ORIGINAL ARTICLES

The Significance of Low Anti-Müllerian Hormone Levels in Young Women Undergoing in Vitro Fertilization

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OBJECTIVE: To determine if young women (aged ≤35 years) with low anti-Müllerian hormone (AMH) levels undergoing their first in vitro fertilization (IVF) cycle have lower pregnancy rates as compared to young women with normal AMH levels.

STUDY DESIGN: Retrospective cohort study.

RESULTS: Thirty-two women with an AMH <1 ng/mL and 130 with an AMH ≥1 ng/mL met study criteria. Patients with AMH <1 ng/mL had higher average FSH levels (8.1 mIU/mL vs. 6.5 mIU/mL) and were slightly older (31.6 vs. 30.4 years). Both groups had comparable numbers of embryos transferred (AMH <1, 1.5±0.6 vs. AMH ≥1, 1.3±0.5). Clinical pregnancy rates per embryo transfer were higher in women with AMH ≥1 ng/mL (47.6% vs. 21.9%). A sensitivity analysis demonstrated lower clinical pregnancy rates in those with AMH <1 ng/mL when excluding those patients with abnormal day 2 FSH or estradiol.

CONCLUSION: AMH levels <1 ng/mL in women ≤35 years old appear to predict lower clinical pregnancy rates in women undergoing IVF, even in the setting of normal day 2-3 ovarian reserve testing. Providers may consider transferring 2 embryos in women ≤35 years with low AMH values. AMH may be used as a sole measure of ovarian reserve in young women if significantly low. (J Reprod Med 2018;63:97–103)

At this point, we would recommend counseling young patients with low AMH levels (<1 ng/mL) to move forward with more aggressive fertility treatments.

Keywords: anti-Mullerian hormone, antimullerian hormone, assisted reproductive techniques, assisted reproductive technologies, diminished ovarian reserve, in vitro fertilization.

The need for in vitro fertilization (IVF) has risen in recent years, in part due to delayed childbearing for personal or professional reasons.[1] Success of IVF depends on several factors, including age and

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Dr. Szabo's work is funded through a Grant from the National Center for Advancing Translational Sciences at the National Institutes of Health (specifically, grant no. 8UL1TR000055 supported the statistical analysis).

Presented as an oral presentation at the American Society for Reproductive Medicine Annual Meeting, Baltimore, Maryland, October 17–21, 2015.

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Financial Disclosure: The authors have no connection to any companies or products mentioned in this article.

0024-7758/18/6303-04–0097/$18.00/0

a woman's ovarian reserve, defined as the number and quality of the remaining ovarian follicles and their corresponding oocytes.[2] Anti-Müllerian (AMH) is a protein secreted by granulosa cells in small antral and preantral follicles and is thought to influence the growth and maturation of the primordial follicles of the ovary, reflecting the remaining ovarian oocyte pool.[3,4]

A review by La Marca et al demonstrated a positive correlation between AMH and the number of oocytes retrieved during controlled ovarian hyperstimulation, making AMH a strong marker for quantitative ovarian response during IVF treatment.[5-8] As chromosomal abnormalities increase with age, oocyte quality decreases and can negatively impact IVF outcome. While some studies have found that the association with AMH and IVF outcome is independent of age, the results are inconsistent.[9,10] In a study by Wang et al the correlation between AMH and IVF outcomes demonstrate a weaker correlation at the extremes of maternal age, presumably due to the impact of oocyte quality on IVF outcome.[11] In one study, women <42 years with extremely low serum AMH levels still demonstrated reasonable pregnancy and live birth rates.[12] The observation that the link between AMH and IVF outcome wanes in the extremes of maternal age would suggest that AMH is a quantitative test of ovarian reserve and does not adequately predict oocyte quality.[13,14] However, in an abstract in 2013 Sherbahn et al examined pregnancy rates in young women with diminished ovarian reserve and found lower pregnancy rates in patients with low AMH who underwent IVF cycles.[15]

With the increased utilization of AMH in the evaluation of the infertile patient, providers are in need of data with which to counsel young patients with low AMH levels. The true clinical significance of a low AMH level in a young patient is not clear. Our hypothesis is that patients <35 years old with low AMH levels typically seen in patients of advanced maternal age (>35 years) will have comparable pregnancy and implantation rates compared to young women with normal AMH levels.

