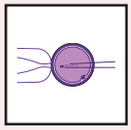
IV

INFERTILITY

Chapter 28: Female Infertility

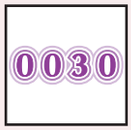
Chapter 29: Male Infertility

Chapter 30: Induction of Ovulation



Chapter 31: Assisted Reproductive Technologies

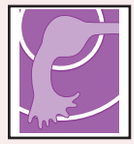
Chapter 32: Fertility Preservation



Chapter 33: Recurrent Early Pregnancy Loss

Chapter 34: Genetics

Chapter 35: Endometriosis



Chapter 36: Ectopic Pregnancy

Female Infertility

28

INTRODUCTION

Infertility has been traditionally defined as 1 year of regular unprotected intercourse without conception.[1] However, this definition dates back to an era when the majority of couples started family building at younger ages compared to today. Currently, it is considered most appropriate for women under age 35. For women older than 35 years, investigations and treatment may begin after 6 months of trying to conceive, and for women over age 40, they can be initiated even earlier.[1] The term **subfertility** is used interchangeably to describe women or couples who may not be sterile but who exhibit decreased reproductive efficiency. **Fecundability** is the probability of being pregnant in a single cycle, and **fecundity** is the probability of achieving a live birth within a single cycle. Approximately 85% to 90% of healthy young couples conceive within 1 year, most within 6 months.[2,3] Infertility, therefore, affects approximately 10% to 15% of couples, and it is estimated that globally, one in six people (17.5%) experience infertility at some stage in their lives.[4] Therefore, infertility represents an important part of clinical practice and has significant social, psychological, and financial implications.[4,5]

Contrary to popular perception, the overall incidence of infertility does not seem to have increased over the past three decades (https://www.cdc.gov/nchs/nsfg/key_statistics/i-keystat.htm#infertility).[6] However, the evaluation and treatment of infertility have changed dramatically during the same period. Three major developments have had the greatest impact. The first was the introduction of in vitro fertilization (IVF) and other assisted reproductive technologies (ART). ART techniques have provided the means to study reproductive processes in new and more revealing ways and have markedly improved the prognosis for a great many infertile couples, particularly those whose infertility relates to severe tubal damage or male factors. Second, changes in population demographics have resulted in greater numbers of women attempting pregnancy at older ages when they are inherently less biologically fertile. Third, advances in ART and concerns about the age-related decline in fertility have combined to attract greater media attention and to raise public awareness of infertility and modern treatments. Consequently, infertile couples are now more likely to seek medical advice, evaluation, and treatment.

THE EPIDEMIOLOGY OF INFERTILITY IN THE UNITED STATES

The first US Census was in 1790. At that time, the crude birth rate was 55 per 1,000 total population; in 2015, it was 12.4 per 1,000 and continued its decline to 11.4 per 1,000 in 2022,[7] representing a record low for the nation with a nearly 80% decline over the past 200-plus years and a 32% decline since 1990 (16.7 per 1,000 population). The general fertility rate (births per 1,000 women aged 15–44) in 2022 was 56, 21% lower than in 1990 (70.9/1,000), 36% lower than in 1970 (87.9/1,000), and 47% lower than in 1950 (106.2/1,000) during the postwar "baby boom."[7–9]

The overall long-term decline in the US birth and fertility rates has been attributed to several factors:

- **Greater interest in advanced education and careers among women**
- **Later marriage and more frequent divorce**
- **Improvements in contraception and access to family planning services**
- **Delayed childbearing**
- **Decreased family size**
- **Rising cost of raising children, including housing, education, and health care**

Attitudes toward women and among women in our society have changed dramatically in many ways over the past 50 years. Expanding opportunities have increased interests

in advanced education and careers among women. The U.S. Census data indicate that in 1970, only 8.2% of women aged 25 and older had completed 4 or more years of college; by 2022, that proportion had increased to 45.2% and exceeded the same rate in men (39.9%).[10] Women have represented the majority of college students in the United States since 1979. In recent years, the majority of bachelor's and more advanced degrees have been awarded to women.[11] In parallel with the increasing rate of completing higher education, the proportion of US women with infant children in the workforce has steadily increased, from 31% in 1976 to 59% in 2016.[10] In 2022, approximately 77% of all women aged 25 to 44 years were in the labor force.[12]

Greater focus on education and careers among women triggered other trends in modern society. Less frequent and later marriage and more frequent divorce were among the most striking. First-marriage rates in the United States peaked after World War II, between 1945 and 1947 (143 per 1,000 single women aged 15–44), and declined about 15% every 10 years and approximately 50% overall over the next five decades.[13] The median age at first marriage for women has been steadily rising since 1960 (20.3 years) and reached 26.5 years in 2009 and 28 years in 2016. The proportion of never-married women aged 25 to 29 has also steadily increased from 26.9% in 1986 to 46.8% in 2009 and 59.7% in 2016.[14] The probability of future marriage for women decreases as age increases: 84% at age 25, 72% at age 30, 52% at age 35, and 41% at age 40.[15] If and when women do marry, they are also more likely to divorce than in the past.[13,15–17] Divorce rates among women of reproductive age rose quickly after 1960 to more than double by 1980 (40 per 1,000 married women aged 15–44) and have declined only slightly over the last 30 years. The National Center for Health Statistics estimates that approximately one-third of new marriages among younger people will end in divorce within 10 years and 43% within 15 years. Once-married women are also increasingly less likely to remarry. Remarriage rates peaked in 1968 (166 per 1,000 divorces or widowed women aged 15–44) as divorce rates began to rise but have since declined steadily by more than one-third, in parallel with first-marriage rates.[13–17]

The postwar "baby boom" generation, those born between 1946 and 1964, was the first to be afforded the means to safely and effectively control their fertility. Expanding contraceptive options and access to family planning and legalized abortion services over the past five decades has definitely contributed to declining US birth and fertility rates. Their effects have been direct, by reducing the number of unplanned pregnancies and births, and indirect, by helping women to avoid pregnancy until their education and career goals were met, and marriage and family became a priority.

The net result of all of these societal changes was a trend to delayed childbearing among American women. The mean age at first live birth has been continuously rising, from 21.4 years in 1970 to 26.4 years in 2015 and to an all-time high of 27.4 years in 2022 (6 years' increase in five decades). The percentage of first births occurring to women aged 30 years or older also increased drastically between 1970 and 2019[7,18]; 24.9% of first births occurred after age 30 among married women in 2019.[19]

Between 1970 and 2015, per the latest data available while writing the former edition of this book, birth rates fell for women aged 15 to 19 (68.3 vs 22.3/1,000), 20 to 24 (167.8 vs 76.8/1,000), and 25 to 29 (145.1 vs 104.3/1,000) and increased for women aged 30 to 34 (73.3 vs 101.5/1,000), 35 to 39 (31.7 vs 51.8/1,000), and 40 to 44 (8.1 vs 11.0/1,000).[7,9] Based on 2022 data, birth rates continued to decline since 2015 for women aged 15 to 19 (13.6/1,000), 20 to 24 (57.5/1,000), 25 to 29 (93.5/1,000) and 30 to 34 years (97.5/1,000), but not for 35 to 39 (55.3/1,000) and 40 to 44 years (12.6/1,000). Predictably, increasing age at first birth and declining fertility rates combined to result in fewer births per woman. At the height of the postwar baby boom, the US total fertility rate (births by age 45) reached a modern high of 3.7 births per woman (1957). Thereafter, the total fertility rate declined to a low of 1.8 in 1976 and has remained stable with some fluctuations.[7] In 2023, the total fertility rate was 1.6, under the population replacement rate, as has consistently been the case since 2007. Likewise, the total fertility rates in most industrialized countries are inadequate to ensure replacement of the population.[20]

Trends in the incidence of infertility among US women have been difficult to define confidently. The earliest national estimate of infertility, from the 1965 National Survey of Family Growth (NSFG), was 11.2%. In 1982, 8.5% of married American women were infertile, and in 2002 7.4% were infertile, representing a 10% decrease over the intervening 20 years.[21] The latest figure from 2019 is 8.7%, demonstrating a plateau.[22] A similar trend is observed worldwide. An analysis of 277 demographic and reproductive health surveys from 173 countries showed statistically similar prevalence of infertility in 1990 and 2010.[23]

The array of infertility services, and their availability, has increased dramatically over the last 40 years. The public has a greater awareness of infertility and modern treatments, largely due to the increased media attention, good and bad, surrounding the advances and controversies relating to ART. As infertility has become more visible, and more socially acceptable, couples have become more comfortable to seek evaluation and treatment.

