Exposure of dairy cows to high environmental temperatures and their lactation status impairs establishment of the ovarian reserve in their offspring

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ABSTRACT

The objectives of this study were to establish if exposure of pregnant dairy cows to high environmental temperatures and humidity during the first trimester of pregnancy impairs the establishment of the ovarian reserve (total number of healthy follicles and oocytes in ovaries) and fertility in their offspring. Serum anti-Müllerian hormone (AMH) concentrations and number of follicles ≥3 mm (antral follicle count; AFC) were assessed on a random day of the estrous cycle in 310 sixteen-month-old dairy heifers. Based on season of their conception and early fetal life, heifers were separated into 2 groups: summer (mean monthly temperature-humidity index = 69.33 ± 2.6) and winter (temperature-humidity index = 54.91 ± 1.08). The AMH and AFC were lower in summer (419.27 ± 22.81 pg/mL and 9.32 ± 0.42 follicles, respectively) compared with winter heifers (634.91 ± 47.60 pg/mL and 11.84 ± 0.46 follicles, respectively) and were not influenced by farm and age at sampling. Heifers born to dams that were not being milked during gestation had lower AMH and AFC compared with offspring of cows on their first lactation, whereas no difference was detected between offspring of cows on their first and subsequent lactations. Summer and winter heifers had similar age at first service and at first calving, and similar number of services per conception. Regardless of season in early fetal life, heifers were classified into 3 groups based on AMH and AFC (low = 20%, intermediate = 60%, high = 20%). Heifers with the lowest AMH were older at first service compared with herd mates with intermediate AMH, but age at first calving and number of services per conception were similar among AMH categories. No difference was detected in any of the fertility measures among AFC categories. Heifers born to mothers exposed to high environmental temperatures in early gestation had smaller ovarian reserves compared with herd mates conceived in winter, but no association between season of early fetal life and fertility at first conception was established. Season of conception and maternal lactation status affect the size of the ovarian reserve, but not fertility, at first conception in the progeny.

Key words: anti-Müllerian hormone, antral follicle count, fetal programming, dairy cow

INTRODUCTION

The ovarian reserve is the total number of healthy oocytes and follicles in the ovary of a female mammal, which declines during her reproductive life span and is never replenished (Evans et al., 2010; Ireland et al., 2011). In cattle, gonadal development occurs during fetal life, and primordial follicles form between d 70 to 100 of gestation and initiate their irreversible growth by d 90 to 140 of pregnancy (Rüssel, 1983; Yang and Fortune, 2008; Burkhart et al., 2010; Fortune et al., 2013). Also, the peak in number of germ cells is reached within the first 3 mo of gestation (Erickson, 1966), thus the first trimester of fetal life is a critical window for the establishment of the ovarian reserve in cattle. Understanding the regulation of follicular formation is relevant because the variation in the size of the ovarian reserve may be among the main factors that contribute to the high variation in fertility among young, adult female cattle (Evans et al., 2010; Ireland et al., 2011; Mossa et al., 2017). Two markers have been identified to reliably estimate the size of the ovarian reserve in cattle: antral follicle count (AFC) and circulating concentrations...
of anti-Müllerian hormone (AMH). The AFC is the total number of ovarian antral follicles equal to or larger than 3 mm in diameter on both ovaries and is determined by ultrasonography; AMH is a glycoprotein exclusively produced by healthy, growing ovarian follicles (Monniaux et al., 2008). Both AFC and AMH are positively associated with the size of the ovarian reserve (Burns et al., 2005; Ireland et al., 2008; Rico et al., 2009; Batista et al., 2014; Ribeiro et al., 2014) and are highly repeatable within the same animal during the same or multiple estrous cycles (Burns et al., 2005; Rico et al., 2009; Ireland et al., 2011; Monniaux et al., 2012; Mossa et al., 2012; Pfeiffer et al., 2014; Souza et al., 2015). However, they are highly variable among individuals (Baldrighi et al., 2014; Ribeiro et al., 2014). Studies conducted using either AFC, AMH, or both indicate that the ovarian reserve is positively associated with several measures of fertility (Jimenez-Krassel et al., 2009; Mossa et al., 2012; Ribeiro et al., 2014; Martinez et al., 2016; McNeel et al., 2017) and with the response to ovarian hormonal stimulation (Singh et al., 2004; Ireland et al., 2007; Rico et al., 2009; Hirayama et al., 2012; Silva-Santos et al., 2014; Souza et al., 2015; Santos et al., 2016; Mossa and Ireland, 2019).

