Impacts of vitamin C and E injections on ovarian structures and fertility in Holstein cows under heat stress conditions

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Abstract: This study evaluated the effect of injecting vitamin C (VC) and E (VE) on size of the preovulatory follicle, volume of the corpus luteum, and pregnancy rates in Holstein cows under heat stress conditions (temperature humidity index > 74). Sixty-two cows were randomly assigned to one of four treatments: 1, control, n = 15: cows were not supplemented with vitamins; 2, VCG, n = 15: cows were simultaneously injected i.v. with 500 and s.c. with 2500 mg of VC before and after estrus; 3, VEG, n = 15: cows were injected with VC and VE in the same way and doses as in treatments 2 and 3. Treatment did not significantly affect any of the measured variables, despite a numerical increase in pregnancy rates in cows injected with vitamins (7.5 ± 7.3%, 9.6 ± 9.4%, 15.1 ± 10.0%, and 18.34 ± 9.9% for control, VCG, VEG, and VCEG, respectively). In conclusion, injections of vitamin C and E did not affect either the development of the preovulatory follicle and the corpus luteum or pregnancy rates in Holstein dairy cattle under heat stress conditions.

Key words: Corpus luteum, dairy cattle, heat stress, pregnancy rate, vitamin C, vitamin E

1. Introduction
Heat stress induces behavioral, metabolic, and hormonal changes in dairy cattle, resulting in poor reproductive performance (1). To date, there is no treatment that can fully restore the fertility of dairy cattle under heat stress. However, supplementation of antioxidants could be a feasible way to improve fertility in cows under such conditions (2). This seems logical, since blood concentrations of antioxidants, such as vitamins C and E, are diminished by heat stress (3,4).

Vitamin C and E are necessary for normal reproduction in cattle (5,6) and required for follicle and corpus luteum (CL) development (7,8). Low fertility in dairy cattle may be a consequence of smaller preovulatory follicle and CL size compared to animals without heat stress (9–11). We speculated that cows exposed to heat stress conditions and supplemented with vitamin C and E have larger preovulatory follicles and CL, resulting in higher pregnancy rates compared to cows that did not receive vitamin supplementation.

2. Materials and methods
2.1. Animal welfare
All technical and management procedures were performed based on the guidelines set by the Canadian Council on Animal Care (12).

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2.2. Location
The experiment was conducted at the dairy farm “18 de Julio” of the Universidad Autónoma Chapingo. The farm is near Tlahualilo, Durango, México, located at 25°54’N and 103°35’W, 1137 m above sea level. The climate of the region is semiarid, with a mean annual temperature of 21.1 °C and 239 mm of rainfall per year (13). The experiment was conducted during the third week of August and the first week of September 2014.

2.3. Animals, treatments, and experimental design
Multiparous Holstein dairy cows (n = 62) with an average of 188.75 ± 15.90 days in milk and producing 37.50 ± 1.13 liters of milk per day, were randomly assigned to one of four treatments: 1, control, n = 15: cows were not injected with vitamins; 2, VCG, n = 15: cows received a total dose of 3000 mg of vitamin C (ascorbic acid, Q.P., Reasol; 500 mg via i.v. and 2500 mg via s.c.) at night on day –5 (day 0 was the day of progesterone release device (CIDR) removal), immediately after estrus detection and 2 days after artificial insemination; 3, VEG, n = 15: cows received a single i.m. injection of 3000 IU of vitamin E ((±)α-tocopherol, Sigma-Aldrich) at night on day –5; 4, VCEG, n = 17: cows were injected with both vitamins on the same days and doses
IU of vitamin E (56.5 kg day⁻¹ cow⁻¹) of a total mixed
The animals received a diet formulated to provide 1600
2.5. Nutrition and feeding
The follicular wave of the cows was synchronized with
a CIDR containing 1.9 g of progesterone (CIDR 1900
CATTLE INSERT, Zoetis), inserted intravaginally for 8
days, and an i.m. injection of 250 µg of GnRH analogue
(GnRH, Sanfer). Estrus behavior was induced by an i.m.
injection of 500 µg of cloprostenol (Celosil, MSD Animal
Health) at CIDR removal. Once the CIDR was withdrawn,
the animals were constantly monitored by direct
observation for signs of standing estrus. The cows were
artificially inseminated 12 h after standing estrus with a
single dose (approximately 2 × 10⁶ spermatozoa) of semen
from a single bull of proven fertility. To induce ovulation,
an injection of 250 µg of GnRH analogue was given to each
cow immediately after AI.

