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Association between ambient air temperature, ovarian reserve, and outcomes of ovarian stimulation among oocyte donors

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Association between ambient air temperature, ovarian reserve, and outcomes of ovarian stimulation among oocyte donors

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May 2020

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An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Epidemiology

2023

Abstract

Association between ambient air temperature, ovarian reserve, and outcomes of ovarian stimulation among oocyte donors

By Meg McAloon

Objective: To examine the relation of ambient temperature with ovarian reserve and outcomes of ovarian stimulation among young, healthy females.

Methods: We included 589 oocyte donors who underwent 943 ovarian stimulation cycles at a fertility clinic in Atlanta, Georgia, USA (2008-2020). Daily residential ambient temperatures were estimated from a spatially refined gridded climate data set beginning three months prior to ovarian stimulation through oocyte retrieval. Antral follicle count (AFC) was assessed with transvaginal ultrasonography and mature oocyte count was assessed following oocyte retrieval. Poisson regression models with robust standard errors were used to estimate the associations of ambient temperature with AFC and oocyte count adjusted for age, education level, race, state of residence, year of retrieval, month of retrieval, and body mass index (BMI).

Results: The mean (standard deviation) age of donors was 25.2 (2.8) years and 27% were racial/ethnic minorities. Overall, there were no statistically significant associations between average ambient temperature exposures (average, maximum, minimum, or apparent) in the 2 weeks, 1 month, or 3 months prior to scan and AFC. While there was a suggestion of a negative association between higher ambient temperatures in the 3 months prior and lower AFC, particularly for maximum temperature (% change: -2.6 per interquartile range increase, 95% CI -9.3, 4.6), the association was not significant. The observed associations of average ambient temperature with total and mature number of oocytes retrieved were also negative across all three time points; however, confidence intervals were imprecise. For instance, an interquartile range increase in average maximum temperature in the 3 months prior, one month prior, and two weeks of ovarian stimulation was associated with -4.2% (95% CI -11.7%, 3.9%), -3.8% (95% CI -9.2%, 2.1%) and -3.4% (95% CI -8.2%, 1.8%) fewer mature oocytes retrieved, respectively.

Conclusions: In our cohort of young, healthy women residing in the Southeastern United States, we found little evidence for an association of ambient temperatures with ovarian reserve and outcomes of ovarian stimulation. Although the effect estimates were small in magnitude and imprecise, they were most commonly negative which may indicate a potentially harmful effect of ambient temperatures on ovarian function.

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Introduction

Climate change is widely recognized as the greatest threat humanity faces today. As greenhouse gas concentrations continue to increase, the planet continues to warm (1). The changing climate has increased the frequency and intensity of extreme weather events such as hurricanes, droughts, and heat waves (2). According to NOAA, the top ten hottest years have occurred in the last 20 years (3). The Lancet Countdown states that "from 2000 to 2021, populations were exposed to an average increase in summer temperature two times higher than the global mean" (4)

In particular, heat waves can have detrimental effects on human health (5). Studies have shown an association between exposure to extreme heat and acute kidney injury, adverse pregnancy outcomes, worsened sleep patterns, negative mental health impacts, and worsening cardiovascular and respiratory diseases (4). A meta-analysis including 70 studies found that preterm births were more common at higher than lower temperatures (6). The same meta-analysis found that higher temperatures were associated with reduced birth weight and an increased risk of stillbirths. Though there are many studies on the connection between higher temperatures and birth outcomes, there are very few that have evaluated the association between higher temperatures and human fertility.

In animals, it is well documented that ovarian function, including folliculogenesis and oogenesis, are negatively impacted by heat stress (7). For instance, higher ambient temperatures have been shown to result in a reduced number of oocytes retrieved and a poorer developmental ability following in vitro fertilization (8, 9). Chronic heat stress also reduces the production of hormones needed to stimulate follicular and oocyte growth (10, 11). These effects of heat on ovarian

function are likely a key pathway explaining the decreased birth rates observed during summer months in many animal species (12, 13).

