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The Effects of Evaporative Cooling on Folliculogenesis and Ovarian Hormones during the Estrous Cycle of Dairy Holstein Cows during the Summer Months in Saudi Arabia

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Abstract

Heat stress is identified as a major cause of lower productive and reproductive performance in animal farming. This situation is more common in Saudi Arabia than in other countries because of extremely high ambient temperatures experienced during the summers. The evaporative cooling cow group had significantly increased serum E2 concentrations on days 7 and 14 - 19 of their estrous cycles, and significantly decreased serum P4 concentrations on days 7 - 18 of their estrous cycles. Evaporative cooling plus shade lowered ambient temperatures (41.80 ± 0.74 versus 47.40˚C ± 0.84˚C) have increased the relative humidity (0.33 ± 0.01 versus 0.24 ± 0.01) and decreased the temperature humidity index (80.24 ± 0.60 versus 84.77 ± 0.68) when compared with shade alone. However, cows under shade alone, compared to cows under evaporative cooling with shade, had a larger number of small (10.04 ± 0.54 versus 4.72 ± 0.58), medium (2.80 ± 0.21 versus 1.79 ± 0.17), and large follicles (6.82 ± 0.28 versus 5.66 ± 0.22). These results demonstrated that while evaporative cooling had positive effects on dairy cows in this experiment, changes could have been more profound, if installations of curtains and fans were implemented, which might need further investigation.

Keywords

Evaporative Cooling, Folliculogenesis, Heat Stress, E2 and P4

1. Introduction

Evaporative cooling lowers ambient and body temperature, as a result, improves the production and reproduction of dairy cows. Large and small dairy farms specially in arid and semi-arid areas response to heat stress (HS) by providing shade and evaporative cooling to increase the productivity of their dairy herds.

Average summer temperatures in semi-arid Saudi Arabia can reach 43˚C, and it is not uncommon for temperatures to reach up to 54˚C [1] [2]. The resulting HS affects milk production and conception rate [3] [4], reduced feed intake [5] [6], respiratory rate, heart rate, sweating, blood chemistry and hormone levels [7] and follicular growth during the estrous cycle [4] in cows. During hot weather, HS interferes with the hypothalamo-hypophyseal-ovarian axis [8] that suppresses the release of luteinizing hormone (LH) and results in an earlier pre-ovulatory surge of LH compared with cool seasons [9]. This negative effect of HS on LH release might in turn affect ovulation and the timing between estrus and ovulation [10], which is one of the causes that has been implicated to explain the poor conception rate during the summer months of the year.

The number of follicles and their functions are affected by HS. Heat stress compromises follicular growth [11] and alters gene expression profiles in the granulosa cells of large pre-ovulatory follicles, which can affect follicular growth [12]. Follicles and oocytes have been damaged by HS, and cows needed 2 - 3 estrous cycles to recover from HS effects [13]. The sizes of large follicles were reduced during HS [14]. In addition, HS has been shown to increase the number of medium-sized follicles, which are associated with decreased plasma inhibin and increased follicle-stimulating hormone (FSH). Furthermore, small antral follicles have also been reported to be very sensitive to HS [15]. The increased FSH during HS affects the reproductive efficiency of dairy cows [16]. However, the effects of HS on the number of small follicles remain unclear; some authors have reported a decrease in their number [17], while others have reported an increase [18].

Ovarian secretions of estradiol (E2) and progesterone (P4) in heat-stressed cows are also affected [19]. Follicular E2 was much higher in winter than in summer [20]. Other study stated no differences were observed in follicular E2 concentrations between heat-stress and non heat-stressed cows [12]. Heat-stressed cows with lowered E2 during the second half of their cycle [21] or during their entire estrous cycle [11] exhibited reduced pre-ovulatory surges of E2. Serum concentrations of P4 during HS have been associated with conflicting results. Some authors have reported an increase in P4 [20]; some have reported a decrease [10]; and some have reported no changes at all [19]. *In vitro* studies have indicated that high temperatures suppressed P4 production; luteal cells produced less P4 during incubation at 38˚C [22].

To the best of our knowledge, no studies have been performed to identify the effect of HS on folliculogenesis in Holstein dairy cattle in Saudi Arabia. In addition, most studies that have been performed about HS did not involve cows experiencing extreme HS, as that which occurs in Saudi Arabia during the summer. It would be beneficial to test the effect of HS on folliculogenesis comparing the effect of evaporative cooling on folliculogenesis, E2 and P4.

