Caitlin Dunne, MD, FRCSC

Infertility: Testing and diagnosis for the community physician

The workup for couples who fail to conceive should include confirmation that ovulation is occurring, measurement of hormone levels, hysterosalpingography, and semen analysis.

ABSTRACT: Infertility is a condition commonly encountered by family physicians in the community. Timely diagnosis and treatment of infertility can help to mitigate the clinical and emotional consequences for the patient and her partner. Investigations for infertility should be initiated after a year of trying without pregnancy, although a community physician would be advised to order testing before a year of trying in four common clinical situations: female age over 35 years, presence of oligomenorrhea, presence of risk factors for tubal disease, and suspicion of male factor infertility. Standard investigations include ovulation testing such as cycle tracking, use of ovulation predictor kits, basal body temperature charting, and serum progesterone measurement. Ovarian reserve testing should be undertaken to assess the monthly cohort of hormone-responsive (pre-antral and antral) follicles. The most common endocrine ovarian test involves measuring follicle-stimulating hormone levels on day 3 of the menstrual cycle. Additional ovarian reserve tests such as anti-Müllerian hormone assay and/or antral follicle count (done by ultrasound in a fertility clinic) can improve sensitivity, specificity, and convenience. Uterine-tubal evaluation may be undertaken with hysterosalpingography while hysteroscopy or sonohysterography can be used to further investigate the endometrial cavity as needed. Semen analysis is a fundamental part of the workup because the male factor accounts for approximately 35% of infertility. Infertility investigations should start after 6 months of trying for women over 35 years, and for women over 40 years investigations should be initiated immediately. Consultation with a gynecologist or fertility specialist is covered by provincial health insurance and should be considered for couples with abnormal test results and for couples who fail to conceive despite normal test results.

This article has been peer reviewed.

Infertility is defined as the failure to achieve a pregnancy after 12 months of unprotected intercourse. It is a prevalent condition that affects about 15% of couples[1] and is commonly encountered by family physicians in the community. The majority of couples conceive within the first 3 months of trying, after which time the chances of pregnancy decline substantially.[1] After 1 year, 85% of couples will have achieved a pregnancy, while after another year only an additional 5% to 8% of couples will become pregnant.[2]

Peak fecundability occurs during the fertile window, which encompasses the 6 days up to and including the day of ovulation.[1] In a study of 221 couples, similar pregnancy rates were achieved with daily intercourse (37%) and intercourse every other day (33%).[1] When frequency of sexual

Dr Dunne is a co-director at the Pacific Centre for Reproductive Medicine and a clinical associate professor at the University of British Columbia. She is certified by the Royal College of Physicians and Surgeons in both obstetrics and gynecology and in reproductive endocrinology and infertility.

intercourse was reduced to weekly, the pregnancy rate fell to 15%.[3] Pregnancies were recorded with sperm as old as 3 days, although the highest chances of conception were seen with intercourse 2 days before ovulation and on the day of ovulation itself.[1,3] Sperm has been found to survive for up to 7 days in the cervical mucus and to retain the ability to fertilize a human egg in vitro after 5 days.[4,5] Following ovulation, an egg may be ready for fertilization within 20 minutes and remains usable for 12 to 24 hours.[6] In the fertility clinic, we often tell patients that "The sperm should be waiting for the egg." A period of abstinence longer than 5 to 10 days can have detrimental effects on sperm motility and concentration.[7] Conversely, normal sperm counts can be maintained even with daily ejaculation.[7] Patients can therefore be advised that intercourse every day or every other day during the late follicular phase will optimize their chances of conceiving. Coital position does not affect the chances of conceiving and women can be safely reassured that they do not need to remain supine for any length of time after intercourse.[8] Sperm have been found within the cervical canal within seconds and in the fallopian tube within minutes of ejaculation.[8] Thereafter, the cervix may serve as a reservoir for sperm and fertilization will remain possible in the following days.

Detecting ovulation

The surest sign of ovulation is a regular menstrual cycle of 21 to 35 days. However, many women choose adjunctive methods to help them detect ovulation in order to better predict the most fertile time of each month. Numerous devices and products are available for detecting ovulation. However, if a patient finds the process stressful she should be reassured that ovulation tracking is not a requirement for conceiving and be directed to simply have regular intercourse around mid-cycle.

