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Age at Menarche in Relation to Oocyte Quality and Ovarian Reserve

In Young, Healthy Oocyte Donors

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Abstract

Age at menarche in relation to oocyte quality and ovarian reserve in young, healthy oocyte donors

By Chengcheng Hu

Introduction. Infertility, defined as the failure to achieve pregnancy after 12 months or more of trying, affects approximately 19% of heterosexual, reproductive-aged women in the United States. Assisted reproductive technologies (ART) such as in vitro fertilization (IVF) are used to address infertility, but live birth rates remain low. The relationship between age at menarche and oocyte quality and ovarian reserve in oocyte donors has not been extensively investigated.

Objective. This study aimed to explore the associations of oocyte donors' age at menarche with ovarian reserve and oocyte quality to better understand potential early-life reproductive factors affecting young-adult fertility parameters.

Methods. A retrospective cohort study was conducted using data from 597 non-identified vitrified oocyte donors who underwent 973 oocyte retrieval cycles at Reproductive Biology Associates (RBA) in Atlanta, GA, USA. Age at menarche (in years) was self-reported and ovarian reserve and oocyte quality were assessed through anti-Müllerian hormone (AMH) levels, antral follicle count (AFC), and the number of total and mature oocytes retrieved per cycle. Generalized estimating equations with Poisson distribution, log link, and robust standard errors were used to estimate the associations of age at menarche with AFC and oocyte counts adjusted for age, BMI, race, year, and education.

Results. The median age of donors was 25 years and 27% were racial/ethnic minorities. There were no statistically significant associations of age at menarche with ovarian reserve and oocyte quality markers, including AMH and AFC, or the number of total or mature oocytes retrieved. For instance, the adjusted AFC was 39.4, 39.2, 38.0, and 38.5 for women with an age at menarche of ≤11, 12, 13, or ≥14 years (p-trend=0.87). Similarly, the adjusted number of mature oocytes retrieved was 25.4, 26.4, 25.3, and 24.9 among women with an age at menarche of ≤11, 12, 13, or ≥14 years (p-trend=0.64).

Conclusion. In our large cohort of young, healthy oocytes donors, age at menarche was not significantly associated with oocyte quality or ovarian reserve.

Age at menarche in relation to oocyte quality and ovarian reserve in young, healthy oocyte donors

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Introduction

Infertility, defined as the failure to achieve pregnancy after 12 months or more of trying, is a prevalent issue affecting approximately 19% of heterosexual women aged 15-49 years with no previous births in the United States, according to the Centers for Disease Control and Prevention (CDC). The increasing demand for assisted reproductive technologies (ART), such as in vitro fertilization (IVF), reflects the growing need to address infertility and help individuals and couples achieve their family-building goals. Data from the CDC's 2020 Fertility Clinic Success Rates Report indicate that 326,468 ART cycles were performed in 449 reporting clinics across the United States, resulting in 75,023 live birth deliveries and 79,942 live-born infants [1]. Despite the extensive use of ART, the live birth rate remains relatively low, warranting further investigation into the underlying causes of this phenomenon.

Oocyte quality and ovarian reserve play crucial roles in determining ART success [2, 3], and a range of factors, including age, lifestyle, and genetics, can influence these outcomes [4-6]. Several previous studies have observed a link between age at menarche and time to pregnancy [7, 8], suggesting that it may be associated with other markers of female fertility such as ovarian reserve or oocyte quality. Furthermore, a Mendelian randomization analysis from the UK Biobank also identified several potential causal relationships between reproductive factors, including the age at menarche and several fertility endpoints, suggesting a genetic correlation between the two [9].

Thus, the primary aim of our study was to elucidate the associations between age at menarche and ovarian reserve and oocyte quality. Gaining insight into this relationship could provide valuable information on the potential impact of early-life reproductive factors on later-life fertility and may contribute to a better understanding of the underlying biological mechanisms connecting age at menarche with fertility outcomes, potentially guiding future research in reproductive health. [10].

Materials and Methods

Study Design.