Based on 2015–2019 NSFG data, 12.2% of women aged 15 to 49 in the United States reported having ever used infertility services.[24] This percentage remained consistent across previous survey periods (2006–2010 and 2011–2015). Compared to the general population, women who sought infertility services were more likely to be older (aged 35–44 years; 43% vs 36%), nulliparous (36% vs 16%), married (79% vs 64%), relatively affluent (61% vs 51%), and to have health care insurance (83% vs 74%).[25] Among surveyed women, 12% reported receiving drugs to improve ovulation, 5% underwent intrauterine insemination (IUI), and 1.5% utilized ART.[24]

AGING AND FERTILITY

The effects of aging on female fertility are perhaps best revealed by the results of studies in "natural" populations, wherein couples reproduce without voluntary restrictions[26]; the Hutterites in North America are a classic example. Contraception is condemned in the sect, which emigrated originally from Switzerland in the 16th century and settled ultimately in communal colonies in South Dakota in the late 19th century. Studies of fertility in the Hutterites illustrate how fertility declines with advancing age.[27] Whereas only 2.4% of Hutterite women were infertile, 11% bore no children after age 34, 33% were infertile by age 40, and 87% were infertile at age 45. **Although revealing, these and other data derived from studies in natural populations may not reflect true biologic reproductive potential, for several reasons:**

- **Women who have children when young may be less inclined to conceive again in later life.**
- **Coital frequency often declines as age increases, reflecting decreasing desire or lack of a partner.**
- **The incidence of subclinical abortion is unknown.**
- **The cumulative impact of other diseases or conditions that can adversely affect fertility (eg, pelvic infections, leiomyomata, endometriosis) is greater in older women.**

Taken together, data from studies in the Hutterites and other natural populations suggest that fertility in women peaks between the ages of 20 and 24, decreases relatively little until approximately age 30 to 32, and then declines progressively. **Overall, fertility rates are 4% to 8% lower in women aged 25 to 29 years, 15% to 19% lower in those aged 30 to 34, 26% to 46% lower in women aged 35 to 39, and as much as 95% lower for women aged 40 to 45 years**.[28,29]

Variations in fertility rates among natural populations could reflect differences in genetic factors or socioeconomic conditions at different times and in different places.

Other evidence for the adverse effect of aging on fertility derives from numerous studies of cumulative conception rates among women attempting pregnancy by artificial insemination with donor sperm. Since women in donor insemination programs are less likely to have other infertility factors and carefully timed inseminations eliminate the confounding effects of decreasing coital frequency with increasing age, donor insemination studies provide good estimates of female fertility. In a French study involving more than 2,000 women across up to 12 insemination cycles, conception rates were highest in those aged 25 or younger (73%) and aged 26 to 30 (74%), 16% lower (62%) in women aged 31 to 35, and 27% lower in those over age 35 (54%).[30] An American donor insemination study yielded similar results, observing lower overall conception rates and a 2-fold higher number of inseminations per conception in women over age 35.[31] A Dutch study observed that the probability of a healthy live birth decreased by approximately 3.5% per year after age 30.[29] In a large British study of nearly 3,000 donor insemination cycles from a single center, cumulative conception rates in women over age 30 were 20% to 35% lower than in younger women after 3 (17% vs 21%), 6 (26% vs 40%), and 12 insemination cycles (44% vs 62%).[32]

Success rates achieved with ART using nondonor eggs also decline as age increases. The numbers of oocytes retrieved and embryos available and implantation, pregnancy, and live birth rates are lower in older than in younger women.[33] Annual reports derived from registry data collected by the Centers for Disease Control and Prevention (CDC) in the United States since 1989 demonstrate consistently that age is the single most important factor affecting the probability of success with ART. Pregnancy and live birth rates for ART cycles using fresh, nondonor eggs or embryos vary little for women under age 32 but thereafter decrease steadily in an almost linear fashion as age increases. Regardless of whether success rates are calculated per cycle, per oocyte retrieval, or per embryo transfer, the result is the same. In the 2021 US national summary, the live birth rate per intended egg retrieval after all resulting embryo transfers for a given cycle was 54.0% for women under age 35, 40.5% for ages 35 to 37, 26.0% for ages 38 to 40, 13.3% for ages 41 to 42, and 4.0% for women aged over 42 years.[33]

The age-related decline in ART live birth rates reflects not only decreasing fertility but also increasing pregnancy wastage. Just as fertility decreases with increasing age, the incidence of clinically recognized pregnancy loss rises as age advances. **Pregnancy loss rates in natural conception cycles are generally low before age 30 (7–15%) and rise with age, only slightly for ages 30 to 34 (8–21%) but to a greater extent for ages 35 to 39 (17–28%) and ages 40 and older (34–52%)**.[28,34–36] The same pattern is observed in pregnancies resulting from ART. In the 2016 US national summary of IVF outcomes, pregnancy loss rates were below 15% for women under age 35, almost 30% at age 40, and 60% for women aged 44.[37] Longitudinal studies of healthy young women, wherein daily urine samples were monitored for the appearance of human chorionic gonadotropin (hCG), have revealed that true spontaneous pregnancy loss rates (also including clinically unrecognized "biochemical" pregnancies) are substantially higher.[38–40] *Up to 60% of all conceptions miscarry within the first 12 weeks of gestation, and 20% to 40% of all early pregnancy losses go unrecognized.* Whether the incidence of occult early pregnancy loss is also higher in older women than in young women has not been determined. If so, the relationship between true spontaneous pregnancy loss rates and age may be even more dramatic. Even if not, the overall risk of pregnancy loss (recognized and unrecognized) in women over age 40 approaches or exceeds 75% (**Figure 28.1**).[39,41]

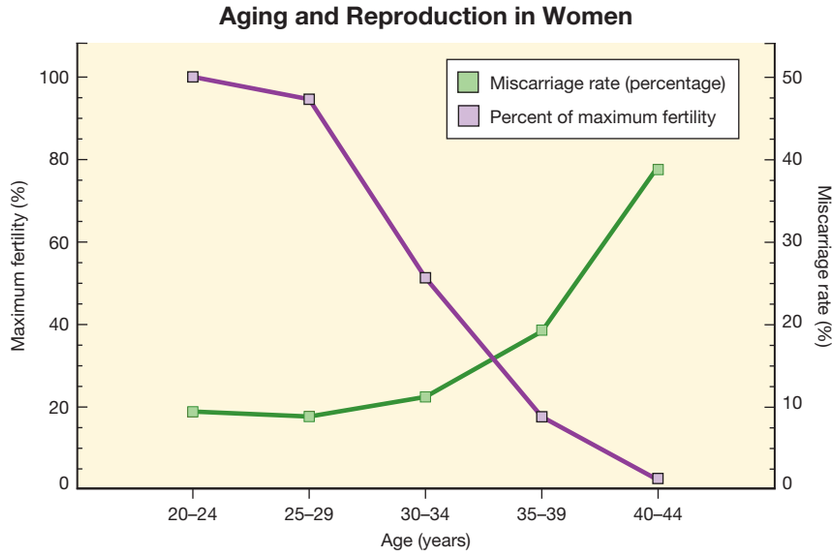


FIGURE 28.1

Physiology of Reproductive Aging

The age-related decrease in female fertility and the societal trends toward delayed childbearing have focused a great deal of attention on the physiology of reproductive aging. Consequently, our understanding of the mechanisms that govern the pace of follicular depletion, the endocrinology of reproductive aging, the age-related changes in follicular dynamics and oocyte quality, and the genetic determinants of ovarian aging has advanced greatly over the past 30 years. We long ago recognized the changes in menstrual cycle characteristics that accompany advancing age but now much better understand the mechanisms responsible for those changes. We long ago recognized that fertility declines as age increases but now have measures of reproductive aging that help to guide our efforts to overcome its limitations. We may not be able to prevent aging, but now we can better help women to set and to realize their reproductive goals.

Follicular Depletion

In humans, the number of oocytes peaks around the 20th week of gestation when approximately 6 to 7 million oocytes arrested at the first meiotic prophase are found in the ovarian cortex.[42–44] Afterward, regulated apoptosis starts an irreversible decline in the germ cell population.[45] The number of oocytes declines to 1 to 2 million at birth[46] and to 300,000 to 400,000 by puberty.[43,47] Over the next 35 to 40 years of reproductive life, only about 400 oocytes will ovulate, the rest being lost through atresia. By age 40, the number of follicles shrinks to approximately 25,000, and at menopause, there remain less than 1,000 follicles.[48–51]

Accurate modeling of the pattern of follicle depletion in the human ovary is important because the ability to measure reproductive aging or to predict the number of remaining follicles—to tell time on the biologic clock—would help women make informed decisions about their reproductive plans.[52] However, for obvious reasons, accurate measures of the numbers of primordial follicles across a human female reproductive life span are difficult to obtain.