Materials and Methods

Inclusion Criteria

The Medical College of Wisconsin Institutional Review Board approved our study. Data from IVF cycles completed at the Reproductive Medicine Center in Milwaukee, Wisconsin, were evaluated via retrospective chart review from January 2010 to May 2014, as AMH was only first routinely utilized in the clinic as of January of 2010. Inclusion criteria included all women presenting to the clinic who had an AMH level assessed within the previous 12 months before their IVF cycle start and who were undergoing their first cycle of IVF. Exclusion criteria included any woman who had only 1 ovary, any Fragile X carrier, any history of possible iatrogenic damage to the ovary including radiation therapy and/or chemotherapy, and patients who were not planning to have a fresh embryo transfer (i.e., fertility preservation for cancer, patients undergoing preimplantation genetic screening or diagnosis, cycles canceled prior to embryo transfer due to poor response or other factors).

Specimen Handling and Laboratory Analysis

All blood specimens collected for AMH measurements were drawn at the Dynacare Laboratory (Milwaukee, Wisconsin), where serum was immediately frozen to –20°C and then shipped to the Esoterix, Inc. testing laboratory where they could be processed. Samples were batched and testing was performed once weekly at this outside facility utilizing the Generation I Diagnostic System laboratories (DSL) ELISA (Beckman Coulter, Inc.). The DSL immunoassay was run per instrument protocol. The lowest detectable level of AMH was 0.16 ng/mL. Because of the infrequency of samples found in this range, all low levels that were ≤0.16 ng/mL were reported as such. Intraassay and interassay variability reported by the Esoterix lab was 6–9%.

Study Protocols

Regimens utilized during the study included either a "short" or a "long" protocol of downregulation with a GnRH agonist followed by stimulation via gonadotropins or stimulation with gonadotropins followed by pituitary suppression with a GnRH antagonist.

Stimulation proceeded until at least 2 leading follicles reached 17–18 mm in size with appropriate estradiol levels. Discussion of risks or continuation versus cycle cancellation occurred with any of the following: poor response with small follicle size and/or number with fewer than 3 follicles or with low estradiol levels (<500 pg/mL) or excessive response resulting in signs and symptoms concerning for ovarian hyperstimulation syndrome (OHSS).

Ovulation was triggered via hCG. For GnRH antagonist protocols in which patients were deemed to be at high risk of OHSS, ovulation was triggered

by 2 mg leuprolide acetate administered with low dose (1,500 IU) hCG or leuprolide acetate alone.

Under conscious sedation, oocyte retrieval was performed 34–36 hours after hCG administration via transvaginal ultrasound-guided needle aspiration. The oocytes were then fertilized using either intracytoplasmic semen injection (ICSI) or by microinsemination, depending upon patient preference, physician discretion, and/or male factor. For luteal support, patients started either daily intramuscular progesterone (at 50 ng/mL) or vaginal gel the day after oocyte retrieval. In cases where leuprolide acetate and low-dose hCG trigger were used, the luteal phase was supported with 2 mg oral estradiol twice daily and one of the progesterone preparations. In cases at high risk for OHSS no luteal support was given and all embryos were cryopreserved.

Embryo transfer was performed 3–5 days later depending upon number of embryos and embryo development. Our departmental policy is to perform a day 5 transfer if there are at least 5 normally fertilized embryos that develop well. Embryo transfer was performed by 1 of 3 different attending physicians, and all were done under ultrasound guidance utilizing either the Sydney catheter or SureView Wallace catheter. Pregnancy tests for β-hCG levels were performed 14 days after oocyte retrieval.

Statistics

Our null hypothesis is that patients <35 years old with low AMH levels typically seen in patients of advanced maternal age (>35 years) will have comparable pregnancy and implantation rates as compared to young women with normal AMH levels. Because there is not a plethora of data on the impact of low AMH values on pregnancy outcomes in women under 35 years of age, for power calculations we used pregnancy rates from a population with comparable AMH levels in which there are some data, that is, women of advanced maternal age.