This retrospective cohort study utilized data on women undergoing non-identified vitrified oocyte donation between 2008 and 2022 at Reproductive Biology Associates (RBA) in Atlanta, GA, USA. The study included all ovarian stimulation cycles where all oocytes from a donor were cryopreserved through vitrification for storage in an oocyte bank and subsequently thawed in separate cohorts for recipient use. Initially, data was abstracted on 662 oocyte donors who underwent a total of 1082 retrieval cycles. Retrievals lacking information on year and cycles prior to 2008 were excluded (n=17 donors and 35 retrievals), as were cycles employing Lupron protocols for ovarian stimulation (n=5 donors and 7 retrievals). After further exclusion of retrieval cycles with missing data on key outcomes (number of total oocytes retrieved, n=5 donors and 7 retrievals, and number of mature oocytes retrieved, n=11 donors and 18 retrievals), as well as missing data on age at menarche (n=27 donors and 42 retrievals), 597 oocyte donors who underwent a total of 973

oocyte retrieval cycles remained in the study. The Emory Institutional Review Board granted ethical approval for this study before initiation (IRB00080463).

Data Collection.

Study data were collected through medical record review by trained research assistants. All data were stored in a REDCap electronic database hosted at Emory University [11]. Oocyte donors were screened in accordance with clinic protocol per ASRM recommendations[11]. Data collected on the donors included demographics (e.g., age, race/ethnicity, education, and smoking status) and reproductive history (e.g., gravidity/parity), which were self-reported at the time of their first retrieval. At the donor's first clinic visit, height and weight were measured with a standardized scale and stadiometer. Body mass index was calculated by dividing weight (in kg) by height (in meters) squared. Age at menarche was self-reported in discrete years.

For each retrieval, ovarian reserve data and ovarian stimulation data were collected [12]. 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 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 [13]. Mature oocytes were vitrified within 39-40 hours of trigger using a standard protocol. Additional information on ovarian stimulation parameters, including gonadotrophin dose, number of days of stimulation, number of large follicles (>14 mm) at trigger shot, peak estradiol levels, and trigger type were also abstracted.

Statistical Analysis.

Demographic, reproductive history, and ovarian stimulation parameters of oocyte donors at the time of their first retrieval were compared based on self-reported age at menarche, utilizing the following categories: ≤11 years, 12 years, 13 years, and ≥14 years. These categories were selected according to the distribution of participants in our cohort. To evaluate differences across groups, chi-square tests were employed for categorical variables, and Analysis of Variance (ANOVA) was utilized for continuous variables.

Primary outcomes encompassed baseline anti-Müllerian hormone (AMH) levels and antral follicle count as well as total count of oocytes retrieved and the quantity of mature oocytes retrieved. AMH was not routinely measured before 2012, which resulted in 201 donors (296 cycles) lacking an AMH value. Baseline AFC was missing in 38 donors (62 cycles) and were not included in these analyses. Since donors could contribute more than one oocyte retrieval cycle to the analysis, all models took repeated observations into account. The relationship between age at menarche and the continuous variable AMH was examined using generalized estimating equations (GEE), employing normal distribution and identity

link function. For the discrete outcomes, such as AFC, the total number of oocytes, and the number of mature oocytes, GEE models with Poisson distribution, log link function, and robust standard errors were utilized. The analysis of donor age at menarche was conducted as both continuous and categorical variable. Data are presented as the crude and adjusted mean of each outcome across the four age at menarche categories at the mean level of continuous covariates and the most common level of categorical covariates. Linear trend tests were performed using the median values of each age at menarche category as a continuous variable.

Potential confounders were evaluated using prior knowledge and descriptive statistics from the cohort through the use of directed acyclic graphs (DAGs). Covariates retained in the final multivariable models included donor age, BMI, race (Northern European, African-American, Hispanic or Latino, Asian, or Other), retrieval year (2008–2011, 2012-2014, 2015-2017, 2018-2020), and education (Completed high school, GED, or some college; Completed technical school or 2-year college degree; Currently in college; Completed 4-year college degree; Pursuing or completed an advanced degree). Missing data on covariates was rare (<5%); however, in these instances single imputation was performed where the mean value or the most common category was used. All tests of statistical significance were two-sided, and a significance level of $P < 0.05$ was used. Data analysis was performed using SAS 9.4 (SAS Institute Inc, Cary, NC, USA).