2. Materials and Methods

Two groups of Holstein dairy cows (n = 6 each) were examined on a dairy farm in the Riyadh region. All of the cows were cycling, at least 45 days after parturition and were milked twice daily. Evaporative cooling was turned on during the day and ran from 8:00 am until 8:00 pm. All of the cows were kept for two weeks in open shade without evaporative cooling before the beginning of the experiment. One group was assigned to shade only (S) and kept in pen 1, and the second group was assigned to evaporative cooling (EC) and kept in pen 2.

All of the cows were provided with water at libitum and fed according to the National Research Council (NRC) protocols for dairy cattle. The experiment period included the hottest months of the year, July and August. The study conducted a rectal ultrasonography of each cow on a daily basis for a full estrous cycle and all follicles were measured, using an LCD Ultrasound Scanner (Animalprofi 2, Draminski S.A., Poland) equipped with a 5 MHz probe (frequency range: 3.5 - 7 MHz), and the data were recorded.

The study has measured the ambient temperature (At) and relative humidity (Rh) inside the pens on a daily basis using a data logger (Pocket Logger, Pace Scientific Inc., Data Loggers, USA). The study further measured the number and size of all of the follicles (height and width), and divided the follicles into three groups according to their diameters: 1) small follicles: <5 mm, 2) medium follicles: 6 - 9 mm, 3) large follicles: >9 mm. Temperature Humidity Index (THI) was used to estimate the environmental severity, which was calculated using the following adapted equation: $THI = Tdb(^\circ C) + 0.36 \times Tdp(^\circ C) + 41.2$ [23]. This formula uses dry bulb temperature (Tdb, ˚C) and Rh. The Rh was divided by 100 to express the percentage in decimals.

Estrous synchronization using intramuscular injections of a 2 ml $PGF_{2\alpha}$ analog, Prosolvin (Virbac, UK), was applied on days 1 and 11. The cows were observed for estrus signs twice a day, one hour in the morning and one hour at sunset. A cow was considered to be in estrus, when it stood still; while being mounted by another cow. One cow was removed from S group for having cystic follicles. Blood samples were collected daily via tail vein into vacutainer tubes (Becton, Dickinson and Company, NJ, USA) for 22 days from the start of estrus. The serum was separated by centrifugation at 2000 g for 30 min at 4˚C, transferred into 1.5-ml Eppendorf tubes and stored at −20˚C until being assayed for E2 and P4 using ELISA kits (Cayman Pharma. s.r.o, Neratovice, Czech Republic). The samples were analyzed in duplicate within the same assay. The study statistically analyzed the data, using the GLM procedure of the Statistical Analysis System (SAS) to determine the effect of evaporative cooling on serum P4, E2 and ovarian folliculogenesis.

3. Results

The study has analyzed 11 lactating dairy cows during July and August, which are the hottest months of the year in central Saudi Arabia. **Figure 1** shows At

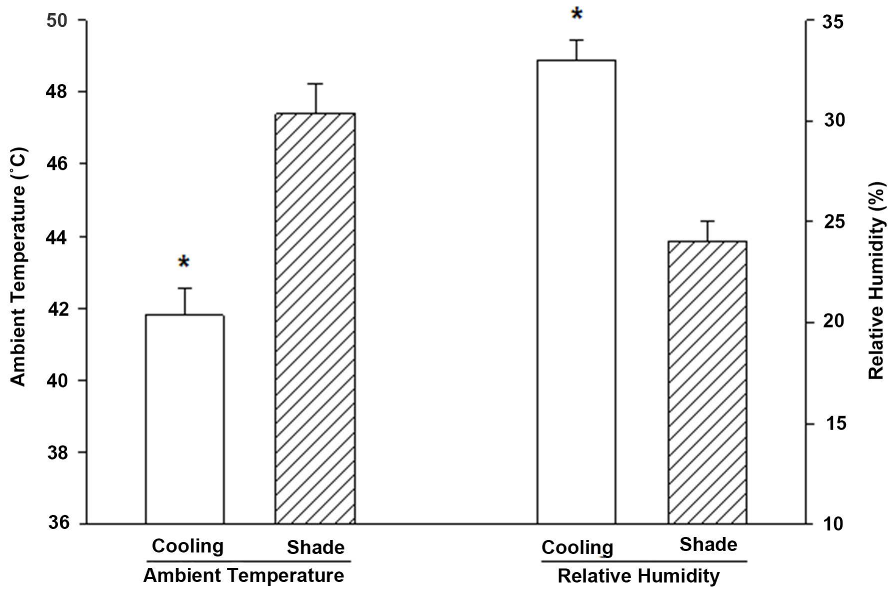

Figure 1. Ambient temperatures and relative humidity in the two pens, evaporative cooling and shade only. Values represent means ± SE. An asterisk (*) denotes a statistically significant difference (P < 0.01) in ambient temperature or relative humidity between the groups.