Women with AMH levels <1.0 ng/mL are considered to have diminished ovarian reserve.[16] Based upon a large study evaluating mean AMH levels among women presenting to an infertility clinic, women aged ≥42 years had mean AMH levels <1 ng/mL.[17]

The power calculations were based on frequencies published by the Society for Assisted Reproductive Technology (SART) 2012 clinic summary report.[18] In 2012 women aged 41–42, who typically have AMH levels <1 ng/mL, had a clinical pregnancy rate of 19.8%, and women under age 35 had a clinical pregnancy rate of 46.7%. The prevalence of the diagnosis of diminished ovarian reserve was 17%. To detect a difference in anticipated pregnancy rates of 20% vs. 45% between young women with low and normal AMH levels, we needed a total of 170 cycles (34 cycles with low AMH and 136 cycles with normal AMH), for a power of 80% and an α of <0.05.

Patient demographic information, including age, race, and primary diagnosis, was summarized overall and stratified based on AMH levels. Wilcoxon's rank-sum test was used to compare continuous characteristics, and Pearson's χ^2 test was used for discrete variables, including the primary outcome (clinical pregnancy rates), between AMH level groups. A p value <0.05 was considered statistically significant. Logistic regression was used for a multivariate analysis adjusted for age, day 3 FSH, day 3 estradiol, mode of ovulation trigger (hCG versus Lupron plus hCG), day 3 versus day 5 transfer, number of embryos transferred, and BMI. The linearity of the effect of continuous predictors was evaluated using a cubic spline transformation. For the implantation rate (number of gestational sacs as a proportion of the number of embryos transferred) overdispersed logistic regression via quasi-likelihood was used. A subgroup sensitivity analysis was then performed by removing those patients with day 3 FSH >10 mIU/mL or estradiol >70 pg/mL in order to assess the sole predictive value of AMH.[19-22] All statistical analyses were performed using R 3.1.2. (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 201 subjects who completed IVF at the Reproductive Medicine Center from January 2010 to May of 2014 were included in the analysis. As seen in Figure 1, after screening data for exclusion criteria, a total of 32 subjects with AMH <1 ng/mL and a total of 130 subjects with AMH ≥1 ng/mL were included in the data analysis. Secondary to either data located outside of the EPIC system, or due to ongoing pregnancy, live-birth outcome data were missing for 39 subjects, 3 of whom had AMH <1 ng/mL and 35 of whom had AMH ≥1 ng/mL.

Table I displays the demographics of the study population, stratified by AMH level. In those subjects with AMH ≥1, nearly half were male factor as

