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Observational Study on the Heat Detection, Pregnancy, and Conception Rates of Cooled High-Yielding Holstein Cows

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Simple Summary: In dairy cows, current methods for managing reproduction still need improvement. Future advancements will require new strategies to minimize additional interventions and maintain reproduction at thermo-neutral level in high yielding dairy cows. As a result, the development of new strategies in dairy cows industry poses a significant challenge for improving reproductive performances. Our study suggests that high-yielding dairy cows raised in a temperate continental climate can experience a lower pregnancy rate during the summer season. This effect on the conception rate can last into autumn, even when a standard cooling system is used.

Abstract: In dairy cows, future advancements will require new strategies to minimize additional interventions and maintain reproduction at thermo-neutral level in high yielding dairy cows. Thus, this observational study used data from a high-yielding dairy farm in northeastern Romania over two consecutive years. In both years an automated cooling system was used, in order to maintain the temperature humidity index within the range of 65-75 when environmental conditions rise above this value. The study found that heat detection rates were high in both years and the values are similar ($P > 0.05$). However, the probability of conception/pregnancy was negatively affected by the cow's parity, season, and repeat breeding. As a result, pregnancy and conception rates showed a consistent trend over the two years and the values are similar. According to the observational data from the current study, we observed that current cooling systems are insufficient for maintaining reproduction at a thermo-neutral level for high yielding dairy cows. Thus, conception and pregnancy rates were significantly affected during the summer ($P < 0.05$). Notably, the effects on conception rates extended into autumn ($P < 0.05$) across both years. As expected, multiparous and repeat-breeding dairy cows had the lowest reproductive performance ($P < 0.05$).

Keywords: cooling; high yielding cows; heat stress; reproductive parameters

1. Introduction

Cattle reproduction and management are experiencing significant changes worldwide, particularly in dairy production. Several factors are driving this transformation, including the rapid advancement of technologies designed to enhance dairy cow reproductive management, removing quotas in Europe, and the substantial growth in herd and farm sizes [1]. Additionally, climate change increases the pressure on dairy cattle industry. Recent estimates of annual financial losses from heat stress primarily stem from seasonal infertility, reduced milk quality and yield, and increased veterinary costs [2–4].

The reproductive performance of dairy cattle is negatively affected during hot season due to both direct and indirect impacts on their reproductive systems [2]. Until the early 2000s, dairy genetic

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selection programs in dairy-producing countries primarily focused on increasing milk yield, often neglecting other important dairy traits such as fertility and health [5,6].

Seasonal patterns of estrus detection, time to first service, and conception rates in dairy cows show consistently lower rates during summer compared to winter [7]. Additionally, the effects of heat stress on fertility seem to extend into the autumn [8–10]. The low fertility typical of the warm months (June to September) persists into the autumn (October and November), even though the cows are no longer exposed to heat stress [11]. It is unclear if the reproduction of dairy cows is affected during the summer and autumn seasons on farms in temperate continental climates that use standard cooling systems. Thus, this observational study evaluates how season, parity, and the number of artificial inseminations impact heat detection, conception, and pregnancy rates in cooled, high-yielding dairy cows raised in a temperate continental climate.

2. Materials and Methods

2.1. Ethical Approval

All dairy cows were handled in accordance with the directives of the European Union on the protection of animals used for scientific purposes (Dir 2010/63/EU). The study protocol was approved by the Ethics Committee of Faculty of Veterinary Medicine, University of Life Sciences, 700489 Iasi, Romania. Efforts were made to minimize animal handling and stress.

2.2. Cattle herd Management and Study Design

A Northeastern Romanian Holstein-Friesian dairy herd (n = 2000) served as the subject of this study. The average number of lactating cows in the herd was 780 during the study period, and the average milk output per cow was 14,030 kg/305 days. The number of heifers was roughly 865 in 2022 and 1043 in 2023, with a 14-month waiting period.

The cows were housed in free-stall barns with concrete floors, and straw beds and fed a Total Mixed Ration twice per day with ad libitum water access, according to the level of milk production and cow size. To keep the animals healthy, standard management practices, including a cooling system coupled with a weather station (CMPimpianti, Calvisano (BS) - Italy) for hot conditions, were followed. The coolers turn on automatically when the THI reaches 65, operating at low speeds. The speeds increase as THI values rise, reaching a maximum at 75. Additionally, water sprayers begin operating around this value. The farm milked about 780 cows three times a day at 0400 h, 1200 h, and 1900 h, for a daily average of 46 kg milk/cow/day.

