Antimüllerian Hormone as a Predictor of Natural Fecundability in Women Aged 30–42 Years

Anne Z. Steiner, MD, MPH, Amy H. Herring, ScD, James S. Kesner, PhD, Juliana W. Meadows, PhD, Frank Z. Stanczyk, PhD, Steven Hoberman, MS, and Donna D. Baird, PhD, MPH

OBJECTIVE: To generate estimates of the association between markers of ovarian aging and natural fertility in a community sample at risk for ovarian aging.

METHODS: Women aged 30–44 years with no history of infertility who had been trying to conceive for less than 3 months provided early-follicular phase serum and urine (N=100). Subsequently, these women kept a diary to record menstrual bleeding and intercourse and conducted standardized pregnancy testing for up to 6 months. Serum was analyzed for estradiol, follicle-stimulating hormone (FSH), antimüllerian hormone, and inhibin B. Urine was analyzed for FSH and estrone 3-glucuronide. Diary data on menstrual cycle day and patterns of intercourse were used to calculate day-specific fecundability ratios.

RESULTS: Sixty-three percent of participants conceived within 6 months. After adjusting for age, 18 women (18%) with serum antimüllerian hormone levels of 0.7 ng/mL or less had significantly reduced fecundability given intercourse on a fertile day compared with women with higher antimüllerian hormone levels (fecundability ratio 0.38; 95% confidence interval [CI] 0.08–0.91). The day-specific fecundability for women with early-follicular phase serum FSH values greater than 10 milli-international units/mL compared with women with lower FSH levels was also reduced, although nonsignificantly (11% of women affected; fecundability ratio 0.44; 95% CI 0.08–1.10). The association with urinary FSH was weaker (27% women affected; fecundability ratio 0.61; 95% CI 0.26–1.26), and the associations for the other markers were weaker still.

CONCLUSION: Early-follicular phase antimüllerian hormone appears to be associated with natural fertility in the general population.

(Obstet Gynecol 2011;117:798–804)
DOI: 10.1097/AOG.0b013e3182116bc8

LEVEL OF EVIDENCE: II

Societal and behavioral shifts in recent years have resulted in a trend toward delayed childbearing. Women are choosing to delay attempts to conceive until they reach their 30s and 40s, ages associated with a lower probability of conceiving.[1] The observed decline in fecundability is the result of reproductive aging, a natural progression through stages of puberty, fertility, subfertility, the menopause transition, and finally, menopause.[2] The rate of movement through these stages varies among individuals; women of the same chronologic age clearly differ in their reproductive potential, just as age at menopause varies between individuals. Despite variability in the age of onset of subfertility, no validated biomarkers exist to monitor an individual woman's fertility.

Ovarian aging is associated with a decline in oocyte number reflected by a decline in circulating levels of antimüllerian hormone and inhibin B and a rise in early-follicular phase follicle stimulating hormone (FSH) and estradiol levels in blood.[3–8] Although these markers are physiologically associated with

From the Departments of Obstetrics and Gynecology and Biostatistics, University of North Carolina, Chapel Hill, North Carolina; the National Institute for Occupational Safety and Health, Cincinnati, Ohio; the Department of Obstetrics and Gynecology and Preventive Medicine, University of Southern California, Los Angeles, California; and the Epidemiology Branch, National Institute of Environmental Health Science/National Institutes of Health, Research Triangle Park, North Carolina.

Supported by National Institutes of Health (NIH) R21 HD060229 and 5 K12 HD050113 (University of North Carolina Women's Reproductive Health Research Center). Also supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (ie, support for coauthor D.B.).

Corresponding author: Anne Z. Steiner, MD, MPH, University of North Carolina, CB #7570, 4001 Old Clinic Building, Chapel Hill, NC 27599; e-mail: asteiner@med.unc.edu.

Financial Disclosure
The authors did not report any potential conflicts of interest.