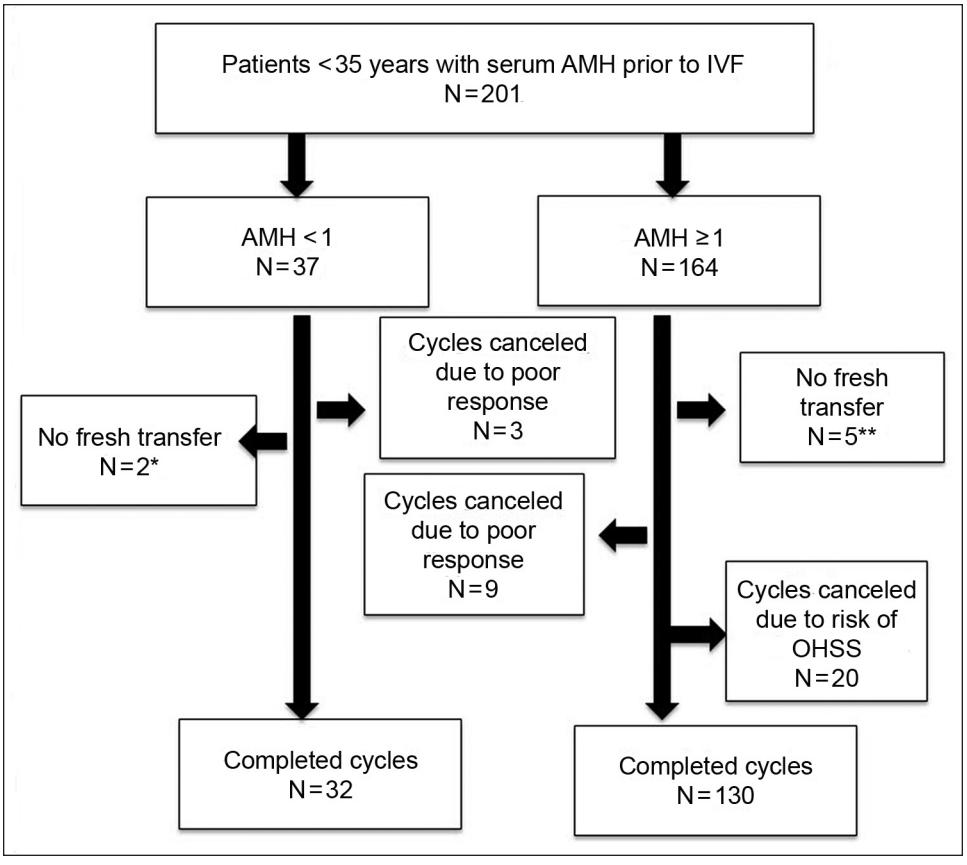

Figure 1
Final data analysis subject numbers after screening for exclusion criteria, stratified by AMH <1 ng/mL and ≥1 ng/mL.
*One subject requested a frozen embryo transfer, and the other subject had a planned frozen embryo transfer for preimplantation genetic diagnosis.
**Reasons include: planned preimplantation genetic diagnosis, frozen embryo transfer by request and for a gestational carrier, and 2 subjects who had an endometrial polyp seen on ultrasound during retrieval.

primary diagnosis, as compared to <15% of those in the AMH <1 group (p<0.0001). Patients with AMH <1 ng/mL and ≥1 ng/mL were similar in terms of percent nulliparity (84.4% and 85.2%, respectively) and mean BMI (25.9 vs. 26). Table II shows the stimulation parameters for each group, stratified by those with AMH <1 ng/mL and ≥1 ng/mL. A higher percentage of patients in the low AMH group were treated with antagonist protocols as compared to the AMH >1 group (83.9% vs. 63.5%, p=0.05).

The mean number of embryos transferred was comparable between those with AMH ≥1 ng/mL and AMH <1 ng/mL (1.3±0.5 and 1.5±0.6, respectively). The low AMH group had a near-equal dis-

Table I *Mean Population Demographics*

	AMH <1 ng/mL	AMH ≥1 ng/mL	p Value
Age (yrs)	31.6 (±2.2)	30.4 (±2.3)	0.010
AMH (ng/mL)	0.6 (±0.2)	4.0 (±2.7)	0.01
BMI (kg/m^2)	25.9 (±4.8)	26 (±5.5)	0.88
Day 2 FSH (mIU/mL)	8.1 (±2.2)	6.5 (±1.6)	0.0003
Day 2 estradiol (pg/mL)	44.6 (±19.7)	39.7 (±22.1)	0.057

Values shown as mean (±SD). p Values are from Wilcoxon rank sum test.

Table II *Mean Stimulation Parameters*

	AMH <1 ng/mL	AMH ≥1 ng/mL	p Value
Days of stimulation	10.6 (±1.6)	10.0 (±1.6)	0.04
Ampules of gonadotropins	47.0 (±22.1)	29.3 (±13.2)	<0.0001
Oocytes retrieved	7.9 (±3.8)	13.6 (±5.3)	<0.0001
Mature oocytes retrieved	6.0 (±3.5)	10.5 (±4.4)	<0.0001
No. of embryos created	4.6 (±2.7)	7.8 (±3.9)	<0.0001
No. of embryos transferred	1.5 (±0.6)	1.3 (±0.5)	0.16

Values shown as mean (±SD). p Values are from Wilcoxon rank sum test.

tribution between those with 1 embryo transferred and those with 2–3 embryos transferred (56.2% and 43.8%, respectively). This is compared to those with AMH ≥1 ng/mL, where 68.8% of subjects had only 1 embryo transferred. In those with a normal AMH, there was a trend towards more day 5 transfers (78%), as compared to those with AMH <1 ng/ML, in whom half were day 5 transfers (p=0.003).

Overall, among those who completed an embryo transfer, women with higher AMH levels were more likely to have a positive pregnancy test following IVF (55.9% vs. 31.2%, p=0.022). In addition, clinical pregnancy rates (47.6% vs. 21.9%, p=0.015) were also higher in those women with AMH ≥1 ng/mL. Implantation rates were also higher in patients with higher AMH levels (43.3% vs. 21.9%, p=0.02). Of note, the lowest AMH value at which clinical pregnancy was observed was 0.45 ng/mL.

