomes functional (Perry, 2004).

Diestrus

During diestrus, the longest stage of the estrous cycle, P4 secretion is elevated (Larson and Randle, 2008). Colloquially known as the pregnancy hormone, P4 makes the uterine environment more favorable for the establishment and maintenance of pregnancy as well as preventing estrus and ovulation (Lamb et al., 2010b). Luteolysis ends diestrus, P4 concentrations decrease, and allows for a second or third wave of follicles to continue growth (Webb et al., 2004).

Follicle recruitment

There are two different pools of follicles on the ovaries of cattle: non-growing (primordial follicles) and growing (primary, secondary, and tertiary follicles). Follicles are continually being recruited from the non-growing group to join the growing group. Once follicles are recruited, they eventually either ovulate or undergo atresia. Recruitment is thought to begin with an increase in circulating FSH (Webb et al., 2003). As follicles shift from primordial to tertiary, notable changes include development of mRNA coding for gonadotropin receptors and steroidogenic enzymes.

A new follicular wave emerges approximately every 10 days (Ginther et al., 1997), but a developing antral follicle takes at least 40 days to progress to ovulation (Wathes et al., 2007). One wave is usually composed of 3-5 follicles three or more millimeters in diameter that begin in a common growth phase (Webb et al., 2003). During the common growth phase, there is no difference in growth rates, but the future dominant follicle may already be larger than the others (Kulick et al., 1999). Follicles that are small (<3mm) or not observed at the start of the common growth phase do not generally reach a size greater than 5 mm in diameter or become a dominant follicle (Webb et al., 2003).

Follicle Selection and Dominance

At a size of approximately 5mm, follicles are able to secrete E2 and inhibin, both of which suppress FSH release (Webb et al., 2003). Inhibin can also have a paracrine effect on surrounding follicles to slow or prevent their growth. The production of E2 remains steady until a dominant follicle has been selected, then begins to increase (Kulick et al., 1999). Selection of the dominant follicle happens at a follicular size of 6-8mm. A slight increase in circulating LH is also seen during this time and is believed to continue to stimulate growth of the selected follicle. Approximately 24 hours following the LH increase, a deviation in growth rate emerges between a selected follicle and the others (Ginther et al., 1997; Kulick et al., 1999). This occurs as early as 2.7 days after the initiation of the follicular wave. The selected larger follicle acquires LH receptors and the capacity to continue to grow in an environment with continually decreasing FSH concentrations. Within the first five days of the wave, the dominant follicle will be 2-3mm or larger than others and will continue to grow until it reaches ovulatory capacity. Accordingly, subordinate follicles will begin to regress by around day 5.5 after the wave emerges due to a lack of FSH to stimulate their continued growth (Ginther et al., 2001).

It is thought that bovine follicles acquire ovulatory capacity at about 10 mm, but require an increased concentration of LH to ovulate at this size than when they become larger (Sartori et al., 2001; López-Gatius et al., 2022). In this work, cows with known follicular diameters were given a 4 or 24 mg dose of LH when the largest follicle reached 10mm diameter. A 4mg dose on this size follicle only caused 1/13 follicles to ovulate. Of the cattle given a 24mg dose, 9/13 ovulated. In a following trial, cows were given 40mg of LH when follicles reached 7mm, 8.5mm, or 10mm. This dosage did not have an ovulatory effect on the cows with 7mm or 8.5mm follicles. However, it caused 80% of the cows with 10mm follicles to ovulate. If a smaller follicle is present with an ovulatory follicle, it is generally considered to be atretic. These smaller (7-9mm) follicles can sometimes ovulate alone or concurrently with a larger follicle when subject to a synchronization protocol (López-Gatius et al., 2022). Smaller follicles that ovulated yielded smaller CL in a study where follicle and CL position were tracked (López-Gatius et al., 2022).

Depending on which follicular wave is occurring, the dominant follicle can ovulate or undergo atresia. In the presence of a functional CL and P4, ovulation will not occur. If the follicular wave is destined to ovulate, E2 continues to increase as the dominant follicle continues to grow (Webb et al., 2003). Cytochrome p450 aromatase (p450arom), located in granulosa cells, is the enzyme responsible for the conversion of androgens to estrogen (Gervásio et al., 2014). Feedback from E2 causes the release of GnRH, which, in turn, drives pulsatile LH release. In the absence of a functional CL and P4, LH will peak around day six of the follicular wave and day 20 of the estrous cycle (Perry, 2004). It has been demonstrated that LH and E2 both are related to increasing blood flow to the follicular wall (Acosta and Miyamoto, 2004). Without pharmaceutical intervention, a dominant follicle will take three to four more days after selection to reach a size of 12-20mm prior to ovulating (Kanitz, 2003). Ultimately, ovulation occurs on average at day 21 of the estrous cycle when the follicle ruptures and the oocyte is released along with the cumulus cells surrounding it. The corresponding cells left in the ovary will begin formation of a CL rapidly.

Corpus luteum formation

The preovulatory LH surge begins the process of luteinization: the transition of follicular cells to luteal cells. Directly after ovulation, a corpus hemorrhagicum, caused by the breakage of follicular wall vessels, will form and can be seen from the time of ovulation until about day three of the estrous cycle (Senger, 2012). Another early process in CL formation is angiogenesis, the formation of new blood vessels leading to the area. The ovary is one of the only places in a mature animal that has the capacity to undergo angiogenesis aside from wound healing and cancerous tissues (Berisha et al., 2016). From days three to five, what was the corpus hemorrhagicum will change in appearance to a larger structure: the corpus luteum. The luteal cells have been present but are just becoming macroscopically visible on the surface of the ovary. IGF-1 promotes the proliferation and differentiation of cells in the forming CL, as well as P4 secretion (Berisha et al., 2016). mRNA coding for IGF-1, IGF-2, and the IGFR-1 was seen at peak expression during days one to four of CL formation in luteal cells (Berisha et al., 2016).

Corpus luteum function

By day five of the estrous cycle, rising P4 levels can be detected (Perry, 2004). Circulating P4 depends on production by the CL and metabolism by the liver (Wiltbank et al., 2014). The CL producing P4 is composed of large and small luteal cells formed from the granulosa and thecal cells, respectively, of the follicle (Weber et al., 1987). Large luteal cells can increase in size, but do not generally multiply, so they are dependent on the number of granulosa cells “donated” from the follicle. Both large and small luteal cells can synthesize P4 from cholesterol and secrete it (Fitz et al., 1984; Senger, 2012). In the small luteal cells, LH is the main driver of P4 production (Schams and Berisha, 2004). There is a notable increase in the number of LH receptors on the CL during early development (SpicerIreland and Roche, 1981). Growth hormone (GH) is a main stimulus of large luteal cells. The large luteal cells are responsible for 80% of the P4 produced in the CL and have the capability to produce oxytocin as well (Fitz et al., 1984; Schams and Berisha, 2004). Serum P4 has been shown to be positively associated with increased CL weight in oophorectomized heifers (SpicerIreland and Roche, 1981). In another experiment done, serum P4 increased by 7x from day 1.9 to 4.5 after the LH surge (SpicerIreland and Roche, 1981). Prostaglandin E2 (PGE2) is a luteotropic agent, which has also been shown to increase P4 secretion by the CL (Fitz et al., 1984). PGE2 can also be beneficial to oocyte competence in vitro (Boruszewska et al., 2020). Maternal recognition of pregnancy occurs around day 15 of the estrous cycle via interferon tau (INFT) secreted by the conceptus (HansenSinedino and Spencer, 2017). If fertilization occurs, instead of regressing the CL will remain intact and secreting P4, which is responsible for the early maintenance of pregnancy via stimulating the uterus to release factors that are beneficial to the conceptus. In the absence of pregnancy, P4 concentrations naturally decline around day 17 of the estrous cycle as a result of functional luteolysis (Perry, 2004).

Luteolysis

All mechanisms that affect luteolysis are not yet defined, but P4, E2, oxytocin, and PGF2α have notable effects. Externally, P4 from the CL limits the contractile function of the uterine myometrium via binding to membrane receptors and inhibiting calcium influx (Senger, 2012). Myometrial P4 receptors are expressed at a higher rate early in the luteal phase (Kowalik et al., 2022). In part, it is believed that P4 binding to the uterine receptors blocks the production of PGF2α from the uterus. Later in the luteal phase, uterine P4 receptors are downregulated, allowing for a higher level of PGF2α production (OkudaMiyamoto and Skarzynski, 2002). Along with producing P4, large luteal cells synthesize oxytocin. Oxytocin has a stimulatory effect on PGF2α release from the uterus (OkudaMiyamoto and Skarzynski, 2002). When in the presence of P4, rising E2 levels from growing follicles has been shown to increase both basal and oxytocin-regulated PGF2α release in mid-luteal phase cows (OkudaMiyamoto and Skarzynski, 2002).

Following release from the uterus, PGF2α binds to receptors on the membrane of large luteal cells and is thought to cause apoptotic effects through a decrease in blood flow (Fitz et al., 1984; Acosta and Miyamoto, 2004). Small luteal cells have fewer receptors for PG, and therefore, are less affected by the increasing circulations (KnickerbockerWiltbank and Niswender, 1988). In cows where a complete hysterectomy was performed, CL were maintained. It was seen where parts of the uterus were left, there was a corresponding CL regression (Wiltbank and Casida, 1956). An injection of a PGF2α analog has also been shown to cause a significant decrease in P4 concentration in mid to late cycle cows (SpicerIreland and Roche, 1981). This happens in as few as 30 minutes: prior to when a CL volume change or blood flow differences are detectable (Acosta and Miyamoto, 2004). Volume and blood flow changes were detected by Doppler ultrasound at approximately eight hours post PG injection in cows with a mid-cycle CL. There was no effect shown in cows with an early cycle CL (Acosta and Miyamoto, 2004). Doppler ultrasound can be used to measure blood flow to the ovary and structures composing it as well as detect early pregnancy success or failure (Acosta and Miyamoto, 2004; Fontes and Oosthuizen, 2022). In the instance of failure, apoptotic regression of the CL is followed by a primary follicle becoming the dominant endocrine structure on the ovary, a rise in circulating E2, standing estrus, and ovulation (Perry, 2004). A corpus albicans will be left in the place of the regressed CL. Latin for white body, it’s a small spot of scar-like connective tissue on the surface of the ovary.