ISSN: 0029-7844/10

changes in ovarian function, their ability to predict fertility in the general population has never been rigorously examined. Previous research included only infertile patients, measured outcomes only after treatment with assisted reproductive technology, or used menopause as the outcome. Despite limited supporting evidence, FSH levels are frequently measured at doctors' offices and in commercial home kits marketed in the United States as a method of assessing one's fertility. Commercial, dipstick kits measure FSH in urine. A positive test is noted when urinary values exceed the cutoff value equivalent to a serum value of 10 milli-international units/mL. These unproven tests have the potential to lead to unnecessary treatment or false reassurance, induce anxiety, and result in inappropriate costs and inconvenience to consumers if the tests are not valid.[9]

Fertility of individuals can be compared a number of ways. Relative fecundability, the difference in fecundability (probability of conceiving in a menstrual cycle) of one group compared with another, is estimated by analyzing data on time to pregnancy, the number of menstrual cycles required to conceive. Because retrospective assessments of time to pregnancy are subject to strong bias effects,[10] prospective studies are preferable. If information on menstrual bleeding and intercourse is also collected, time to pregnancy studies can be used to compare day-specific probabilities of pregnancy for affected and unaffected individuals or the relative probability of pregnancy given an act of intercourse on a fertile day.

Because specific endocrinologic changes appear to reflect ovarian aging, which is the presumed major cause of decline in fecundability, we sought to estimate the association between endocrine indices of ovarian aging (early-follicular phase FSH, estradiol, antimüllerian hormone, and inhibin B in serum as well as FSH and the estrogen metabolite, estrone 3-glucuronide in urine) and fecundability in a community sample at risk for ovarian aging.

MATERIALS AND METHODS

Time to Conceive, a time to pregnancy study, was approved by the institutional review board of the University of North Carolina. English-speaking women between 30 and 44 years of age, who were attempting to conceive for 3 months or less or were about to start trying to conceive, were eligible for participation in the study. Women with a history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded from participation. Eligible women were enrolled and provided informed consent at their study visit, which was scheduled for the second, third, or fourth menstrual cycle day (first day defined as the first day of menses) after determination of eligibility. Women who were determined to be eligible while using contraception were enrolled in the menstrual cycle immediately after cessation of birth control.

At the study visit, women provided a urine and blood sample. To prevent breakdown of FSH that otherwise occurs in frozen samples, urine (5 mL) was transferred to a polypropylene vial containing glycerol (7% final glycerol dilution).[11] The serum and urine samples were stored frozen at −80°C until analysis. At the study visit, participants were also provided and instructed on the use of the study diary, which was designed to collect daily information on vaginal bleeding, intercourse, pregnancy test results, and medication use. Participants were asked to complete the diary daily until pregnancy was detected or three menstrual cycles had passed. In addition, women were provided with free home pregnancy tests (sensitivity=20 milli-international units human chorionic gonadotropin/mL) and instructed to use them at the time of missed menses.

Women were instructed to inform study staff of a positive pregnancy test. Women were provided a free pregnancy ultrasonography to encourage notification of pregnancy results. Women who did not report a positive pregnancy test were contacted at 3 and 6 months after the study visit. Women were followed until a positive pregnancy test or until 6 months of attempt after the study visit.

Urine was shipped frozen to the National Institute for Occupational Safety and Health Reproductive Endocrinology Laboratory, where the samples were assayed for FSH, estrone 3-glucuronide, and creatinine. Urinary FSH concentrations were assayed in duplicate using a noncompetitive, two-site time-resolved immunofluorometric assay.[12] Urinary estrone 3-glucuronide concentrations were measured in triplicate using competitive double-antibody time-resolved fluoroimmunoassay developed and characterized in the National Institute for Occupational Safety and Health laboratory.[13] Urinary creatinine concentrations were measured using a Vitros 250 Chemistry Analyzer that uses a slide composed of a dry, multilayered analytical element coated on a polyester support. Urinary endocrine values (FSH and estrone 3-glucuronide) were divided by the respective sample's creatinine concentration to adjust for urine flow rate. All samples were measured in one assay per analyte. Intra-assay coefficients of variation were 3.5%

for FSH, 16.1% for estrone 3-glucuronide, and 1.1% for creatinine.

Serum was shipped frozen to the University of Southern California Reproductive Endocrinology Laboratory, where the samples were assayed by sensitive and specific assays for FSH, estradiol, antimüllerian hormone, and inhibin B. FSH was measured by a direct immunochemiluminometric assay using the automated Immulite system. Estradiol was measured by radioimmunoassay after an organic solvent extraction step. Antimüllerian hormone and inhibin B assays used a monoclonal two site enzyme-linked immunosorbent assay. Interassay coefficients of variation ranged from 7% to 11%.