To determine effect on pregnancy outcomes, a logistic regression was performed adjusting for the following independent variables: AMH, age, day 3 FSH, day 3 estradiol, ovulation trigger (hCG versus Lupron plus hCG), day 3 versus day 5 transfer, number of embryos transferred, and BMI. When controlling for these variables, AMH ≥1 ng/mL was still a significant predictor of implantation rates (OR=2.74, 95% CI 1.11–6.77, p=0.04), positive pregnancy test (OR=2.72, 95% CI 1.04–7.47, p=0.05), and clinical pregnancy (OR=3.22, 95% CI 1.17–9.65, p=0.03). When controlling for all other factors, including AMH level, day of transfer did not affect pregnancy rates but had an effect on implantation rates that was not statistically significant (OR=2.03, 95% CI 1.00–4.11, p=0.06). The effect of BMI was found to be nonlinear, thus it was modeled with a cubic spline. Interestingly, as seen in Figure 2, there appears to be a decrease in probability of pregnancy at the extremes of BMI, regardless of AMH level.

In a per-embryo transfer analysis, on average there were significantly fewer subjects in the AMH <1 ng/mL group who had supernumerary embryos to cryopreserve at the blastocyst stage (46.9% vs. 73.4%, p=0.008). Among those who were able to cryopreserve embryos, there was a trend towards fewer number of supernumerary embryos in those subjects with an AMH <1 ng/ mL; 33.3% of subjects with AMH <1 ng/mL had only 1 embryo, compared to only 18.1% of those with AMH levels ≥1 (p=0.087, referring to the number of supernumerary embryos as a continuous variable).

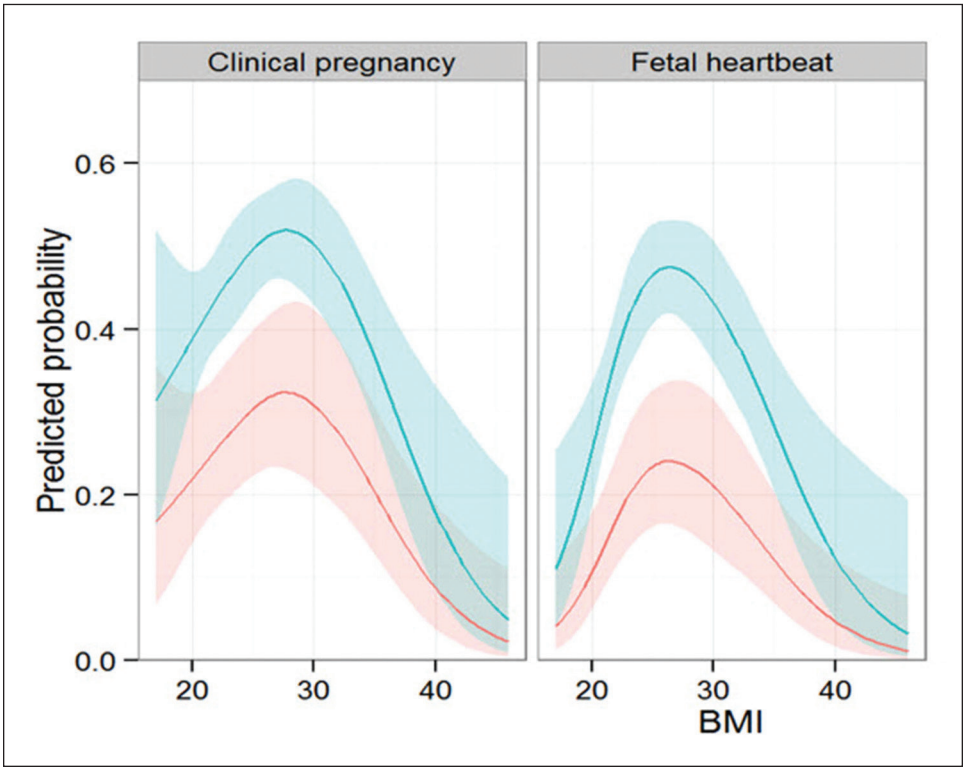

Figure 2
Logistic regression based on predicted probabilities of pregnancy for a patient with average age, FSH, and estradiol, triggered with hCG only on day 5 and 1 transferred embryo, by AMH category and BMI. Bands represent standard errors. Red = AMH <1 ng/mL, Blue = AMH ≥1 ng/mL.