During the study period from January 2022 to December 2023, the cows were grouped after calving based on days in milk (DIM), milk production, and parity. Calving dates, breeding dates, and DIM were obtained from the AfiMilk management software (AfiMilk, Kibbutz Afikim, Israel). The voluntary waiting period was set at 90 days during studied period. After the end of the voluntary waiting period, the cows were artificial inseminated (AI) at natural estrus and the anestrous cows were subjected to timed artificial insemination (TAI) protocol. The TAI protocol consisting in administering the GnRH (dephereline: gonadorelin acetate [6-DPhe]; Gonavet Veyx, Veyx-Pharma GmbH, Schwarzenborn, Germany) in day 0, PGF2α (Cloprostenol, PGF Veyx, Veyx-Pharma GmbH, Schwarzenborn, Germany) in day 7 and 8, GnRH in day 9 and AI after 12 h by the GnRH treatment. From January 2022 to December 2022, approximately all the cows, starting with the first AI, were included in the G0 group, which received the above reproductive program. For the period from January 2023 to December 2023, each cow in the herd, starting with the first AI, was included in the G5 group, and received the same reproductive program as in 2022 but in addition a single dose of GnRH (dephereline: gonadorelin acetate [6-DPhe]; Gonavet Veyx, Veyx-Pharma GmbH, Schwarzenborn, Germany) on day 5 after AI to induce ovulation and aCL formation. About 75% of artificial inseminations occurred during natural estrus in both years, with the remaining 25% occurring during a timed artificial insemination program (TAI).

Estrous cows were identified using the AfiMilk (AfiMilk, Kibbutz Afikim, Israel) daily estrus report, and an experienced veterinarian team examined each one. Signs such as attempting to mount

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other cows, chasing herds mates, restlessness, chin resting, sniffing herd mates' vaginas, bellowing, congestion, relaxation, and mucus discharge from the vulva were considered suggestive of estrus. Additionally, the visualization of a cow standing while being mounted was regarded as a definitive sign of estrus. Afimilk recorded the cows that did not exhibit any signs of estrous, and these cows were added to the anestrous group.

The veterinary team performed the ultrasound examinations and hormone injections. Each animal in the G5 group received one dose of GnRH after each AI, regardless of the number of AIs. A 5–7.5 MHz rectal convex probe (BCF EasyScan, BCF Ultrasound Australasia, Mitcham, Victoria, Australia) was used to scan the uterus and diagnose pregnancy. Pregnancy confirmation was based on visualizing an anechoic fluid-filled uterine horn and the presence of an embryo, along with a corpus luteum (CL) on the same side of the uterus. The first pregnancy diagnosis was performed using transrectal ultrasonography on day 30 after AI and confirmed on day 60. The non-pregnant cows on day 30 after artificial insemination were included in the days open group and underwent the same TAI protocol as anestrus cows.

The following reproductive parameters were evaluated:

- Heat detection rate: percentage of cows inseminated over a 21-day period divided by the number of open cows during those 21 days.

- Conception rate: percentage of pregnant cows divided by the number of cows inseminated during a 21-day period.

- Pregnancy rate: proportion of eligible cows that become pregnant in a 21-day period.

2.3. Statistical Analysis

For statistical analyses, cow parity was classified as either heifers, primiparous or multiparous. The calving season was categorized as spring (March to May), summer (June to August), autumn (September to November), or winter (December to February). Statistical analyses were conducted using the SAS program (ver. 9.4; SAS Institute, USA). The odds ratio (OR) and 95% confidence interval (CI) were calculated during the logistic regression. The results are presented as proportions and OR with their respective 95% CI. A t-test was used to identify significant main effects. A p-value of < 0.05 was considered significant.

3. Results

Throughout the period under consideration, both groups displayed a high heat detection rate, and the values are similar (P > 0.05, Table 1, Table 2). The probability of pregnancy/conception following artificial insemination (AI) was impacted by the cow's parity, season and repeat breeding. In both groups, the likelihood of conception was lower (P < 0.05) during the summer and autumn seasons, and pregnancy rates were negatively impacted (P < 0.05) only by the summer season (Tables 1 and 2). Additionally, in both groups, heifers and primiparous cows exhibited a higher conception rate compared to multiparous cows. In both groups, the 4+ AIs affected the likelihood of conception, and the values are similar (Tables 1 and 2).