and Rh during the experiment period under S and EC. There was a significant (P < 0.01) difference between the two pens; the EC pen had lower At (41.8˚C ± 0.7˚C) when compared to the S pen (47.4˚C ± 0.8˚C). At the same time, Rh was significantly (P < 0.01) higher in the EC pen in comparison to the S pen (0.33 ± 0.01 vs. 0.24 ± 0.01). These decreases in At due to EC were demonstrated by the THI; it was lower (P < 0.01) in the EC pen compared with the S pen (80.24 ± 0.60 vs. 84.77 ± 0.68).

The numbers of small (<5 mm diameter) follicles, medium (6 - 9 mm diameter) follicles and large (>10 mm diameter) follicles (**Figure 2**) were significantly (P < 0.01) smaller in the EC group than in the S group (4.72 ± 0.58 vs. 10.04 ± 0.54 for small follicles, 1.79 ± 0.17 vs. 2.80 ± 0.21 for medium follicles and 5.66 ± 0.22 vs. 6.82 ± 0.28). Serum E2 concentrations (**Figure 3**) were significantly lower (P < 0.01) in the S group than the EC group on days 14 - 19 of the estrous cycle. In contrast, serum P4 concentrations (**Figure 4**) were significantly higher (P < 0.05) in the S group than the EC group on days 7 - 18 of the estrous cycle.

4. Discussion

This study has demonstrated that EC lowered At significantly (from 47.4 to 41.8˚C), but not sufficiently. Animals are considered to be under HS when At reaches 24˚C - 27˚C, regardless of the Rh [24]. At the same time, Rh increased from 24% under S to 33% under EC, and the THI decreased from 84.99 to 80.24 because of EC that was implemented in comparison to the shade-only pen. The increase in THI indicates that animals are under more HS and that EC lowers the THI significantly. Estrus and ovulation are interconnected and regulated by

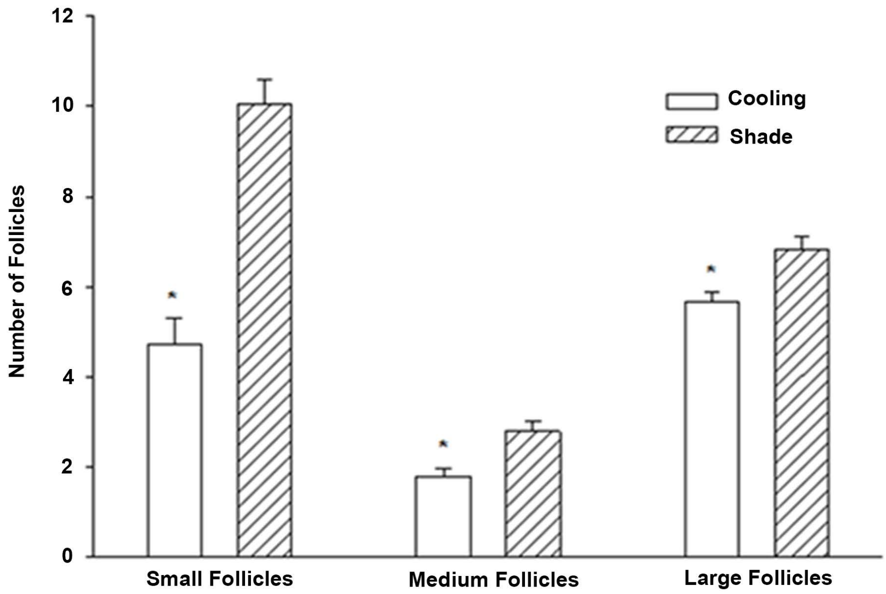

Figure 2. Average number of follicles (small, medium and large) during estrous cycle between the evaporative cooling and shade groups. Values represent means ± SE. An asterisk (*) denotes a statistically significant difference (P < 0.01) in the average number of follicles between the groups.