Nevertheless, what determines the size of the ovarian reserve in cattle is unclear. While recent studies report that AFC and AMH are moderately heritable traits in dairy cattle (Walsh et al., 2014; Nawaz et al., 2018; Gobikrushanth et al., 2019), evidence also indicates that the ovarian reserve might be influenced by the environment encountered during fetal life (Sloboda et al., 2011; Evans et al., 2012; Mossa et al., 2015; Mossa et al., 2019). Growing evidence indicates that a ruminant’s maternal nutritional status can influence the development of the reproductive system in female (Borwick et al., 1997; Rae et al., 2001; Sullivan et al., 2009a,b; Mossa et al., 2013; Weller et al., 2016; Smith et al., 2019) and male offspring (Alejandro et al., 2002; Kotsampasi et al., 2009; Mossa et al., 2018). Other environmental conditions may influence the establishment of the ovarian follicular population; for instance, cows with a chronic mammary gland infection during gestation gave birth to heifers with reduced AMH concentrations (Ireland et al., 2011).

Maternal exposure to high environmental temperatures and humidity may be among the factors that have an effect on the establishment of the ovarian reserve, and evidence indicates that heat stress affects dairy cows in several parts of Italy. Negative effects on productive performances (Bernabucci et al., 2014), metabolic and hormonal acclimation (Bernabucci et al., 2010), nonreturn rate (Biffani et al., 2016), and mortality (Vitali et al., 2009) in cattle have been reported in Italy. Significant increases in rectal temperature and respiratory rates, as well as reductions in DMI have been documented in Italian Holsteins exposed to high air temperatures (Nardone et al., 1997; Bernabucci et al., 1999). Moreover, high humidity and temperatures have been recorded in Sardinia, the region where we conducted this study (Atzori and Cannas, 2011). In dairy cows, heat stress during gestation adversely affects birth weight, immunocompetence, and growth of the calves (Tao and Dahl, 2013; Tao et al., 2014; Monteiro et al., 2016). Furthermore, heifers born to cows exposed to heat stress during late gestation require more services per conception (Monteiro et al., 2016). Recent evidence suggests that cows exposed to heat stress during the second and third trimester of gestation produce female offspring with lower AMH concentrations (Akbarinejad et al., 2017).

Based on the evidence that identifies the first trimester of gestation as crucial for follicular formation (Erickson, 1966; Rüsse, 1983; Tanaka et al., 2001; Burkhart et al., 2010), we tested the hypothesis that high environmental temperatures during the first trimester of fetal life are negatively associated with the establishment of the ovarian reserve and fertility in Holstein Friesian female cattle.

**MATERIALS AND METHODS**

All animal experiments were performed in accordance with DPR 27/1/1992 (Animal Protection Regulations of Italy) in conformity with European Community regulation 86/609, and were approved by the local Committee for the Animal Welfare of the University of Sassari.