To date, there is only one study in humans that examines the association between ambient temperature and ovarian function. It was conducted in a cohort of women presenting for infertility treatment in Boston, Massachusetts, USA (14). The authors found that women who had exposure to higher maximum ambient temperatures in the three months prior to their scan had lower antral follicle counts. Additionally, there were weaker associations for higher temperatures in the month and two weeks prior versus the three months prior. This suggests that cumulative heat exposure that occurs during the preantral to preovulatory stages of follicular development may be more detrimental than shorter-term exposures in the final stages of antral development. However, the study lacked generalizability because the study population was limited to older, sub-fertile women residing in the Northeastern United States, who were predominantly white and of higher socioeconomic status.

Thus, the aim of this study was to build upon the existing literature and evaluate whether exposure to ambient temperatures impacts markers of fertility in a cohort of young, racially, and socioeconomically diverse oocyte donors residing in the Southeastern United States.

Study Population

This was a retrospective study using data from a national oocyte bank based at Reproductive Biology Associates (RBA) in Atlanta, GA (2008-2020). The data collection project was approved through the institutional review board (IRB) of Emory University (IRB00080463). Ovarian stimulation cycles included in this study were those in which oocytes were cryopreserved via vitrification for use in an oocyte bank. Of the 1,068 ovarian stimulation cycles

(from 659 unique donors) that were initially eligible for this analysis, we excluded stimulation cycles that occurred prior to 2008 to limit variation in stimulation protocols (n=25). We further excluded 100 ovarian stimulation cycles that were missing data on day 3 antral follicle count (n=68), total number of oocytes retrieved (n=8) or number of MII oocytes retrieved (n=24), our primary outcomes of interest. Finally, donors residing in states outside of the Southeast (e.g. Georgia, Alabama, Tennessee, Florida, North Carolina, and South Carolina) were excluded to limit temperature variation (n=11). The final dataset contained 943 ovarian stimulation cycles from 589 women.

Exposure Assessment

We estimated daily residential ambient temperatures beginning three months prior to the woman's AFC date, as this roughly corresponds to the preantral to preovulatory stages of follicular development (approximately 2-4 months) (15). The patient's residential addresses were collected from their medical record and geocoded using ArcGIS. If the address changed over time, this was noted, along with the year of move. The patient's geocoded address was then linked to ambient temperature data from the Parameter-elevation Regressions on Independent Slopes Model (PRISM), which provides daily estimates of the following: average ambient temperature (Tavg), minimum temperature (Tmin), maximum temperature (Tmax), and mean dew point temperature (Tdew) at a 4 km^2 spatial resolution (16, 17, 18). The gridded Parameter-elevation Regressions on Independent Slopes Model data set allows for more spatially explicit meteorologic exposures than observations from individual weather stations.

Relative humidity (RH) was calculated using the Magnus approximation:

RH = 100xe^(cb(Tdew - Tavg)c + Tavg)(c + Tdew))

Where b = 17.625 and c = 243.04.

Apparent temperature (Tapp), defined as a person's perceived air temperature, was calculated using the following formula: $Tapp = -2.653 + (0.994 \times Tavg) + (0.0153 \times Tdew2)$

For the outcome of AFC, the three overlapping exposure windows were examined: 3 months prior, 1 month prior, and 2 weeks prior to scan. These were selected a priori based on the timeframe of follicular development in humans and previous literature on bovines that demonstrated primordial and primary follicles are heat resistant. The 3-month time point represents exposures during the preantral to the preovulatory stages of development. The 1-month time point represents exposure during the early antral to preovulatory stages of development. The 2-week time point represents exposures during the final stages of antral development. Similar time windows of exposure were chosen for the outcomes of oocyte counts: 3 months and 1 month prior to oocyte retrieval and exposure during ovarian stimulation (8-14 days) following similar biological rationale.

Outcome Assessment

Ovarian AFC, defined as the sum of antral follicles in both ovaries, was measured by a reproductive endocrinologist using transvaginal ultrasonography on the 3rd day of an unstimulated menstrual cycle. Immediately following AFC assessment, the antagonist protocol was employed for the oocyte donors' ovarian stimulation. After eight to fourteen days of ovarian stimulation, oocyte retrieval was performed using a transvaginal ultrasound guided aspiration. Embryologists classified the retrieved oocytes as a germinal vesicle, metaphase I, metaphase II (MII) or degenerated. Total oocyte yield was defined as the sum of all oocytes retrieved regardless of type. Mature oocyte yield was the sum of all MII oocytes.