The first attempt to define the age-related pattern of follicular depletion was based on an analysis of combined data from older morphometric studies and yielded a biexponential model of ovarian aging, describing a biphasic pattern of oocyte depletion, with a distinct increase in the rate of decline beginning at approximately age 37.5 years.[42,48,53,54] The biphasic model was widely accepted, despite the biologic implausibility of an abrupt, population-wide, physiologic shift in the rate of follicular depletion.[55,56] The model is still cited frequently,[57,58] but subsequent work has demonstrated that a simpler, more biologically plausible, exponential[49,59] or power function[60] conforms best with available human data and current concepts regarding the mechanisms that govern the rate of follicular depletion.[52,61] **The current working model describes a gradually increasing rate of follicular depletion in which the pace of decline increases as the number of follicles remaining decreases, supported by evidence that paracrine factors secreted by primordial follicles inhibit recruitment and regulate the size of the resting follicular pool.**[52,61–63] The model describes the mean trajectory of follicular depletion, but a great deal of population variation remains unexplained. Some of the variation among individuals doubtless relates to differences in the size of the initial follicular pool, which could be random but is likely genetically determined, and on lifestyle factors. The current model of reproductive aging is still evolving and does not yet have any real clinical utility because it cannot predict the reproductive life span for an individual woman.[52,60]

Endocrinology of Reproductive Aging

Toward the end of the reproductive period, serum follicle-stimulating hormone (FSH) levels begin to rise, while luteinizing hormone (LH) concentrations remain unchanged. This occurs before any discernible change in menstrual regularity. The subtle "monotropic" rise in circulating FSH concentrations is most apparent during the intercycle transition, when the corpus luteum regresses and menses begins and could result from age-related changes in the pattern of pulsatile kisspeptin and gonadotropin-releasing hormone (GnRH) secretion or from progressive follicular depletion and decreased inhibitory feedback by ovarian hormones. The weight of available evidence supports the second explanation.[64,65]

A variety of studies in animals and women have identified changes in the patterns of hypothalamo-pituitary hormone secretion across the menopausal transition. In rodents, an age-related decrease in pulsatile GnRH and LH secretion and a loss of positive estrogen feedback have been observed, before the follicular pool is exhausted.[66–69] In nonhuman primates, pulsatile GnRH release increases during the perimenopause, and the positive feedback response remains intact.[70] Studies in perimenopausal and postmenopausal women have yielded conflicting results. Whereas some have observed changes in sensitivity to estrogen feedback signals[71,72] or in LH pulse amplitude or frequency,[73–78] others have not.[79–81] The response to exogenous GnRH stimulation is also inconsistent.[77,82,83] On balance, these data strongly suggest that age-related changes in pulsatile LH secretion and gonadotropin concentrations merely reflect changes in ovarian feedback signals and do not result from aging of the hypothalamo-pituitary axis.

The bulk of available evidence indicates that the progressive increase in FSH concentrations associated with reproductive aging results from a progressive decrease in the levels of feedback inhibition from the smaller cohorts of follicles recruited from a shrinking follicular pool. Circulating follicular phase inhibin B levels (derived primarily from smaller antral follicles) decrease as or even before FSH concentrations begin to increase.[64,84–91] Inhibin A levels also decline, but only in the later stages of reproductive aging, after the onset of menstrual irregularity.[88,92–95] Both inhibins selectively inhibit pituitary FSH secretion. Consequently, FSH levels rise progressively as inhibin production from smaller cohorts of aging follicles decreases, most noticeably in the early follicular phase. Whereas declining inhibin production could also reflect a decrease in the functional capacity of older follicles,[96] the observation that preovulatory follicular fluid inhibin concentrations are similar in young and older cycling women suggests that the number of remaining follicles is more important.[84] Ovarian steroid hormones do not play a major role. The initial rise in FSH levels precedes any measurable decrease in estradiol levels, by several years.[65,97] Follicular phase estradiol levels in older cycling women are generally similar to those in younger women and often even higher.[84,98] Luteal phase estrogen and progesterone levels also do not seem to change consistently with advancing age.[64,86,88,99–102] Moreover, in sporadic ovulatory cycles in aging women, serum concentrations of estradiol and progesterone are comparable to those observed in younger women.[103]

As age and FSH levels increase, the follicular phase becomes shorter[104–106]; LH levels and luteal phase duration remain unchanged. As the follicular phase shortens, estradiol levels rise earlier, suggesting that higher FSH levels stimulate more rapid follicular development.[64] **However, careful studies have shown that the earlier rise in estradiol levels results not from accelerated follicle growth but from advanced follicular development at the beginning of the cycle and earlier selection of the dominant follicle**.[99,105,107] The earlier increase in follicular phase FSH level also frequently results in more than one dominant follicle,[108–110] explaining the higher prevalence of dizygotic twinning in older cycling women.[99,108,111]

Reproductive aging is already quite advanced when the first clinical sign appears. Cycles remain regular, but overall cycle length and variability decrease gradually, reaching a nadir at an average age of 42 years,[104,112] when fertility is at or near an end. However, women generally take notice only when cycles become irregular, marking the beginning of the menopausal transition.[113] The age of menopause, recognized only in retrospect, averages 51 years but ranges widely between ages 40 and 60 years.[114–120] The variation in menopausal age is very similar across populations and generally follows a normal distribution that is slightly skewed to younger ages.[10–122] (refer to Chapter 21 for a detailed discussion of menopausal transition).

Genetics of Reproductive Aging

Barring any disease that destroys or causes the removal of ovarian tissue and any important environmental insults, the total number of follicles at birth, and the age when the supply is exhausted, is genetically determined.[47,120,123–130]

There is good correlation between menopausal age in mothers and daughters and between sisters, suggesting that genetic factors play an important role in determining menopausal age.[131–133] **Approximately 10% of women become menopausal by age 45,**[114,120] **probably because they were endowed with a smaller than average ovarian follicular pool that is functionally depleted at an earlier age. When menopause occurs prior to age 40, it is termed premature ovarian insufficiency (POI, also called primary ovarian failure and primary ovarian failure). POI is defined by the presence of spontaneous amenorrhea or irregular menstrual cycles for at least 4 months and an elevated FSH concentration >25 IU/L before age 40.**[134,135] **The reported prevalence of noniatrogenic POI varies from approximately 1% in older studies to 3.5% in recent publications.**[135] Pedigree analysis has revealed that the genetic features of

early menopause (age 40–45 years) and POI are similar, suggesting a dominant pattern of inheritance through maternal or paternal relatives.[136,137] The same genetic factors that determine the age at menopause also likely determine the age of reproductive milestones preceding the menopause.[138] In natural populations, the age at the last birth varies as widely as the age at menopause but occurs on average 10 years earlier.[47] Moreover, women who repeatedly respond poorly to exogenous gonadotropin stimulation also tend to have an earlier menopausal transition,[138–141] suggesting their poor response reflects an advanced stage of follicular depletion, beginning years sooner than would be anticipated normally.[138]

Genes affecting reproductive hormones (FSH, FSHR, LH, LHR, CYP17, CYP19) or involved in the initial growth of follicles (GDF9, BMP15, GPR3) impact follicular function; mutations are rare in humans, but polymorphisms could influence the rate of follicular recruitment and depletion and thereby affect the length of reproductive life.[142] Variations in other genes encoding DNA-binding proteins and transcription factors (NOBOX, LHX8) and RNA-binding proteins (EPAB, CPEB, NANOS) expressed during oogenesis, and mitochondrial genes regulating energy metabolism in oocytes and early embryos (CLPP, MFN1, MFN2) could affect germ cell formation; mutations causing POI have been identified in a few women.[143–148] Variations in other genes with links to POI might also affect the rate of follicular depletion in normal women (ADAMts9, FOXL2).[149,150] In a Dutch cohort study, common polymorphisms in the gene encoding the receptor for antimüllerian hormone (AMHR2) were associated with menopausal age,[151] implicating a decrease in AMH signaling that would weaken its paracrine inhibition of primordial follicle recruitment, leading to more rapid follicular depletion.

Candidate gene approaches are based on hypotheses regarding the genes or pathways that are most likely to be associated with a certain phenotype. This approach is limited by our incomplete understanding of the biology of reproductive aging and its genetic regulation. Genome-wide association studies (GWAS), on the other hand, aim to discover novel susceptibility loci for any disease as well as traits like age at menopause, without the limitation of a preconceived hypothesis. The first GWAS performed to identify loci involved in reproductive aging enrolled approximately 17,000 women and identified 13 loci clustered at chromosomes 5, 6, 19, and 20, associated with age at natural menopause.[152] A subsequent GWAS also identified the loci on chromosomes 19 and 20 and, in addition, another locus at chromosome 13, associated with the age at natural menopause.[153] Studies with similar approaches in 6,500 women with African ancestry and 3,500 Chinese women were consistent with the findings of earlier reports, suggesting that the timing of menopause is largely regulated in a similar fashion in different ethnic populations.[154,155] Subsequently, some of the genetic variants identified by these GWAS were found to be associated with early menopause in an independent cohort, suggesting that common genetic variants influencing natural menopause also constitute risk factors for early onset of menopause.[156] A more recent meta-analysis of age at natural menopause in nearly 40,000 women of European ancestry from 22 studies with replication in an additional 14,000 women confirmed four of the previously reported loci and identified 13 novel loci. The identified genes were not implicated in follicle development; instead, they were associated primarily with DNA repair and maintenance and immune function.[157] It is noteworthy that recent studies using whole exome sequencing identified mutations in homologous recombination and dsDNA break repair genes MCM8 and MCM9 in women with POI.[158,159] Careful examinations of these and other candidate genes yet to be identified will likely yield new insights and further our understanding of the mechanisms governing reproductive aging.