**Animals and Assessment of AFC and Peripheral AMH Concentrations**

This study was conducted on 4 commercial dairy farms located in Sardinia, Italy (farm 1: 39°44′ N, 8°34′ E; farm 2: 39°44′ N, 8°31′ E; farm 3: 40°35′ N, 8°53′ E; farm 4: 39°44′ N, 8°34′ E). The herds ranged in size from 90 to 480 cows, with an average annual milk yield of 8,800 to 9,900 kg/cow. All animals (both cows and heifers) were housed in freestall barns with roofs and open side walls. No cooling systems operated in the farms during the study. Holstein Friesian heifers (n = 310) born between February 2016 and May 2017 were studied from August to September 2017, November 2017 to April 2018, and June to September 2018 (16.1 ± 1.32 mo of age; mean ± SD). To estimate the size of the ovarian reserve, peripheral AMH concentrations (n = 310) and AFC (n = 258) were determined. The ani-
mals subjected to AFC evaluation were less than those submitted to AMH dosage due to a technical problem that occurred with the transrectal ovarian ultrasonography. On a random day of the estrous cycle, a single blood sample (10 mL) was collected from the coccygeal vein using an additive-free tube. Samples were refrigerated (4°C) for 24 h and centrifuged at 2,000 × g at 4°C; serum was removed and stored at −20°C until assayed. Serum AMH concentrations were measured with an AMH (bovine) ELISA commercial kit (Ansh Labs, Webster, TX) with an analytical sensitivity of 11 pg/mL. All assays were performed in duplicates, and mean values were calculated. If a difference greater than 10% was detected between duplicates of the same samples, samples were re-analyzed. The intra- and interassay coefficients of variation were 3.8 and 9.5%, respectively.

On the same day of blood sampling, AFC was assessed (n = 258) by transrectal ovarian ultrasonography (MyLabOne, Esaote, Genova, Italy) performed by the same operator. Each ultrasonography was recorded, and the number of antral follicles ≥3 mm in diameter was counted (Burns et al., 2005; Ireland et al., 2008; Mossa et al., 2010a; Mossa et al., 2012) on farm and on video.

Reproductive Management and Measures

Heifers were inseminated either during the same estrous cycle in which AMH concentrations and AFC were assessed or in a subsequent cycle. Heifers were artificially inseminated by the same technician following detection of estrus. Animals detected in estrus before morning milking (0630 h) were inseminated that afternoon, whereas heifers detected later in the day were inseminated the following morning. Pregnancy diagnosis was performed using ultrasonographic examinations of the reproductive tracts at approximately 25 to 36 and 60 to 66 d post-AI. The following reproductive variables were calculated: age at first insemination, age at first conception, age at first calving, and number of services per conception.

Climate During the First Trimester of Fetal Life

Dates of conception of each heifer were retrieved from farm records. Based on the season from conception to the end of the first trimester of their fetal life, heifers were placed into 2 groups: heifers born to mothers that conceived and spent the first trimester of pregnancy during May through August of 2015 and 2016 (summer group) or November 2015 through March 2016 (winter group). To test whether exposure of dams to high environmental temperatures during early gestation resulted in diminished AFC and AMH and impaired fertility in their progeny (as young adult heifers), climatic conditions during the months of interest were determined using data collected from 2 weather stations of the Sardinian Regional Agency for the Environment Protection (Agenzia Regionale per la Protezione dell’Ambiente della Sardegna, ARPAS) for the years 2015 to 2016. The first climatic station was located within 10 km of farms 1, 2 and 4; the second climatic station was approximately 54 km away from farm 3. Daily minimum and maximum air temperature (Ta; expressed as °C) and relative humidity (RH; expressed as percentage) were used to calculate the mean monthly temperature-humidity index (THI; °C) using the following formula (Johnson et al., 1964):

\[
\text{THI} = [1.8 \times \text{Ta} - (1 - \text{RH/100}) \times (\text{Ta} - 14.3)] + 32.
\]

A THI ≥68 was considered an indicator of high environmental temperatures and potential heat stress (Zimbelman et al., 2009; De Rensis et al., 2015). The experimental protocol is summarized in Figure 1.

Statistical Analysis

All data were analyzed with MiniTab and SAS University Edition version 3.6 (SAS Institute Inc., Cary, NC). The normal distribution of data was investigated by Kolmogorov-Smirnov normality test with MiniTab. As most of the data were not normally distributed, data were log-transformed, but natural numbers are reported in the text. Relations among variables were analyzed with Pearson Correlation with SAS.