Results

Demographic Characteristics

The study involved 597 non-identified vitrified oocyte donors who underwent one to six oocyte retrieval cycles (total number of cycles was 973). The majority of donors completed one cycle (n=389, 65%), while only three donors completed six cycles. The median age of the donors was 25 years old and the median BMI was 22.5 kg/m^2. Most of the donors were White (73%) and had a 4-year college degree as their highest level of education (32%). The median age at menarche was 13 years (Range: 9 to 18 years) with 105 (18%) reporting an age at menarche ≤11 years and 155 (26%) reporting an age at menarche ≥14 years. None of the demographic or reproductive characteristics of donors varied across age at menarche categories (**Table 1**). Donors with the youngest age at menarche (≤11 yrs) were more likely to have fewer (8-9 days) and more (11-12 days) days of stimulation compared to the other age at menarche groups.

Ovarian Reserve

The median AMH value in our donors was 4.8 ng/mL (IQR: 3.3 to 4.8 ng/mL) and the median AFC was 36 (IQR: 29 to 46). There was no statistically significant association between donor's age at menarche and markers of ovarian reserve, including AMH and AFC (**Table 2**). For example, the adjusted geometric mean of AMH for each age at menarche group was 5.0, 5.1, 4.9, and 5.3 ng/mL in donors with an age at menarche of ≤11, 12, 13, or ≥14 years (p-trend=0.58). The adjusted AFC by age at menarche group was 39.4, 39.2, 38.0, and 38.5 for women with an age at menarche of ≤11 , 12, 13, or ≥14 years (p-trend=0.87).

Outcomes of Controlled Ovarian Stimulation

The median total oocytes retrieved per cycle in our donors was 32 (IQR: 24 to 41) and the median mature oocytes was 24 (IQR: 18 to 31). There was also no statistically significant association between donor's age at menarche and outcomes of controlled ovarian stimulation (**Table 3**). The adjusted mean number of total oocytes retrieved across categories of age at menarche was 35.6, 38.3, 37.2, and 36.1 for women whose first menarche was at ≤11, 12, 13, or ≥14 years (p-trend=0.82). Similarly, the adjusted number of mature oocytes retrieved was 25.4, 26.4, 25.3, and 24.9 among women with an age at menarche of ≤11, 12, 13, or ≥14 years (p-trend=0.64).

Discussion

In our large cohort of healthy, young oocyte donors, we observed limited evidence for a relation between age at menarche and ovarian reserve and oocyte quality.

Our null results on age at menarche and ovarian reserve are congruent with one but not the majority of previous investigations on this topic. The most similar study to date was conducted by Bragg and colleagues and focused on the relation between age at menarche and AMH in 294 young Filipina women (average age 21 years) [14]. The authors found that after accounting for age, BMI, smoking status, and parity, a one-year increase in age at menarche was associated with a ~12% of a SD reduction in AMH. Jung et al. conducted a cross-sectional study among late premenopausal women (median age 40 years) that investigated the relation of age at menarche with AMH [15]. These authors observed that

women who entered menarche at <12 years had a lower AMH concentration (0.90 ng/mL) than women with an age at menarche ≥14 year (1.12 ng/mL) [15]. Another investigation into this topic by Weghofer et al. focused on 502 women experiencing infertility (mean age 39 years) and evaluated the association between age at menarche and diminished functional ovarian reserve (DFOR), defined as abnormally low age-specific AMH levels [16]. These authors found that a diagnosis of DFOR occurred in 63% of women with an early age at menarche (<13 years) compared with 51% of women with a later age at menarche (≥13 years). Finally, similar to our findings, a cross-sectional study conducted by Dólleman and colleagues among 2,320 premenopausal Dutch women (median age 37 years), found no association between the age of menarche and AMH levels (p=0.09) [17].