The Aging Follicle and Oocyte

Whereas the number of remaining ovarian follicles steadily declines with increasing age, observations in stimulated cycles suggest that aging follicles also become progressively less sensitive to gonadotropin stimulation. As age increases, the total dose and duration of treatment required to stimulate multiple follicular development increase. The rate of rise and the peak in estradiol levels decrease, reflecting the smaller cohorts of follicles that can be recruited. However, the amount of estradiol secreted by the follicles that do emerge and grow to maturity appears comparable to that in younger women.[160] Although a decrease in exogenous hCG-induced ovarian androgen production can be demonstrated before age 30, circulating estradiol levels remain normal throughout and beyond the reproductive years, probably because rising FSH levels are able to compensate.[161] Studies of ovarian follicular development and preovulatory follicular fluid hormones in older and younger cycling women do not suggest any age-related decline in follicular function, once growth and development begin. Preovulatory follicles in older and younger women are similar in size and inhibin content, and follicular fluid progesterone levels and estrogen/androgen ratios are even higher in older than in younger women.[84]

Older cycling women ovulate as regularly as and more frequently than younger women. Their rising FSH levels apparently compensate quite effectively for any decrease in follicular sensitivity to gonadotropin stimulation. Preovulatory follicles in older cycling women get an earlier start but grow at a normal pace and reach a normal size; their follicular fluid characteristics suggest they are also quite healthy. Why, then, does fertility in women decline progressively with age? **The available evidence indicates that both the age-related decline in female fertility and the increase in risk of pregnancy loss can be attributed to an increase in the proportion of abnormal oocytes in an aging and shrinking follicular pool.**

As the number of follicles decreases, oocyte quality also declines, primarily because of an increase in meiotic nondisjunction, resulting in an increasing rate of oocyte and embryo aneuploidy in aging women.[50,162–164] A wide variety of techniques has been used to study the chromosomal composition of human oocytes. The best available evidence, derived from a detailed cytogenetic analysis of oocytes retrieved for IVF that failed to fertilize, suggests that the global rate of oocyte aneuploidy increases with advancing maternal age.[165,166]

As previously discussed (Chapter 2), meiosis involves a single round of DNA replication (in the premeiotic S phase), followed by two cell divisions to form gametes, each with a half (or haploid) set of chromosomes. In the first meiotic division (meiosis I), duplicated chromosomes, consisting of pairs of sister chromatids, pair up with their homologues, and a synaptonemal complex is formed between them; recombination then occurs between nonsister homologous chromatids. At anaphase I, the two homologue chromosomes migrate to opposite poles. The second meiotic division (meiosis II) then follows without additional DNA replication. The pairs of sister chromatids align on the metaphase II spindle, and at anaphase II, the two sister chromatids finally separate to opposite poles, resulting in daughter cells with a haploid set of chromosomes. Oocyte aneuploidy results primarily from premature separation of sister chromatids during meiosis I or from whole chromosome nondisjunction during meiosis II.[166] The prevalence of both types of meiotic segregation errors increases progressively with age and makes the greatest contribution to the age-dependent increase in the prevalence of aneuploidy in human embryos.[165–169]

The European Society for Human Reproduction and Embryology (ESHRE) set up a pilot study of polar body biopsy and aneuploidy analysis in women of advanced maternal age (mean age 40.0).[170] Intracytoplasmic sperm microinjection was performed, and both polar bodies were biopsied and assessed simultaneously to identify errors in meiotic divisions. If aneuploidy was detected in one or both polar bodies, the corresponding presumed aneuploid fertilized oocyte or zygote was tested independently to confirm concordance with the polar body testing. This study revealed a high incidence of aneuploidies (72%), often multiple aneuploidies per zygote, resulting from an approximately equal proportion of errors in both meiotic divisions. While all chromosomes (except chromosome 3) had errors, the incidence of aneuploidy was higher for those with shorter and acrocentric chromosomes, particularly in chromosomes 11–22.

The pattern of copy number changes demonstrated that most meiosis I errors resulted from premature separation of sister chromatids as originally observed two decades ago[167] and that only 3% were caused by classical nondisjunction. In about half of cases where there had been an error in meiosis I, this error was balanced in meiosis II. Therefore, in the zygote, there were more aneuploidies resulting from errors in meiosis II than meiosis I. This pattern of multiple meiotic errors caused by premature separation of sister chromatids, and a net excess of meiosis II errors in zygotes following assisted conception, contrasts with the low incidence of multiple aneuploidies detected in pregnancies following natural conception and the predominance of female meiosis I errors in trisomies.[35,163] While, this could suggest that the use of high doses of exogenous hormones for ovarian stimulation in older patients may perturbs the process of meiosis., evidence from well-designed studies in assisted reproduction does not support a relationship between aneuploidy and gonadotropin use.[171,172]

An analysis of trophectoderm biopsies from more than 15,000 human blastocysts also showed that aneuploidy rate increases with age.[169] This is true for all chromosomes and highest for chromosomes 15, 16, 18, 19, 21, and 22.[173] The age-related increase in aneuploidy was most pronounced for acrocentric chromosomes (where centromere is located near one end of the chromosome—eg, chromosome 21).[173]

The age-related decrease in the proportion of normal oocytes (23,X) and the corresponding increase in the proportion of aneuploid embryos bear striking similarity to the age-related decrease in fertility and increase in the incidence of spontaneous pregnancy loss in women. Fertility and the prevalence of euploid oocytes decrease progressively with age. *The risk of pregnancy loss and the prevalence of aneuploid oocytes are relatively low and change little until approximately age 35 and then increase progressively, reaching virtually 100% after age 45.*[165,170] These observations offer a logical explanation for the age-related increase in the prevalence of aneuploidy in spontaneous abortuses. Whereas at least half of all clinically recognized pregnancy losses exhibit an abnormal karyotype and the frequency of both euploid (normal) and aneuploid (abnormal) abortuses increases with maternal age, the probability that an abortus will be chromosomally abnormal increases with age, from less than 35% at age 20 to nearly 80% over age 42.[35] Trisomies are by far the most common abnormality observed, followed by polyploidies and monosomy X (45,X).

Studies of meiotic segregation have revealed that factors predisposing to nondisjunction relate to the disruption of chromosomal pairing and recombination.[174,175] Various mechanisms have been implicated, but all involve an age-dependent deterioration in cellular factors required for proper spindle formation and function.[176] Molecular investigations of chromatid cohesion and separation have implicated cohesins, a specific class of proteins that maintain cohesion between sister chromatids and oppose the splitting forces mediated by the microtubules of the meiotic spindle.[177–180] An age-related premature degradation or deficiency of cohesins may result in unstable bivalent chromatid structures and predispose to premature separation of sister chromatids before they align on the meiotic spindle. The smaller chromosomes appear more prone to premature chromatid separation, possibly because they have fewer of the chiasma that help to prevent such dissociation.[167,181,182] Other studies using high-resolution confocal microscopy to examine the meiotic spindle in human oocytes have revealed that abnormalities of the cleavage spindle microtubular matrix or chromosome

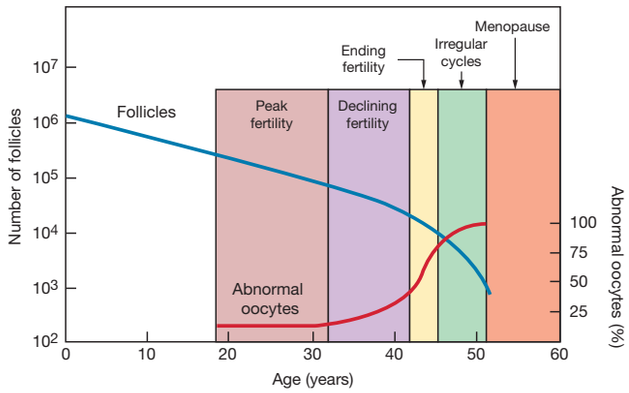


FIGURE 28.2 Adapted from The World Factbook. Country comparison—total fertility rate. https://www.cia.gov/the-world-factbook/field/total-fertility-rate/country-comparison/; Desai S, Wood-Trageser M, Matic J, et al. MCM8 and MCM9 nucleotide variants in women with primary ovarian insufficiency. *J Clin Endocrinol Metab*. 2017;102:576.

alignment during meiosis II are 4 to 5 times more common in older cycling women (age 40–45) than in younger women (age 20–25).[50] These and other observations of cultured human oocytes collected from unstimulated ovaries further indicate that the meiotic competence of oocytes declines with age.[183] **In sum, accumulated evidence strongly suggests that the primary cause of the age-dependent decrease in fecundability and increase in the incidence of pregnancy loss is an increasing prevalence of aneuploidy in aging oocytes, resulting at least in part from disordered regulatory mechanisms governing meiotic spindle formation and function** (Figure 28.2).