Table 1. Odds ratio (OR) of variables included in the logistic regression model of the probability of heat detection/pregnancy/conception in G0 group.

Variable level	Reproductive parameter	OR	95% CI	*p* value
AI season	**Heat detection rate, % (number of cows)**			
Spring	68.5 (417/609)	Reference		
Summer	65.1 (413/634)	0.86	0.679-1.089	>0.05
Autumn	71.8 (587/817)	1.17	0.934-1.477	>0.05
Winter	72.9 (468/642)	1.24	0.970-1.581	>0.05
AI season	**Conception rate, % (number of cows)**			

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Spring	48 (193/402)	Reference		
Summer	36.9 (148/401)	0.63	0.477-0.839	<0.05
Autumn	40.7 (222/545)	0.74	0.574-0.965	<0.05
Winter	43.8 (195/445)	0.84	0.644-1.107	>0.05
Al season	**Pregnancy rate, % (number of cows)**			
Spring	32.5 (193/594)	Reference		
Summer	24.5 (148/604)	0.67	0.523-0.868	<0.05
Autumn	28 (222/792)	0.81	0.642-1.019	>0.05
Winter	31.5 (195/619)	0.95	0.751-1.216	>0.05
Cow parity	**Conception rate, % (number of cows)**			
Heifers	54.7 (473/865)	Reference		
1st lact.	49.6 (373/752)	0.82	0.671-0.992	>0.05
2nd lact.	40.2 (199/495)	0.56	0.445-0.697	<0.05
3+lact.	37.1 (246/664)	0.49	0.404-0.611	<0.05
No. of AI	**Conception rate, % (number of cows)**			
1 AI.	52.4 (724/1381)	Reference		
2 AIs.	46.7(319/684)	0.79	0.660-0.952	>0.05
3 AIs.	46.4 (172/371)	0.78	0.623-0.986	>0.05
4+AIs.	34.7 (118/340)	0.48	0.377-0.617	<0.05

Table 2. Odds ratio (OR) of variables included in the logistic regression model of the probability of heat detection/pregnancy/conception in G5 group.

Variable level	Reproductive parameter	OR	95% Cl	*p* value
Al season	**Heat detection rate, % (number of cows)**			
Spring	67.2 (518/771)	Reference		
Summer	60.8 (513/844)	0.76	0.617-0.928	>0.05
Autumn	71.5 (590/825)	1.23	0.991-1.518	>0.05
Winter	69.5 (533/767)	1.11	0.897-1.379	>0.05
Al season	**Conception rate, % (number of cows)**			
Spring	48.3 (208/431)	Reference		
Summer	30.3 (144/476)	0.47	0.354-0.610	<0.05
Autumn	39.9 (217/544)	0.7	0.543-0.904	<0.05
Winter	44.4 (219/493)	0.86	0.661-1.111	>0.05
Al season	**Pregnancy rate, % (number of cows)**			
Spring	27.6 (208/753)	Reference		
Summer	17.6 (144/820)	0.56	0.438-0.709	<0.05
Autumn	27.5 (217/789)	0.99	0.794-1.242	>0.05
Winter	29.8 (219/736)	1.11	0.886-1.389	>0.05
Cow parity	**Conception rate, % (number of cows)**			
Heifers	58.7 (612/1043)	Reference		
1st lact.	45.8 (207/452)	0.59	0.743-0.476	>0.05
2nd lact.	36.5 (262/717)	0.41	0.333-0.493	<0.05
3+lact.	37.6 (264/703)	0.6	0.348-0.515	<0.05
No. of AIs	**Conception rate, % (number of cows)**			
1 AI.	52.3 (760/1452)	Reference		
2 AIs.	44.1 (311/706)	0.72	0.598-0.858	>0.05
3 AIs.	45.6 (181/397)	0.76	0.610-0.953	>0.05
4+AIs	38.1 (137/360)	0.55	0.442-0.709	<0.05