To investigate the effect of the environmental conditions during gestation on the establishment of the ovarian reserve, serum concentrations of AMH, AFC, number of services per conception, age at first service, and age at first conception in the heifers were analyzed with a mixed model (Proc MIXED procedure of SAS) considering the main effects of season during the first trimester of fetal life (summer vs. winter), age at sampling and ultrasonography, maternal lactation status (nonlactating heifers vs. lactating cows), lactation number of the dam (0, 1, or ≥2), and their interaction. The farm (1, 2, 3 or 4) was included as a random factor. Tukey test was used for comparisons in all the models. To test the hypothesis that cattle with a high ovarian follicular population are more fertile than herd mates with a smaller ovarian reserve, heifers were ranked into 3 groups (low = 20%, intermediate = 60%, high = 20%) based on AMH concentrations and AFC, respectively.
Differences among groups in age at first service, number of services per conception, and age at first conception were analyzed with ANOVA. Differences with $P \leq 0.05$ were considered significant. All results are expressed as mean ± standard error.

**RESULTS**

**The AFC and Peripheral AMH Concentrations in Young Adult Heifers**

The mean circulating AMH concentrations per heifer (±SEM) were $512.48 \pm 25.01$ pg/mL, ranging from 16.83 to 3909.44 pg/mL per animal ($n = 310$). The mean AFC was $10.61 \pm 0.32$, and individual AFC ranged from 3 to 36 ($n = 258$). Distributions of heifers by AMH concentrations (panel A) and AFC (panel B) are presented in Figure 2. Serum AMH concentrations and AFC were highly positively correlated ($R = 0.70$; $P < 0.0001$; Figure 3).

**The THI During Summer and Winter**

The mean monthly THI during the months of the study are reported in Figure 4. During the first trimester of fetal life of heifers in the summer group, the average monthly THI was $69.78 \pm 2.58$ from May to August 2015 and $68.89 \pm 2.63$ from May to August 2016, respectively. During the season of conception of the winter group, the mean monthly THI was $54.91 \pm 1.08$.

**Influence of Season on AFC and Peripheral AMH Concentrations in Offspring**

Both serum AMH concentrations (Figure 5, panel A) and AFC (Figure 5, panel B) were lower ($P < 0.0001$) in summer compared with winter heifers. Neither AMH nor AFC were influenced by farm and by age at sampling and ultrasonography.

**Influence of Lactation Status of Dams on AFC and Serum AMH Concentrations in Offspring**

Heifers born to dams that were not being milked during gestation had lower AMH concentrations and AFC compared with offspring of cows on their first lactation ($P < 0.05$), whereas no difference was detected with daughters of cows on second or greater compared with first lactation (Table 1). The AMH and AFC were similar between offspring of cows on their first and second or greater lactation (Table 1).

**Influence of Season on Offspring Fertility**

Heifers in the summer and winter groups had a similar age at first service (summer $15.40 \pm 0.16$; winter $14.43 \pm 0.15$ mo), at first conception (summer $16.13 \pm 0.20$; winter $15.29 \pm 0.19$ mo), and at first calving (summer $25.18 \pm 0.18$; winter $24.43 \pm 0.20$ mo). No difference was observed between groups in the number of services per conception (summer $1.48 \pm 0.07$; winter $1.54 \pm 0.08$).
**Fertility, AFC, and AMH**

When they were inseminated for the first time, heifers with low AMH concentrations were older compared with herd mates with intermediate AMH concentrations (Table 2), but no difference was detected between the low and high AMH group in age at first service. Age at first conception and at first calving, and the number of services per conception were similar among heifers with different AMH concentrations (Table 2). No difference was detected in any of the fertility measures among AFC groups (data not shown).

![Figure 2](image_url)
DISCUSSION

The most relevant findings of this study were that (1) AMH and AFC (2 markers of the number of healthy follicles) were lower in young adult heifers exposed to high environmental temperatures during the first trimester of fetal life compared with heifers that were not exposed to high environmental temperatures and humidity in their early uterine life, (2) AMH and AFC were lower in heifers born to nonlactating dams compared with daughters of lactating cows, and (3) despite the negative effect of environmental temperature and humidity and lactation on size of the ovarian follicular population, fertility at first conception was not impaired.