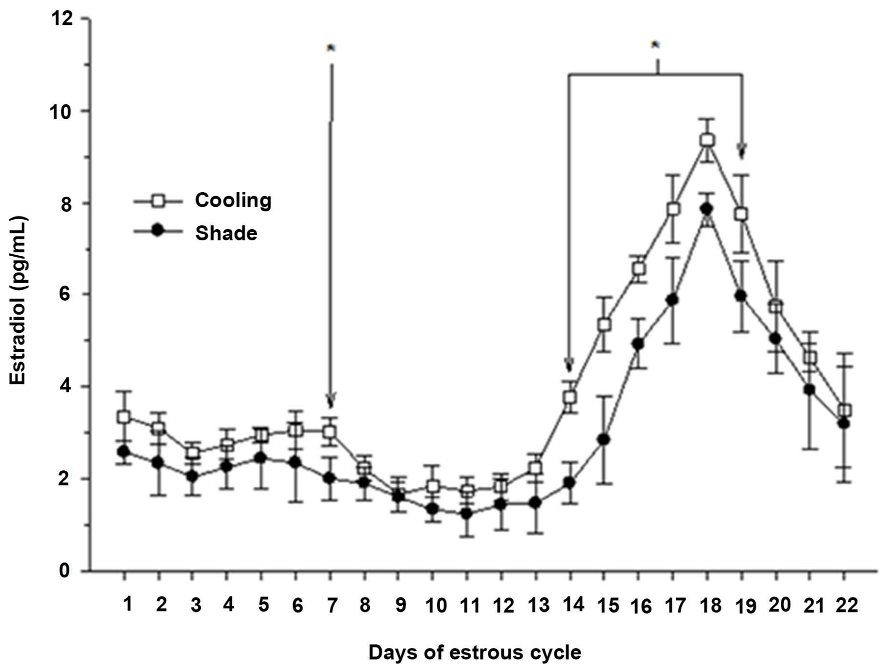

Figure 3. Average serum estradiol concentrations (pg/ml) during the estrous cycle between the evaporative cooling and shade groups. Values represent means ± SE. An asterisk (*) denotes a statistically significant difference (P < 0.01) in estradiol concentrations between the groups.

the hypothalamo-hypophyseal-ovarian axis in which the feedback system accepts hormonal signals from the hypothalamus, pituitary gland and ovaries. In Saudi Arabia, HS during the summer has delayed days to first service, increased

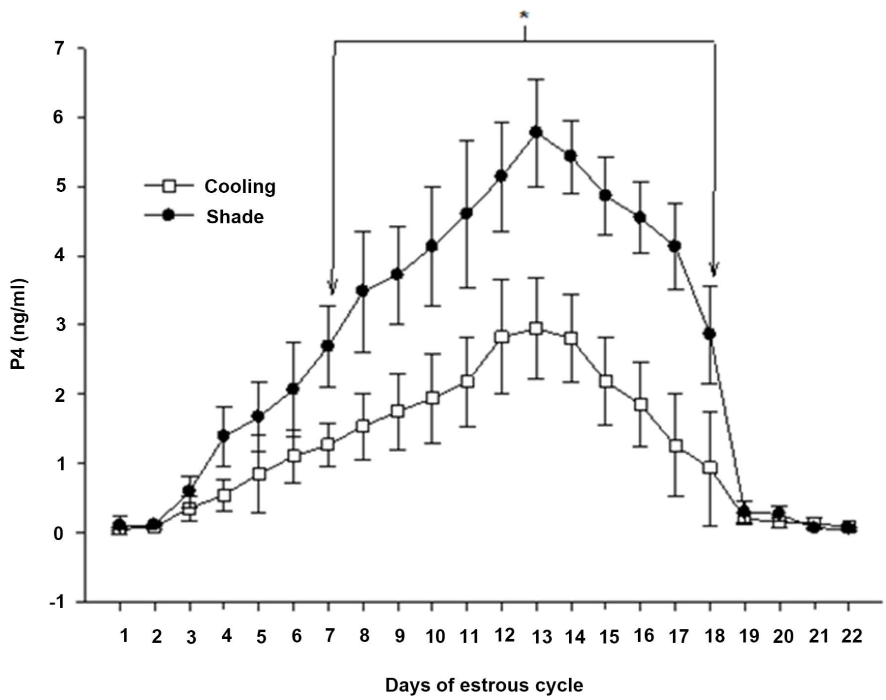

Figure 4. Average serum progesterone concentrations (ng/ml) during the estrous cycle between the evaporative cooling and shade groups. Values represent means ± SE. An asterisk (*) denotes a statistically significant difference ($P < 0.01$) in progesterone concentrations between the groups.

days open, and increased the number of services per pregnancy [25]. Due to the increased At and decreased Rh in the Riyadh region of Saudi Arabia, dairy farms use water sprinklers to decrease the impact of HS on animal productivity. While these results show that EC improved At, Rh and THI, these changes were meant to alleviate the effect of HS rather than remove it completely. Even with the use of EC during hot summers in Saudi Arabia, animals are considered to be under HS.