Initially Pearson's correlation coefficients and *P* values were calculated to estimate correlation between hormone levels and between the hormone levels and age. Subsequently, we evaluated the associations between the hormone levels of interest and day-specific probabilities of pregnancy. Pregnancy was defined by the report of a positive home pregnancy test. Because of the relatively small sample size, maternal age was dichotomized at a cut point of 35 years of age. All hormone levels were dichotomized at standard levels used in clinical practice. When clinical cutoff values were not available (like with serum estradiol and inhibin B and urinary estrone 3-glucuronide), cut points were based on quartiles of the data.[14] Specifically, we dichotomized serum FSH at a cut point of 10 milli-international units/mL, antimüllerian hormone at 0.7 ng/mL, inhibin B at the 25th percentile (21.9 pg/mL), and estradiol at the 75th percentile (54.6 pg/mL). Creatinine-corrected urinary FSH was dichotomized at a cut point of 11.5 milli-international units/mg creatinine (equivalent to serum value of 10 milli-international units/mL). Creatinine-corrected urinary estrone 3-glucuronide was dichotomized at the 75th percentile (13.5 ng/mg creatinine). In a sensitivity analysis, urinary cut points for FSH of 10 and 13 milli-international units/mg creatinine were also analyzed.

Information from the diary on days of menstrual bleeding, days of intercourse, and results of pregnancy tests were used to estimate day-specific probabilities of pregnancy (probability of pregnancy given an act of intercourse on a fertile day). Ovulation was assumed to have occurred 14 days before the first day of menses or the first positive home pregnancy test with the fertile window designated as extending from 6 days before to 5 days after day of ovulation based on the standard days method.[15] The day specific-probabilities model by Scarpa and Dunson[16] was used to generate day-specific fecundability ratios. A fecundability ratio less than 1.0 suggests reduced fecundability. The model accommodates for multiple acts of intercourse during the fertile window. The model assumes independence between acts of intercourse. In the model, the first observed menstrual cycle is entered at the appropriate attempt time (cycle 1 for those who enrolled when they started trying to conceive, cycle 2–4 for those who enrolled later in their attempt). Prospectively observed cycles were included in the model for up to three observed cycles or until pregnancy was detected. There were 224 cycles available for analysis. We fit both unadjusted models and age-adjusted models for each hormone of interest. Data from cycles without diary information were used to provide descriptive information, eg, the percentage of women achieving pregnancy within 6 months.

RESULTS

A total of 100 women were enrolled in the study, providing blood and urine. Follow-up was available for 98 women. Thirty percent of women were 35 years of age or older, and 5% were 40 years old or older. Participants tended to be nulliparous (63%), white (86%), highly educated (61% with graduate or professional degrees), and to have normal body mass indexes (62% between 18.5 and 24.9; calculated as weight (kg)/[height (m)]^2). Twelve percent of participants were obese (body mass index higher than 30). Median male partner age was 33.5 years (interquartile range 31–37 years; range 27–56 years). Women reported approximately two acts of intercourse per week (interquartile range 1.5–3.0; range 0.2–6). Thirty-eight percent of women enrolled during their first cycle of attempt to become pregnant. By 6 months of enrollment, 63.6% of women had conceived. Eighty percent of women completed at least one cycle of daily fertility diaries.

Median serum values were 7.1 milli-international units/mL FSH (interquartile range 6.0–8.7 milli-international units/mL; range 3–51 milli-international units/mL), 1.7 ng/mL antimüllerian hormone (interquartile range 0.8–3.0 ng/mL; range less than 0.06–8.3 ng/mL), 27.9 pg/mL inhibin B (interquartile range 14.9–46.6 pg/mL; range less than 10–120 pg/mL), and 42.8 pg/mL estradiol (interquartile range 35.2–54.6 pg/mL; range 15–437 pg/mL). Antimüllerian hormone and inhibin B values below the limit of detection (n=3 and 15, respectively) were imputed as the limit of detection (0.06 ng/mL and 10 pg/mL, respectively) divided by the square root of 2, following standard practice.[17] Median creatinine-corrected urinary FSH and estrone 3-glucuronide were 9.1