Covariate Assessment

At the patient's initial visit, data was collected on date of birth, self-identified race/ethnicity, education, and reproductive history. Weight and height were measured using standardized procedures to calculate body mass index (BMI; kg/m^2). For each retrieval the donor underwent, we collected ovarian stimulation data including gonadotropin dose, number of days of stimulation, peak estradiol level, number of follicles >14mm at trigger, and trigger type.

Statistical Analysis

Among the oocyte donors, we compared demographic, reproductive, and ovarian stimulation parameters at their first retrieval across quintiles of ambient temperature exposure in the 3 months prior to AFC. Chi-square or Kruskal-Wallis tests were used to compute the differences across quintiles. Generalized estimating equations with Poisson distribution, log link, and robust standard errors were used to estimate the association between the temperature exposures and outcomes - AFC, number of oocytes, and number of MII – taking into account the potential for repeated observations per woman. Results are presented as percentage change in the outcome per 1-degree Celsius increase in temperature or per interquartile range (IQR) increase in temperature. Confounding was assessed based on biological relevance and descriptive statistics from our study population. Final models were adjusted for donor age, race, education level, and BMI. We also adjusted for the month of AFC to account for seasonal changes in temperature since our goal was to assess the effect of deviations in temperature from the monthly average rather than seasonal differences. We adjusted for year of AFC/retrieval to account for protocol differences as well as any time trends in temperature.

Since there is evidence that exposure to higher temperatures may be more detrimental to health outcomes outside of the summer season due to acclimatization, we evaluated effect modification according to the month and season of antral follicle scan by adding cross product terms to the final multivariate model. Non-linearity was assessed with restricted cubic splines, which used the likelihood ratio test comparing the model with the linear term to the model with the linear and the cubic spline terms.

Results

The 589 oocyte donors included in our analysis underwent a total of 943 oocyte retrievals. The majority had one (58%) retrieval while 21% had two, and 21% had three or more retrievals during our study period. The median baseline AFC was 36 (minimum = 10; maximum = 101). The median oocyte count was 32 (min=9; max=108) and the median MII count was 24 (min=5; max=79). All three outcomes – AFC, oocytes retrieved, and MII retrieved – varied slightly across categories of age, year of retrieval, season, state of residence, race, education level, and BMI (**Supplemental Table 1**).

Supplemental Table 1: Distribution of outcomes across confounding variables

Age Group	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
21-23	38.33	33.46	25.27
24-26	38.86	33.73	25.32
27-29	37.96	34.58	25.64
30-33	39.69	35.31	26.59
Year of Retrieval	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
2008-2011	34.24	28.78	21.81
2012-2015	38.47	34.70	26.18
2016-2020	42.44	37.83	27.93

Season	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
Fall	37.57	33.60	25.15
Spring	40.23	34.87	26.42
Summer	38.08	33.51	24.84
Winter	38.43	34.39	25.85
State of Residence	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
Georgia	38.51	33.81	25.27
Other states	38.98	36.54	28.14
Race	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
Caucasian	38.28	33.85	25.54
African American	39.23	36.39	25.91
Asian-American	39.74	32.74	24.90
Hispanic or Latinx	42.36	38.67	29.60
Other	36.83	30.49	22.43
Education level	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
Completed high school, received GED, or completed some college	42.11	38.71	28.47
Currently enrolled in college	40.20	35.08	26.36
Completed technical school or 2-year degree	38.72	33.16	24.75
Earned 4-year degree	37.51	32.67	24.94
Completed or pursuing advanced degree	37.03	34.09	24.96
BMI	**Mean AFC**	**Mean # of Oocytes**	**Mean # of MII**
<= 21	37.03	32.82	24.35
21.1-24.9	39.07	34.50	26.07
>= 25	39.32	34.63	25.60

The mean (standard deviation) age of donors was 25.2 (2.8) years. The majority of donors were Caucasian (72.7%), normal weight (52.8% had a BMI 21.1-24.9 kg/m^2), had a college education

or higher (53.5%), and were nulligravid (67.6%) (**Table 1**). Our donors resided in Georgia (90.2%), South Carolina (4.0%), Alabama (3.0%), Tennessee (1.3%), Florida (0.3%), and North Carolina (0.2%). The majority of demographic, reproductive, and ovarian stimulation characteristics did not differ across quintiles of mean ambient temperature exposure in the 90 days prior to AFC. The exception was year and season of retrieval and state of residence. Donors residing in Southeastern US states outside of Georgia and those undergoing stimulation in the summer and fall had higher average mean temperature exposures. Unexpectedly, women in the second quintile of mean temperature exposure were less likely to have undergone ovarian stimulation in the earliest years of our study, 2008-2011.