There are several factors such as the age and race/ethnicity of study population, sample size, geographical location, and variation in menarche age that may explain the discrepancies between study findings. First and foremost, the two previous studies that found a positive association between later age at menarche and higher ovarian reserve were among older premenopausal women. Given the large difference in average participant age, there was also a notable difference in the median AMH levels, with a relatively low value of 1 ng/mL in the Jung et al. study compared to 4.8 ng/mL in our investigation. Similarly, none of the women in our study would have been classified as having DFOR compared to over half of the women in the Weghofer et al. study. If age at menarche has a stronger impact on ovarian reserve during the later reproductive years, when it starts to markedly decline, then this could be one reason why we failed to observe an association in

our study. Second, while the Bragg et al. study was similar to ours in terms of average age and AMH level of women, all of the study participants were from the Philippines whereas very few of our women were of Asian ancestry. If there are differences in the association between age at menarche and ovarian reserve by race (as a social or biological construct) then this could be a reason for our divergent findings.

While there may be no true association between age at menarche and markers of ovarian reserve and oocyte quality, there are several other explanations that may potentially account for the lack of significant findings in our study. First, all of the women in our study were young and healthy with no evidence of infertility. If age at menarche influences fertility through any of these pathways, which served as exclusion criteria for being an oocyte donor – for example, the development of gynecological conditions such as PCOS or endometriosis or high body mass index – then this would have biased our results towards the null [18, 19] [20, 21]. Additionally, because of our donor's young age, the average AMH in our study was high and may have less direct clinical utility for predicting ovarian reserve or fertility as existing evidence often uses a cut-off point below 1 ng/mL [22].

Second, our sample size might not have been large enough to detect a small but clinically relevant effect size, particularly for the outcome of AMH, which was only routinely measured after 2012. Third, the measures used to assess ovarian reserve and oocyte quality might not have been sensitive enough to detect subtle variations related to age at menarche. We selected AMH and AFC as markers of ovarian reserve based on several recent

studies supporting their use for predicting ovarian reserve with varying degrees of precision[13] [23]. However, the lack of an international standard for AMH limits the comparison between AMH assays, and little is known about endogenous and exogenous factors that influence serum AMH levels, limiting the proper interpretation of AMH values in a clinical setting.[24] We considered the number of oocytes retrieved, especially the number of mature oocytes retrieved, as a measurement of oocyte quality. As oocyte competence is well defined as the ability of the oocyte to complete maturation and undergo successful fertilization, poor oocyte quality might be clinically represented by a lower number of MII oocytes retrieved [18] [25, 26]; however, it is possible that this was not the best metric to assess oocyte quality. Finally, because this was an observational study, it is possible that other unmeasured confounding factors or effect modifiers might have influenced the results, masking any true associations between the variables.

Despite these limitations, our study has several strengths, such as the use of a well-characterized cohort of oocyte donors without common infertility diagnoses. Most existing studies focus on older, subfertile women, which may not be as applicable to relatively young and healthy women. We were also able to include multiple markers of ovarian function including AMH, AFC, and oocyte counts, which is well beyond what previous studies were able to evaluate. We also adjusted for several potential confounders, including age and race, which are known to be related to oocyte quality and ovarian reserve [27, 28]. In addition, we adjusted for the year of retrieval, considering that the number of oocytes retrieved can be affected by oocyte retrieval techniques and simulation protocols, which have changed

over the years of our study [29, 30]. Education was also adjusted for as a proxy for socioeconomic status, which may affect external factors such as maternal nutrition, stress, and environmental exposure that are related to oocyte quality and ovarian reserve [5]

To further explore the relationship between age at menarche and oocyte quality and ovarian reserve, future research should address the limitations of our study by employing larger sample sizes, more sensitive measures of oocyte quality and ovarian reserve. The ultimate aim of our study was to assess the relationship between age at menarche and ART pregnancy outcomes. Future studies could investigate live birth rates, miscarriage rates, and other pregnancy-related outcomes to provide a more comprehensive understanding of how age at menarche influences the success of ART treatments. This information could potentially inform clinical decision-making, donor selection, and counseling for patients undergoing assisted reproductive procedures, ultimately contributing to improved reproductive health outcomes for individuals and couples seeking to build their families through ART.