In cattle, high temperatures and humidity interfere with follicular development, peripheral hormonal concentrations, and uterine environment, thus impairing oocyte competence and early embryonic development (Zeron et al., 2001; De Rensis and Scaramuzzi, 2003; Dash et al., 2016; Santana et al., 2017). Also, maternal exposure to heat stress during gestation can affect development of the conceptus with long-term consequences after birth. In dairy cows, maternal heat stress in late gestation induced low birth weight, reduced total plasma protein concentrations and hematocrit, and impaired the immunocompetence of the calves (Tao and Dahl, 2013; Tao et al., 2014; Monteiro et al., 2016). For the first time, we provided evidence that high air temperatures and humidity in the preconception period and in the earliest stages of gestation are linked to a small population of healthy ovarian follicles in the offspring of female cattle. The developing gonad may be highly sensitive to external stimuli during this early window of exposure because it coincides with the peak in number of germ cells in the bovine fetus (Erickson, 1966; Rüsse, 1983; Tanaka et al., 2001). This finding is in contrast with recent evidence of the lack of difference in AMH concentrations between cows born to dams exposed to heat stress in the first trimester of gestation compared with unexposed mothers (Akbarinejad et al., 2017). Such discrepancy between the 2 studies may be due to the differences in the number of animals enrolled and in the age at AMH assessment (heifers vs. cows). Here, AMH and AFC were assessed when animals were 1.4 yr old, sexually mature, yet nulliparous. This postpubertal time frame was chosen because AMH concentrations vary in prepubertal heifers (Monniaux et al., 2011; El-Sheikh Ali et al., 2017; Mossa et al., 2017), but are stable during estrous cycles (Ireland et al., 2011; Monniaux et al., 2012; Ribeiro et al., 2014). In women, higher AMH concentrations were recently observed in early postmenarchal girls compared with adult women with regular, ovulatory cycles (Ortega et al., 2020), but others report that AMH concentrations are stable from childhood to early adulthood (Hagen et al., 2011; Kelsey et al., 2011). In cattle, there is no evidence indicating that AFC or AMH are affected by the number of estrous cycles since puberty. Also, neither

Figure 3. Association between anti-Müllerian hormone (AMH) peripheral concentrations and the total number of follicles $\geq 3$ mm in diameter (antral follicle count; AFC) detected with ovarian ultrasonography on a random day of the estrous cycle in 16-mo-old Holstein heifers ($n = 258$). Serum AMH concentrations and AFC were highly positively correlated ($r = 0.70; P < 0.0001$).
AMH nor AFC were influenced by age at sampling and ultrasonography and by farm in our study, indicating that the observed differences in the size of the ovarian follicular population may be attributed to the environmental conditions in early fetal life.

Moreover, a stringent correlation between AMH and AFC was observed, as previously reported (Ireland et al., 2008; Rico et al., 2009; Baldrighi et al., 2014; Batista et al., 2014; Ghanem et al., 2016), providing further evidence for their reliability as markers of the number of healthy ovarian follicles and oocytes. Nevertheless, irrespective of season of conception, AMH peripheral concentrations in all heifers were highly variable among individuals and similar to previously reported values in Holstein cattle (Guerreiro et al., 2014; Ribeiro et al., 2014), whereas the mean AFC was lower compared with other reports (Mossa et al., 2012; Martinez et al., 2016). Indeed, in our previous studies the mean AFC ranged from 4 to 61 per cow, whereas in the present work AFC varied from 3 to 36 follicles per heifer. The reason for this narrow AFC range is unclear. It could be due to (1) an inherent variation in the AFC among genetic strains (Baldrighi et al., 2014; Batista et al., 2014), similar to the racial and ethnic differences observed in women (Schuh-Huerta et al., 2012; Tal and Seifer, 2013), (2) differences in the detection of the antral follicles by ultrasonography among operators, or (3) the presence of numerous early follicles with a reduced diameter that, although producing AMH, may not be visualized by ultrasonography.

