



## Commentary

# Anti-Müllerian Hormone: A Potential New Tool in Epidemiologic Studies of Female Fecundability

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The objective of the present commentary is to suggest that epidemiologists explore the use of anti-Müllerian hormone (AMH) as a new measurement tool in fecundability studies. The authors briefly summarize the advantages and limitations of the 3 current approaches to studies of fecundability. All 3 approaches involve the collection of time-to-pregnancy or attempt-time data, and most are limited to participants who plan their pregnancies. AMH is produced by ovarian follicles during their early growth stages and is measured clinically to assess ovarian reserve (the number of remaining oocytes). Unlike time to pregnancy, serum AMH level can be assessed regardless of pregnancy-attempt status. Measurements are not significantly affected by phase of the menstrual cycle, oral contraceptive use, or early pregnancy. The authors suggest that AMH measurement can be a valuable addition to traditionally designed fecundability studies. In addition, this hormone should be investigated as an independent measure of fecundability in studies that focus on exposures hypothesized to target the ovary.

anti-Mullerian hormone; epidemiology; fertility; research design

Abbreviation: AMH, anti-Müllerian hormone.

For successful reproduction, numerous biologic processes in both the male and female partners must facilitate fertilization, blastocyst formation, implantation, trophoblast invasion, and early development of the embryo. Yet, it was not until the 1980s that epidemiologists began to study the ability to conceive, which operationally measures the majority of these processes. In this commentary, we briefly summarize the current approaches to studying fecundability (the probability of conceiving in a given menstrual cycle) and suggest exploration of anti-Müllerian hormone (AMH) as a new measurement tool.

An early approach to studying fecundability involved the use of a case-control design. In 2 such studies, investigators reported associations between the use of an intrauterine device and tubal infertility. These results eventually led to withdrawal of the Dalkon Shield from the market (1–3). However, the limitations of such studies were apparent. Clinical case-control studies of infertility identify patients who seek treatment, but such couples are not representative of all persons experiencing fertility problems. In a Danish study conducted

at approximately the same time, researchers examined occupational and lifestyle factors that might affect ability to conceive by collecting information from pregnant women about the duration of their attempts to conceive (time to pregnancy or waiting time to pregnancy) (4, 5). The methodology for using time-to-pregnancy data to estimate the effect of exposures on fecundability was subsequently presented (6), and further refinement of analytic methods has continued (7).

Time-to-pregnancy data can be collected in a prospective, retrospective, or cross-sectional manner (8). Prospective studies of time to pregnancy follow women/couples during their attempts to conceive. These studies provide accurate time-to-pregnancy data and include women who never successfully conceive. However, these studies are nearly always restricted to participants who plan their pregnancies and can identify when they began attempting to conceive. In the United States, approximately half of pregnancies are unplanned (9), so limiting a study to persons who planned their pregnancies can result in significant selection bias.

In studies with a retrospective design, women are asked about past attempts to conceive; these may include unsuccessful attempts but more often are limited to attempts ending in a pregnancy (time to pregnancy). Again, the data analysis is usually restricted to persons who planned their pregnancies because those who did not are usually unable to provide time-to-pregnancy data. However, in this design, exposure data can be collected from persons who did not plan their pregnancies, so potential planning bias can be evaluated. A disadvantage is that the time-to-pregnancy data are self-reported and unverifiable by medical records except in the small subset of participants who seek fertility services. (For most couples, pregnancy attempts are a personal matter, not a medical matter.) The results of studies designed to evaluate the reliability and validity of retrospective time-to-pregnancy data have generally been reassuring (10). Furthermore, an increased sample size can compensate for outcome misclassification (11), but differential misclassification for some exposures is likely (12). Additional potential biases inherent in retrospective designs have been described elsewhere (13, 14), and design and analysis strategies to evaluate them must be included and clearly presented in publications.