Conclusion

In conclusion, our study found no associations between age at menarche and ovarian reserve and oocyte quality among young, healthy oocyte donors. While our results do not support a strong relationship between age at menarche and ovarian function in young women, further research is needed to clarify the potential influence of early-life

reproductive factors on fertility outcomes and to guide the optimization of ART and donor selection strategies.

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Table 1: Characteristics of oocyte donors by age at menarche, 2008-2020

		Age at Menarche, years				
	Total	⩽11	12	13	⩾14	p-value
Number of women	597	105	162	175	155	
Age at first retrieval (yr)						
21-23	195(32.7)	32(30.5)	58(35.8)	57(32.6)	48(31.0)	0.98
24-26	190(31.8)	35(33.3)	50(30.9)	55(31.4)	50(32.3)	
27-29	166(27.8)	28(26.7)	41(25.3)	51(29.1)	46(29.7)	
30-32	46(7.7)	10(9.5)	13(8.0)	12(6.9)	11(7.1)	
Year of retrieval						
2008-2011	184(30.8)	26(24.8)	56(34.6)	56(32.0)	46(29.7)	0.45
2012-2014	144(24.1)	21(20.0)	40(24.7)	45(25.7)	38(24.5)	
2015-2017	175(29.3)	42(40.0)	43(26.5)	45(25.7)	45(29.0)	
2018-2020	94(15.8)	16(15.2)	23(14.2)	29(16.6)	26(16.8)	
Race/Ethnicity*						
Northern European	434(72.7)	66(64.1)	115(71.4)	139(80.4)	114(74.5)	0.17
African-American	65(10.9)	12(11.7)	20(12.4)	14(8.1)	19(12.4)	
Hispanic or Latino	26(4.4)	8(7.8)	6(3.7)	5(2.9)	7(4.6)	
Asian	29(4.9)	8(7.8)	12(7.5)	5(2.9)	4(2.6)	
Other	36(6.0)	9(8.7)	8(5.0)	10(5.8)	9(5.9)	
BMI (kg/m^2)*						
⩽21.0	167(28.0)	24(23.1)	43(26.5)	55(31.4)	45(29.0)	0.70
21.1-24.9	316(52.9)	59(56.7)	89(54.9)	84(48.0)	84(54.2)	
⩾25.0	113(18.9)	21(20.2)	30(18.5)	36(20.6)	26(16.8)	
Donor Education*						
Completed high school, GED, or some college	49(8.3)	6(5.7)	13(8.2)	12(7.0)	18(11.7)	0.17
Completed technical school or 2-year college degree	67(11.4)	13(12.4)	16(10.1)	21(12.3)	17(11.0)	
Currently in college	160(27.2)	36(34.3)	44(27.9)	42(24.6)	38(24.7)	
Completed 4-year college degree	188(32.0)	22(21.0)	57(36.1)	64(37.4)	45(29.2)	
Pursuing or completed an advanced degree	124(21.1)	28(26.7)	28(17.7)	32(18.7)	36(23.4)	
Donor Smoking Status*						
Never smoker	539(90.7)	97(92.4)	149(92.6)	158(90.3)	135(88.2)	0.54
Former or current smoker	55(9.3)	8(7.6)	12(7.5)	17(9.7)	18(11.8)	
Gonadotropin dose (IU)						
⩽2,500	331(55.4)	61(58.1)	93(57.4)	90(51.4)	87(56.1)	0.63
>2,500	266(44.6)	44(41.9)	69(42.6)	85(48.6)	68(43.9)	
Days of stimulation*						
8-9	108(18.1)	27(25.7)	34(21.1)	19(10.9)	28(18.3)	0.03
10-11	384(64.3)	57(54.3)	105(65.2)	123(70.3)	99(64.7)	
11-12	102(17.1)	21(20.0)	22(13.7)	33(18.9)	26(17.0)	
Number of follicles >14mm at trigger						
<12	43(7.2)	8(7.6)	14(8.6)	12(6.9)	9(5.8)	0.51
13-24	342(57.3)	57(54.3)	95(58.6)	106(60.6)	84(54.2)	
25-40	191(32.0)	38(36.2)	46(28.4)	54(30.9)	53(34.2)	
41-55	21(3.5)	2(1.9)	7(4.3)	3(1.7)	9(5.8)	
Peak estradiol (pg/mL)						
<2,000	124(20.8)	14(13.3)	37(22.8)	34(19.4)	39(25.2)	0.10
2,001-4,500	242(40.5)	41(39.1)	65(40.1)	81(46.3)	55(35.5)	
4,501-6,000	106(17.8)	20(19.1)	23(14.2)	33(18.9)	30(19.4)	