The surest sign of ovulation is a regular menstrual cycle of 21 to 35 days.

Cycle tracking

Cycle tracking, otherwise known as the calendar method, is one of the oldest ways to determine when ovulation is likely to occur. A review of the normal physiology of the corpus luteum (CL) permits a better understanding of this method.

The granulosa cells of a dominant follicle—the cells responsible for making estradiol in response to follicle-stimulating hormone (FSH) in the follicular phase—undergo several important changes around the time of ovulation. First, they acquire luteinizing hormone (LH) receptors in the late follicular phase to enable them to respond to the mid-cycle LH surge and ovulate. Second, they become vascularized and therefore capable of transforming cholesterol into the principal steroid of the corpus luteum: progesterone.[6] Peak production of progesterone is achieved approximately 8 days after ovulation; at this moment the CL is one of the most vascular areas of the body.[6] The luteal phase usually lasts 14 days from the time of the LH surge. Therefore, unless the CL receives ongoing stimulation in the form of beta-human chorionic gonadotropin from a pregnancy, progesterone production ceases and menses ensues. The calendar method assumes that if you count backwards 14 days from the first day of menses you can estimate the date of ovulation in retrospect and use this information to predict future cycles. Some smart phone apps allow a woman to record her menses and then use this information to predict her fertile window using a personalized monthly average cycle length.

Use of ovulation predictor kits

Ovulation predictor kits (OPKs) available from pharmacies and online can be used to detect a high amount of luteinizing hormone in a urine sample. Follicle rupture occurs 34 to 36 hours after the onset of the LH surge at mid-cycle and LH is generally detectable in the urine for most of this time.[9] Patients are advised to test morning urine, which is the most concentrated. The LH surge is responsible for maturation of the oocyte through resumption of meiosis (from prophase I to metaphase II) and for release of the oocyte from the dominant follicle.[6] Digital ovulation kits purport to have increased accuracy by adding daily detection of a urinary metabolite of estrogen, estrone-3-glucuronide (E3G). Some brands use a smiley face to indicate when E3G levels are high (correlating with a growing dominant follicle) to identify the fertile window leading up to the LH surge and ovulation.

Basal body temperature charting

Basal body temperature (BBT) charting requires that a woman measure her temperature orally each morning before rising and before eating or exercising. The thermometer should be capable of detecting 0.1 °C increments. Daily temperature can be charted on a preprinted graph (many are available online) or using a smart phone app. A biphasic monthly temperature pattern indicates ovulation. Typically, a rise in body temperature of 0.5 °C can be seen after ovulation owing to the production of progesterone. Although this rise in BBT means a woman's most fertile days have passed, she can use this information to predict ovulation in future cycles. Around the time of ovulation a woman may also observe egg white cervical mucus that thickens and turns yellow after progesterone is produced. In mid-cycle some women experience mittelschmerz, one-sided lower abdominal pain associated with ovulation.

Serum progesterone measurement

In the mid-luteal phase (day 21 to 23 of a typical cycle) a serum progesterone level greater than 10 nmol/L is evidence of ovulation. Because progesterone is released in response to pulsatile stimulation by LH, which in turn is influenced by progesterone exposure at the level of the hypothalamus, values can fluctuate throughout the luteal phase.[6,10] Patients are thus encouraged not to dwell on the absolute value of a progesterone measurement as long as it is above 10 nmol/L.

Investigations for infertility

In addition to confirming ovulation, the basic workup for infertility includes ovarian reserve testing with a follicle-stimulating hormone test, uterine-tubal evaluation with hysterosalpingography, and semen analysis (**Box**).

It is generally recommended that investigations for infertility be initiated after a year of trying without a pregnancy. There are, however, four common clinical situations where a community physician would be advised to order testing before a year of trying: female age over 35 years, presence of oligomenorrhea, presence of risk factors for tubal disease, and suspicion of male factor infertility.