Table 1: Characteristics of oocyte donors by mean ambient temperature exposure in the 90 days prior to baseline AFC measurement, 2008-2020

		Quintile of Mean Temperature Exposure in 90 days prior to AFC					
	Total Count (%)	Q1 0-9.2	Q2 9.3-14.2	Q3 14.3-21.5	Q4 21.6-24.7	Q5 24.8-28.2	p-value
Number of women	589	109 (18.5)	123 (20.9)	124 (21.1)	115 (19.5)	118 (20.0)	0.86
Age at first retrieval Mean (std dev)	25.2 (2.8)						0.44
21-23 years	195 (33.1)	33 (30.3)	43 (35.0)	41 (33.1)	39 (33.9)	39 (33.1)	
24-26 years	189 (32.1)	43 (39.5)	39 (31.7)	36 (29.0)	32 (27.8)	39 (33.1)	
27-29 years	161 (27.3)	30 (27.5)	27 (22.0)	39 (31.5)	35 (30.4)	30 (25.4)	
30-33 years	44 (7.5)	3 (2.8)	14 (11.4)	8 (6.5)	9 (7.8)	10 (8.5)	
Year of retrieval							<.0001
2008-2011	199 (33.8)	46 (42.2)	19 (15.5)	38 (30.7)	46 (40.0)	50 (42.4)	
2012-2015	216 (36.7)	35 (32.1)	52 (42.3)	50 (40.3)	45 (39.1)	34 (28.8)	
2016-2020	174 (29.5)	28 (25.7)	52 (42.3)	36 (29.0)	24 (20.9)	34 (28.8)	
Season of retrieval							<.0001
Winter	107 (18.2)	37 (33.9)	50 (40.7)	19 (15.3)	1 (0.9)	0	
Spring	153 (26.0)	72 (66.1)	69 (56.1)	12 (9.7)	0	0	
Summer	174 (29.5)	0	4 (0.7)	68 (54.8)	68 (59.1)	34 (28.8)	
Fall	155 (26.3)	0	0	25 (20.2)	46 (40.0)	84 (71.2)	
State of Residence							0.006
Georgia	531 (90.2)	105 (96.3)	116 (94.3)	110 (88.7)	102 (88.7)	98 (83.1)	
Other SE state	58 (9.9)	4 (3.7)	7 (5.7)	14 (11.3)	13 (11.3)	20 (17.0)	
Race/Ethnicity							0.34
Caucasian	428 (72.7)	83 (76.2)	85 (69.1)	85 (68.6)	83 (72.2)	92 (78.0)	
African American	66 (11.2)	6 (5.5)	17 (13.8)	13 (10.5)	14 (12.2)	16 (13.6)	