The development of follicles has been reported to be damaged [26] or altered [27] by HS during folliculogenesis, with a delayed negative effect on ovarian function. Decreased LH pulse and frequency have been reported because of HS [28]. The study has found that the number of small follicles was decreased in the HS group of cows. Small antral follicles have been reported to be very sensitive to HS [17]. There was a carryover effect of HS on small follicular growth and development [4], which led to reduced oocyte competence in Gir cows (an indigenous Indian cattle breed) under HS [29]. In Saudi Arabia, HS during the summer has delayed days to first service, increased days open, and increased the number of services per pregnancy [24]. Due to the increased at and decreased Rh in the Riyadh region of Saudi Arabia, dairy farms use water sprinklers to decrease the impact of HS on animal productivity. While these results show that EC improved At, Rh and THI, these changes were meant to alleviate the effect of HS rather than remove it completely. Even with the use of EC during hot summers in Saudi Arabia, animals are considered to be under HS.

In cycling goats, HS led to more follicles at the recruitment stage becoming atretic during subsequent development stages [30]. As a result, FSH secretion was elevated under HS, likely due to the inhibition of negative feedback from small follicles, which in turn affected the cows' reproductive efficiency [16]. In addition to the reduced number of small follicles, the findings showed that HS negatively affected the number of medium follicles (6 - 9 mm diameter), which is consistent with other studies [15]. The decreased number of medium follicles is an indication that HS depresses follicular dominance in the first and second follicular wave [26]. Mediums follicles are sensitive indicators of follicular dominance since the dominant follicle inhibits the growth of subordinate follicles [31]. It is reported [32] that FSH triggers the emergence of follicular waves as the main factor responsible for follicular turnover, since the number of medium follicles increases when FSH concentrations increase.

The number of large follicles was decreased in the HS group. It was found that the number of large follicles tended to be larger, but not significantly when compared with cooled cows [15]. Other studies [33] have demonstrated an increase of 50% in the number of large follicles (>10 mm) in cows in HS environments. Furthermore, it was reported that HS increased ($P < 0.05$) the number of large follicles in Bos indicus cattle [29]. Other studies have found that the diameter of large ovulatory follicles [27] was greater ($P < 0.05$) in November (18.0 ± 0.8 mm) than in April (14.0 ± 0.8 mm) and that the number of large follicles increased more in summer than in winter. These studies, which have mostly indicated an increased number of large follicles in HS cows, could not explain the underlying cause of such a phenomenon, since serum and follicular fluid E2 concentrations were increased in winter rather than summer, even in the presence of large follicles. While our findings agree with these results, we did not compare the size of large follicles between the EC and HS groups.

Serum P4 concentrations in cows under HS are controversial. Some studies have reported that serum P4 concentrations were decreased [34], did not change [19] or increased [18] when compared with cows not under HS. The study has demonstrated increased serum P4 concentrations in heat-stressed cows. Reports have noted decreased serum P4 concentrations in cows under HS during their estrous cycle; HS compromises follicular development and leads to abnormal oocyte maturation and early embryonic death [35]. However, HS might be related to increases in endometrial secretions of $PGF_{2\alpha}$, which leads to premature luteolysis [36]. The study has shown that P4 concentrations were higher during HS. These results imply that there were other intrinsic and extrinsic factors that interfered with fertility other than the low serum P4 concentrations during the estrous cycle. These factors include the rate of P4 secretion associated with variables such as blood luteal blood flow [37], P4 metabolism in liver, blood volume, hyperthermia, nutrition and stage of lactation [38].

5. Conclusion

Under the conditions of this study, evaporative cooling helped lower ambient temperature and increased relative humidity. At the same time, evaporative cooling resulted in lowered small, medium and large follicle numbers in cows during estrous cycle when compared to cows under shade only. There are conflicting results regarding the effect of heat stress on the number and quality of follicles during estrus. This study, while looked into the average number of follicles during esturs, the number of cows was not large, which is a short coming of this study. In addition, these results demonstrated that while evaporative cooling had positive effects on dairy cows in this experiment, changes could have been more profound, if installations of curtains and fans were implemented, which might need further investigation.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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