Advancing female age is becoming an increasingly prevalent cause of infertility. British Columbia has the highest age of first birth in Canada at 30.5 years versus 30.3 years in Ontario.[11] Over the past 3 decades the industrialized world has seen a dramatic increase in the age of first birth.[12,13] According to Statistics Canada, 2010 marked the first time in our history that more women in their 30s were having children than women in their 20s.[14] In 2011, there were 52.3 babies born per 1000 women age 35 to 39, compared to 45.7 per 1000 women age 20 to 24.[15] In BC, the percentage of live births to women age 35 years and older rose from 11% in 1990 to 23% in 2011, while the percentage of live births to women age 20 to 34 fell from 83% to 74% over the same period.[16] By far the most common reason women reported for not pursuing childbearing earlier was lack of a partner.[13]

The consequences of delaying childbearing are increasing rates of infertility, embryo aneuploidy, and miscarriage. These are largely attributed to aging oocytes with failing meiotic spindles and other ooplasm deficiencies such as mitochondrial dysfunction. Oocyte aging and the resulting chromosomal errors explains why miscarriage rates in natural pregnancies for women younger than age 30 are only 7% to 15% and become marginally higher for women age 30 to 34 at 8% to 21%. By age 35 to 39 the rate is 17% to 28%, and over age 40 the rate is 34% to 52%.[6] According to a computer simulation model, 32 is the maximum age at which couples should start trying to conceive in order to have a 90% chance of having a one-child family; for a two-child family the maximum age is 27; and for a three-child family the maximum age is 23.[17] For women age 35 to 40, fertility investigations are indicated after 6 months of trying and for women over 40 years they should be initiated immediately.[1]

Box. Workup for infertility

When to investigate by female age:
- After 1 year for patients < 35 years.
- After 6 months for patients 35–40 years.
- Immediately for patients > 40 years.

Clinical factors warranting earlier investigation:
- Female age > 35 years.
- Oligomenorrhea.
- Tubal risk factors.
- Male infertility risk factors.

Most common causes of infertility:
- Male factor.
- Ovulatory dysfunction.
- Tubal/pelvic disease.
- Advanced female age.

Standard investigations:
- Ovulation confirmation (serum progesterone > 10 nmol/L).
- Cycle day 3 follicle-stimulating hormone (< 7–10 IU/L) + estradiol (< 200 pmol/L).
- Hysterosalpingography.
- Semen analysis.

Additional ovarian reserve testing with anti-Müllerian hormone (AMH) assay:
- AMH reported in pmol/L in Canada and ng/mL in the US.
- Testing can be done on any day of the cycle.
- Patients must pay privately ($70).

Oligomenorrhea warrants early investigation for infertility because it is almost always the result of anovulation. If a woman's intermenstrual interval is greater than 35 to 40 days, she may be ovulating infrequently, unpredictably, or not at all. The most common causes of oligomenorrhea are polycystic ovary syndrome, perimenopause, endocrine disturbances such as thyroid disease, and endometrial pathology such as polyps, fibroids, or hyperplasia.

Ovarian reserve testing

Ovarian reserve testing aims to estimate the number of oocytes a woman has remaining. It is more accurately termed functional ovarian reserve testing because we cannot actually count the number of nongrowing primordial follicles in vivo.[18] Therefore, contemporary ovarian reserve tests assess the monthly cohort of hormone-responsive (pre-antral and antral) follicles to obtain a more accurate reflection of the true ovarian reserve.[19,20]

Female age is still one of the best predictors of oocyte quality and quantity. A female attains her lifetime maximum of oocytes (6 to 7 million) at around 20 weeks gestational age in utero.[6] By the time she is born that number has already dropped to 1 million and by the time she reaches puberty it is less than half that.[18] Oocyte number declines throughout life, dropping more rapidly after age 35 until the menopause threshold, when approximately 1000 oocytes remain.[18]

Follicle-stimulating hormone measured on day 3 of the menstrual cycle is the most common endocrine ovarian test. FSH is a gonadotropin produced by the anterior pituitary and it acts on granulosa cells in women to stimulate folliculogenesis and estrogen production.[6] Elevations in FSH were first described as a marker of ovarian aging over 40 years ago.[21] As follicular growth progresses in the early menstrual cycle, production of estradiol and inhibin B results in a negative feedback loop with the pituitary, and FSH secretion declines.[6] For this reason, it is customary to avoid falsely reassuring results when measuring day 3 FSH by checking that the estradiol level is low (less than 200 pmol/L, approximately) and FSH is not being suppressed. At menopause, when the follicular pool is depleted, FSH is no longer suppressed by estradiol and inhibin B and therefore remains indefinitely elevated. When FSH is high (above 20 IU/L) it is a reliable indicator of severely diminished ovarian reserve or perimenopause. A day 3 FSH level in the normal range (less than 10 to 15 IU/L) is not specific. While some researchers have reported on FSH thresholds, there is no level of FSH that can be considered definitively reassuring for confirming fertility potential. In a study of 3519 subfertile women, FSH levels above 8 IU/L were associated with a reduced probability of spontaneous pregnancy in the next 12 months (HR 0.93 per IU/L).[22] In cycles of in vitro fertilization (IVF), the live birth rate was maximal when the FSH level was less than 7 IU/L at all ages, and the live birth rate was below 2% when the FSH level was above 18 IU/L.[23] Measuring FSH can be inconvenient for patients since levels must be obtained on cycle days 2 to 4 and are prone to intercycle fluctuations. For this reason, offering additional ovarian reserve tests such as anti-Müllerian hormone (AMH) assay or antral follicle count (done by ultrasound in a fertility clinic) can improve sensitivity, specificity, and convenience.