Table 1. Estimated Correlation Between Markers of Ovarian Aging*

		Serum Markers				Urinary Markers (Creatinine-Adjusted)	
	Age	FSH	AMH	Inhibin B	Estradiol	FSH	$E_1$3G
Age	1.00	0.13	−0.21†	−0.04	0.01	0.21†	−0.02
Serum markers							
FSH		1.00	−0.28‡	−0.08	0.07	0.85‡	−0.06
AMH			1.00	0.23†	−0.08	−0.36‡	−0.04
Inhibin B				1.00	0.08	−0.16	−0.01
Estradiol					1.00	0.10	0.78‡
Urinary markers (creatinine-adjusted)							
FSH						1.00	−0.06
$E_1$3G							1.00

FSH, follicle-stimulating hormone; AMH, antimüllerian hormone; $E_1$3G, estrone 3-glucuronide.
* Pearson's correlation coefficients presented.
† $P<.05$.
‡ $P<.01$.

milli-international units/mg creatinine (interquartile range 6.7–11.7 milli-international units/mg; range 4.1–14.0 milli-international units/mg) and 9.9 ng/mg creatinine (interquatile range 7.4–13.5 ng/mg; range 2.3–77.3 ng/mg), respectively. Urinary and serum FSH were highly correlated (r=0.85, $P<.001$). Regression analysis revealed that the creatinine-corrected urinary FSH value comparable to a serum FSH value of 10 milli-international units/mL was 11.5 milli-international units/mg creatinine. Eleven percent of women had serum FSH values over 10 milli-international units/mL and 27% of women had urinary FSH values over 11.5 milli-international units/mg creatinine. Serum estradiol and urinary estrone 3-glucuronide were also highly correlated (r=0.78, $P<.001$). Correlation statistics between all marker values are presented in Table 1.

Age was a strong predictor of the day-specific probability of pregnancy; women who were aged 35 or older had significantly reduced fecundability compared with younger women given an act of intercourse on a fertile day (fecundability ratio 0.42; 95% confidence interval [CI] 0.15–0.85). After adjustment for serum hormone measures, the age effect was even more pronounced with a corresponding fecundability ratio of 0.32 (95% CI 0.01–0.75). Antimüllerian hormone was strongly associated with day-specific fecundability (Table 2). Women with antimüllerian hormone levels of 0.7 ng/mL or less had significantly lower fecundability compared with their counterparts

Table 2. Estimated Associations of Endocrine Markers With Day-Specific Probability of Pregnancy

	Number (%) of Affected Participants	Day-Specific Fecundability Ratio* (95% Confidence Interval)	
		Unadjusted	Age-Adjusted
Serum measures			
FSH greater than 10 milli-international units/mL	11 (11)	0.47 (0.08–1.15)	0.44 (0.08–1.10)
AMH 0.7 ng/mL or less	18 (18)	0.36 (0.01–0.84)	0.38 (0.08–0.91)
Inhibin B 21.9 pg/mL or less	20 (20)	0.82 (0.35–1.55)	0.83 (0.35–1.55)
Estradiol 54.6 pg/mL or greater	23 (23)	0.74 (0.27–1.47)	0.71 (0.25–1.42)
Urinary measures			
FSH 11.5 milli-international units/mg creatinine or greater	26 (27)	0.59 (0.25–1.22)	0.61 (0.26–1.26)
$E_1$3G 13.5 ng/mg creatinine or greater	24 (24)	0.61 (0.27–1.15)	0.62 (0.27–1.16)

FSH, follicle-stimulating hormone; AMH, antimüllerian hormone; $E_1$3G, estrone 3-glucuronide.
* Interpreted as relative probability of pregnancy given an act of intercourse on a fertile day. A fecundability ratio less than 1.0 suggests reduced fecundability. Analyzed 221 cycles from 98 participants for urinary hormones; 184 cycles from 78 participants for serum hormones.

with higher antimüllerian hormone levels (fecundability ratio 0.36; 95% CI 0.01–0.84) given an act of intercourse on a fertile day; this effect remained after adjustment for age (fecundability ratio 0.38; 95% CI 0.08–0.91). After adjusting for age, women with early-follicular phase serum FSH values greater than 10 milli-international units/mL had an estimated fecundability ratio of 0.44 (95% CI 0.08–1.10) compared with women with lower values. Women with urinary values of FSH over a serum equivalent of 10 milli-international units/mL (11.5 milli-international units FSH/mg creatinine) exhibited 40% reduced day-specific fecundability compared with women with normal urinary values. A higher cutoff value (13 milli-international units/mg creatinine) resulted in fewer affected women (n=16) but did not strengthen the association (fecundability ratio 0.61; 95% CI 0.21–1.37). The estimated fecunability ratios for the remaining endocrine markers (serum inhibin B, serum estradiol, and urinary estrone 3-glucuronide) were also not statistically significant, and CIs were even broader than for urinary FSH.