2.4. Estrus synchronization and breeding
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cow immediately after AI.

2.5. Nutrition and feeding
The animals received a diet formulated to provide 1600
IU of vitamin E (56.5 kg day⁻¹ cow⁻¹) of a total mixed
ratio: alfalfa, 15.0; corn silage, 26.5; steam corn, 6.0; and
concentrate, 9.0 kg as fed. The food composition of the
concentrate mixture was as follows: cottonseed, 1.099;
walnut, 0.895; soybean, 2.049; wheat bran, 1.911; molasses,
1.431; soy plus (cooker-expeller soybean, Soyplus), 1.135;
magnesium oxide, 0.0162, sodium bicarbonate, 0.135;
lactomil (bypass fat, Lactomil), 0.054; calcium carbonate,
0.189, microminerals, 0.030; vitamins, 0.030; Ganadero
plus (Saccharomyces cerevisiae, Biotecap), 0.019; and
maxifolipol (Flavosofolipol, Pisa), 0.002 kg as fed).

2.6. Response variables
To assess the effects of vitamin C and E injections on
follicle and CL development and, subsequently, on the
fertility of Holstein dairy cattle, the following variables
were evaluated: diameter of the largest follicle at CIDR
removal (DFP0, mm) and at estrus detection (DFP1, mm),
time of estrus after CIDR removal (h), growth rate of the
largest follicle (mm day⁻¹), volume of the CL (cm³), and
pregnancy rate (%). Temperature and relative humidity in
the stable were recorded each day from day –4 to 4 days
after AI. Daily temperature humidity index (THI) values
were calculated using the equation given by Mader et
al. (14): THI = 0.8 × ambient temperature + [(% relative
humidity ÷ 100) × (ambient temperature − 14.4)] + 46.4.

The cows were considered to be exposed to heat
stress when THI values exceeded 74. In addition, body
temperature was recorded in the morning and afternoon
for the same period of time as in THI. The parameters
DFP0, DFP1, and volume of CL were measured by real-
time ultrasonography (Medison SonoVet 2000, 7.5 MHz,
linear-array transducer; Universal Medical Systems Inc.,
Bedford Hills, NY, USA). The diameter of the largest
follicle was calculated by the average of horizontal and
vertical measurements, while the volume of CL was
calculated directly via ultrasound. The location of the
largest follicle at CIDR removal was recorded and its
diameter was measured once again at estrus detection.
The growth rate of the largest follicle was calculated taking
into consideration the difference in size between DFP0
and DFP1 and the time from CIDR removal to estrus
detection. The pregnancy test was performed 33 days after
AI by ultrasonography.

2.7. Statistical analysis
Of the total initial 62 cows, only 52 showed estrus and
were therefore considered in the analysis. The number of
cows that completed the study for each of the treatments
was control = 13, VCG =10, VCE = 12, and VCEG = 17. All
measured variables were subjected to analysis of variance
under the following mixed linear model:

Yijk = μ + Ti + Cj + b1(Xijk – X̄jk) + eijk

where μ is the overall mean, T is the effect of the
i th treatment (i = control, VCG, VEG, VCEG); Cj is the
random effect of the jth cow (j = 1, ..., 52) ~ NI (0, σc²), b1 is
the respective coefficient of linear effect of milk production
level (Xj); < 35, 35–40, and >40 L day⁻¹ for low, medium,
and high level, respectively, and eijk is the residual ~ NI (0,
σe²).

Covariates that did not show significant effects (P >
0.05) were deleted from the final model. Means for the main
effect were calculated by least squares. The variables were
analyzed using the Glimmix procedure of the statistical
package SAS (Statistical Analysis System, version 9.3). In
the case of variables considered with normal distribution,
such as DFP0, DFP1, time to estrus after CIDR removal,
growth rate of the largest follicle, and CL volume, the
function link used was identity. The variable pregnancy
rate was analyzed considering a binary distribution and
using logit as the function link. In all cases, P ≤ 0.05
was considered significant. Means of all variables were
compared using Tukey’s test.