Anti-Müllerian hormone has been called the "holy grail" of ovarian reserve testing.[24] The hormone was initially described in the 1940s regarding its role in sexual differentiation of the male embryo.[25] Specifically, AMH production by testis Sertoli cells in the late first trimester was shown to result in regression of the Müllerian ducts, while persistence of the Wolffian ducts was shown to result in formation of the internal male structures (epididymis, seminal vesicles, and vas deferens).[6] In 2002 it was discovered that AMH is closely correlated with the number of oocytes retrieved during an IVF cycle.[26] This led to a huge resurgence of interest in the hormone for assessing women's reproductive physiology. Although AMH is a functional ovarian reserve test, it represents a very accurate assessment of a woman's remaining egg number.[20]

A blood sample is required for an AMH assay, and in BC this test is not covered by provincial health insurance. The cost per assay is typically $70, which is paid to the collecting outpatient laboratory. AMH can be measured on any day of the menstrual cycle because it is only produced by the pre-antral and antral follicles, not the dominant follicle.[27] Some cycle-to-cycle variability of AMH does occur, but it is not significant enough to warrant repeated measurement.[28] One study found that AMH was 19% lower in users of the oral contraceptive pill compared with nonusers.[29] Other patient characteristics and lifestyle factors associated with lower AMH levels include pregnancy, African-American and Hispanic ethnicity, and obesity.[30-32] Interestingly, smoking has been consistently associated with earlier menopause but not with lower AMH values.[33,34] Research studies have incorporated AMH to improve menopause forecasting but the wide confidence intervals and marked variation between women make it difficult to use clinically.[35] The principal

utility of AMH is in assessing ovarian reserve and predicting a woman's response to controlled ovarian stimulation for an IVF cycle. AMH measurement has also been used to record ovarian reserve before and after treatments known to damage the ovary, such as chemotherapy, radiation, and ovarian surgery.

The normal values of AMH are highly age-specific and require careful interpretation. It is also important to note that Canadian labs report AMH in pmol/L, which can be multiplied by 0.14 for conversion to the American units of ng/mL. Although there is no universal definition of high AMH, a level above 21.0 pmol/L (3.0 ng/mL) is considered by many as a risk factor for hyper-response to IVF stimulation.[36] There is no upper level of AMH that is diagnostic of polycystic ovary syndrome. AMH levels below 8.0 pmol/L (0.7 to 1.1 ng/mL) are considered low and can be a marker for poor egg yield during the IVF process.[36]