Pharmacological Control of the Estrous Cycle

Prostaglandin F2α

Modern-day estrous synchronization protocols rely on the use of three hormones, prostaglandin F2α (PGF2α), gonadotropin releasing hormone (GnRH) and Progesterone (P4). PGF2α is endogenously produced by the uterus and has a luteolytic effect in cattle, meaning that if a CL is present PGF2α will induce regression (Wiltbank and Casida, 1956). One of the first experiments done to explore the synchronization of estrous was published by Lauderdale and others in 1974. Cattle were palpated and given PGF2α upon detection of a CL, then observed for signs of estrus. Following estrus detection, they were bred either once at detection or twice at 72 and 90 hours post estrus. Of the animals that exhibited estrus over the 7 days post PGF2α injection, almost half (49%) showed estrus on day 3. Less than 5% of cows exhibited estrus on days 1, 5, 6, or 7. Days 2, 3, and 4 combined were when 88% of the cows showed estrus. Pregnancy rates were similar between groups, whether they were bred off estrus detection or at a set number of hours following PGF2α (Lauderdale et al., 1974). The efficacy of this protocol depends on the stage of the estrous cycle that is occurring at the time of the injection. The introduction of PGF2α will only act to regress a CL if one is present and has acquired luteolytic capacity. Animals with no CL present, such as those that have not yet become pubertal or those in a period of anestrous, would not be affected in the same manner.

Gonadotropin Releasing Hormone

Gonadotropin releasing hormone (GnRH)**,** a hypothalamic hormone, regulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) via a feedback loop. GnRH or an analogue is commonly utilized in estrous synchronization protocols to control follicular waves. By stimulating the release of LH and FSH, an injection of GnRH can be used to cause ovulation if a follicle with LH receptors is present. On average, GnRH will cause ovulation 25-33 hours post injection (Kanitz, 2003). It can be used in combination with PGF2α to synchronize ovulation in a group of cattle (PursleyMee and Wiltbank, 1995). In their 1995 research with dairy cows, the following synchronization protocol was used. On day zero, 1 100 µg injection was given of a GnRH analogue; on day 7, 1 35 mg injection of PGF2α was given; 48 hours later on day 9, a second 100 µg injection of a GnRH analogue was given; 24 hours later on day 10, the cattle were inseminated. On day 32 following insemination, they were ultrasounded to determine pregnancy. A similar synchronization protocol was used on heifers enrolled in the experiment. However, instead of a second GnRH shot at 48 hours after PGF2α, the time between the two was 24 hours. Serum P4 was also monitored 3x/week beginning on day zero of synchronization and ending 4 weeks after ovulation. It was found that 90% of the cows ovulated in response to the first GnRH injection, resulting in the beginning of a new follicular wave emerging on average 2.5 days later. The PGF2α given was successful in causing the regression of the CL in all cows and 18/24 heifers. All those animals ovulated between 24 and 32 hours following the second GnRH shot. P4 concentrations differed between pregnant and non-pregnant animals past 14 days after AI (PursleyMee and Wiltbank, 1995). This protocol, later known as OvSynch, was the first to condense synchronized ovulation into a time period as short as 8 hours. A modified version of the protocol, where the second GnRH shot is given at the time of AI, was later developed and is known as CO-Synch.

Progesterone

The inclusion of a source of P4 was later added to synchronization protocols. This serves to better control follicular waves. The CIDR, an intravaginal P4 insert, was approved for use in beef cattle in May 2002 and is now the most common P4 device used (Lamb et al., 2010a). The 7-day CO-Synch + CIDR protocol developed as a result of the CIDR's use in synchronization. It was shown to increase pregnancy rates when compared to the CO-Synch protocol (Lamb et al., 2001). Both protocols include fixed-time AI. The CIDR has also proven useful and is FDA approved for returning anestrous cows to cyclicity. When cows were non-cyclic at the beginning of the synchronization protocol, the CO-Synch + CIDR resulted in increased pregnancy rates compared to the CO-Synch protocol (59% vs. 39%) (Lamb et al., 2001). It has also been shown when using the 7 day co-synch + CIDR or 14 day CIDR protocol that estrus expression is associated with an average of 27% improvement in attainment of pregnancy (Richardson et al., 2016).

Advantages of Estrous Synchronization

A main advantage of utilizing an estrous synchronization protocol is the ability to have large numbers of cows be exposed to AI at the same time. From a management standpoint, this is valuable for recordkeeping and labor purposes. Fixed-time AI (FTAI) allows for the insemination of these large groups of cattle without the need for estrus detection and breeding at multiple times. Synchronization protocols utilizing FTAI have the potential to increase profitability for beef producers in the United States when compared to natural service (Rodgers et al., 2012). Cows that underwent synchronization and FTAI calved earlier in the season (days 1-20) and had heavier weaning weights per cow exposed. In turn, using a 5 year mean price, the FTAI group would have a projected average profit of $49.14 more per cow exposed despite added labor and equipment costs (Rodgers et al., 2012). In this project, the handling facilities were already in place. Adequate facilities could pose a major obstacle to the implementation of this technology, especially for smaller producers.

Nutrition and Reproduction Interactions

Body Condition Score

Body condition score (BCS) is a parameter used to assess the fat reserves of cattle. It does not require any equipment, only training, and can be performed with ease. The BCS scale for beef cattle spans from 1 to 9(Wagner et al., 1988). A BCS of 1 indicates that the animal is emaciated and weak while a score of 9 is very obese with possible limited mobility (Whitman et al., 1975; Thomas, 2021). It's expected that BCS will fluctuate throughout the production cycle, but it is beneficial to manage cows to be at a score of 4 or above during breeding. Cows with a BCS less than 4 are less likely to display outward signs of estrus when compared to cows with a BCS greater than 4 (Richardson et al., 2016). A decreasing body condition score over time correlates to weight loss in that animal. Weight loss can be detrimental to the health of the cattle, among other things. Cows at an increased BCS at calving are more likely to return to estrous earlier than cows at a decreased BCS and body weight at calving (Saha et al., 2015). Cows being bred at a increased BCS are also more likely to become pregnant (Lamb et al., 2001). While BCS is often used as an indicator of the nutritional status of cattle, it does not tell the entire story. It is possible for cows to fall within an appropriate range of scores, but still be in a negative energy balance.

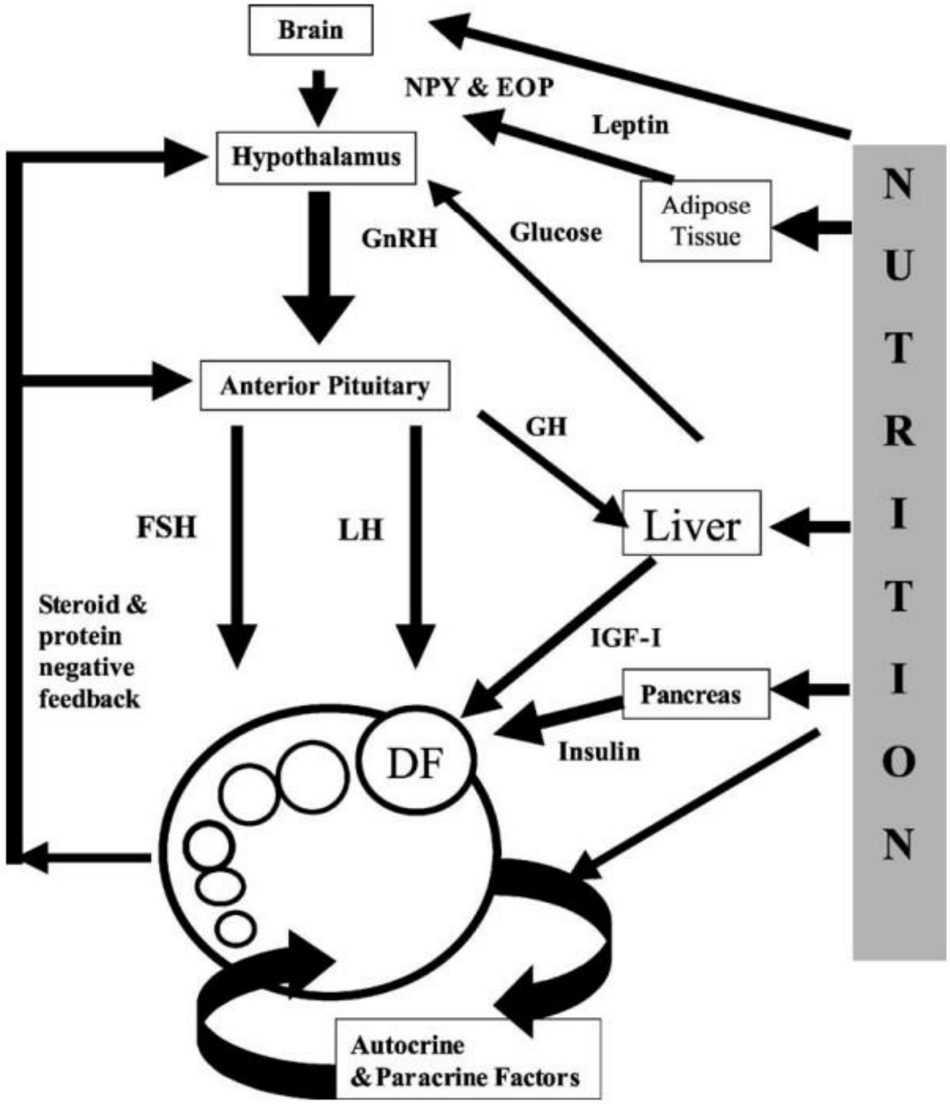

Figure 2. *Diagrammatic representation of possible mechanisms by which nutrition could affect ovarian follicular function (Diskin et al., 2003).*