DISCUSSION

In this study, we found that serum antimüllerian hormone levels were strongly and significantly associated with natural fertility as measured by day-specific probabilities of pregnancy. Of the remaining markers, early-follicular phase serum FSH values over 10 milli-international units/mL were most closely associated with reduced fertility; however, the CI was broad and the result not statistically significant. With urinary FSH cutoff values equivalent to a serum cutoff of 10 milli-international units/mL, we did not see a significant association between elevated urinary FSH and reduced fertility. Serum analytes (estradiol and FSH) were significantly and strongly correlated with their urinary analytes; however, correlation between different hormones was low (generally less than 0.3).

Of all the serum and urinary markers of ovarian aging that were tested, serum antimüllerian hormone levels were most strongly associated with fecundability. Previous studies have shown that antimüllerian hormone declines with age,[18] predicts stages of the menopause transition,[19] and is associated with the probability of conceiving after in vitro fertilization.[20] Measurement of antimüllerian hormone may have value in future epidemiologic studies as a measure of diminished fertility resulting from ovarian aging or diminished oocyte number (ovarian reserve). Antimüllerian hormone serum values are relatively unaffected by cycle day[21] and appear to have value as an ovarian index independent of the woman's age. We are not aware of a urinary assay for antimüllerian hormone; however, dried blood spots have been a mechanism for measuring other reproductive hormones in population studies and may be useful for antimüllerian hormone measurements.[22]

Elevated serum FSH (greater than 10 milli-international units/mL, a commonly used cutoff value) was seen infrequently in our cohort. Women in this group also tended to have reduced day-specific fecundability; however, in our small cohort, the association was not statistically significant. As a result of the relatively small sample size, the predictive value of antimüllerian hormone and FSH (or other markers) could not be compared with formal statistical analyses. Although early-follicular phase FSH has been used for years as a marker for staging the menopause transition[23] and as a predictor of pregnancy after in vitro fertilization,[24] it has only recently been evaluated as a marker of natural fertility,[25] albeit among subfertile women. Our study is unique in that it enrolled women without a history of subfertility. One other study examined pregnancy in relation to FSH in women older than 30 years without a history of subfertility but was designed to compare FSH levels for women with early pregnancy loss and women with ongoing pregnancies.[26] Although no formal analysis of the time to pregnancy was conducted, they found no significant differences in FSH between women who conceived and those who did not.

Commercial urinary fertility kits use a cutoff level equivalent to 10 milli-international units/mL serum FSH.[27] Thus, for this study, we choose a urinary FSH cutoff value of 11.5 milli-international units/mg creatinine as a cutoff, which was our equivalent to a serum FSH value of 10 milli-international units/mL based on regression analysis. Although serum and urinary FSH levels are highly correlated (r=0.85), an elevated urinary FSH (greater than 11.5 milli-international units/mg creatinine) was only weakly and nonsignificantly associated with fecundability. Selecting an even higher urinary FSH cutoff value (13 milli-international units/mg) did not strengthen the association. Our findings raise concern about the commercial use of urinary FSH test kits to predict fertility. Urinary FSH should be tested further to better understand its relationship to fecundability in the general population.

Given their age, this cohort was at higher risk for ovarian aging than the general population trying to conceive. Still, the majority of participants were in their early 30s, and only 11–18% of the women had abnormal measures of ovarian aging as measured by

early-follicular phase serum FSH levels over 10 milli-international units/mL or serum antimüllerian hormone levels under 0.7 ng/mL—commonly accepted clinical cutoff values. We also noted that although statistically significant, the correlation between the markers was surprisingly low (typically less than 0.3), indicating that these markers are not redundant to each other. Finally, adjustment for age did not significantly alter the measures of association between the various endocrine measures and day-specific probabilities of pregnancy. It is possible that these markers reflect biologic phenomena associated with reproductive aging independent of chronologic age. If so, a combination of indices, including age, may have greater potential than any single biologic marker or age alone for predicting a decline in fecundability resulting from reproductive aging.