The feasibility of the cross-sectional study design has only recently been demonstrated (15). Women were asked if they were currently having unprotected intercourse (intercourse with no attempt to prevent pregnancy) and for how long they had been doing so. These data are useful because even though time to pregnancy is not measured, persons who report longer times will on average have longer times to pregnancy. Exposure and confounder information, including frequency of intercourse, is determined at the same time. This design has the advantage of not restricting analyses to persons who planned their pregnancies, as it only excludes those who become pregnant while using contraception. Thus, for exposures that might be much more common in persons who did not plan their pregnancies (e.g., binge drinking), this design could be especially useful.

Time-to-pregnancy studies have led to important public health discoveries. One of the first factors shown to reduce fecundability was cigarette smoking (5, 16). Another lifestyle factor that has been linked to reduced fecundability is increased female body mass index. Anovulation in obese women had been recognized for decades (17), but the more subtle subfecundity effects have only recently been appreciated (18). Furthermore, the fact that these effects can be reversed with weight loss (19, 20) supports a causal association. Numerous other factors have also been linked to reduced fecundability, including prenatal factors (e.g., exposure to diethylstilbestrol (21)), occupational exposures (e.g., glycol ether exposure in the semiconductor industry (22, 23)), and environmental exposures (e.g., low-level ionizing radiation from cobalt-60-contaminated building materials (24)).

As with any epidemiologic data, causal inference depends not only on the strength of individual study findings but also on coherence of findings and biologic plausibility. Thus, studies of laboratory animals that provide biologic plausibility are adjuncts to time-to-pregnancy studies. An important new direction is the growing research effort to examine the impact of exogenous exposures on the probability of successful conception with in vitro fertilization. Although these studies are

limited in their ability to examine exposures that affect gamete production and transport, they can begin to isolate effects in humans on critical events, such as fertilization and implantation (25). Coordinating population studies with in vitro fertilization studies (26), as well as with laboratory animal research, can provide more convincing evidence for exposure effects.

Perhaps the most exciting potential new approach for fecundability studies is measurement of AMH. Also called Müllerian-inhibiting substance, AMH is a dimeric protein produced by the granulosa cells of preantral and small antral follicles (27). Its primary function is to inhibit the early stages of follicular development, that is, to maintain follicles in the resting stage (27).

Serum AMH levels in females are very low (often nondetectable) at birth, rise during the early years, peak in the teen years, and decline to nondetectable levels by menopause (27–29). AMH levels in adult women are correlated with ovarian reserve (assessed by ultrasound methods and histologic sectioning of ovaries) (30). During the past decade, AMH has been increasingly used in assisted reproduction programs as a predictor of the number of eggs that will be retrieved after ovarian stimulation (27).

Although the vast majority of research on AMH has been focused on infertile women, there is an increasing number of studies in nonclinic populations. Data from these studies also show a decline in AMH with age and suggest that AMH might be used to assess reproductive aging. Prospectively collected data have confirmed the validity of low AMH levels as a marker of menopausal transition (31, 32). Our recent findings in a non-clinic-based population also provide tantalizing preliminary evidence for AMH as a marker of female fecundability (33). Data from this small, prospective time-to-pregnancy study among women who were 30 years of age or older showed a significant association between fecundability and both crude and age-adjusted AMH levels (33).

The influences of 2 known ovarian toxicants on AMH levels have been examined. Antineoplastic drug treatment has been associated with reduced AMH levels in multiple studies (34, 35), and smoking also appears to affect AMH levels. Though cross-sectional analyses in which AMH levels in smokers and nonsmokers have been compared showed mixed results, studies that have compared age-specific AMH levels have consistently found that the age-related decline in AMH is steeper among smokers than among nonsmokers (36, 37, and unpublished reanalysis of data from 38 (E. T. Golub, Johns Hopkins University, personal communication, 2011)).