The biological pathways by which high air temperatures and humidity may have negatively influenced fetal follicular development were beyond the scope of this study. Nevertheless, we speculate that high THI during summer may have increased the body temperature of pregnant dams, resulting in hyperthermia. Cattle exposed to heat stress reduce their feed intake (Ominsksi et al., 2002), and we have previously demonstrated that heifers born to nutritionally restricted mothers during the first trimester of gestation had a diminished ovarian population of healthy follicles and oocytes (Mossa et al., 2013). This is demonstrated by consistently lower circulating AMH concentrations from 4 mo to 1.8 yr of age, lower AFC (number of antral follicles growing during follicular waves) from 7 wk to 1.6 yr of age, and increased follicle-stimulating hormone concentrations, a phenotypic characteristic of cattle with a low AFC (Burns et al., 2005; Ireland et al., 2007; Jimenez-Krassel et al., 2009; Mossa et al., 2010b). Thus, it is plausible that the exposure to high air temperatures and humidity may have impaired feed intake in the mothers during early gestation, thus reducing the energy supply during fetal gonadal development. Indeed, other factors associated with summer may have influenced fetal follicular development. Dietary ration may have been different in summer versus winter, as the TMR was composed of home-grown forage crops, which were harvested once a year and either ensiled (corn) or stored as hay. Silage storage may have influenced the nutritional quality of the ration offered to dams.
and offspring in the different seasons (Bernardes et al., 2018), and moderate variations in ration formulation may have occurred during the duration of the study as part of the routine herd management practices. Other management practices, such as number and time of milking, were constant through the summer and winter. Nevertheless, because the experimental design of this study did not allow to control for several managerial and environmental variables, the observed differences in the ovarian follicular population of the offspring cannot be exclusively attributed to the environmental conditions in early pregnancy.

Vitamin D is a steroid hormone whose deficiency has been associated with a 25% fertility reduction in rats (Halloran and DeLuca, 1980), and recent evidence suggests that low vitamin D levels have a negative effect on the ovarian reserve in women (Shahrokhi et al., 2018). Cattle synthesize vitamin D₃ (cholecalciferol) following exposure to sunlight, and obtain both vitamin D₂ (ergocalciferol) and D₃ through dietary sources (Hidiroglou et al., 1985; Hymøller and Jensen, 2010). Although animals enrolled in this study were permanently housed in freestall barns with a shed, the increase in the number of hours of daylight during summer may have led to higher peripheral concentrations of vitamin D in early gestation in dams of the summer group. Vitamin D significantly decreased AMH expression from hen granulosa cells of small growing follicles and caused a significant increase in the expression of FSHR (Wojtusik and Johnson, 2012). Because AMH is considered to reduce premature exhaustion of the ovarian reserve by inhibiting primordial follicular growth (Dewailly et al., 2014), the AMH decrease in the first trimester of pregnancy of dams in the summer group may have resulted in a premature activation of follicular growth and consequent early follicular depletion in fetal ovaries.

Another important finding of this work was that AFC and AMH were lower in heifers born to nonlactating dams compared with daughters of cows in their first lactation, irrespective of season of early fetal development. This finding is in accordance with evidence from our previous study conducted on Irish Holstein Friesian cattle in a pasture-based system, where daughters of heifers had on average 3 follicles less than daughters of lactating dams (Walsh et al., 2014). Similarly, AMH concentrations were greater in Holstein heifers born to multiparous cows compared with nulliparous heifers (Akbarinejad et al., 2018), and beef heifers born to cows had more antral follicles than herd mates born to cows (McNeel et al., 2017). The repeatability of this finding across independent studies conducted in different countries and farming conditions is remarkable;

### Table 1. Effect of lactation number of the dam at conception on the mean (±SEM) peripheral concentrations of anti-Müllerian hormone (AMH) and total number of follicles ≥3 mm in diameter (antral follicle count; AFC) detected on a random day of the estrous cycle in 16-mo-old Holstein heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>Lactation 0</th>
<th>Lactation 1</th>
<th>Lactation ≥2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of heifers</td>
<td>147</td>
<td>76</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>AMH (pg/mL)</td>
<td>441.53 ± 28.63ᵃ</td>
<td>612.75 ± 63.05ᵇ</td>
<td>543.99 ± 48.91ᵇ</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number of heifers</td>
<td>124</td>
<td>63</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>AFC</td>
<td>9.64 ± 0.43ᵃ</td>
<td>11.79 ± 0.80ᵇ</td>
<td>11.22 ± 0.54ᵇ</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ᵃᵇDifferent superscripted letters within the same row indicate statistical differences (P < 0.05).