The primary limitation of this study is its relatively small sample size. This limited our ability to adjust for a broad range of potential covariates (including male factors) and to explore in detail the relationship among the markers of ovarian aging. Women with partners who had known fertility problems were excluded from the study, but no semen analyses were performed. Such a study requirement reduces participation in community studies, potentially affecting generalizability. Because many women conceive in their first three cycles of attempt, our sample, which included some women who failed to conceive in these first few cycles, can be assumed to be less fertile than the general population attempting pregnancy. Our time to pregnancy analysis did allow for variation of estimates by time of enrollment. In addition, we screened out women with strong risk factors for infertility, creating a more homogeneous cohort to study the specific effect of ovarian aging. The homogeneity of the cohort, a largely white and highly educated group, strengthens the internal validity of our findings but may reduce generalizability. However, measures of ovarian aging have not been consistently shown to differ by ethnic group or socioeconomic status. The small sample of women in their 40s also may limit generalizability to this age group.

This study did use daily diaries to collect information on bleeding and intercourse patterns, allowing us to calculate day-specific probabilities of pregnancy, which adjusts for intercourse patterns. Adjustment for intercourse frequency is important, because frequency of intercourse is reported to decline with age.[28] A larger study is needed to estimate if the strength of these associations differs by age or parity. In addition, precision of day-specific probability estimates are strengthened with the use of ovulation predictors (either cervical mucus or urinary kits), which this study did not use.

The prospective design of our study is vital for an analysis of the relationship between markers of ovarian aging and fertility because women, who may never conceive are included. A retrospective study of time to pregnancy within a pregnancy cohort, a commonly used design, would miss women who do not conceive. Such women represent a large percentage of the population in older age groups. In this study, women were provided free home pregnancy tests and instructions as to when to test for pregnancy. Therefore, differential detection of early pregnancy is unlikely. In addition, this study evaluated multiple endocrine markers, both in urine and in serum, as indices of ovarian aging and used models to adjust for age and intercourse patterns.

Urinary home kits generally call for first morning urine but do not adjust for urine flow rates. In this study, first morning urine was not collected; however, we adjusted for urine flow rates using urinary creatinine concentrations in the samples. Like in previous studies,[29] our creatinine-corrected FSH values were highly correlated with serum FSH values. Creatinine-corrected gonadotropin values have been shown to improve measurement precision[30] and have been used in previous epidemiologic studies.[31,32]

In summary, serum antimüllerian hormone appears to be a predictor of age-related reductions in fecundability in the general population. Urinary FSH, the marker used in commercially available kits for women's self-assessment, may only be weakly predictive. Larger studies are needed to confirm these findings and to explore the way the different endocrine markers interact as potential joint predictors of fertility.

REFERENCES

1. Abma JC, Chandra A, Mosher WD, Peterson LS, Piccinino LJ. Fertility, family planning, and women's health: new data from the 1995 National Survey of Family Growth. Vital Health Stat 23 1997;19:1–114.
2. Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, et al. Executive summary: Stages of Reproductive Aging Workshop (STRAW). Fertil Steril 2001;76:874–8.
3. Backer LC, Rubin CS, Marcus M, Kieszak SM, Schober SE. Serum follicle-stimulating hormone and luteinizing hormone levels in women aged 35–60 in the U.S. population: the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994). Menopause 1999;6:29–35.
4. Kim YK, Wasser SK, Fujimoto VY, Klein NA, Moore DE, Soules MR. Utility of follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol and FSH:LH ratio in predicting reproductive age in normal women. Hum Reprod 1997;12:1152–5.