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4. Discussion

High submission rates of artificial insemination are crucial for achieving a 365-day calving interval in seasonal calving herds, and an effective and practical method to identify each cow in estrus is necessary [1]. Standing to be mounted is the primary behavioral sign that indicates an estrous period, and it is used to determine the optimal time for insemination [12]. However, the difficulty in detecting estrus in dairy cows, visually, during summer is a recognized issue among veterinarians [13]. Thus, one factor that increases the calving-conception interval of dairy cows during the hot season is poor estrus detection [7]. Currently, there are no published studies evaluating the effects of seasons on estrus detection by AFIMILK sensors concerning summer infertility in high-yielding dairy cows raised in climate-controlled environments. Surprisingly, in this study, the estrus detection rates were higher in both years with only minor variations, particularly during the summer. This confirms a high management standard of the herd which is clearly reflected in the high estrus detection rate. According to the activity monitoring system, 95% of the cows that were identified to be in estrus ovulated, whereas only 5% did not ovulate within seven days of the induction of luteolysis. This means that 95% of the cows which the activity monitoring system identified as estrus ovulated [14]. Heat detection rates, or submission rates, differ among herds, with 30% to 70% of cows showing estrous behavior being detected in estrus [1]. In high-yielding Holstein cows, the percentage of cows that exhibit standing heat, where they allow themselves to be mounted by other cows, has decreased. This reduction makes it more challenging to detect estrus [15]. A study conducted by Roelofs et al. [15] (2005) found that only 58% of cows were observed in standing estrus. This finding aligns with the heat detection rate recorded during the summer in this study (65.1% in 2022 and 60.8% in 2023); however, it differs from the higher heat detection rates observed in the other three seasons (more than 67%). One possible explanation for these differences is that, on the studied farm, the cooling system activates the coolers at a Temperature-Humidity Index (THI) of 65. This activation occurs not only during the summer season and this enhances the comfort of the cows in all seasons, leading to an increase in their estrus behaviors.

However, during the summer season, which is the hottest period in Romania, both groups experienced lower pregnancy and conception rates despite the intensive use of standard cooling devices in the shelter. Additionally, the studied dairy farm observed a negative impact on conception rates in the autumn of both years, although pregnancy rates remained unaffected. Possible, the cooling system cannot supply the cows needed taking into account the high milk production (> 14,000 kg milk/305 days) or the mechanism which affect reproduction in this time period is unknown. Intensive cooling, which requires ten cooling periods, is essential to prevent a decline in milk production among high-yielding cows (those producing over 13,000 kg/year) [16]. A study comparing five and eight cooling periods, each lasting 45 minutes, found that eight cooling periods led to better physiological and behavioral responses. These improvements were indicated by lower rectal temperatures, reduced respiratory rates, and increased time spent lying down and ruminating [17]. Furthermore, the eight cooling periods increased dry matter intake (27.0 kg/day compared to 24.7 kg/day) and improved milk production (40.1 kg/day versus 36.6 kg/day). However, in this study, the coolers operated continuously after the THI rose up to 65. Low reproductive performance in the summer and autumn seasons on the studied dairy farm is due to the different thermo-neutral levels of high-yielding dairy cows. While we can reduce heat stress impact on reproduction, complete elimination is not possible. Thus, data from the Israeli dairy herd in 2019 indicate that with efficient cooling management, the conception rate during the summer is 18.5% lower compared to winter (22.5% in summer versus 41.0% in winter), resulting in a winter-to-summer ratio of 0.55 (Israeli Herd Book, 2019). Future studies are essential to assess how heat stress impacts reproduction in cooled high yielding dairy cows, as well as to develop new cooling systems for dairy farms.

The low conception rates during autumn may be due to how prolonged heat stress affects fertilization, rather than the reproductive management practices on the dairy farm. It has been suggested that heat stress during the hot months could have a lasting effect on the antral follicles, which will develop into large dominant follicles, 40–50 days later [18,19]. The effects of heat stress extend beyond

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the hot months, impacting the subsequent cooler months (autumn) and leading to long-term consequences throughout the year [20,21]. It takes a period of two to five estrous cycles (40 to 105 days) for recovery from summer heat stress and for competent oocytes to appear in the following autumn [19,22]. This prolonged impact of heat stress on the oocytes may explain the reduced conception observed in autumn when cows are no longer exposed to environmental thermal stress.