Asian	30 (5.1)	7 (6.4)	5 (4.1)	8 (6.5)	6 (5.2)	4 (3.4)	
Hispanic or Latino	24 (4.1)	4 (3.7)	9 (7.3)	7 (5.7)	3 (2.6)	1 (0.9)	
Other	41 (7.0)	9 (8.3)	7 (5.7)	11 (8.9)	9 (7.8)	5 (4.2)	
Donor Education*							0.14
Completed high school or some college	43 (7.5)	4 (3.7)	10 (8.5)	8 (6.5)	6 (5.4)	15 (12.9)	
Currently enrolled in college	156 (27.1)	32 (29.6)	41 (34.8)	32 (26.0)	26 (23.4)	25 (21.6)	
Completed technical school or 2-year college degree	67 (11.6)	10 (9.3)	12 (10.2)	20 (16.3)	14 (12.6)	11 (9.5)	
Earned 4-year degree	192 (33.3)	44 (40.7)	34 (28.8)	40 (32.5)	36 (32.4)	38 (32.8)	
Completed or pursuing an advanced degree	118 (20.5)	18 (16.7)	21 (17.8)	23 (18.7)	29 (26.1)	27 (23.3)	
BMI*							0.18
<= 21 kg/m^2	167 (28.6)	34 (31.2)	28 (22.8)	32 (25.8)	41 (36.0)	32 (27.8)	
21.1-24.9 kg/m^2	309 (52.8)	60 (55.1)	74 (60.2)	61 (49.2)	52 (45.6)	62 (53.9)	
>= 25 kg/m^2	109 (18.6)	15 (13.8)	21 (17.1)	31 (25.0)	21 (18.4)	21 (18.3)	
Gravidity							0.53
0	398 (67.6)	73 (67.0)	87 (70.7)	82 (66.1)	70 (60.9)	86 (72.9)	
1	105 (17.8)	21 (19.3)	23 (18.7)	21 (16.9)	22 (19.1)	18 (15.3)	
2	86 (14.6)	15 (13.8)	13 (10.6)	21 (16.9)	23 (20.0)	14 (11.9)	
Gonadotropin total dose							0.089
≤1,500 IU	18 (3.1)	6 (5.5)	1 (0.8)	4 (3.2)	7 (6.1)	0	
1,501-2,500 IU	314 (53.3)	60 (55.1)	69 (56.1)	62 (50.0)	62 (53.9)	61 (51.7)	
2,501-3,500 IU	238 (40.4)	40 (36.7)	52 (42.3)	52 (41.9)	40 (34.8)	54 (45.8)	
3,501-5,000 IU	19 (3.2)	3 (2.8)	1 (0.8)	6 (4.8)	6 (5.2)	3 (2.5)	
Days of stimulation							0.83
8-9	111 (18.9)	21 (19.3)	17 (13.8)	22 (17.7)	25 (21.7)	26 (22.0)	
10-11	383 (65.0)	72 (66.1)	83 (67.5)	80 (64.5)	73 (63.5)	75 (63.6)	
12-14	95 (16.1)	16 (14.7)	23 (18.7)	22 (17.7)	17 (14.8)	17 (14.4)	
Number of follicles >14mm at trigger							0.76
≤12	40 (6.8)	8 (7.3)	7 (5.7)	9 (7.3)	6 (5.2)	10 (8.5)	
13-24	344 (58.4)	63 (57.8)	64 (52.0)	74 (59.7)	72 (62.6)	71 (60.2)	
25-40	184 (31.2)	35 (32.1)	49 (39.8)	36 (29.0)	31 (27.0)	33 (28.0)	
41-55	21 (3.6)	3 (2.8)	3 (2.4)	5 (4.0)	6 (5.2)	4 (3.4)	
Peak estradiol							0.068
<2,000 pg/mL	127 (21.6)	24 (22.0)	15 (12.2)	29 (23.4)	25 (21.7)	34 (28.8)	
2,001-4,500 pg/mL	236 (40.1)	48 (44.0)	47 (38.2)	49 (39.5)	50 (43.5)	42 (35.6)	
4,501-6,000 pg/mL	101 (17.2)	19 (17.4)	21 (17.1)	24 (19.4)	19 (16.5)	18 (15.3)	
>6,000 pg/mL	125 (21.2)	18 (16.5)	40 (32.5)	22 (17.7)	21 (18.3)	24 (20.3)	

Maturation trigger type*							0.26
hCG	33 (5.7)	8 (7.3)	3 (2.5)	7 (5.7)	5 (4.4)	10 (8.6)	
Lupron	449 (77.0)	82 (75.2)	101 (84.9)	89 (72.4)	87 (75.7)	90 (76.9)	
Ovidrel	101 (17.3)	19 (17.4)	15 (12.6)	27 (22.0)	23 (20.0)	17 (14.5)	

*13 observations were missing donor education data; 4 observations were missing BMI data; 6 observations were missing maturation trigger data

Overall, there were no statistically significant associations between average ambient temperature exposures (average, maximum, minimum, or apparent) in the 2 weeks, 1 month, or 3 months prior to scan and AFC (**Table 2**). While there was a suggestion of a negative association between higher ambient temperatures in the 3 months prior and lower AFC, particularly for max temperature (% change: -2.6 per IQR increase, 95% CI -9.3, 4.6), the association was not significant. Effect estimates for the association between temperature exposures in the 1 month and 2 weeks prior to AFC were very close to zero, indicating little evidence of a statistically or clinically significant effect. The restricted cubic splines model of the association between average ambient temperature exposure in the past 3 months and AFC shows there is no evidence of a non-linear relationship (p-for-non-linearity = 0.82).