Uterine-tubal evaluation

Estimates suggest that tubal and pelvic disease cause 35% of infertility.[6] In BC the most readily available test for tubal patency is hysterosalpingography (HSG), which involves transcervical instillation of radiopaque fluid and use of fluoroscopy to visualize the internal contour of the uterus and the spill of fluid through the fallopian tubes into the pelvis. HSG is generally scheduled in the follicular phase to avoid interfering with a pregnancy. Many facilities require the patient to phone for an appointment when a menstrual period begins and to perform a pregnancy test the day before HSG. For women who do not have a regular menstrual cycle, exogenous progestin can be used to induce a withdrawal bleed (e.g., 10 mg medroxyprogesterone acetate PO for 10 days). If the patient has risk factors for postprocedure infection such as hydrosalpinx or previous pelvic inflammatory disease, then antibiotic prophylaxis is recommended (e.g., 100 mg doxycycline PO, twice daily for 3 to 5 days, beginning the day before the procedure).[37] HSG is a good test for ruling out tubal pathology such as obstruction or hydosalpinx. One meta-analysis reported 65% sensitivity and 83% specificity for tubal obstruction.[38] HSG is less specific for endometrial pathology such as polyps, submucous fibroids, and adhesions. Diagnostic tests in the form of hysteroscopy or sonohysterography can be done to further investigate the endometrial cavity as needed. When a hysterosalpingogram suggests bicornuate uterine configuration, imaging of the uterine corpus must be done to differentiate between septate and bicornuate Müllerian anomalies. This can be done with 3D ultrasound, magnetic resonance imaging, or concurrent hysteroscopy with laparoscopy. Some studies have reported an increase in spontaneous pregnancy rates following HSG with water-based contrast medium,[39] although historically this benefit has been attributed to oil-based contrast HSG.[40] A recent study followed over 1000 infertile women randomly assigned to undergo HSG with water-based or oil-based contrast.[41] There were significantly more live births in the oil-based group (39% versus 28%, OR 1.38, 95% CI 1.17–1.64). The underlying mechanism for this possible benefit may involve dislodging of mucus plugs and endometrial or immunomodulatory effects.[41] In BC hysterosalpingography is usually performed with water-based contrast. The gold standard for tubal and pelvic evaluation is laparoscopy with chromopertubation. Because of the inherent risks of surgery and long wait times, however, it is not commonly performed for tubal assessment without other indications for surgery.

Semen analysis

Semen analysis is a fundamental part of the workup because the male factor accounts for at least 35% of infertility.[6] The process of spermatogenesis takes approximately 70 days and continues throughout a man's lifetime, allowing many men to maintain fertility in perpetuity.[6] Once a spermatozoon is deposited in the vagina, it must separate itself from the seminal fluid (a product of the seminal vesicles and prostate gland) and swim through the cervical mucus and endometrial cavity to wait for the oocyte in the fallopian tube. A complex set of activities in the sperm is required for successful fertilization, including capacitation and the acrosome reaction, which allow for penetration of the cumulus oophorus and zona pellucida (egg shell).[6] Once the sperm head fuses with the oolemma it will undergo nuclear decondensation to form the male pronucleus and eventually fuse with the female pronucleus to create an embryo.[6]

An optimal sample for semen analysis is obtained after 2 to 5 days of abstinence and processed for analysis after 15 to 30 minutes of observed liquefaction. The normal values for semen parameters in the current World Health Organization (WHO) laboratory manual (5th edition) were obtained from a retrospective examination of fertile men whose partners conceived within 12 months.[42] The lowest fifth percentile was used as a cutoff. A semen analysis uses one-sided lower limits for reference: volume (1.5 mL), concentration (15 M/mL), total sperm count (39 M), total motility (40%), progressive motility (32%), vitality (58%), and morphology (4%).[43] For fertility, the chief

prognostic parameters are sperm concentration and motility, with morphology being of lesser importance. Sperm concentration can vary substantially from sample to sample in both fertile and infertile men.[42] When the concentration is lower than 15 M/mL fertility is reduced, while increases above this level are not consistently associated with better pregnancy rates.[42] Sperm motility can be affected by many factors, including duration of abstinence, age, health status, length of time to processing, and exposure to heat or toxins.[42] An abnormal test result warrants repeat testing, allowing a break of at least 2 to 3 months for any intervention aimed at improving sperm quality. Assessing morphology involves examination of at least 200 sperm under 400x or 1000x magnification to consider the head, mid-piece, and tail according to the Kruger criteria.[42] The first edition of the WHO laboratory manual required 80.5% normal-shaped sperm; over subsequent editions this was reduced to 50.0% (2nd edition), 30.0% (3rd edition), and 15.0% (4th edition). It is important when counseling patients to reassure them that even 0% normal sperm morphology does not preclude a pregnancy.[44]