To determine the predictive value of the AMH level alone on pregnancy outcomes in women, a sensitivity analysis was performed removing those subjects with elevated day 3 ovarian reserve testing (FSH >10 mIU/mL or estradiol >70 pg/mL). These findings were consistent with analysis of the full population as there was a higher clinical pregnancy rate in those with an AMH level ≥1 ng/mL (46.8% vs. 20.8%, p=0.035).

Discussion

Our study demonstrates lower pregnancy rates in young women with low AMH levels. Young patients in both AMH stratifications had comparably low numbers of embryos transferred.

While AMH has been shown to be a strong marker for quantitative ovarian response during ovarian hyperstimulation, the utility of AMH as a marker of qualitative ovarian response and of pregnancy outcome has been questioned.[4-7,23-25] Some studies have demonstrated a positive, linear correlation with AMH level and live birth rates; however, this predictability has shown variability at the extremes of maternal age. While Wang et al found only a positive correlation between AMH levels and clinical pregnancy rates in women between the ages of 34–41, our data show a statistically significant difference in clinical pregnancy rates between those with an AMH <1 ng/mL vs. ≥1 ng/mL in women under the age of 35. Additionally, due to an increased number of cryopreserved embryos, patients with AMH >1 were more likely to have additional opportunities to attain pregnancy from a given IVF cycle, thereby increasing the cumulative pregnancy rate relative to those with an AMH <1 ng/mL.[9,10,26]

In a study by Rosen et al (2012) the change with age in ovarian reserve markers was compared to follicle counts observed histologically.[27] Interestingly, while both estradiol and FSH mirrored the known decrease in ovarian reserve seen in the late 30s to early 40s, AMH levels appeared to show a decline earlier, beginning in the early 30s.[28] This early decline in AMH levels was also seen with antral follicle count, a well-established and reliable marker of ovarian reserve.[29] Overall, our data suggest that AMH may be a more sensitive predictor of ovarian reserve in young women. Changes in AMH alone, prior to changes seen in day 3 ovarian reserve testing, may warrant earlier interventions than may previously have been considered.

The most compelling strength of our study is the comparably low number of embryos transferred in both groups, allowing for appropriate comparison. Women were treated with hormonal stimulation by a small number of providers with similar approaches to hormonal stimulation. We included only patients undergoing their first cycle of IVF.

The weaknesses of our study include the retrospective nature of the study as well as the limited data regarding live birth rates. Given the small overall sample size, especially of the low AMH patients, there is a possibility of a Type I error. Additionally, the AMH assay used is not FDA approved and is intended for experimental use. The Esoterix laboratory utilized the Generation I ELISA through mid-2014, when our patient review stopped. Thus, all patients included in our study had AMH levels measured by this ELISA, allowing for consistency in comparison. However, we also acknowledge that the results in our study are from a specific assay that is no longer utilized and that has shown varying results from newer assays.[30,31] Thus, the generalizability of our study may be limited and future studies with the newer assays are warranted.

It is recommended that clinicians consider patient age, history, and embryo morphology when determining the appropriate number of embryos to transfer. Although it is the standard in our practice to recommend single-embryo transfer in young women undergoing a first IVF cycle, based upon the findings in our study, it may be appropriate to consider transferring 2 embryos in IVF cycles in young women with low AMH levels. More research on differences in implantation rates based upon the number of embryos transferred and day of transfer in this patient population is needed to make definitive recommendations.

Our data suggest that for young patients with abnormally low AMH levels, their success rates with IVF are lower, regardless of their day 2-3 FSH and estradiol levels. The decrease in AMH appears to portend a true decline in qualitative and quantitative ovarian reserve. As AMH testing has become widely clinically available within the past decade, it is important to have appropriate data with which to counsel patients. An abnormally low AMH in the setting of normal day 2-3 FSH and estradiol appears to be clinically significant, thus demonstrating the utility of evaluating AMH levels even in young patients. At this point, we would recommend counseling young patients with low AMH levels (<1 ng/mL) to move forward with more

aggressive fertility treatments. Additionally, there must be continued research to establish optimal IVF protocols and procedures, including number and stage of embryos to transfer, in this challenging population.

Acknowledgment

The authors would like to thank Amy Granlund, B.S., for her assistance with data collection.

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