**Figure 5.** Mean (±SEM) peripheral concentrations of anti-Müllerian hormone (AMH; panel A) and total number of follicles ≥3 mm in diameter (antral follicle count; AFC; panel B) on a random day of the estrous cycle in 310 sixteen-month-old Holstein Friesian dairy heifers that were conceived and spent the first trimester of their fetal life in May through August (summer; n = 176) or November through March (winter; n = 134). Different letters a,b indicate a significant difference (P < 0.0001).
Table 2. Reproductive performance in Holstein heifers (mean ± SEM) with low (20%), intermediate (60%), and high (20%) peripheral anti-Müllerian hormone (AMH) concentrations

<table>
<thead>
<tr>
<th>Item</th>
<th>AMH category</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (pg/mL)</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Number of heifers</td>
<td>61</td>
<td>186</td>
</tr>
<tr>
<td>AMH</td>
<td>155.08 ± 6.37</td>
<td>398.24 ± 9.74</td>
</tr>
<tr>
<td>Age at first insemination (mo)</td>
<td>15.59 ± 0.26(^a)</td>
<td>14.85 ± 0.15(^c)</td>
</tr>
<tr>
<td>Age at first conception (mo)</td>
<td>16.02 ± 0.32</td>
<td>15.73 ± 0.18</td>
</tr>
<tr>
<td>Age at first calving (mo)</td>
<td>25.29 ± 0.31</td>
<td>24.82 ± 0.18</td>
</tr>
<tr>
<td>Number of services per conception</td>
<td>1.45 ± 0.12</td>
<td>1.56 ± 0.07</td>
</tr>
</tbody>
</table>

\(^ {a,b}\)Values within a row with no common superscript differ (P < 0.05).

thus, it is likely that nutrients are partitioned toward maternal rather than fetal growth in pregnant heifers (Scholl et al., 1994), resulting in the impairment of the ovarian follicular reserve in the progeny. This possibility is further confirmed by the lack of association between dam milk production before conception and during pregnancy with daughter AFC in Holstein cows (Walsh et al., 2014). Nevertheless, in the present study, heifers born to cows in their second or later lactation had similar AFC and AMH compared with daughters of nulliparous and primiparous dams. Thus, the potential association between lactation number of the dam and the ovarian follicular population of the progeny is not clear and warrants further investigation.

The lack of association between season of conception and fertility contradicted our initial hypothesis. Due to the project time frame, we only collected reproductive measurements from the first calving of the progeny, but the potential decrease in fertility in animals exposed to heat stress as fetuses may be evident when cows are lactating, and thus more prone to reproductive failure. It is also possible that the number of animals enrolled in this study was insufficient to detect differences in fertility. Indeed, heifers in the winter group were on average 22 d younger at first calving compared with animals in the summer group, yet no statistically significant difference was observed. It should also be noted that heifers in the summer group received their first service during months with higher THI and longer days (March–September) compared with heifers in the winter group, which were inseminated for the first time from September to April, when days were shorter and air temperatures were lower. It is likely that heifers in the summer group were exposed to heat stress at the time of their first service, but because the months at first insemination partially overlapped between groups, the potential cumulative effect of heat stress occurring both before and after birth on a heifer’s fertility may have not been detected. Therefore, it should be investigated with controlled experiments. Indeed, recent evidence from more than 600,000 lactation records indicates that cows conceived in winter were on average 10 d younger at their first calving compared with herd mates conceived in summer (Pinedo and De Vries, 2017).