>6,000	125(20.9)	30(28.6)	37(22.8)	27(15.4)	31(20.0)	
Maturation trigger type*						
hCG	124(20.8)	17(16.5)	39(24.2)	37(21.6)	31(20.0)	0.49
GnRH Agonist (Lupron)	466(78.1)	86(83.5)	122(75.8)	134(78.4)	124(80.0)	

*Variables with missing data: race/ethnicity (n=7), BMI (n=1), donor education (n=9), donor smoking status (n=3), days of stimulation (n=3), Maturation trigger type (n=7).

Table 2: Association between donor age at menarche and markers of ovarian reserve.

	Anti-Mullerian Hormone (ng/mL)				Antral Follicle Count			
Donor Age at Menarche	**No. of Women (n=396)**	**No. of Cycles (n=677)**	**Unadjusted Mean (95% CI)**	**Adjusted Mean (95% CI)***	**No. of Women (n=559)**	**No. of Cycles (n=911)**	**Unadjusted Mean (95% CI)**	**Adjusted Mean (95% CI)***
≤11 yrs	75	135	4.8 (4.1,5.5)	4.7 (4.0,5.6)	95	156	39.4 (36.8,42.3)	41.1 (37.2,45.5)
12 yrs	100	182	4.9 (4.3,5.4)	4.9 (4.2,5.8)	152	259	39.2 (37.1,41.4)	41.5 (37.8,45.5)
13 yrs	115	178	4.6 (4.2,5.1)	4.8 (4.2,5.4)	168	254	38.0 (36.2,39.8)	40.6 (37.3,44.2)
≥14 yrs	106	182	4.9 (4.5,5.3)	5.0 (4.4,5.7)	144	242	38.5 (36.7,40.5)	41.1 (37.8,44.8)
P-trend			0.89	0.58			0.45	0.87

*Adjusted for: race, age at retrieval, BMI, year of retrieval, and education.

Table 3: Association between donor age at menarche and outcomes of controlled ovarian stimulation.

	Total Oocytes Retrieved				Mature Oocytes Retrieved			
Donor Age at Menarche	**No. of Women**	**No. of Cycles**	**Unadjusted Mean (95% CI)**	**Adjusted Mean (95% CI)***	**No. of Women**	**No. of Cycles**	**Unadjusted Mean (95% CI)**	**Adjusted Mean (95% CI)***
≤11 yrs	105	173	33.8 (31.9,35.8)	34.7 (31.8,37.9)	104	172	25.4 (23.8,27.1)	26.6 (24.2,29.3)
12 yrs	162	276	35.5 (33.3,37.9)	37.5 (34.2,41.2)	162	276	26.4 (24.7,28.2)	28.3(25.4,31.6)
13 yrs	175	263	34.1 (32.2,36.2)	36.7 (33.8,39.8)	175	263	25.3 (23.7,27.0)	27.5(25.1,30.2)
≥14 yrs	155	261	33.4 (31.1,35.9)	35.2 (32.0,38.7)	155	261	24.9 (23.2,26.7)	26.7(24.1,29.5)
p-trend			0.48	0.82			0.39	0.64

*Adjusted for: race, age at retrieval, BMI, year of retrieval, and education.