5. Ng EH, Yeung WS, Fong DY, Ho PC. Effects of age on hormonal and ultrasound markers of ovarian reserve in Chinese women with proven fertility. Hum Reprod 2003;18: 2169–74.
6. Tufan E, Elter K, Durmusoglu F. Assessment of reproductive ageing patterns by hormonal and ultrasonographic ovarian reserve tests. Hum Reprod 2004;19:2484–9.
7. Broekmans FJ, Scheffer GJ, Bancsi LF, Dorland M, Blankenstein MA, te Velde ER. Ovarian reserve tests in infertility practice and normal fertile women. Maturitas 1998;30:205–14.
8. van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, et al. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril 2005;83:979–87.
9. Barratt A, Irwig L, Glasziou P, Cumming R, Raffle A, Hicks N, et al. Moving from evidence to action: recommendations about screening. User's guides to the medical literature. Chicago (IL): AMA Press; 2002. p. 583–97.
10. Bonde JP, Joffe M, Sallmen M, Kristensen P, Olsen J, Roeleveld N, et al. Validity issues relating to time-to-pregnancy studies of fertility. Epidemiology 2006;17:347–9.
11. Kesner JS, Knecht EA, Krieg EF Jr. Stability of urinary female reproductive hormones stored under various conditions. Reprod Toxicol 1995;9:239–44.
12. Kesner JS, Knecht EA, Krieg EF Jr. Time-resolved immunofluorometric assays for urinary luteinizing hormone and follicle stimulating hormone. Analytica Chimica Acta 1994;285: 13–22.
13. Kesner JS, Knecht EA, Krieg EF Jr, Barnard G, Mikola HJ, Kohen F, et al. Validations of time-resolved fluoroimmunoassays for urinary estrone 3-glucuronide and pregnanediol 3-glucuronide. Steroids 1994;59:205–11.
14. O'Brien SM. Cutpoint selection for categorizing a continuous predictor. Biometrics 2004;60:504–9.
15. Arevalo M, Sinai I, Jennings V. A fixed formula to define the fertile window of the menstrual cycle as the basis of a simple method of natural family planning. Contraception 1999;60: 357–60.
16. Scarpa B, Dunson DB. Bayesian selection of predictors of conception probabilities across the menstrual cycle. Paediatr Perinat Epidemiol 2006;20(suppl 1):30–7.
17. Barr DB, Landsittel D, Nishioka M, Thomas K, Curwin B, Raymer J, et al. A survey of laboratory and statistical issues related to farmworker exposure studies. Environ Health Perspect 2006;114:961–8.
18. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril 2002;77:357–62.
19. Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 2008;93:3478–83.
20. Wunder DM, Guibourdenche J, Birkhauser MH, Bersinger NA. Anti-Müllerian hormone and inhibin B as predictors of pregnancy after treatment by in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2008;90:2203–10.
21. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, te Velde ER, Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;91:4057–63.
22. Shirtcliff EA, Reavis R, Overman WH, Granger DA. Measurement of gonadal hormones in dried blood spots versus serum: verification of menstrual cycle phase. Horm Behav 2001;39: 258–66.
23. Freeman EW, Sammel MD, Gracia CR, Kapoor S, Lin H, Liu L, et al. Follicular phase hormone levels and menstrual bleeding status in the approach to menopause. Fertil Steril 2005;83: 383–92.
24. Toner JP, Philput CB, Jones GS, Muasher SJ. Basal follicle-stimulating hormone level is a better predictor of in vitro fertilization performance than age. Fertil Steril 1991;55: 784–91.
25. van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Broekmans FJ, et al. Predictive value and clinical impact of Basal follicle-stimulating hormone in subfertile, ovulatory women. J Clin Endocrinol Metab 2007;92:2163–8.
26. van Montfrans JM, van Hooff MH, Huirne JA, Tanahatoe SJ, Sadrezadeh S, Martens F, et al. Basal FSH concentrations as a marker of ovarian ageing are not related to pregnancy outcome in a general population of women over 30 years. Hum Reprod 2004;19:430–4.
27. US Food and Drug Administration. Fertell Female Fertility Test: 510(k) Substantial Equivalence Determination Decision Summary. December 2, 2005 [cited December 1, 2009]. Available at: www.accessdata.fda.gov/cdrh_docs/reviews/K032002.pdf. Accessed December 1, 2009.
28. Hayes R, Dennerstein L. The impact of aging on sexual function and sexual dysfunction in women: a review of population-based studies. J Sex Med 2005;2:317–30.
29. Liu JH, Kao L, Rebar RW, Muse K. Urinary beta-FSH subunit concentrations in perimenopausal and postmenopausal women: a biomarker for ovarian reserve. Menopause 2003;10: 526–33.
30. Kesner JS, Knecht EA, Krieg EF Jr, Wilcox AJ, O'Connor JF. Detecting pre-ovulatory luteinizing hormone surges in urine. Hum Reprod 1998;13:15–21.
31. De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, et al. High frequency of luteal phase deficiency and anovulation in recreational women runners: blunted elevation in follicle-stimulating hormone observed during luteal–follicular transition. J Clin Endocrinol Metab 1998;83:4220–32.
32. Reutman SR, LeMasters GK, Knecht EA, Shukla R, Lockey JE, Burroughs GE, et al. Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. Environ Health Perspect 2002;110:805–11.