Energy Balance

A negative energy balance (NEB) is a state in which the energy expenditure exceeds the energy gained from the diet. Some effects of NEB are visualized in Figure 1. Most cattle encounter times during the production cycle where they may undergo a short-term negative energy balance and continue to perform, although it may be at a reduced level of their full potential. Energy in cattle is utilized for bodily tasks in the order of basal metabolism, activity, growth, basic energy reserves, pregnancy, lactation, additional energy reserves, estrous cycles, and excess reserves (Short et al., 1990). Thus, in a time of restriction, excess reserves would be the first to have nutrients partitioned away closely followed by estrous cycles. A loss of 24.0 ± .9% of body weight was shown to cause 91% of non-lactating Hereford females to become non-cyclic (RichardsWettemann and Schoenemann, 1989). In postpartum crossbred anestrous cows, a restricted intake scheme led to an increase in the days to first cycle and first ovulation (Stagg et al., 1995). Restricted cows also tended to have more medium follicular waves and needed more follicular waves to achieve ovulation.

Prolonged and or severe NEB can result in a loss of body mass among other things such as reproductive failure. If cattle encounter a nutrient restricted situation early in life, it has the potential be detrimental the rest of their productive life, particularly to health and fertility (Wathes et al., 2007). Even in mature females, a severe long-term NEB will likely have effects. A below-recommended plane of nutrition during early growth will delay the onset of puberty and thus all reproductive events, such as ovulation, breeding, and calving (SchilloHall and Hileman, 1992). As seen in Figure 2, the effects of NEB on fertility are numerous, particularly on ovarian dynamics. A decreased plane of nutrition can lead to lesser synthesis of GnRH and thus release of FSH and LH. Lesser FSH in circulation may have a negative effect on follicular size while lower levels of LH have been noted to be less effective at stimulation of ovulation (Sartori et al., 2001). Smaller follicles will likely produce less estradiol, impacting outward signs of estrus (Nigussie, 2018). When combined, these effects of NEB can result in delayed or possibly no ovulation. This can lead to less calves over the productive lifespan of the animal.

A lesser-than necessary level of energy intake has a notable endocrine effect as well. Angus and Hereford crossed post-partum cows fed to gain more weight (0.45 kg/d vs .90 kg/d) for the first 71 days after calving returned to cyclicity faster and had an improved pregnancy rate at first estrus (Ciccioli et al., 2003). These cows were also shown to have more circulating IGF-1, insulin, and glucose than their counterparts receiving less feed (Ciccioli et al., 2003). Insulin stimulates glucose absorption by cells in the body and can also act on antral follicles. It has been shown to have a lesser effect than IGF-1 at stimulating growth, but a similar effect in regards to E2 production (Wathes et al., 2007). Infusion of insulin into beef heifers increased the diameter of the dominant follicle (Simpson et al., 1994) and ovulation rate in energy deprived beef heifers (Harrison and Randel, 1986). Nutrient restriction has been shown in crossbred heifers to negatively affect maximum follicle size and persistence (Murphy et al., 1991). In this instance, however, the follicular growth rate and P4 concentrations were not different between low, moderate, or high levels of nutrition. In opposition, feed intake restriction was been shown to decrease the luteal activity, as measured by plasma P4, in Hereford cows (RichardsWettemann and Schoenemann, 1989). The same cows showed decreased plasma glucose and insulin levels as well as increased concentrations of non-esterified fatty acids (NEFAs) (RichardsWettemann and Schoenemann, 1989).

Non-Esterified Fatty Acids

The concentration of NEFAs in plasma can determine the energy status of an animal. When metabolic energy is required, NEFAs are released from lipid stores, taken up by the liver, and partially or fully oxidized to create ketone bodies or acetate. They can also be esterified to create triacylglycerols. Larger concentrations of NEFAs and cholesterol concentrations have also been associated with an increased risk of metritis and retained placentas in post-partum Holstein cows (Kaneene et al., 1997). In other work, decreased serum NEFAs and increased glucose concentrations have been associated with a greater pregnancy rate at first insemination in postpartum dairy cows (Garverick et al., 2013). Betahydroxybutyrate (BHB) is the main ketone body in blood. Its concentration is an index of fatty acid oxidation. In examining BHB concentrations of nutrient restricted cows, the BHB between maintenance fed Angus cows and nutrient restricted (70%) cows was not found to be different at day 19 post ovulation (Fontes et al., 2021). As mentioned, the liver plays a role in metabolism of NEFAs as well as other lipid digestion via bile salt synthesis, secretion, and reabsorption. The liver is able to synthesize cholesterol as well (FrandsonWilke and Fails, 2016). A state of NEB also affects the liver, although all downstream changes haven't yet been well defined. 524 liver genes were found to be differentially expressed in severe vs mild NEB cows, with 10% being related to metabolism (Wathes et al., 2007). These included up-regulation of genes for lipid catabolism and gluconeogenesis as well as IGFBP-2. As seen in Figure 1, genes for IGF-I, GH-R, IGF binding protein-3 (IGFBP-3) were simultaneously downregulated (Wathes et al., 2007).

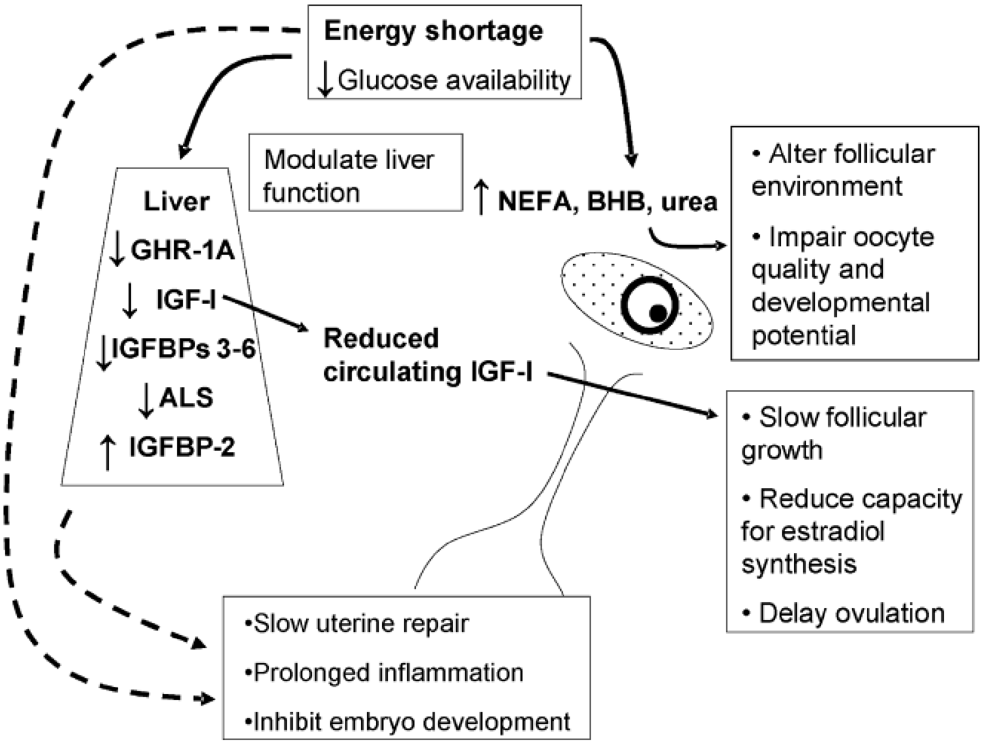

Figure 3. *Influences of NEB on reproductive function in cattle (Wathes et al., 2007).*

There are many endocrine factors involved in digestion and energy balance, but some of the prevalent ones are growth hormone (GH), IGFs, and insulin. Ruminant GH stimulates lipolysis and induces peripheral insulin resistance. This action allows for glucose to be used in the brain, and during pregnancy, the placenta. It's widely accepted that more circulating GH stimulates gonadal function (Scaramuzzi et al., 1999). In female cattle, GH stimulates folliculogenesis and has an effect up to the size of approximately 5 mm in diameter. GH can stimulate the production of IGF-1, but the release of GH can also be influenced by IGF via negative feedback to the hypothalamus and adenohypophysis. The major intrafollicular IGF regulating the growth of bovine antral follicles is IGF-II (Webb et al., 2004).

Insulin-like Growth Factors

Insulin-like growth factors 1 and 2 (IGF-1; IGF-2) are produced and released mainly by cells in the liver (VelazquezSpicer and Wathes, 2008). Circulating IGF-II is mainly observed in prenatal growth while IGF-1 is the main post-natal form. Circulating IGF is almost always coupled with a binding protein. There are 6 members of the insulin-like growth factor binding protein (IGFBP) family. These serve to increase the half-life of IGF in circulation and prevent it from binding to a receptor. It is possible for IGFs to bind to insulin receptors as well as their own. Binding protein -2 is produced locally in many cells, notably during myogenesis (Allard and Duan, 2018). The most prevalent IGFBP in the serum is IGFBP-3 (Allard and Duan, 2018). The liver and many other tissues can secrete IGFBP-3 in reaction to GH levels. It is also able to enter and act in the cell nucleus, along with BP-5. The pituitary gland has a noted prevalence of IGF-1 receptors as well as binding proteins -2, -3, and -5.