Aging and the Uterus

Aging does not appear to have any significant adverse effect on the uterus. Even though benign uterine pathology (leiomyomata, endometrial polyps, adenomyosis) is more common in older women,[184–186] there is limited evidence to suggest that uterine age itself has a major impact on fertility.[187–190] Age also does not appear to adversely affect endometrial development or function in response to steroid stimulation.[191] The most reliable evidence comes from comparing outcomes in nondonor and donor oocyte IVF cycles. Whereas early studies suggested that donor oocyte IVF pregnancy and delivery rates decreased modestly with the age of the recipient,[192–194] the bulk of more recent experience refutes those conclusions.[195,196] In the national summary of ART success rates for the year 2015, live birth rates declined progressively with increasing age for nondonor egg cycles, as expected. In contrast, the overall live birth per transfer rate in donor egg IVF cycles was over 50% and did not vary significantly with the age of the recipient.[33] **Live birth rates in donor egg IVF cycles relate to the age of the donor, not the age of the recipient.** In one large series, pregnancy loss rates increased from 14% in women matched with egg donors aged 20 to 24 to 44% for women whose donors were over age 35.[197] However, perinatal outcomes are improved when a gestational carrier is used rather than transfer of donor embryos to an infertile patient's own uterus, even when adjusted for age.[198,199]

Recent research by Reig et al found live birth rates after euploid embryo transfers to decline with advancing maternal age.[200] This finding, supported by data from the Society for Assisted Reproductive Technology for the 2014 to 2020 period and a meta-analysis, suggests a potential age-related decline in uterine function. However, the possibility of reduced viability of euploid embryos with age cannot be ruled out. There seem to be defects in the infertile uterus, as suggested previously, that may impact fertility and tend to accumulate with age, but aging itself is not thought to be a significant factor influencing uterine receptivity.

Aging and Male Fertility

The relationship between age and fertility in men is discussed in detail in Chapter 29 and summarized here. Several studies have shown that semen volume, sperm motility, progressive motility, and morphologically normal sperm decrease significantly with male age.[201–204] The data also suggest a decrease in sperm concentration and count.[202] However, semen characteristics generally do not accurately predict fertilizing capacity[205–208]; nor do endocrine parameters.[209,210] When the effect of male partner age on pregnancy rates is investigated, obvious confounders are female partner age and lower coital frequency due to aging. Among the few studies that have controlled for female age, pregnancy rates for men over 50 have been 23% to 38% lower than for men under age 30.[201] A British study that examined the effect of men's age on the time to conception (adjusting for the confounding effects of both partner's age and coital frequency) found that increasing men's age was associated with increasing time to conception and declining overall pregnancy rates; the time to conception was 5-fold greater for men over age 45 than for men under age 25, and restricting the analysis to men with young partners yielded similar results.[211] Results of two studies that controlled for female partner age have suggested that male fertility may start to decline earlier, beginning in the late 30s.[212,213] A 2023 systematic review, including 20,527 oocyte donation cycles in 11 studies, reported a significant linear decline in live birth rates with advancing paternal age.[214] Paternal age ranged between 22 and 81 years, with a mean of 41.5 years. However, the effect was minimal, and no specific threshold that precluded live birth was identified.

Similarly, a female age-adjusted analysis of fresh, nondonor, noncanceled ART cycles performed in the United States in 2017 reported a significant decline in live birth rate per cycle (RR = 0.76, 95% CI = 0.72–0.84) and per transfer (RR = 0.82, 95% CI = 0.77–0.88) with a paternal age of ≥46 years compared to ≤45 years.[215] In addition, a 2020 systematic review reported a significant increase in the risk of pregnancy loss with advanced paternal age. Compared to 25 to 29 years,

pooled risk estimates for pregnancy loss for paternal age categories 30 to 34, 35 to 39, 40 to 44 and ≥45 were 1.04 (95% CI = 0.90, 1.21), 1.15 (0.92, 1.43), 1.23 (1.06, 1.43), and 1.43 (1.13, 1.81), respectively.[216]

There are several possible biologic mechanisms that might explain an age-related decline in male reproductive potential. Sperm chromosomal abnormalities may increase in frequency with age and adversely affect early embryonic development.[217] In addition, there are epigenetic changes resulting in altered sperm DNA methylome in aging men,[218,219] and sperm microRNA expression is reported to vary with advancing age.[220] Average FSH levels in men increase during their 30s,[221] suggesting that age-related changes in the hypothalamo-pituitary-gonadal axis may begin during midlife.[222] The testes and prostate also exhibit morphologic changes with aging that might adversely affect both sperm production and the biochemical properties of semen.[223] Whatever the mechanism, decreasing fertility with increasing male age in healthy couples suggests that normal sperm overproduction may not fully buffer the effects of increasing age.

Overall, the available evidence suggests a negative correlation between male age and pregnancy rates. The time to conception increases with male age. However, because there is little or no overall measurable decline in male fertility before age 45 to 50, male factors generally contribute relatively little to the overall age-related decline in fertility.

Ovarian Reserve and Its Assessment

The term ovarian reserve refers to (1) the size and quality of the remaining ovarian follicular pool and (2) the ability of the ovaries to respond to exogenous gonadotropin stimulation, which are related concepts. Since the major effect of aging on a woman's reproductive potential is through declining oocyte number and increased oocyte aneuploidy, the concept of ovarian reserve is relevant for female reproductive aging.

The primary reason for ovarian reserve testing is to gain prognostic information regarding the likelihood of successful response to ovarian stimulation in women undergoing infertility treatment. Although ovarian reserve tests have also been used to predict fecundity (the probability that a cycle will result in a live birth), accumulating evidence consistently shows that these tests fail to provide reliable predictions in regularly menstruating women and, as such, do not help explain the etiology of infertility.[224–228]

Like all screening tests, ovarian reserve tests are aimed at identifying individuals at risk for a disease, in this case "diminished ovarian reserve" (DOR). While DOR is commonly used as a diagnostic term in women undergoing IVF in the United States, it remains poorly defined. In Europe, POR (poor ovarian response) is used instead. With the aim of establishing a standardized definition for POR, the European Society of Human Reproduction and Embryology (ESHRE) published the Bologna criteria in 2011.[229] In this publication, age, ovarian reserve testing, and response to stimulation in a previous cycle were identified as the best indicators of POR, and cutoff values were established. More recently, and following criticism toward the aforementioned criteria for encompassing a population with an extremely poor response, a group of researchers and clinicians from seven countries developed and published the "Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number" (POSEIDON) classification system for POR.[230] This new system subdivided predicted poor responders into four groups based on age and ovarian reserve markers, as well as the presence of prior poor response.[231] However, it is noteworthy that none of these diagnostic criteria for POR or DOR have been widely adopted.

It is important to emphasize that ovarian reserve tests, whether used individually or in combination, cannot reliably predict the size of the ovarian follicular pool. Instead, these tests only identify women who are more likely to exhibit a poor response to gonadotropin stimulation and who may have a lower likelihood of achieving pregnancy with treatment. The value of a screening test depends on its validity, describing its ability to correctly categorize individuals as affected (sensitivity) or unaffected (specificity). The sensitivity and specificity of a screening test will vary with the chosen threshold value. A choice intended to maximize sensitivity minimizes the number of false-negative results (patients with DOR categorized as normal) but increases the number of false-positive results (patients with a normal ovarian reserve categorized as having DOR). Conversely, a threshold value that maximizes specificity minimizes false positives but increases false-negative results. **For measures of ovarian reserve, test threshold values should have high specificity for DOR so as to decrease false-positive results (incorrectly categorizing a patient with a normal ovarian reserve as having DOR), thereby avoiding overly aggressive treatment or inappropriate recommendations to abandon treatment or pursue adoption or oocyte donation. Treating women with unrecognized DOR (the consequence of maximizing specificity) is undesirable but a less serious error.**

The most important test characteristics of a screening test are its positive predictive value (PPV) and negative predictive value (NPV), which vary with the prevalence of the disease of interest—that is, DOR, in the test population. PPV describes the probability that a woman with a positive test truly has DOR, and NPV is the probability that a woman with a negative test truly has a normal ovarian reserve. If the prevalence of DOR is low, as in young women, the PPV will be low, even if sensitivity and specificity are high. Conversely, if the prevalence of DOR is high, as in older women, the PPV will be high if a highly specific threshold value is chosen. **If the purpose of ovarian reserve testing is to correctly identify women with DOR, it will be most useful in women at high risk for DOR. When applied in a low prevalence population, many women with a normal ovarian reserve will have a false-positive result and be categorized as having DOR.**

Ovarian reserve tests include both biochemical and ultrasonographic measures of the size and (by inference) the quality of the ovarian follicular pool. Biochemical tests include both basal measurements, such as FSH, estradiol, inhibin B, and antimüllerian hormone (AMH), and provocative tests, such as the clomiphene citrate challenge test (CCCT). Ultrasonographic measures of ovarian reserve include the antral follicle count (AFC) and ovarian volume.