AMH has many appealing characteristics as a biomarker in epidemiologic studies. There is evidence for the reliability of a single measurement of AMH (i.e., measurements from the same woman over time are highly correlated) (39, 40). AMH levels have little variability across the menstrual cycle, so blood could be collected without regard to cycle day (41, 42), and neither oral contraceptives nor early pregnancy significantly affects levels (43, 44). AMH levels appear to decline in the second and third trimesters of pregnancy (45), but it is not known whether relative differences among women remain stable during this time. In addition, AMH levels appear to be stable for years in frozen samples (46), and blood-spots can be used as a sample source

(Thomas McDade, Northwestern University, personal communication, 2011).

Nelson and La Marca (47) traced the history of AMH measurement over the past 20 years. Initially, individual laboratories developed their own assays. Eventually, 2 commercial assays became available, but both have been discontinued. There is now a single assay, the enzyme-linked immunosorbent assay, that uses previously validated detection and capture antibodies from one of the discontinued assays. Automation is planned for the future (47), but currently the assay is conducted manually. Although the new assay was further validated and the values it produced were found to be highly correlated with those from both previous assays (48), most published literature and clinical cut-off values were derived using one of discontinued assays.

We propose that AMH level be included as an ancillary outcome measure in traditional fecundability studies. It can be used to evaluate the mechanism by which an exposure affects fecundability. An example was given in a recent study of adolescent estrogen exposure (28). In many countries, very tall adolescent females have been prescribed estrogen treatment to limit their growth. In a previous study in Australia, Venn et al. (49) reported reductions in fecundability associated with such treatments. In a recent study in the Netherlands, those findings were replicated, and researchers found that treated participants had reduced AMH levels (50). This strongly suggests that adolescent estrogen exposure targets the ovaries. AMH might also serve as a control variable in studies of exposures that are hypothesized to reduce fecundability through a mechanism other than ovarian toxicity. For example, in studies of male exposures, investigators could collect blood from the men's partners to help control for ovarian factors.

AMH level also should be investigated as a possible primary outcome measure when studying the effects on fecundability of exposures that are suspected to target the ovary. Its major advantage as an outcome measure is that study participants would not need to be limited to persons who were trying to conceive or to women who had planned pregnancies. This could eliminate the major selection biases that can arise in time-to-pregnancy studies. Even women who were taking oral contraceptives or were in their first trimester of pregnancy could be included. Selection issues make it difficult, if not impossible, to use traditional time-to-pregnancy studies to examine time trends in fecundability (51). However, it is possible to examine trends over time in age-specific AMH levels, and these data might begin to address speculation about declines in fertility. In addition, AMH could be measured in stored blood samples that were collected in previous studies in which occupational or environmental exposures (e.g., exposure to pesticides, phthalates, or polychlorinated biphenyls) were measured.

AMH has been studied in thousands of patients seeking treatment for infertility (e.g., Seifer et al. (52) recently reported an age-related decline in over 17,000 patients). Its usefulness in the care of patients seeking treatment for infertility is clear, but whether or not it could serve as a valid biomarker of female fecundability in the general population has yet to be determined. We expect it to reflect effects of factors affecting the ovaries but not other factors, such as sexually transmitted

diseases, that result in tubal damage. Given the limited data from general-population samples, more research is needed to identify methodological limitations for the use of AMH level in epidemiologic research on suspected ovarian toxicants. Existing data indicate that age and smoking are important confounders, but ethnic differences might also exist (38). Another factor that would need to be considered is polycystic ovaries. Women with this condition have excessive numbers of small growing follicles, and their AMH levels are significantly elevated (53). Women with this condition would need to be excluded from AMH studies of fecundability.

We encourage further research to elucidate the potential use of AMH in epidemiologic studies of fecundability. It could be measured in prospective time-to-pregnancy studies when possible. Not only might AMH be an important ancillary outcome measure in such studies, but study data could also be used to help evaluate its usefulness as an independent outcome. AMH levels should also be investigated in relation to numerous covariates in general population samples, such as reproductive-age women in the National Health and Nutrition Examination Survey. In summary, we think that AMH may prove to be a useful outcome measure for studies of potential ovarian toxicants. If so, it could greatly advance our understanding of female fecundability.

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