An alternative method to enhance fertility in heat-stressed dairy cows is the use of hormones to stimulate reproduction. In this regard, the study by Schmitt et al. [23] (1996) examined the induction of an accessory corpus luteum (CL) using a GnRH agonist (Buserelin, 8 mg) or hCG (3,000 IU) to increase plasma progesterone levels and improve conception rates in heifers and lactating cows. The study found that both hormones produced similar results in accessory corpus lutem formation and subsequently increasing progesterone concentrations but did not result in improved pregnancy rates in heifers and dairy cows during the summer season. However, several studies have shown that administering GnRH between days 5 and 15 post-AI can increase conception rates during the warm season by approximately 15 percentage points [24,25]. In these studies, the average milk production was around 11000 kg/305 days, which was lower than that of the farm being investigated. This treatment may not improve reproductive rates on high-yield dairy farms, especially those that require an additional cooling system during very hot periods. Various risk factors can lead to these hormonal imbalances and the occurrence of reproductive and breeding management syndrome. Such risk factors include age, parity, body condition, milk yield, environmental conditions, and peri- and postpartum imbalances [26]. However, our previous study [27], demonstrated that administering the GnRH 7-14 days after AI improved reproductive activity in repeat breeder cows. This finding indicates that administering gonadorelin later in the luteal phase may increase the survival of embryos in repeat breeder dairy cows, or infertile cows [27]. According to the results of Doležel et al. [28] (2017), inducing aCL formation through gonadorelin administration was found to be more efficient in cows treated on day 5 following AI, as compared to cows treated on days 6 or 7. Yan et al. [29] (2016) discovered that administering progesterone to the dairy cows between 3 and 7 days after AI yielded benefits, while supplementation given earlier or later did not. Other studies have discovered that treatment with GnRH 10 days after AI is also conducive to pregnancy after AI [30]. In this regard, the farm owner administered the GnRH agonist on day 5 after artificial insemination in 2023, but reproduction did not improve in any season, as the reproductive parameters remained approximately the same compared with previous year in which this strategy was not used. In addition, in current study, the 2023 conception rate in repeat breeder dairy cows which received GnRH in day 5 after artificial insemination was similar with previous year in which this treatment was not used. Similar findings have been observed with exogenous progesterone administered through the CIDR intravaginal delivery device [31].

In this study, the conception rates were influenced by parity and the number of artificial inseminations performed. The results showed that the conception rate was higher in heifers and primiparous cows compared to multiparous cows in both years, with similar values observed. This aligns with the findings of Fodor et al. [32] (2019), which reported a significantly better conception rate in primiparous cows compared to multiparous cows. This difference may be related to the larger uterine size in multiparous cows compared to heifers and primiparous cows as was revealed by the study of Koyama et al., [33] (2023). Although the interval from estrus to ovulation is similar among heifers, primiparous, and multiparous cows, the optimal timing for insemination in multiparous cows is more restricted than in heifers and primiparous cows. Therefore, to improve the conception rate in multiparous cows, it is crucial to identify the optimal time for artificial insemination [33]. There is a general consensus that fertility is higher in heifers, and primiparous cows compared to multiparous cows. The high-yielding dairy cow and their conception rates vary with the number of artificial inseminations. As expected, the cows with 4 and more artificial insemination presented a low conception rate compared with first 3 artificial insemination in both years. Hormonal alterations are significant factors contributing to subfertility in repeat breeder heifers. Key issues include elevated progesterone levels, abnormal follicular dynamics, delayed ovulation, and reduced oocyte quality [34]. The subfertility of repeat breeder cows may be linked to low progesterone (P4) concentrations during the early stages of embryo development or to poor oocyte quality in the late luteal phase [35].

For many years, researchers have sought a "magic bullet" to reduce the harmful effects of heat stress. The tested strategy has yielded varying degrees of success, but no single strategy has successfully restored its reproductive or productive performance to thermo-neutral levels [36].

5. Conclusions

Reproductive responses to heat stress in high-yielding dairy cattle are complex, and it is unlikely that a single solution will solve this issue. Standard cooling systems are inadequate for high-yielding dairy cows in temperate continental climates to maintain reproduction to thermo-neutral level. Thus, the heat detection rate is not affected by the season; however, the pregnancy rate does decline during the summer. This negative impact on conception rates can persist into the autumn for cooled high-yielding dairy cows. Additionally, both parity and repeat breeding adversely affect reproduction in these cows.

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