In terms of reproduction, it's been speculated that circulating IGF-1 can be used as an indicator of future reproductive success (VelazquezSpicer and Wathes, 2008). In sheep, IGF-1 infusion was effective at stimulation of E2 production during the follicular phase (Scaramuzzi et al., 1999). Nutrient restriction in Angus cows has been shown to decrease plasma insulin and IGF-1 on day 19 following ovulation (Fontes et al., 2021). Other work corroborates that cows in an acute state of NEB have shown a reduction in IGF-I synthesis and availability (Webb et al., 2004).

Overnutrition

Overnutrition can also be detrimental to the health of animals, particularly reproductive performance. Heifers with different original BCS were energy restricted to achieve anestrous, then stepped up to regain cyclicity (Cassady et al., 2009). It was found that the heifers originally classified as fat (7.0 BCS) had a decreased circulating LH level as well as LH pulse frequency and amplitude compared to moderate (5.0 BCS) cattle during the restriction phase of the study. Both groups of heifers showed reduced GH circulation during this time. After the heifers regained cyclicity, the fat heifers had decreased circulating IGF-1, glucose, and insulin concentrations when compared to moderate heifers. An increased plane of nutrition at puberty can also negatively affect mammary development and later milk yield (Sejrsen, 1994).

Summary and Research Goals

The profitability of producers in the United States beef cattle industry hinges on successful reproduction. Cattle reproduction is intricately influenced by numerous endocrine and environmental factors. Notably, feed intake and nutrient availability exert direct effects on reproductive function. In the forthcoming research chapter, we aim to investigate the impact of nutrient restriction on ovarian function, encompassing follicle and CL growth, as well as P4 production. Our hypothesis posits that nutrient restriction to 70% of maintenance levels will impede follicle growth, diminish ovulatory follicle diameter, and subsequently reduce CL volume, thus affecting the capacity for P4 production.

Ovulatory Follicle and Corpus Luteum Development of *Bos taurus* Cows Under Nutrient Restriction

Materials & Methods

This experiment was conducted at the Virginia Polytechnic Institute and State University – Shenandoah Valley Agricultural Research and Extension Center, McCormick Farm, located in Raphine, VA, USA. All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Virginia Polytechnic Institute and State University – Institutional Animal Care and Use Committee (#20-178). The research was conducted from May 1, 2022 to June 14, 2022. The high, low, and average temperatures from this time period were 30.6, -10, and 12°C respectively with 27.3 cm of precipitation recorded.

Animals

A total of 26 Angus multiparous cows were enrolled in this experiment. Cows were stratified based on body weight and randomly assigned to 1 of 4 half-covered, concrete-floor pens (8.4 x 12 m) in a feeding facility equipped with a Calan gate system. Each pen contained 12 Calan gates for individual feed delivery. For 21 days prior to the beginning of the experimental period (days -51 to -30) cows were acclimated to pens, feed bunks and diets. Body condition scores (BCS; Scale of 1-9) were recorded on days -78, -10, -3, 0, 5, 7, 9, and 16 (Whitman et al., 1975) while body weights (BW) were recorded using a smart scale system and a traditional scale on days -78, -10, -3, 0, 1, 2, 3, 4, 5, 6, 7, 9, 16, 19, and 26. Initial BW and BCS were averaged on day -78.

Nutritional Treatments

Following the acclimation period, within pen cows were stratified by BW and randomly assigned to two nutritional treatments: 1) Feed intake restricted to achieve 70%

of energy requirements based on BW (REST, n = 13) or 2) Feed intake to achieve 100% maintenance of energy requirements based on BW (MTN, n = 13). The Beef Cattle Nutrient Requirement Model (BCNRM) computer software was used for these calculations using equations proposed by the National Research Council (2000). This was done by calculating a maintenance level of dry matter intake for all cows, then restricting REST cows to 70% of that intake. Cows were fed the same dietary composition across treatments. All cows had ad libitum access to water during the entire experimental period.

Table 1. *Nutrient analysis of dried corn silage, soybean meal, and mineral total mixed ration fed to both MTN and REST cows for the duration of the trial.*

Item	**As-fed**	**Dry Matter**
Ingredient, % (as-fed basis)		
Corn Silage		88
Soybean Meal		10
Mineral		2
Analysis		
% Moisture	8.9	--
% Dry Matter	91.1	
% Crude Protein	11.9	13.1
% Adjusted Crude Protein	11.9	13.1
% ADF	21.5	23.6
% aNDF	33.5	36.7
% TDN	64	70
Net Energy for Lactation, Mcal/Lb	0.68	0.75
Net Energy for Maintenance, Mcal/Lb	0.68	0.75
Net Energy for Growth, Mcal/Lb	0.43	0.47

Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY). MTN: Diet formulated to meet 100% of the daily nutrient requirements; REST: Diet formulated to meet 70% of daily nutrient requirements.

A corn silage, soybean meal, and mineral total mixed ration (TMR) was fed for the duration of the experiment. The TMR was offered twice daily at 0800 h and 1600 h. The ration was mixed as a batch and weighed out individually.

Feed Sampling

If there was residual feed remaining directly prior to the next feeding, it was collected and weighed. Feed samples were taken weekly and dried in a forced-air oven at 55°C for 72h to determine dry matter and stored for nutritive analyses. Individual feed intakes were recalculated weekly using updated silage dry matter analyses and body weights in order to achieve the 70% and 100% of energy requirements for the respective treatment. Following the conclusion of the experiment, the dried weekly feed samples were pooled and sent for analysis of crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA) as described by (Silva et al., 2018). The results are seen in Table 1. As noted, the samples were dried prior to being sent for nutrient analysis, which affects the dry matter represented in Table 1, but not the nutrient composition on a dry matter basis.

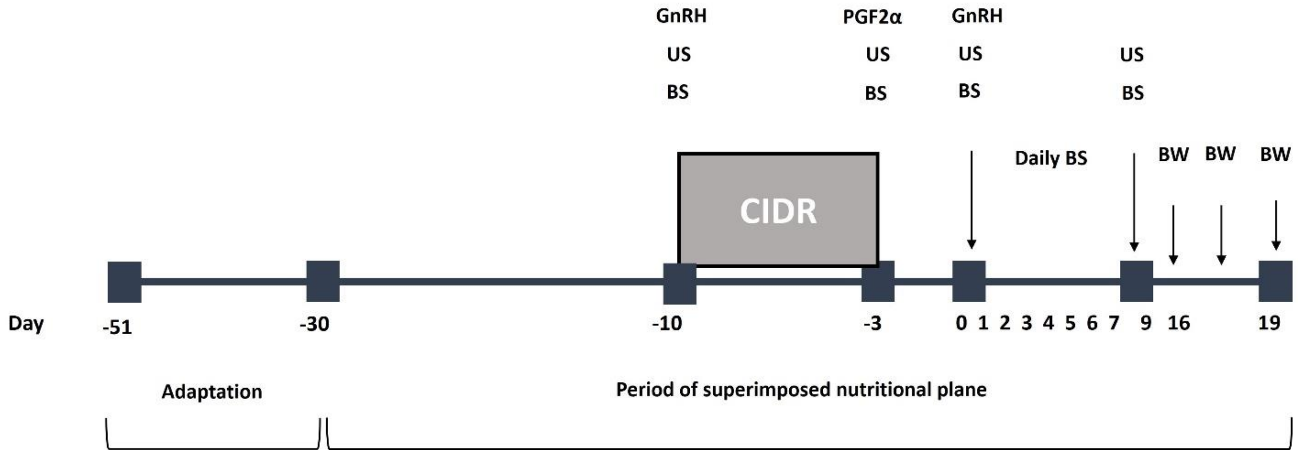

Figure 4. *Experimental timeline. Cattle underwent an acclimation period of 21 days and were exposed to nutritional treatments for 30 days prior to estrous synchronization. Body weight was measured daily using an automated scale and a conventional livestock scale at the beginning and end of the experiment. Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4). GnRH: 100 µg of Gonadotropin releasing hormone (2 mL Factrel; Zoetis Animal Health, Florham Park, NJ); US: Transrectal ultrasonography performed (Ibex portable ultrasound, 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO); BS: Blood sample was collected into commercial blood collection tubes (Vacutainer, 10mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing 143 IU of freeze-dried sodium heparin; BW: Body weight was measured using a smart scale system; PGF2α: 25 mg of Prostaglandin* $F_{2\alpha}$ *(5 mL Lutalyse; Zoetis Animal Health); CIDR: Controlled Internal Drug Release (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health.*

Estrous Synchronization and Ultrasonography

The REST and MTN nutritional treatments were fed for 21 days prior to cows being enrolled in an estrous synchronization protocol. All cows were enrolled in a 7-day CO-Synch + CIDR protocol (Figure 5). Cows underwent an estrous synchronization protocol commonly known as the 7-day CO-synch + CIDR (Larson et al., 2006). In short, cows received 100 µg of GnRH (2 mL Factrel; Zoetis Animal Health, Florham Park, NJ) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion followed in 7 d with 25 mg of PGF (5 mL Lutalyse; Zoetis Animal Health) at CIDR removal. 60-66 hours later, a second 100 µg injection of GnRH was given. Estrus detection aids were used to determine expression of estrus behavior (Estrotect Breeding Indicator, Rockway Inc., Spring Valley, WI).