It should be noted that both biochemical and ultrasonographic markers of ovarian reserve have poor predictive value for conception. Women should not be told that they cannot conceive spontaneously or should not be denied fertility-promoting treatments, including ART, based solely on a finding of decreased ovarian reserve.

Basal FSH and Estradiol Concentrations

Serum FSH concentration was one of the earliest and commonly used tests of ovarian reserve. Since an isolated increase in FSH levels is one of the earliest indications of reproductive aging in women, it was an obvious choice for identifying women with DOR. As a marker of ovarian reserve, serum FSH concentration is best obtained during the early follicular phase (cycle days 2–4).

However, serum FSH concentration has wide intra- and intercycle variation, limiting its value as a screening test. Moreover, FSH values vary with the assay method; although values obtained with different assays correlate very well, absolute values can differ significantly. Values also vary with the reference standard, previously an International Reference Preparation of Human Menopausal Gonadotropin (IRP-HMG) and now the World Health Organization Second International Reference Preparation (IRP 78/549).

Numerous studies have investigated the relationship between cycle day 3 FSH concentrations or FSH/LH ratios and IVF cycle outcomes, all observing that these measures correlate with the ovarian response to exogenous gonadotropin stimulation and, to a lesser extent, with the likelihood for success. As FSH values increase, the peak in estradiol levels, the number of oocytes retrieved, and the probability for pregnancy or live birth steadily decline.[232–238] **With current assays (using IRP 78/549), FSH levels greater than 10 IU/L (10–20 IU/L) have high specificity (80–100%) for predicting poor response to stimulation, but their sensitivity for identifying such women is generally low (10–30%) and decreases with the threshold value.**[239] Although most women who are tested (including those with DOR) will have a normal result, the test is still useful because those with abnormal results are very likely to have DOR.

Because FSH levels can vary significantly, many clinicians prefer to repeat the test. Not surprisingly, consistently high values are associated with a poor prognosis, but a single elevated FSH concentration (>10 IU/L) does not have high specificity for predicting poor response to stimulation or failure to achieve pregnancy.[240] Serial testing in efforts to select the ideal cycle for treatment does not improve outcomes in women with fluctuating FSH concentrations.[241,242]

The basal serum estradiol concentration, by itself, has little value as an ovarian reserve test[243–246] **but can provide additional information that helps in the interpretation of the basal FSH level.** An early elevation in serum estradiol reflects advanced follicular development and early selection of a dominant follicle (as classically observed in women with advanced reproductive aging) and will suppress FSH concentrations, thereby possibly masking an otherwise obviously high FSH level indicating DOR. When the basal FSH is normal and the estradiol concentration is elevated (>60–80 pg/mL), the likelihood of poor response to stimulation is increased, and the chance of pregnancy is decreased.[247–250] When both FSH and estradiol are elevated, ovarian response to stimulation is likely to be very poor.

Due to their low diagnostic performance, basal FSH and estradiol measurements are being increasingly replaced with serum AMH and AFC in daily practice.

Clomiphene Citrate Challenge Test

The CCCT is a provocative and possibly more sensitive test of ovarian reserve that probes the endocrine dynamics of the cycle under both basal and stimulated conditions, before (cycle day 3 FSH and estradiol) and after (cycle day 10 FSH) treatment with clomiphene citrate (100 mg/d, cycle days 5–9).[251]

The smaller follicular cohorts in aging women produce less inhibin B and estradiol, resulting in less negative feedback inhibition on clomiphene-induced pituitary FSH release, causing an exaggerated increase in FSH concentrations.[85,252] Consequently, a frankly elevated cycle day 10 FSH concentration can identify women with DOR who might otherwise go unrecognized if evaluated with basal cycle day 3 FSH and estradiol levels alone.[253,254]

In studies evaluating CCCT results, stimulated concentrations of FSH, estradiol, and inhibin B have varied widely, limiting the value of the test.[255–257] A 2006 systematic review of the predictive value of the CCCT over a range of day 10 FSH concentrations (10–22 IU/L) in women at low, average, and high probability of DOR concluded that the test had a 47% to 98% specificity and a 35% to 93% sensitivity for predicting poor response to stimulation and a 67% to 100% specificity and a 13% to 66% sensitivity for predicting treatment failure.[258] **Overall, the additional value of CCCT over basal FSH and AFC is marginal, and it is being used less often in daily practice.**

Inhibin B

Inhibin B is secreted primarily during the follicular phase by the granulosa cells of smaller antral follicles. Its concentrations increase in response to exogenous GnRH or FSH stimulation and show wide intra- and intercycle variation.[245,259]

Although inhibin B levels are generally lower in women who respond poorly to exogenous gonadotropin stimulation

than in those who respond normally,[260,261] even low threshold values (40–45 pg/mL) have only a 64% to 90% specificity and a 40% to 80% sensitivity for predicting poor response. Inhibin B has a relatively low PPV (19–22%) but a relatively high NPV for detecting DOR in a general IVF population.[259,262–265] **Overall, inhibin B is not regarded as a reliable measure of ovarian reserve.**

Antimüllerian Hormone

AMH is produced by the granulosa cells of preantral and small antral follicles, beginning when primordial follicles start developing into primary follicles and ending when early antral follicles reach a diameter of 2 to 6 mm.[266–270] Small antral follicles are likely the primary source because they contain larger numbers of granulosa cells and a more developed microvasculature.[271,272] Although it functions primarily as an autocrine and paracrine regulator of follicle development, AMH appears in measurable amounts in the serum.[273] The number of small antral follicles correlates with the size of the residual follicular pool, and AMH levels decline progressively, becoming undetectable near the menopause.[274–277]

Because AMH derives from preantral and small antral follicles, levels were thought to be gonadotropin-independent and exhibit little variation within and between cycles.[278–280] However, recent studies suggest AMH levels decrease with the use of oral contraceptives and GnRH agonists.[281] In early clinical studies, AMH has been assayed using two different commercial assay kits, and although the results were highly correlated, their standard curves were not parallel, and there was no applicable conversion factor; one comparative study observed that concentrations measured with one kit were more than 4-fold lower than those measured with the other.[282] Even though the two manufacturers of AMH assays merged to form a third company and a new two-step, sandwich-type enzymatic microplate assay that uses a more stable antibody was introduced, the assay was calibrated to the standards of one manufacturer, and results remained systematically different, that is, 40% higher, than the previous version of the other manufacturer. Moreover, AMH readings are affected by the storage and handling conditions of the samples. Consequently, when applying results in clinical practice, it is important to know which assay method was used to measure AMH. Current commercial assay kits yield consistent results with low interassay variation (<10%).[283]

The performance of AMH as a screening test of ovarian reserve has been examined in the general IVF population and in populations of women at low or high risk for DOR. Overall, lower AMH levels have been associated with poor response to ovarian stimulation and low oocyte yield, embryo quality, and pregnancy rates,[259,260,284–286] but studies correlating mean AMH levels with IVF outcomes have not yielded threshold values that can be applied confidently in clinical care,[243,260,262,284] and more recent studies failed to show an effect of low AMH levels on pregnancy rate, when corrected for age.[287,288] **In the general IVF population, low AMH threshold values (0.2–0.7 ng/mL) have had a 40% to 97% sensitivity, a 78% to 92% specificity, a 22% to 88% PPV, and a 97% to 100% NPV for predicting poor response to stimulation (≤3 follicles or ≤2–4 oocytes) but have proven neither sensitive nor specific for predicting pregnancy.**[259,289–291]

AMH is a very useful screening test for DOR but is likely to be more useful in a general IVF population or in women at high risk for DOR than in women at low risk for DOR. Low threshold values have a good specificity for poor response to ovarian stimulation but not for predicting pregnancy.

Antral Follicle Count

Reproductive-aged women have an estimated 20 to 150 growing follicles in the ovaries at any 1 time, although only those large enough to be imaged (>2 mm) can be visualized by transvaginal ultrasonography (TVUS).[292–294] Antral follicles are FSH sensitive and can progress to more advanced stages of development when stimulated with exogenous FSH. **Histologic studies have revealed that the number of small antral follicles in the ovaries is proportional to the number of primordial follicles remaining.**[295] **Therefore, as the supply of primordial follicles decreases, the number of visible small antral follicles also declines.** The AFC (total number of antral follicles measuring 2–10 mm in both ovaries) thus provides an indirect but useful measure of ovarian reserve.[293,296–299]

AFC correlates with onset of the menopausal transition, indicating that it relates to the number of follicles remaining.[275] Some, perhaps as many as half, of the antral follicles that can be imaged are probably in the process of atresia, but there is no way other than observing their response to FSH stimulation to distinguish them from viable growing follicles.[300] However, AFC correlates well with oocyte yield in IVF cycles,[301] suggesting that gonadotropin stimulation can still rescue follicles that may be in the early stages of atresia.[302] Several studies have observed a relationship between the AFC and response to ovarian stimulation in IVF cycles. In the general IVF population, including women at low and high risk for DOR, an AFC threshold value of three to four follicles has a high specificity (73–100%) for predicting poor response to ovarian stimulation and failure to conceive (64–100%), but a relatively low sensitivity for both end points (9–73% for poor response, 8–33% for failure to conceive).[245,301,303–308] The PPV and NPV of AFC have varied widely in studies.