Table 2: Association between average ambient temperature exposure prior to scan and antral follicle counts.

	Adjusted % Change in AFC·	
	per 1°C increase	**Per IQR increase**
3 Months Prior		
Average Temperature, °C	-0.2 (-0.7, 0.3)	-2.5 (-9.2, 4.7)
Maximum Temperature, °C	-0.2 (-0.7, 0.3)	-2.6 (-9.3, 4.6)
Minimum Temperature, °C	-0.2 (-0.7, 0.3)	-2.3 (-8.9, 4.7)
Apparent Temperature, °C	-0.1 (-0.5, 0.3)	-1.8 (-8.5, 5.4)
1 Month Prior		
Average Temperature, °C	0.1 (-0.3, 0.4)	1 (-4.1, 6.3)
Maximum Temperature, °C	0.1 (-0.3, 0.4)	0.9 (-3.6, 5.7)
Minimum Temperature, °C	0.1 (-0.3, 0.4)	0.9 (-4, 6.1)
Apparent Temperature, °C	0 (-0.2, 0.3)	0.9 (-4, 6.1)
2 Weeks Prior		
Average Temperature, °C	0.1 (-0.3, 0.4)	0.7 (-3.7, 5.3)
Maximum Temperature, °C	0.1 (-0.3, 0.4)	1 (-3.2, 5.4)

Minimum Temperature, °C	0 (-0.3, 0.3)	0.4 (-4.2, 5.2)
Apparent Temperature, °C	0 (-0.2, 0.3)	0.6 (-4, 5.4)

Abbreviations: AFC = antral follicle count
*Adjusted for donor race, age, BMI, education level, state of residence, year of AFC, and month of AFC

The observed associations of average ambient temperature with total and mature number of oocytes retrieved were negative across all three time points (**Table 3**); however, confidence intervals were imprecise, and none obtained conventional levels of statistical significance. Effect estimates also tended to be slightly larger in magnitude for the associations with mature as compared total oocytes. For instance, 3 months prior to retrieval, every IQR increase in average maximum temperature was associated with a -1.4% (95% CI -8.6%, 6.5%) and -4.2% (95% CI -11.7%, 3.9%) fewer total and mature oocytes retrieved, respectively. An IQR increase in average ambient apparent temperature exposure in the one month prior to and the two weeks of ovarian stimulation, was associated with a -3.8% (95% CI -9.2%, 2.1%) and -3.4% (95% CI -8.2%, 1.8%) fewer mature oocytes retrieved. There was no evidence of a non-linear association.

Table 3: Association between average ambient temperature exposure prior to retrieval and outcomes of controlled ovarian stimulation.

	Adjusted % Change in Total Oocytes Retrieved		**Adjusted % Change in Mature Oocytes Retrieved**	
	per 1°C increase	**Per IQR increase**	**per 1°C increase**	**Per IQR increase**
3 Months Prior				
Average Temperature, °C	-0.1 (-0.6, 0.5)	-1.4 (-8.6, 6.5)	-0.3 (-0.9, 0.3)	-3.9 (-11.3, 4.2)
Maximum Temperature, °C	-0.1 (-0.6, 0.5)	-1.4 (-8.7, 6.6)	-0.3 (-0.9, 0.3)	-4.2 (-11.7, 3.9)
Minimum Temperature, °C	-0.1 (-0.6, 0.4)	-1.3 (-8.5, 6.4)	-0.3 (-0.8, 0.3)	-3.5 (-10.9, 4.6)
Apparent Temperature, °C	-0.1 (-0.5, 0.4)	-1 (-8.4, 6.9)	-0.2 (-0.7, 0.3)	-3.5 (-11.1, 4.7)
1 Month Prior				
Average Temperature, °C	-0.1 (-0.5, 0.2)	-1.8 (-6.9, 3.5)	-0.2 (-0.6, 0.2)	-3.4 (-8.7, 2.2)
Maximum Temperature, °C	-0.1 (-0.5, 0.3)	-1.7 (-6.5, 3.4)	-0.3 (-0.7, 0.2)	-3.3 (-8.3, 2)