Transrectal ultrasonography (Ibex portable ultrasound, 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO) was performed on days -10, -3, 0, 5, and 7 to determine the diameter of the dominant follicle and corpus luteum (CL) volume. A vertical and perpendicular horizontal diameter of the largest follicle on each ovary and all CL were measured and recorded. Follicle diameter was calculated by the average value for the vertical and horizontal diameters. Volume (V) of CL tissue was calculated using the formula $V = 4/3 \times \pi \times r^3$, in which r = one half of the average value of the vertical and horizontal diameters. In cases where the CL had a fluid-filled cavity, volume of the cavity was subtracted from the total volume of the CL, resulting in a value that reflected the actual volume of luteal tissue present (Mercadante et al., 2015).

Blood Sampling and analyses

Blood samples were collected from all cows on days -10, -3, 0, 1, 2, 3, 4, 5, 6, and 7 via jugular or coccygeal venipuncture. Blood was collected into commercial blood collection tubes (Vacutainer, 10mL; Becton Dickinson, Franklin Lakes, NJ, USA) containing 143 IU of freeze-dried sodium heparin. After collection, the blood samples were placed immediately on ice and centrifuged at 2400 × *g* for 15 minutes for plasma harvest and stored at -20°C immediately after. Samples were analyzed as singcates for concentrations of plasma P4 using a commercially available chemiluminescence assay (Immulite 2000 XPi Immunoassay System, Siemens Healthcare, CA, USA) (Bedford et al., 2018). The intraassay CV was 1.3%.

Statistical Analysis

The SAS (version 9.3; SAS/STAT; SAS Inst. Inc., Cary, NC) statistical package was used for all analyses. Cow was considered the experimental unit for all analyses. The effects of cow BW, BCS, follicle diameter, CL volume and plasma concentrations of P4 were analyzed as repeated measures using the MIXED procedures of SAS. The covariance structure for all the analyses was selected based on the smallest Akaike information criterion for each variable analyzed. The models used for BW and BCS included the fixed effects of diet, day, and diet × day interaction. When analyzing cow BW and BCS, initial BW and BCS were used as covariates for the respective analysis. The models for recipient plasma concentrations of P4 included the fixed effects of diet, day, and all the interactions that included day. Significance was declared at $P \leq 0.05$, $0.05 > P \leq 0.10$ was considered a tendency. Least squares means ± SEM are reported.

Results and Discussion

Body Condition Scores, Average Daily Gain, and Body Weights

The results for initial BCS and BW along with final BW, ADG, and BCS are summarized in Table 2. As designed, at initiation of the experiment, there were no statistical differences detected between treatment groups for BCS and initial BW. Following the implementation of the nutrient restriction scheme, there was a significant difference in final BW and BCS. It is a realistic expectation that this could represent a time point in the production cycle where cows may experience a reduction in BW and BCS, particularly at a time of intense nutritional demand such as lactation (Short et al., 1990). Body condition scores are an external measure of the total energy contained in an animal (Wagner et al., 1988).

Cows calving at an increased BCS have fewer days to first estrous and achieving pregnancy (Stagg et al., 1995; Lamb et al., 2001; Ciccioli et al., 2003; Saha et al., 2015). When approaching breeding, it is also beneficial for cows to be increasing BCS, as they're more likely to become pregnant earlier (SchilloHall and Hileman, 1992). In the current experiment, there was a time of nutritional restriction. This is evidence of the REST cows being in a state of NEB. It has been shown that a BW loss of 24.0 ± .9% caused 91% of non-lactating Hereford females to become anestrous (RichardsWettemann and Schoenemann, 1989). Based on this, there should not have been any incidence of nutritional anestrous. The cows enrolled in the present experiment were purebred Angus, a similar breed type, and did not lose greater than 20% of their original BW.

Ovulatory Follicle size and Estrus Expression

Previous studies have demonstrated a negative impact of nutrient restriction schemes on dominant follicle size and persistence in heifers (Murphy et al., 1991). In the current study, cows exhibited similar dominant follicle diameters between treatments on days -10 and -3, but the REST group displayed significantly smaller ovulatory follicles compared to the MTN group on day zero (Figure 6). Notably, follicles attain ovulatory capacity when their diameter exceeds 10 mm (Sartori et al., 2001). Furthermore, research on estrous synchronization protocols indicates a correlation between dominant follicle size and the likelihood of achieving pregnancy in both heifers and cows (Perry et al., 2005; Perry et al., 2007). Among cows, the highest probability of pregnancy occurred with an ovulatory follicle size of 15.2 mm. All observed pregnancy losses in this study were in cows that ovulated a follicle smaller than 13.5 mm in diameter. The average ovulatory follicle size in the REST group in our study fell below this critical threshold. This suggests that if the REST cows were to conceive, they might experience increased rates of pregnancy loss compared to their MTN counterparts. Further investigation into sustaining restrictions throughout the establishment of pregnancy could elucidate the potential correlation with corresponding pregnancy losses. Estrus expression was numerically higher in the MTN group (Figure 7). Other experiments concur that cows at a higher BCS and increased plane of nutrition will exhibit more outward signs of estrus. Richardson et al. (2016) reported cows above a BCS of 4 are more likely to exhibit estrus. Estrus expression is important, especially in cows being treated with a synchronization protocol. Some protocols require detection of estrus in order to artificially inseminate. If there is a silent ovulation in this instance, the opportunity for the cow to become pregnant at that time may be lost. It has been shown that when using a 7-day CO-Synch + CIDR protocol that estrus expression is associated with 27% increase in average pregnancy rates (Richardson et al., 2016). Establishment and maintenance of pregnancy is central to profitability for US cow-calf producers (Prevatt et al., 2018).

CL Volume and Plasma Progesterone

It has been noted that larger follicles usually yield larger CL as well as the inverse (López-Gatius et al., 2022). However, no discernible treatment difference was observed in the resulting CL volume on days 5 or 7 (Figure 8); however, there was a notable reduction in circulating P4 concentration in the REST group on days 6 and 7 post-ovulation (Figure 9). Previous studies have indicated a positive correlation between CL weight and circulating P4 levels (Spicer-Ireland and Roche, 1981). Therefore, one would expect lower circulating P4 to be associated with decreased CL volume. However, this contradicts the findings of the current study, where the REST group, despite having a similar CL size, exhibited lower circulating P4. It has also been noted that a decreased blood flow rate to the liver affects the rate of cholesterol metabolism, thus decreasing circulating P4 (Wiltbank et al., 2014). If blood flow and metabolism of P4 differed, the concentrations of P4 in blood could have been affected. The discrepancies in CL and P4 findings might also stem from variations in cattle populations subjected to diets with differing nutrient compositions. Future research considerations could involve assessing liver blood flow, cholesterol bioavailability, and conducting CL biopsies for histological observations.

In a separate feeding study involving crossbred heifers, P4 levels also did not differ significantly among groups with low, moderate, or high levels of nutrition (Murphy et al., 1991). Additional research demonstrated that cows experiencing negative energy balance

(NEB) exhibited decreased luteal activity, as measured by plasma P4 levels before entering anestrous (Richards-Wettemann and Schoenemann, 1989). These findings align with the observed P4 outcomes in the current experiment.

***Table 2**. Effects of dietary treatments[1] on BW, ADG, and BCS.*

Item	**MTN**	**REST**	***P*-Value**
n	13	13	--
Initial Body Weight (kg)	597±32	604±32	0.74
Final Body Weight (kg)	687±24	556±27	<0.001
Average Daily Gain (kg/day)	1.35±0.18	-0.72±0.21	<0.001
Initial BCS	5.62±0.22	5.23±0.22	0.23
Final BCS	5.69±0.29	4.46±0.29	<0.001

[1] *MTN: A diet formulated to meet 100% of the nutritional requirements; REST: a diet formulated to meet 70% of the nutritional requirements of the cow. Intakes were updated weekly based on current weights.*

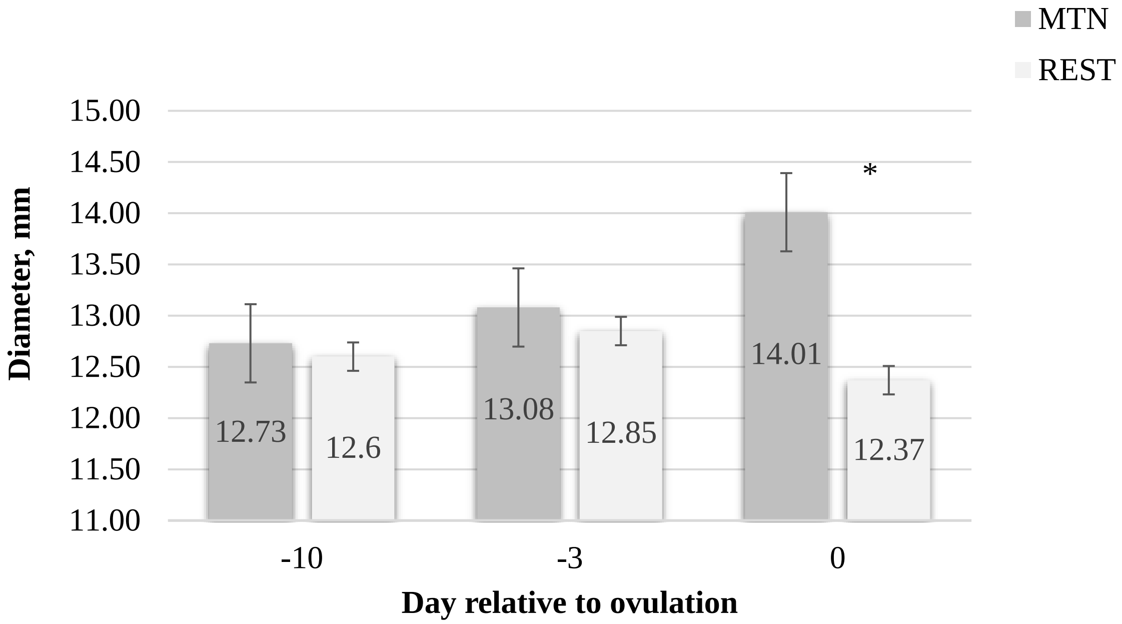

Figure 5. *Effect of dietary treatments on average follicle diameter of MTN and REST cows as detected via transrectal ultrasound on days -10, -3, and 0 relative to ovulation;*

MTN: A diet formulated to meet 100% of the nutritional requirements; REST: a diet formulated to meet 70% of the nutritional requirements of the cow. Intakes were updated weekly based on current weights.