A low AFC has a high specificity for predicting poor response to ovarian stimulation and treatment failure, making it a useful test, but low sensitivity limits its overall clinical utility.

Ovarian Volume

Not surprisingly, ovarian volume decreases with progressive follicular depletion.[309,310] However, the measure has high intercycle and interobserver variability,[245,311–313] and because

most studies of ovarian volume have excluded women with ovarian pathology such as endometriomas and polycystic ovary syndrome, results have limited generalizability.[310,314]

Ovarian volume (length × width × depth × 0.52 = volume) generally correlates with the number of oocytes retrieved, but poorly with pregnancy.[303,308,315–317] A low ovarian volume (<3 mL) has high specificity (80–90%) and widely ranging sensitivity (11–80%) for predicting poor response to ovarian stimulation.[239] The PPV for poor response can be as low as 17% among women at low risk for DOR and as high as 53% in women at high risk.[245] **Overall, ovarian volume has very limited clinical utility as an ovarian reserve test.**

Other Tests of Ovarian Reserve

Numerous other provocative tests of ovarian reserve have been investigated, including exogenous FSH-stimulated estradiol, inhibin B or AMH levels,[284,318–322] and GnRH agonist–stimulated FSH, estradiol, inhibin B, or AMH concentrations.[284,318,323–325] In theory, the ovarian and endocrine response to FSH or GnRH agonist stimulation should provide the best estimate of the number of responsive follicles. **However, the available evidence does not justify the complexity and higher costs of these tests since they are not found to be better predictors of response to ovarian stimulation or pregnancy than basal FSH, AMH, and AFC.**[239]

Combined Tests of Ovarian Reserve

Since none of the ovarian reserve tests alone has 100% sensitivity and specificity, varying combinations of ovarian reserve tests have been tried to improve diagnostic performance. However, as different tests of ovarian reserve are highly correlated, using more than one measure in a prediction model does not necessarily improve its performance.[245,261,303] The use of combined tests will not only increase the cost of testing but also complicate clinical decision-making. A combination of AMH, inhibin B, AFC, and ovarian volume was not found to be a better predictor of response to stimulation than AFC and AMH alone.[306] A meta-analysis of cohort studies investigating the performance of various combinations of tests concluded that models combining tests do not perform significantly better than individual tests such as the AFC.[326]

Summary

Currently, there is no ovarian reserve test that predicts the likelihood of spontaneous conception for a regularly menstruating woman.[224–228] For predicting response to ovarian stimulation, the lack of a uniformly accepted definition of DOR despite a number of attempts remains a problem. In order to predict success of ART, the ideal ovarian reserve test should yield consistent results and be highly specific, to minimize the risk for incorrectly categorizing normal women as having a DOR. Currently, AFC and AMH have replaced basal FSH levels as the most commonly used ovarian reserve test in clinical practice.

Although ovarian reserve tests are capable of predicting response to exogenous gonadotropin stimulation reasonably well, whether the information gained truly affects outcomes is less certain. Individualized FSH dosing based on ovarian reserve testing does not seem to improve live birth rates or decrease cost of treatment.[327–329] Moreover, increasing gonadotropin dosage does not improve response predictably in women with DOR, probably because the small cohort of responsive antral follicles is the limiting factor, and no amount of stimulation can increase that number appreciably.[330–332] Even in women who previously exhibited a poor response to stimulation, changes in treatment regimens have generally not improved response or pregnancy rates in subsequent cycles.[331,333–336]

None of the ovarian reserve tests currently in use are an accurate predictor of pregnancy in IVF cycles, except, possibly, when extreme abnormal threshold values are applied, which results in very low sensitivity for identifying women having a poor prognosis.[337] The tests are adequate for predicting poor response, which does have prognostic value, although not as much in young women as in older women.[338–340] Although ovarian reserve tests have become a routine element of pretreatment evaluation for couples planning IVF, it can be argued that routine testing has limited clinical utility in the large majority of patients and can be misleading, especially in women at low risk for having a DOR.[300]

Ovarian reserve tests have also become a routine element of the diagnostic evaluation for infertility. Advocates for the liberal application of ovarian reserve tests argue that abnormal tests can help to persuade older women to abandon plans to pursue aggressive, costly, and likely futile treatment and can help to convince young women to do just the opposite, to take fullest advantage of a rapidly closing window of opportunity. Others who are more circumspect emphasize correctly that few young women will have an abnormal test and that some of those who do will inevitably be categorized incorrectly, leading to inappropriate counseling and treatment. **The best overall strategy would seem to limit ovarian reserve testing to women at increased risk for having a DOR and to apply highly specific threshold values to minimize the risk for a false-positive result. In this context, ovarian reserve testing can best be justified for women with any of the following characteristics**[138,341–344]:

- **Age over 35**
- **Unexplained infertility to identify unsuspected loss of ovarian reserve**
- **Family history of early menopause**
- **Previous ovarian surgery (ovarian cystectomy or drilling, unilateral oophorectomy), chemotherapy, or radiation**
- **Smoking**
- **Demonstrated poor response to exogenous gonadotropin stimulation**

Ovarian reserve tests should always be interpreted with caution. Rigid application of test results risks inappropriate recommendations for treatment, or for no treatment, and both must be avoided. An abnormal test result does not preclude the possibility of pregnancy. Except perhaps when grossly abnormal, test results should be used not to deny treatment but only to obtain prognostic information that may help to guide the choice of treatment and best use of available resources. Although the probability of pregnancy may be low, many with abnormal test results will achieve pregnancy if afforded the chance, in a given cycle.

GUIDING PRINCIPLES FOR EVALUATION AND TREATMENT OF INFERTILITY

From the beginning, the evaluation of infertility should focus on the *couple* and not on one or the other partner, regardless of past reproductive performance. Ideally, both partners should be present during office visits. This enables the flow of information between the physician and both partners, as well as between the partners. It is equally important to allow each partner to express their own opinion and be able to ask questions directly. This can help increase patient compliance, which is very often a challenging aspect of the management of infertile couples.

The four basic goals of management of infertility are:

- **To identify and to correct specific causes of infertility, when possible. With proper evaluation and treatment, the majority of women will achieve pregnancy.**
- **To provide accurate information and to dispel the misinformation commonly gained from friends, mass media, social media, and the internet.**
- **To provide emotional support during a trying time.**

In many couples, the inability to conceive results in feelings that they have lost control over an important and very personal part of their lives. The process of evaluation and the associated uncertainty significantly adds to that burden. Infertile couples often need the opportunity to express their concerns, frustrations, and fears, and support groups can help to meet that need. Group meetings can help couples to realize that their problem is not unique and to learn how others cope with similar problems. Whereas severe anxieties can have adverse effects on ovulatory function and coital frequency, there is no substantial evidence that the usual anxieties of couples trying to conceive cause or contribute to their infertility.

- **To guide couples failing to conceive with other forms of treatment to alternatives, including IVF, the use of donor gametes (oocytes or sperm), and adoption, and to help those who reject or fail treatment to come to closure.**

Counseling must be an ongoing process during both evaluation and treatment. Regular visits to review and critique results and to outline recommendations for further evaluation and treatment help to ensure that all of the couple's medical, emotional, and financial needs and concerns are addressed effectively in a timely fashion.