Dairy farmers often use lower-cost semen in summer because fertility declines when air temperatures and humidity are high (Collier et al., 2006). This practice may result in lower genetic merit for heifers conceived in summer compared with those conceived in winter. Higher parent-average (estimates of the genetic merit underlying the survival, fertility, and milk yield of the cow based on her sire and dam) for milk, fat, protein, productive life, and net merit have been reported in cows conceived in winter compared with herd mates conceived in summer (Pinedo and De Vries, 2017). Yet, parent-average for daughter pregnancy rate were higher in cows conceived in summer, and authors suggest that the lower performance of cows conceived in summer could not be completely explained by use of lower quality genetics in summer breedings (Pinedo and De Vries, 2017). Although we did not collect genetic data, farmers used the same pool of bull in different seasons; therefore, it is unlikely that the difference in the AFC and AMH between summer and winter heifers could have been influenced by the use of semen with lower genetic merit during the summer months.

Nevertheless, albeit similar in fertility, cattle exposed to high temperatures and humidity in early pregnancy may have a shorter life compared with cattle conceived in winter. We previously tested the hypothesis that AMH in heifers was positively linked to productive herd life (time in herd after calving). Results showed that cows in the quartile (Q) with the lowest AMH concentrations as heifers (Q1) completed fewer lactations compared with Q3 cows and had a 180-d average shorter productive herd life compared with Q2 and Q3 cows. Moreover, the probability of being culled after birth of the first calf was higher for the Q1 compared with Q2, Q3, and Q4 cows, documenting that a single determination of AMH concentrations in young adult Holstein heifers is predictive of their future herd lon-
gevity (Jimenez-Krassel et al., 2015). In the present study, we were not able to investigate the survival of heifers in the herd after their first lactation, but we speculated that animals in the summer group may have had lower longevity compared with cattle in the winter group, possibly due to their impaired ovarian follicular population. Indeed, others report that the odds of survival to a second calving for cows conceived in winter were 1.21 times the odds of survival for cows conceived in summer (Pinedo and De Vries, 2017).

Moreover, we tested the hypothesis that, regardless of season during early fetal development, cattle with a high ovarian follicular population are more fertile than herd mates with smaller ovarian reserve. Age at first service was greater in heifers with low compared with intermediate AMH concentrations, but age at first calving and the number of services per conception were similar among heifers with different AMH concentrations. These findings indicate that the size of the ovarian follicular population in pubertal nulliparous heifers is not predictive of their fertility at first conception, and are in accordance with previous studies that conception rates to first AI, services per conception, and days open after calving until pregnant were similar among Holstein heifers in the different AMH quartiles (Jimenez-Krassel et al., 2015). Further, no association was previously detected between serum AMH, pregnancy outcomes at first service, and pregnancy risk up to 250 d postpartum (Gobikrushanth et al., 2018). On the other hand, we previously observed that dairy cows with a low AFC had a lower conception rate to first AI, greater number of AI to conceive, and higher calving interval compared with cows with an intermediate or high AFC (Mossa et al., 2012). Greater pregnancy rates and lower incidence of pregnancy loss in dairy cows with high AMH (Ribeiro et al., 2014), and lower pregnancy rates in beef heifers (Cushman et al., 2009) and dairy cows with low AFC (Martinez et al., 2016) were also reported. Therefore, the existence of an association between the size of the ovarian reserve and fertility in cattle is yet to be established.

CONCLUSIONS

This study investigated, for the first time, the potential long-term effect of high environmental temperatures and humidity in early pregnancy on gonadal development and subsequent fertility in female offspring. We provided evidence for a negative association between high THI during the first trimester of fetal life and the size of the ovarian reserve in young adult heifers, as assessed by lower serum AMH concentrations and AFC in heifers conceived in summer compared with winter months. Results also showed that the size of the ovarian reserve was lower in daughters of nonlactating dams compared with heifers born to lactating cows. Collectively, these findings indicate that the number of ovarian follicles and oocytes in cattle can be negatively programmed by high temperatures and humidity occurring in the first trimester of fetal life. Although a smaller ovarian reserve did not impair fertility in heifers, the effects of high environmental temperatures and humidity in early pregnancy on offspring gonadal development warrant further research, as the potential decrease in fertility and longevity in animals exposed to heat stress as fetuses may be evident later in life, when cows are lactating and more prone to reproductive failure.

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