*Error bars represent the SEM. * Least square mean difference: P < 0.05.*

Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4).

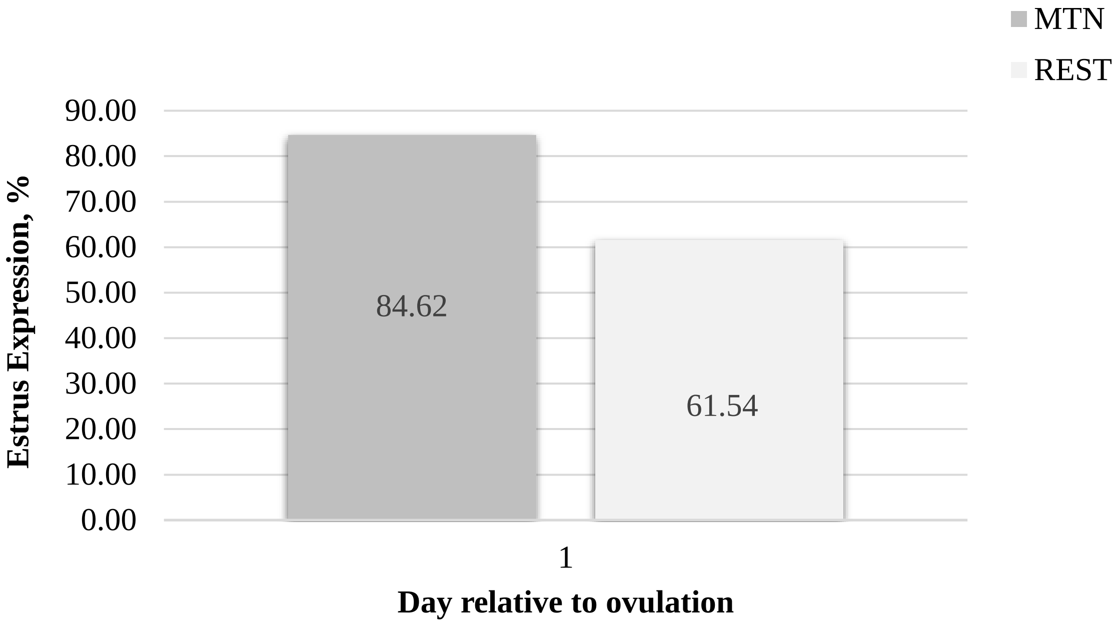

Figure 6. *Effect of dietary treatments on estrus expression of MTN and REST cows on day 1 relative to ovulation; P = 0.842.*

MTN: A diet formulated to meet 100% of the nutritional requirements; REST: a diet formulated to meet 70% of the nutritional requirements of the cow. Intakes were updated weekly based on current weights.

Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4).

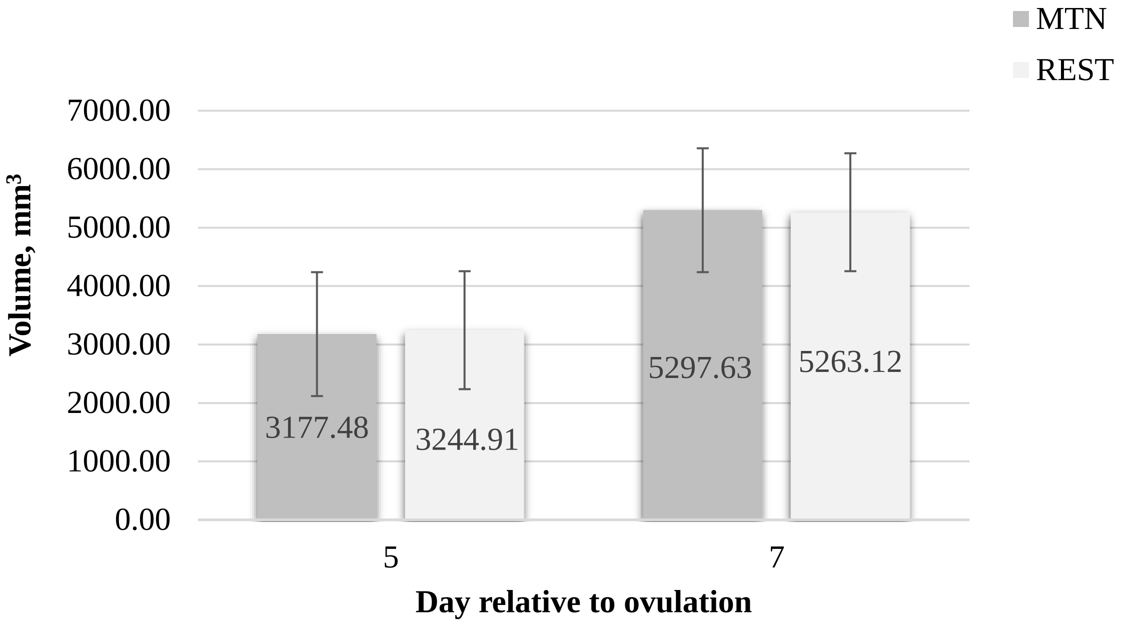

Figure 7. *Effect of dietary treatments on average Corpus luteum volume of MTN and REST cows as detected via transrectal ultrasound on days 5 and 7 relative to ovulation;*

MTN: A diet formulated to meet 100% of the nutritional requirements; REST: a diet formulated to meet 70% of the nutritional requirements of the cow. Intakes were updated weekly based on current weights.

*Error bars represent the SEM. * Least square mean difference: $P < 0.05$.*

Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4).

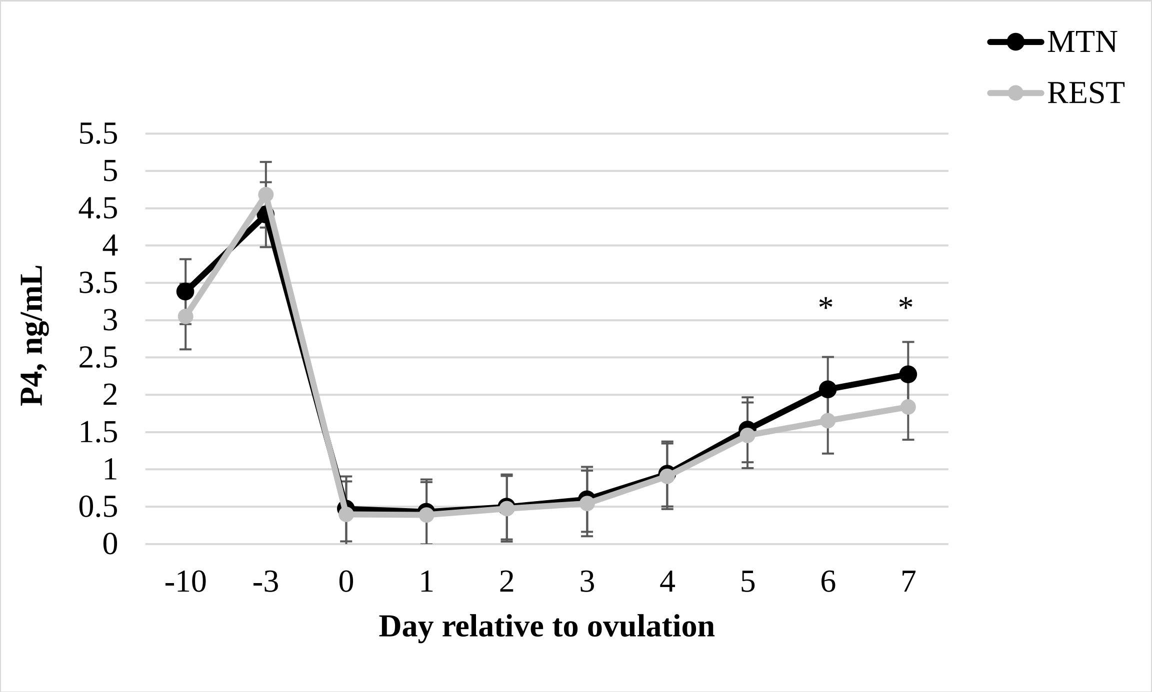

Figure 8. *Effect of dietary treatments on average plasma P4 concentration of MTN and REST cows on days -10, -3, 0, 1, 2, 3, 4, 5, 6, and 7 relative to ovulation;*

MTN: A diet formulated to meet 100% of the nutritional requirements; REST: a diet formulated to meet 70% of the nutritional requirements of the cow. Intakes were updated weekly based on current weights.