Summary

Infertility is a prevalent condition that affects about 15% of couples. In BC women are delaying childbearing longer than anywhere else in the country. This makes our patient population particularly susceptible to age-related infertility. For women over 35 years, infertility investigations should be initiated after 6 months of trying to conceive, and for women over 40 years, investigations should be initiated immediately. The basic workup for infertility includes confirmation of ovulation, measurement of follicle-stimulating hormone level, hysterosalpingography, and semen analysis. Measurement of day 3 FSH should be ordered in conjunction with measurement of estradiol to confirm appropriate timing. Physicians should be aware that because FSH is a late marker of diminished ovarian reserve, there is no level of FSH considered reassuring. AMH is an accurate and convenient test, but interpretation is highly age-specific. HSG is a useful test to rule out tubal obstruction; however, any uterine abnormality should be investigated further with hysteroscopy with laparoscopy or sonohysterography. Semen analysis is a key component of the basic infertility workup, with sperm concentration being the most important predictor for fertility. Consultation with a gynecologist or fertility specialist is covered by provincial health insurance and should be considered for couples with abnormal test results and for couples who fail to conceive despite normal test results. BCMJ

Competing interests

None declared.

References

1. Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Reproductive Endocrinology and Infertility. Optimizing natural fertility: A committee opinion. Fertil Steril 2017;107:52-58.
2. Hoffman B, Schorge J, Schaffer J, et al. Williams Gynecology. 2nd ed. New York: McGraw Hill Medical; 2012.
3. Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of the pregnancy, and sex of the baby. N Engl J Med 1995;333:1517-1521.
4. Perloff WH, Steinberger E. In vivo survival of spermatozoa in cervical mucus. Am J Obstet Gynecol 1964;88:439-442.
5. Cohen J, Fehilly CB, Walters DE. Prolonged storage of human spermatozoa at room temperature or in a refrigerator. Fertil Steril 1985;44:254-262.
6. Fritz MA, Speroff L. Clinical gynecologic endocrinology and infertility. 8th ed. Philedelphia: Lippincott Williams & Wilkins; 2011.
7. Levitas E, Lunenfeld E, Weiss N, et al. Relationship between the duration of sexual abstinence and semen quality: Analysis of 9,489 semen samples. Fertil Steril 2005;83:1680-1686.
8. Kunz G, Beil D, Deininger H, et al. The dynamics of rapid sperm transport through the female genital tract: Evidence from vaginal sonography of uterine peristalsis and hysterosalpingoscintigraphy. Hum Reprod 1996;11:627-632.
9. Pauerstein CJ, Eddy CA, Croxatto HD, et al. Temporal relationships of estrogen, progesterone, and luteinizing hormone levels to ovulation in women and infrahuman primates. Am J Obstet Gynecol 1978;130:876-886.
10. Filicori M, Santoro N, Merriam GR, Crowley WF Jr. Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab 1986;62:1136-1144.
11. MacKenzie E. BC Moms give birth later than the rest of Canada. 24 Hours Vancouver. Accessed 23 June 2017. http://vancouver.24hrs.ca/2016/02/16/bc-moms-give-birth-later-than-rest-of-canada (site discontinued).
12. Mesen TB, Mersereu JE, Kane JB, Steiner AZ. Optimal timing for elective egg freezing. Fertil Steril 2015;103:1551-1556.
13. Hodes-Wertz B, Druckenmiller S, Smith M, Noyes N. What do reproductive-age women who undergo oocyte cryopreservation think about the process as a means to preserve fertility? Fertil Steril 2013;100:1343-1349.
14. Cohn D. In Canada, most babies now born to women 30 and older. Pew Research Center. 10 July 2013. Accessed 1 March 2018. www.pewresearch.org/fact-tank/