3. Results
The study evaluated the effect of vitamin C and E
injections on ovarian follicle structure development
during synchronized estrus as well as pregnancy rates in
Holstein cows under heat stress conditions. Table 1 shows
calculated THI and measured body temperature values. In
general, the parameters DFP0, DFP1, time of estrus after
CIDR removal, growth rate of the largest follicle, and CL
volume were 14.6 ± 0.57 mm, 17.8 ± 0.54 mm, 52.2 ± 2.59
h, 1.8 ± 0.24 mm day⁻¹, and 9.3 ± 0.79 cm³, respectively.
Overall pregnancy rate was 12.8 ± 0.04%. The effects of the
different treatments on each of these variables are shown
in Table 2 and the Figure.
The experimental units (cows) behaved similarly in terms of the evaluated variables, regardless of the experimental group. Although cows injected with both vitamins showed estrus 10 h earlier and 10% higher pregnancy rates compared to animals in the control group, these differences were not statistically significant (P > 0.05).

4. Discussion
Heat stress, as a result of high THI values, causes an increase in normal body temperature. The body temperatures of the cows used in the present study were similar to those reported by Srikandakumar and Johnson (15), who studied cows under heat stress conditions. We therefore assume that the animals in our study were subject to heat stress.

The mechanism by which heat stress diminishes fertility in dairy cattle is not fully understood, but abnormal follicle and CL development, low quality oocytes, and high embryo mortality could be considered the leading factors. To the best of our knowledge, there is no information regarding the effects of supplementing vitamin C and E on preovulatory follicle development in cows under heat stress. However, early onset of estrus in cows receiving vitamins has also been reported in ewes supplemented with vitamin C and E.
vitamin E (16), indicating that vitamin supplementation positively affects some endocrine processes controlling its onset.

The low fertility observed in Holstein dairy cattle under heat stress may be a consequence of ovaries carrying a preovulatory follicle with reduced oxidative status (17). However, this scenario could be reversed by vitamin C and E. On one hand, vitamin C is necessary for normal follicle development (18), and supplementation of this vitamin increases the follicle diameter in a dose-dependent manner (19). However, in this study, vitamin C supplementation did not significantly affect the diameter of the preovulatory follicle. A likely explanation for this may be the small sample size; in addition, the dose of supplemented vitamin C was probably not sufficient to alter the preovulatory follicle diameter.

On the other hand, the effect of vitamin E on fertility is mediated by a direct antioxidant effect on the follicle (20). This is noteworthy, since heat stress impairs fertility by follicle and oocyte disruption (17), probably through inducing oxidative damage (21). Since vitamin C is necessary for reactivating vitamin E functionality (22), these vitamins may act together or separately to improve follicle functionality and oocyte quality.

The suppressed luteal function (low progesterone) caused by heat stress may be responsible for the low fertility in dairy cattle (9). In order to improve fertility in dairy cattle under heat stress, an increase in progesterone production by increasing CL size seems logical. Previous studies have shown a positive correlation between vitamin C supplementation and CL diameter, progesterone concentration (23), and pregnancy rate (24). With regard to vitamin E, there is little information about its effect on CL functionality, but Vierk et al. (25) demonstrated that vitamin E supplementation protects the CL from apoptosis. In the present study, the supplementation of vitamin C and E did not improve the volume of the CL, but it is possible that progesterone production could be affected. However, since we did not measure progesterone concentrations, we cannot confirm such an asseveration.

To the best of our knowledge, there is no previous evidence of the impacts of vitamin C or E supplementation on dairy cattle under heat stress conditions. However, in chickens (26), rabbits (27), and rats (20) under heat stress, supplementation of these vitamins improved reproductive performance.

The results show that vitamin C and E supplementation did not significantly improve pregnancy rates in cows suffering heat stress. The reason for this could be the small sample size used in this study. According to McIntosh (28), several injections of vitamin C are required to improve fertility in cattle. On the other hand, blood concentrations of vitamin E are increased for up to 7 days after parenteral injection (29), and we consider that this period of time is sufficient to affect fertility. The reason for supplementing 3000 IU of vitamin E originates from previous field experience. On the other hand, the dose of vitamin C was chosen based on previous research (30).

The first injections of vitamin C and E were carried out with the objective of protecting both follicle development and oocyte quality. The decision to inject vitamin C at estrus detection was based on results from past research that reported an increase in vitamin C concentrations at this time (30). The third injection of vitamin C was performed
2 days after AI and intended to protect the embryo from heat stress as well as to support CL development.

In conclusion, the supplementation of vitamin C and E before and after synchronized estrus under heat stress conditions did not affect preovulatory follicle or corpus luteum development or pregnancy rates in Holstein cows. However, the reliability of these results is limited by the small sample size. Further studies evaluating the effects of vitamin C and E supplementation on reproductive performance in cows under heat stress conditions should therefore include larger numbers of animals to obtain reliable results.

References