*Error bars represent the SEM. * Least square mean difference: $P < 0.05$;*

Cows were synchronized using a 7-day CO-synch + CIDR protocol beginning on day -10. Ultrasonography of the ovaries was performed at each event of the estrous synchronization protocol on days -10, -3, 0, 5, and 7. Blood samples were taken on days -10, -3, and daily from day 0 through 7 to observe changes in progesterone (P4).

Summary and Conclusions

In summary, this experiment demonstrated that subjecting mature Angus cows to nutrient restriction at 70% of required energy levels led to a reduction in follicle size and circulating P4 levels, while it did not affect CL volume. The observed decrease in follicle size and circulating P4 could potentially impede successful pregnancy establishment, a critical factor in the United States' beef production industry. The insights garnered from this study hold valuable implications for cattle producers. In essence, maintaining cyclic cows on a negative nutritional plane proves detrimental. Furthermore, the multifaceted impact of nutrition on reproductive processes underscores its pivotal role. Effectively managing the reproductive functions of cows necessitates maintaining a ration that fulfills their energy requirements. Further research is warranted to comprehensively understand the additional endocrine effects and underlying mechanisms contributing to these results, particularly the absence of significant differences in CL size despite the disparity in P4 levels between the MTN and REST groups.

References

Acosta, T. J., and A. Miyamoto. 2004. Vascular control of ovarian function: ovulation, corpus luteum formation and regression. Animal Reproduction Science 82-83:127-140. doi: https://doi.org/10.1016/j.anireprosci.2004.04.022

Allard, J. B., and C. Duan. 2018. IGF-Binding Proteins: Why Do They Exist and Why Are There So Many? Frontiers in Endocrinology 9(Review) doi: 10.3389/fendo.2018.00117

Asdell, S. A. 1946. Patterns of mammalian reproduction. Patterns of mammalian reproduction.

Bartlett, J. W., and E. J. Perry. 1939. Lessons Learned from Eighteen Months' Experience with Cooperative Artificial Breeding of Dairy Cattle in New Jersey. Journal of Animal Science 1939:243-245.

Bedford, A., L. Beckett, K. Hardin, N. W. Dias, T. Davis, V. R. G. Mercadante, A. D. Ealy, and R. R. White. 2018. Propionate Affects Insulin Signaling and Progesterone Profiles in Dairy Heifers. Scientific Reports 8(1):17629. doi: 10.1038/s41598-018-35977-1

Berisha, B., D. Schams, D. Rodler, and M. W. Pfaffl. 2016. Angiogenesis in The Ovary – The Most Important Regulatory Event for Follicle and Corpus Luteum Development and Function in Cow – An Overview. Anatomia, Histologia, Embryologia 45(2):124-130. doi: https://doi.org/10.1111/ahe.12180

Boruszewska, D., I. Kowalczyk-Zieba, K. Suwik, J. Staszkiewicz-Chodor, J. Jaworska, K. Lukaszuk, and I. Woclawek-Potocka. 2020. Prostaglandin E(2) affects in vitro maturation of bovine oocytes. Reprod Biol Endocrinol 18(1):40. doi: 10.1186/s12958-020-00598-9

Cassady, J. M., T. D. Maddock, A. DiCostanzo, and G. C. Lamb. 2009. Initial body condition score affects hormone and metabolite response to nutritional restriction and repletion in yearling postpubertal beef heifers1. Journal of Animal Science 87(7):2262-2273. doi: 10.2527/jas.2008-1716

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows1,2. Journal of Animal Science 81(12):3107-3120. doi: 10.2527/2003.81123107x

Diskin, M. G., D. R. Mackey, J. F. Roche, and J. M. Sreenan. 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim Reprod Sci 78(3-4):345-370. doi: 10.1016/s0378-4320(03)00099-x

Fitz, T. A., E. J. Mock, M. H. Mayan, and G. D. Niswender. 1984. Interactions of prostaglandins with subpopulations of ovine luteal cells. II. Inhibitory effects of PGF2α and protection by PGE2. Prostaglandins 28(1):127-138. doi: https://doi.org/10.1016/0090-6980(84)90120-5

Fontes, P. L. P., and N. Oosthuizen. 2022. Applied Use of Doppler Ultrasonography in Bovine Reproduction. Frontiers in Animal Science 3(Review) doi: 10.3389/fanim.2022.912854

Fontes, P. L. P., N. Oosthuizen, F. M. Ciriaco, C. D. Sanford, L. B. Canal, R. F. Cooke, K. G. Pohler, D. D. Henry, V. R. G. Mercadante, A. D. Ealy, S. E. Johnson, N. DiLorenzo, and G. C. Lamb. 2021. Effects of nutrient restriction on the metabolic profile of Bos indicus-influenced and B. taurus suckled beef cows. Animal 15(3):100166. doi: https://doi.org/10.1016/j.animal.2020.100166

Foote, R. 2010. The history of artificial insemination: Selected notes and notables. J. Anim. Sci 80:1-10.

Foote, R. H., and M. T. Kaproth. 2002. Large Batch Freezing of Bull Semen: Effect of Time of Freezing and Fructose on Fertility. Journal of Dairy Science 85(2):453-456. doi: https://doi.org/10.3168/jds.S0022-0302(02)74094-0

Frandson, R. D., W. L. Wilke, and A. D. Fails. 2016. Anatomy and Physiology of Farm Animals. 7th ed. Wiley-Blackwell, Ames, IA.

Frandson, R. D. W., W. Lee; Fails, Anna Dee. 2009. The Ovary and Estrous Cycles, Anatomy and Physiology of Farm Animals. Wiley-Blackwell, Ames, Iowa.

Garcia Buitrago, J. 2021. Teaching Notes on Dairy Production. REPRODUCTIVE MANAGEMENT OF DAIRY CATTLE. ANATOMY OF REPRODUCTIVE TRACT AND ESTROUS CYCLE OF COW.

Garverick, H. A., M. N. Harris, R. Vogel-Bluel, J. D. Sampson, J. Bader, W. R. Lamberson, J. N. Spain, M. C. Lucy, and R. S. Youngquist. 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. Journal of Dairy Science 96(1):181-188. doi: https://doi.org/10.3168/jds.2012-5619

Gervásio, C. G., M. P. Bernuci, M. F. Silva-de-Sá, and A. C. J. d. S. Rosa-e-Silva. 2014. The role of androgen hormones in early follicular development. International Scholarly Research Notices 2014

Ginther, O. J., D. R. Bergfelt, M. A. Beg, and K. Kot. 2001. Follicle Selection in Cattle: Relationships among Growth Rate, Diameter Ranking, and Capacity for Dominance. Biol Reprod 65(2):345-350. doi: 10.1095/biolreprod65.2.345

Ginther, O. J., K. Kot, L. J. Kulick, and M. C. Wiltbank. 1997. Emergence and deviation of follicles during the development of follicular waves in cattle. Theriogenology 48(1):75-87. doi: https://doi.org/10.1016/S0093-691X(97)00192-1

Hansen, T. R., L. D. P. Sinedino, and T. E. Spencer. 2017. Paracrine and endocrine actions of interferon tau (IFNT). Reproduction 154(5):F45-f59. doi: 10.1530/rep-17-0315

Kalbacher, J. Z., and D. Deare. 1986. Farm population of the United States, 1985. Curr Popul Rep Popul Charact (59):i-1-20.

Kaneene, J. B., R. Miller, T. H. Herdt, and J. C. Gardiner. 1997. The association of serum nonesterified fatty acids and cholesterol, management and feeding practices with peripartum disease in dairy cows. Preventive Veterinary Medicine 31(1):59-72. doi: https://doi.org/10.1016/S0167-5877(96)01141-5

Kanitz, W. 2003. Follicular dynamic and ovulation in cattle – a review. Arch. Anim. Breed. 46(2):187-198. doi: 10.5194/aab-46-187-2003

Knickerbocker, J. J., M. C. Wiltbank, and G. D. Niswender. 1988. Mechanisms of luteolysis in domestic livestock. Domestic Animal Endocrinology 5(2):91-107. doi: https://doi.org/10.1016/0739-7240(88)90011-2

Kowalik, M. K., K. Dobrzyn, J. Mlynarczuk, and R. Rekawiecki. 2022. Effect of Steroid Hormones, Prostaglandins (E2 and F2α), Oxytocin, and Tumor Necrosis Factor Alpha on Membrane Progesterone (P4) Receptors Gene Expression in Bovine Myometrial Cells. Animals 12(4):519.

Kulick, L. J., K. Kot, M. C. Wiltbank, and O. J. Ginther. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 52(5):913-921. doi: https://doi.org/10.1016/S0093-691X(99)00182-X

Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, and J. S. Stevenson. 2010a. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle:

A review1. Journal of Animal Science 88(suppl_13):E181-E192. doi: 10.2527/jas.2009-2349

Lamb, G. C., M. F. Smith, G. A. Perry, J. A. Atkins, M. E. Risley, D. C. Busch, and D. J. Patterson. 2010b. Reproductive Endocrinology and Hormonal Control of the Estrous Cycle. The Bovine Practitioner 44(1):18-26. doi: 10.21423/bovine-vol44no1p18-26

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2alpha for ovulation control in postpartum suckled beef cows. J Anim Sci 79(9):2253-2259. doi: 10.2527/2001.7992253x

Larson, R., and R. Randle. 2008. The bovine estrous cycle and synchronization of estrus. Citeseer.