Minimum Temperature, °C	-0.1 (-0.5, 0.2)	-1.9 (-7.2, 3.6)	-0.2 (-0.6, 0.2)	-3.4 (-8.9, 2.4)
Apparent Temperature, °C	-0.1 (-0.4, 0.2)	-2 (-7.3, 3.5)	-0.2 (-0.5, 0.1)	-3.8 (-9.2, 2.1)
During Ovarian Stimulation				
Average Temperature, °C	-0.1 (-0.4, 0.2)	-1.7 (-6.1, 2.9)	-0.2 (-0.6, 0.1)	-3 (-7.7, 1.9)
Maximum Temperature, °C	-0.1 (-0.4, 0.2)	-1.3 (-5.5, 3)	-0.2 (-0.6, 0.2)	-2.6 (-7.1, 2.1)
Minimum Temperature, °C	-0.1 (-0.5, 0.2)	-2 (-6.6, 2.8)	-0.2 (-0.6, 0.1)	-3.3 (-8.2, 1.8)
Apparent Temperature, °C	-0.1 (-0.3, 0.2)	-1.9 (-6.4, 2.9)	-0.2 (-0.5, 0.1)	-3.4 (-8.2, 1.8)

*Adjusted for donor race, age, BMI, education level, state of residence, month of retrieval, and year of retrieval

There was no evidence of effect modification of the associations of average ambient temperature exposure with AFC and oocyte counts by retrieval month or season (**Supplemental Table 2**).

Supplemental Table 2: Effect modification of the associations of average maximum ambient temperature exposure with AFC and oocyte counts by retrieval month.

Month of AFC	Number of Women	Mean (min, max) of Tmax in 3 months prior	Adjusted % change (95% CI) in AFC per 1 degree Celsius	Adjusted % change (95% CI) in Oocytes per 1 degree Celsius	Adjusted % change (95% CI) in MII per 1 degree Celsius
January	36	16.8 (12.7, 20.1)	0.2 (-5.7, 6.5)	-2.4 (-14.4, 11.2)	-3.9 (-16.2, 10.2)
February	48	13.8 (9.8, 18.2)	-4.9 (-10.3, 0.9)	-2.3 (-13.4, 10.2)	-3.6 (-15.1, 9.5)
March	46	13.0 (9.0, 16.2)	1.1 (-4.5, 7)	-3.1 (-14.6, 9.8)	-3.9 (-15.6, 9.5)
April	49	15.0 (12.1, 22.1)	1.2 (-4.2, 6.9)	-2.4 (-13.8, 10.6)	-2.7 (-14.6, 10.8)
May	52	18.8 (14.7, 23.4)	-0.7 (-6, 4.8)	-6.4 (-17.5, 6.1)	-8.3 (-19.7, 4.7)
June	50	23.2 (18.6, 27.7)	0.8 (-4.7, 6.5)	-2.4 (-14.4, 11.3)	-4.6 (-16.4, 9)
July	67	27.9 (24.8, 32.5)	-2.9 (-9.6, 4.3)	-4.6 (-16.2, 8.6)	-6.1 (-18, 7.6)
August	52	30.5 (28.1, 32.8)	-19.7 (-9.2, 5.4)	-8.4 (-19.9, 4.7)	-9 (-21, 4.8)
September	53	31.3 (28.8, 33.9)	-1.5 (-9, 6.7)	-7.1 (-18.8, 6.3)	-9.5 (-21.3, 4.2)
October	57	30.2 (26.6, 32.3)	0.6 (-6.8, 8.6)	-3.2 (-15.7, 11.1)	-3 (-15.9, 12)

November	44	26.8 (21.8, 30.3)	-0.6 (-6.4, 5.6)	-4.5 (-15.8, 8.4)	-4.7 (-16.8, 9.1)
December	19	24.0 (21.0, 28.3)	0.6 (-4, 5.3)	4 (-7.7, 17.1)	4.8 (-7.4, 18.6)

Discussion

In our retrospective cohort of young, healthy non-identified oocyte donors in the Southeastern United States, we found little evidence for an association of exposure to ambient temperatures with ovarian reserve and outcomes of ovarian stimulation. Although the effect estimates were small in magnitude and imprecise, they were most commonly negative which may indicate a possibly harmful association between higher ambient temperatures and lower AFC and poorer ovarian response to stimulation, yet the immediate clinical relevance is likely limited.