2013/07/10/in-canada-most-babies-now-born-to-women-30-and-older/.
15. Statistics Canada. Report on the demographic situation in Canada, 2008 to 2012. Accessed 1 March 2018. www.statcan.gc.ca/daily-quotidien/130709/dq130709a-eng.htm.
16. British Columbia Vital Statistics Agency. Annual report 2011. www2.gov.bc.ca. Accessed 1 March 2018. www2.gov.bc.ca/gov/content/life-events/statistics-reports/annual-reports/2011.
17. Habbema JD, Eijkemans MJ, Leridon H, te Velde ER. Realizing a desired family size: When should couples start? Hum Reprod 2015;30:2215-2221.
18. Hansen KR, Knowlton NS, Thyer AC, et al. A new model of reproductive aging: The decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod 2008;23:699-708.
19. Broekmans FJ, Visser JA, Laven JS, et al. Anti-Müllerian hormone and ovarian dysfunction. Trends Endocrinol Metab 2008; 19:340-347.
20. Anderson RA, Nelson SM, Wallace WH. Measuring anti-Müllerian hormone for the assessment of ovarian reserve: When and for whom is it indicated? Maturitas 2012;71:28-33.
21. Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. J Clin Invest 1975;55:699-706.
22. van der Steeg JW, Steures P, Eijkemans MJC, et al. Predictive value and clinical impact of Basal follicle-stimulating hormone in subfertile, ovulatory women. J Clin Endocrinol Metab 2007;92:2163-2168.
23. Scott RT, Elkind-Hirsch KE, Styne-Gross A, et al. The predictive value for in vitro fertility delivery rates is greatly impacted by the method used to select the threshold between normal and elevated basal follicle-stimulating hormone. Fertil Steril 2008;89:868-878.
24. Lambalk CB. Anti-Müllerian hormone, the holy grail for fertility counselling in the general population? Hum Reprod 2015; 30:2257-2258.
25. Josso N. Professor Alfred Jost: The builder of modern sex differentiation. Sex Dev 2008;2:55-63.
26. Seifer DB, MacLaughlin DT, Christian BP, et al. Early follicular serum müllerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 2002;77:468-471.
27. Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370-385.
28. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103-3107.
29. Birch Petersen K, Hvidman HW, Forman JL, et al. Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan. Hum Reprod 2015;30:2364-2375.
30. Köninger A, Schmidt B, Mach P, et al. Anti-Mullerian-Hormone during pregnancy and peripartum using the new Beckman Coulter AMH Gen II Assay. Reprod Biol Endocrinol 2015;13:86.
31. Seifer DB, Golub ET, Lambert-Messerlian G, et al. Variations in serum müllerian inhibiting substance between white, black, and Hispanic women. Fertil Steril 2009; 92:1674-1678.
32. Steiner AZ, Stanczyk FZ, Patel S, Edelman A. Antimullerian hormone and obesity: Insights in oral contraceptive users. Contraception 2010;81:245-248.
33. Ertunc D, Tok EC, Aytan H, Gozukara YM. Passive smoking is associated with lower age at menopause. Climacteric 2015; 18:47-52.
34. La Marca A, Spada E, Grisendi V, et al. Normal serum anti-Müllerian hormone levels in the general female population and the relationship with reproductive history. Eur J Obstet Gynecol Reprod Biol 2012; 163:180-184.
35. Dólleman M, Verschuren WM, Eijkemans MJ, et al. Added value of anti-Müllerian hormone in prediction of menopause: Results from a large prospective cohort study. Hum Reprod 2015;30:1974-1981.
36. La Marca A, Ferraretti AP, Palermo R, Ubaldi FM. The use of ovarian reserve markers in IVF clinical practice: A national consensus. Gynecol Endocrinol 2016;32:1-5.
37. ACOG Committee on Practice Bulletins–Gynecology. ACOG Practice Bulletin No. 104: Antibiotic prophylaxis for gynecologic procedures. Obstet Gynecol 2009; 113:1180-1189.
38. Swart P, Mol BW, van der Veen F, et al. The accuracy of hysterosalpingography in the diagnosis of tubal pathology: A meta-analysis. Fertil Steril 1995;64:486-491.
39. Cundiff G, Carr BR, Marshburn PB. Infertile couples with a normal hysterosalpingogram. Reproductive outcome and its relationship to clinical and laparoscopic findings. J Reprod Med 1995;40:19-24.
40. Johnson NP, Farquhar CM, Hadden WE, et al. The FLUSH trial–Flushing with lipiodol for unexplained (and endometriosis-related) subfertility by hysterosalpingography: A randomized trial. Hum Reprod 2004;19:2043-2051.
41. Dreyer K, van Rijswijk J, Mijatovic V, et al. Oil-based or water-based contrast for hysterosalpingography in infertile women. N Engl J Med 2017;376:2043-2052.
42. Silverberg K, Turner T. Evaluation of sperm. In: Textbook of assisted reproductive techniques. 4th ed. Vol 1: Laboratory perspectives. Gardner DK, Weissman A, Howels CM, Shoham Z, editors. Boca Raton, FL: CRC Press; 2012:48-60.
43. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO; 2010.
44. Hotaling JM, Smith JF, Rosen M, et al. The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: A systematic review and meta-analysis. Fertil Steril 2011;95:1141-1145.