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of Cattle Following PGF2α Injection. Journal of Animal Science 38(5):964-967. doi: 10.2527/jas1974.385964x

López-Gatius, F., M. Llobera-Balcells, R. J. Palacín-Chauri, I. Garcia-Ispierto, and R. H. F. Hunter. 2022. Follicular Size Threshold for Ovulation Reassessed. Insights from Multiple Ovulating Dairy Cows. Animals (Basel) 12(9)doi: 10.3390/ani12091140

Lyimo, Z. C., M. Nielen, W. Ouweltjes, T. A. M. Kruip, and F. J. C. M. v. Eerdenburg. 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. Theriogenology 53(9):1783-1795. doi: https://doi.org/10.1016/S0093-691X(00)00314-9

McTavish, E. J., J. E. Decker, R. D. Schnabel, J. F. Taylor, and D. M. Hillis. 2013. New World cattle show ancestry from multiple independent domestication events. Proceedings of the National Academy of Sciences 110(15):E1398-E1406. doi: doi:10.1073/pnas.1303367110

Mercadante, V. R. G., L. E. Kozicki, F. M. Ciriaco, D. D. Henry, C. R. Dahlen, M. R. Crosswhite, J. E. Larson, B. E. Voelz, D. J. Patterson, G. A. Perry, R. N. Funston, T. L. Steckler, S. L. Hill, J. S. Stevenson, and G. C. Lamb. 2015. Effects of administration of prostaglandin F2α at initiation of the seven-day CO-Synch+controlled internal drug release ovulation synchronization protocol for suckled beef cows and replacement beef heifers1. Journal of Animal Science 93(11):5204-5213. doi: 10.2527/jas.2015-8967

Moore, S. G., and J. F. Hasler. 2017. A 100-Year Review: Reproductive technologies in dairy science. Journal of Dairy Science 100(12):10314-10331. doi: https://doi.org/10.3168/jds.2017-13138

Murphy, M. G., W. J. Enright, M. A. Crowe, K. McConnell, L. J. Spicer, M. P. Boland, and J. F. Roche. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. J Reprod Fertil 92(2):333-338. doi: 10.1530/jrf.0.0920333

Nigussie, T. 2018. A Review on the Role of Energy Balance on Reproduction of Dairy Cow. Journal of Dairy Research and Technology 1:1-9. doi: 10.24966/DRT-9315/100003

Okuda, K., Y. Miyamoto, and D. J. Skarzynski. 2002. Regulation of endometrial prostaglandin F2α synthesis during luteolysis and early pregnancy in cattle. Domestic Animal Endocrinology 23(1):255-264. doi: https://doi.org/10.1016/S0739-7240(02)00161-3

Perry, G. 2004. The bovine estrous cycle.

Prevatt, C., G. C. Lamb, C. Dahlen, V. R. Mercadante, and K. Waters. 2018. What is the economic impact of infertility in beef cattle? AN208, Rev. 9/2018. EDIS 2018(4)

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44(7):915-923. doi: 10.1016/0093-691x(95)00279-h

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional Anestrus in Beef Cows: Body Weight Change, Body Condition, Luteinizing Hormone in Serum and Ovarian Activity. Journal of Animal Science 67(6):1520-1526. doi: 10.2527/jas1989.6761520x

Richardson, B. N., S. L. Hill, J. S. Stevenson, G. D. Djira, and G. A. Perry. 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Animal Reproduction Science 166:133-140. doi: https://doi.org/10.1016/j.anireprosci.2016.01.013

Roche, J. F., M. A. Crowe, and M. P. Boland. 1992. Postpartum anoestrus in dairy and beef cows. Animal Reproduction Science 28(1):371-378. doi: https://doi.org/10.1016/0378-4320(92)90123-U

Rodgers, J. C., S. L. Bird, J. E. Larson, N. Dilorenzo, C. R. Dahlen, A. DiCostanzo, and G. C. Lamb. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows1. Journal of Animal Science 90(11):4055-4062. doi: 10.2527/jas.2011-4836

Saha, S., M. Alam, M. Shamsuddin, and M. Khatun. 2015. Effects of breed, management system, milk yield and body weight on onset of postpartum ovarian cyclicity in cows. Bangladesh Veterinarian 32(1):27-34.

Sartori, R., P. M. Fricke, J. C. Ferreira, O. J. Ginther, and M. C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol Reprod 65(5):1403-1409. doi: 10.1095/biolreprod65.5.1403

Scaramuzzi, R. J., J. F. Murray, J. A. Downing, and B. K. Campbell. 1999. The effects of exogenous growth hormone on follicular steroid secretion and ovulation rate in sheep. Domest Anim Endocrinol 17(2-3):269-277. doi: 10.1016/s0739-7240(99)00043-0

Schams, D., and B. Berisha. 2004. Regulation of Corpus Luteum Function in Cattle – an Overview. Reproduction in Domestic Animals 39(4):241-251. doi: https://doi.org/10.1111/j.1439-0531.2004.00509.x

Schillo, K. K., J. B. Hall, and S. M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. Journal of Animal Science 70(12):3994-4005. doi: 10.2527/1992.70123994x

Sejrsen, K. 1994. Relationships between nutrition, puberty and mammary development in cattle. Proceedings of the Nutrition Society 53(1):103-111. doi: 10.1079/PNS19940014

Senger, P. L. 2012. Pathways to pregnancy and parturition. 3rd ed. Current Conceptions Pullman, WA, Pullman, WA.

Service, N. A. S. 2023. July 1 Cattle Inventory Down 3 Percent. In: U. S. D. o. Agriculture (ed.). United States Department of Agriculture.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle2. Journal of Animal Science 68(3):799-816. doi: 10.2527/1990.683799x

Silva, L. G. T., R. F. Cooke, K. M. Schubach, A. P. Brandão, R. S. Marques, T. F. Schumaher, P. Moriel, and D. W. Bohnert. 2018. Supplementing a yeast-derived product to enhance productive and health responses of beef steers. Animal 12(8):1576-1583. doi: https://doi.org/10.1017/S1751731117003585

Spicer, L. J., J. J. Ireland, and J. F. Roche. 1981. Changes in Serum LH, Progesterone, and Specific Binding of 125I-hCG to Luteal Cells During Regression and Development of Bovifle Corpora Lutea1. Biology of Reproduction 25(4):832-841. doi: 10.1095/biolreprod25.4.832

Stagg, K., M. G. Diskin, J. M. Sreenan, and J. F. Roche. 1995. Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. Animal Reproduction Science 38(1):49-61. doi: https://doi.org/10.1016/0378-4320(94)01354-O

Thomas, J. B., Eric. 2021. Body Condition Scoring of Beef Cattle. In: U. o. M. Extension (ed.).

Trimberger, G. W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation (Research Bulletin: Bulletin of the Agricultural Experiment Station of Nebraska No. 153).

United Nations Department of Economic and Social Affairs, P. D. 2022. World Population Prospects 2022: Summary of Results. p 52.

USDA-ERS. 2023. Agriculture and its related industries provide 10.5 percent of U.S. employment. In: U. S. D. o. Agriculture (ed.). United States Government.

USDA. 2014. Dairy 2014, "Health and Management Practices on U.S. Dairy Operations, 2014". In: USDA-APHIS-VS-CEAH-NAHMS. (ed.). USDA, Fort Collins, CO.

USDA. 2020. Beef 2017, "Beef Cow-calf Management Practices in the United States, 2017, report 1.". In: USDA-APHIS-VS-CEAH-NAHMS. (ed.) No. #.782.0520, Fort Collins, CO.

Velazquez, M. A., L. J. Spicer, and D. C. Wathes. 2008. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domestic Animal Endocrinology 35(4):325-342. doi: https://doi.org/10.1016/j.domaniend.2008.07.002

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L. E. Walters. 1988. Carcass Composition in Mature Hereford Cows: Estimation and Effect on Daily Metabolizable Energy Requirement During Winter. Journal of Animal Science 66(3):603-612. doi: 10.2527/jas1988.663603x

Wathes, D. C., M. Fenwick, Z. Cheng, N. Bourne, S. Llewellyn, D. G. Morris, D. Kenny, J. Murphy, and R. Fitzpatrick. 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68:S232-S241. doi: https://doi.org/10.1016/j.theriogenology.2007.04.006

Webb, R., P. C. Garnsworthy, J.-G. Gong, and D. G. Armstrong. 2004. Control of follicular growth: Local interactions and nutritional influences1,2. Journal of Animal Science 82(suppl_13):E63-E74. doi: 10.2527/2004.8213_supplE63x

Webb, R., B. Nicholas, J. Gong, B. Campbell, C. Gutierrez, H. Garverick, and D. Armstrong. 2003. Mechanisms regulating follicular development and selection of the dominant follicle. REPRODUCTION-CAMBRIDGE-SUPPLEMENT-:71-90.

Weber, D., P. Fields, L. Romrell, S. Tumwasorn, B. Ball, M. Drost, and M. Fields. 1987. Functional differences between small and large luteal cells of the late-pregnant vs. nonpregnant cow. Biology of reproduction 37:685-697.

Whitman, R. W., J. N. Wiltbank, E. E. Remmenga, R. E. Taylor, and E. J. Carroll. 1975. Weight change, body condition and beef-cow reproduction.

Wilson, L. L., K. G. MacDonald, H. H. Mayo, and K. J. Drewry. 1965. Development of the Beef Cattle Industry. p 4, Historical Documents of the Purdue Cooperative Extension Service.

Wiltbank, J. N., and L. E. Casida. 1956. Alteration of Ovarian Activity by Hysterectomy. Journal of Animal Science 15(1):134-140. doi: 10.2527/jas1956.151134x

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, A. P. Cunha, J. O. Giordano, P. M. Fricke, G. M. Baez, and M. G. Diskin. 2014. Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal 8:70-81. doi: https://doi.org/10.1017/S1751731114000585