Previous studies have indicated that exposure to heat has a negative association with semen quality (19, 20). On the other hand, very little is known about the impact of ambient temperature on female gametes. A previous study, conducted in 2021, studied the impacts of ambient temperature exposure on AFC among women presenting to a fertility clinic in the Northeastern US. Similar negative associations were observed between higher temperatures and lower AFC (14), but the findings were of greater magnitude and more robust than we found in our study. Possible reasons for this discrepancy include differences in underlying fertility, age, location and acclimatization. Gaskins et al. found that women with female factor infertility diagnosis experienced a stronger association between higher ambient temperatures and lower ovarian reserve. Thus, it is possible since all the women in our study did not have an infertility diagnosis, we observed a smaller impact of ambient temperature on ovarian reserve. Additionally, it is well known that age plays a large role in ovarian reserve; in general, our population was much younger (mean age 25 years) than that of Gaskins et al (mean age ~35 years). Therefore, it is

possible that as women age and the number of antral follicles decline, there is a stronger impact of environmental exposures, such as ambient temperature, on ovarian reserve and response to ovarian stimulation (22, 23). Finally, our population resided in the Southeastern United States while the population studied in Gaskins et al. resided in the Northeastern United States. Beyond obvious differences in average temperature between the two metropolitan areas, there may be differences in personal and infrastructural resilience to temperature changes (24, 25). For example, only 44% of households in the Northeast United States have central AC equipment. In contrast, 85% of households in the Southern United States have central AC (26). The greater access to air conditioning could offset the impact temperature has on ovarian reserve.

Numerous animal studies have observed an adverse impact of ambient heat on ovarian function. Heat stress experienced by cows has been observed to lower the number and quality of male cow sperm and female cow oocytes. One study found that during the cool season in Louisiana, Holstein heifers produced 67 oocytes on average compared to the hot season when the same cows produced only 28 oocytes on average (8). Even chickens experienced decreased fertility and reproduction due to higher ambient temperatures. Chickens that were inseminated in the morning had a much higher fertilization rate (and experienced lower temperatures) than those that were inseminated in the afternoon during the hottest point of the day (9). Dairy cows that experienced heat stress had decreased estradiol production, decreased viability of granulosa cells, and decreased androstenedione production by thecal cells (7). While cow models suggest that preantral follicles are heat resistant (11), once antral follicles are damaged by heat stress, it takes two or three estrous cycles before oocyte quality returns to normal (21). Our study also showed stronger associations between temperature and AFC across the 3 months prior to assessment than during the 1 month or 2 weeks prior to AFC scan. This pattern supports the notion that heat

exposures that occur throughout the preantral to preovulatory stages of follicular development are influential rather than just the shorter-term exposures in the final stages of antral development.

Strengths of our study include its large sample size (which included multiple AFC and ovarian stimulation outcomes in many women), robust assessment of ovarian reserve, and our comprehensive adjustment for other reproductive and lifestyle factors that enhanced our ability to adjust for confounding. A major limitation of the study was the use of residence-based ambient temperature data as the exposure; this practice could result in measurement error compared to personal temperature measurements. However, to offset this error, the temperature data was collected via a spatially refined, gridded dataset rather than general area averages provided by airport weather stations. Another potential limitation is the lack of racial and ethnic diversity among the cohort. A large majority of the egg donors self-identified at Caucasian (73%) because of this, results from this study may not directly apply to those who are unlike the study cohort. On the other hand, our study was novel in that it included healthy young people with ovaries that had a diverse range of socioeconomic statuses. This is in contrast to a fertility clinic population that tends to include more older women, with preexisting infertility diagnoses, and with greater access to healthcare. Additionally, the data only includes individuals who resided within the Southeast United States. It is possible that regional differences in infrastructure, individual practices, and weather produce differing impacts of temperature exposure on fertility. Finally, as this was an observation study, there is a possibility of residual confounding that was not accounted for in the model.

In conclusion, our study shows that exposure to ambient temperature was not strongly associated with markers of ovarian reserve and outcomes of ovarian stimulation in our population of young,

healthy oocyte donors. Given the continued warming of our planet due to anthropogenic climate change, further research on the associations between temperature and markers of human fertility are warranted, particularly in diverse populations across the globe.

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