Lifestyle and Environmental Factors

Understandably, all infertile couples are very interested in learning anything they might do to maximize the likelihood of achieving a successful pregnancy. Lifestyle choices and environmental factors influence fertility and deserve consideration and discussion when they are relevant. The United States has the highest obesity rate in the world. Over 40% of American women are obese, and another 30% are overweight.[345] Obesity is defined as a body mass index (BMI) greater than 30 kg/m^2, and overweight is defined as a BMI between 25 and 30 kg/m^2. In women, obesity is associated with menstrual dysfunction, decreased fertility, and increased risks of pregnancy loss and obstetric and neonatal complications. In men, obesity is associated with abnormal semen parameters and can adversely affect fertility.[346]

Substance abuse is one of the few things over which the couple may have specific control, smoking being the most important. Many are not aware of the adverse effects smoking has on fertility and pregnancy outcome.[347] The couple's motivation to maximize their fertility presents a golden opportunity to educate those who smoke and to establish a smoking cessation strategy. Smoking has a well-known adverse impact on pregnancy outcome, and evidence strongly suggests that fertility is lower in both men and women who smoke.[347] The prevalence of infertility is higher, fecundability (probability that a cycle will result in pregnancy) is lower, and the time to conception is longer in smoking than in nonsmoking women, while the effects of passive smoke exposure are only slightly less than those of active smoking by either partner.[348] The available data suggest that the adverse effects of smoking on fertility are dose-dependent.[349–352] The mechanisms involved may include accelerated follicular depletion,[353–355] menstrual cycle abnormalities,[356] or gamete or embryo mutagenesis induced by toxins in cigarette smoke.[357–362] A causal relationship between cigarette smoking and female infertility has not been established. However, based on the results of a meta-analysis including 12 studies (overall OR for risk of infertility in women smokers vs nonsmokers 1.60),[363] and assuming a 10% prevalence of smoking in women of reproductive age, up to 5% of female infertility could be related to smoking.[364,365] Consequently, an active approach to prevention of infertility is justified, discouraging smoking and helping those who smoke to quit.[366]

Marijuana is legalized in many states, and 12% of women reported using marijuana prior to conception.[367] A prospective study including 485 female and 160 male marijuana users from the United States and Canada reported similar

fecundability rates with nonusers in adjusted analyses.[368] A retrospective analysis of data from respondents of NFGS identified 1,076 female and 758 male users, who were found to have similar time to pregnancy with non-users.[367] Nevertheless, the data is limited, and the American College of Obstetricians and Gynecologists recommends against marijuana during the preconceptional period and pregnancy.[347,369] Cocaine use can impair spermatogenesis in men[370,371] and has been associated with a greatly increased risk of tubal disease in women.[372] Heavy alcohol consumption in women may decrease fertility[373–375]; in men, it has been associated with decreased semen quality and impotence.[376] While a prospective cohort study involving 6,120 Danish women between the ages of 21 and 45 years found no discernible effect of less than 14 servings of alcohol per week on fertility, a US prospective cohort study involving 413 women reported that both moderate (3–6 drinks per week) and heavy (>6 drinks per week) alcohol intake during the periovulatory and luteal phase were associated with decreased fecundity.[229,377] A 2017 systematic review involving 19 observational studies and 98,567 women reported that both light drinkers (≤12.5 g/d of ethanol) and moderate to heavy drinkers (>12.5 g/d of ethanol) had significantly lower fecundability compared to nondrinkers (RR = 0.89, 95% CI = 0.82–0.97 and RR = 0.77, 95% CI = 0.61–0.94, respectively). Moreover, a dose–response meta-analysis demonstrated a linear association between increasing alcohol consumption and decreasing fecundability.[378] In both women and men, even modest amounts of alcohol consumption have been associated with lower pregnancy rates in IVF cycles.[379] Although moderate caffeine ingestion (200 mg daily, which is equal to about one 12-ounce cup of coffee) appears not to have any adverse effects on fertility, higher levels of consumption may delay conception[380–382] or increase the risk of pregnancy loss.[383,384] However, a recent prospective cohort study involving 3,628 women planning a pregnancy reported that caffeine intake in excess of 300 mg/d was not associated with a significant decline in fecundability ratio when compared with <100 mg/d (1.04, 95% CI = 0.90–1.21). Likewise, more than three servings per day of coffee consumption was not associated with fecundability as compared with nonusers (1.05, 95% CI = 0.85–1.33).[385] A North American prospective cohort study involving 2,135 women planning a pregnancy and a secondary analysis of the Effects of Aspirin in Gestation and Reproduction trial both reported similar results.[386,387]

Other potentially harmful occupational and environmental exposures, although uncommon, may be identified. Exposures to perchloroethylene in the dry-cleaning industry, toluene in the printing business, ethylene oxide, and mixed solvents have been associated with decreased fecundity. Semen abnormalities have been described in men exposed to radiant heat or heavy metals. Environmental exposure to herbicides or fungicides has been associated with decreased fertility in women[375] and exposure to pesticides and other chlorinated hydrocarbons with an increased risk of pregnancy loss.[388]

For couples attempting to conceive, there is fair evidence to support recommendations for smoking cessation and efforts to achieve a BMI between 20 and 25 kg/m^2. While a healthy lifestyle and diet should always be encouraged, a specific diet or a particular micronutrient is not unequivocally shown to be related to fertility.[389] **Recommendations to avoid alcohol consumption is consistent with available evidence. However, there have been no randomized controlled trials demonstrating that such lifestyle modifications improve fertility.**

Normal Reproductive Efficiency

Explaining the normal human reproductive process helps to provide perspective for infertile couples. Understanding the relative inefficiency of human reproduction can help decrease the couple's stress and set realistic expectations regarding the assessment and treatment process. **In normally fertile couples, cycle fecundability averages 20% and does not exceed approximately 35% even when coitus is carefully timed.**[213,390,391] This information is particularly helpful when discussing and comparing the efficacy of different treatment options, typically viewed in terms of cycle fecundability. It is important to ensure that couples realize that the benchmark for comparison is 20% to 30%, and not 100%.

Given the average 20% cycle fecundability, the cumulative pregnancy rates observed over time in normal fertile couples are easy to understand. The data in **Table 28.1** have been a standard since 1956 and have been confirmed by more recent studies.[3,392,393]

A normal sperm can survive in the female reproductive tract and retain the ability to fertilize an egg for at least 3 and up to 5 days, but an oocyte can be fertilized successfully for only approximately 12 to 24 hours after ovulation.[394] **Consequently, virtually all pregnancies result from intercourse occurring sometime within the 6-day interval ending on the day of ovulation.**[213,391,395] Estimates of when fertility peaks vary with the method used to determine the time of ovulation. When ovulation is assumed to occur on the day before the midcycle rise in basal body temperature (BBT), the day of peak fertility falls 2 days prior to

TABLE 28.1 Time Required for Conception Among Couples Who Will Attain Pregnancy

Months of Exposure	% Pregnant
3 mo	57
6 mo	72
1 y	85
2 y	93

Data from Guttmacher AF. Factors affecting normal expectancy of conception. *JAMA*. 1956;161:855.

ovulation[213]; ovulation generally occurs within 1 day of that predicted.[395] When the time of ovulation is based on daily urine estrogen concentrations, the probability of conception increases steadily as ovulation nears and peaks on the day before and the day of ovulation,[391,395] ranging from about 10% at its low to approximately 33% at its peak. When daily urinary LH excretion is monitored to detect the midcycle surge that triggers ovulation, follicular collapse (as determined by serial TVUS) and, presumably, ovum release generally follow within 14 to 26 hours, and almost always within 48 hours.[396,397] Regardless of the method used, all studies indicate that fertility plummets almost immediately thereafter, declining to near zero within 24 hours after ovulation.

Even though timing coitus to coincide with the most fertile period seems straightforward and is frequently recommended to infertile couples as a means to increase the likelihood of pregnancy, there is very low-quality evidence to support the recommendation.[398] Although BBT and ovulation predictor kits (that measure elevations in urinary LH) can help define the time of ovulation, they should be used only when necessary. Scheduled intercourse clearly adds to the already significant stress of infertility. Moreover, much of the interval of peak fertility during the menstrual cycle may be inadvertently excluded while awaiting the appropriate "signal." **For most couples, the simple recommendation for intercourse approximately once every 2 to 3 days can avoid an unnecessary source of stress while also helping to ensure that coitus occurs during the interval of highest fertility.[399] However, timed coitus may be a reasonable recommendation for couples having infrequent intercourse, by preference or because of circumstance.**

Causes of Infertility

Before any formal investigation begins, the major causes of infertility and the basic components of the infertility evaluation should be outlined for the couple. **The major causes of infertility include ovulatory dysfunction (20–40%), tubal and peritoneal pathology (30–40%), and male factors (30–40%); uterine pathology is relatively uncommon, and the remainder is largely unexplained.** Many couples suffer from multiple etiologies. The prevalence of each cause of infertility varies with age. Ovulatory dysfunction is more common in younger than in older couples, tubal and peritoneal factors have a similar prevalence, and male factors and unexplained infertility are observed somewhat more often in older couples.[400,401] The distribution of causes also varies with the duration of infertility and the level of care.[402–404] Most couples seeking evaluation have been trying to conceive for 2 or more years; therefore, a few will be normally fertile (**Figure 28.3**). Those with longer durations of infertility generally have more severe or multiple problems and tend to congregate in tertiary care centers. The average duration of infertility for couples seen in tertiary care centers (42 months)[404] is twice that for couples seen in the primary care setting (21 months).[402] Predictably, the proportion of couples with easily treatable ovulatory dysfunction decreases from primary to tertiary care, and that with more severe tubal/peritoneal or male factors increases.

The human reproductive process is complex, but for purposes of evaluation, it can be dissected into its most important and basic components.

Key Points: Human Reproductive Process

- Sperm must be deposited at or near the cervix at or near the time of ovulation and must ascend into the fallopian tubes and have the capacity to fertilize the oocyte (male factor).
- Ovulation of a mature oocyte must occur, ideally on a regular and predictable basis (ovarian factor).
- The fallopian tubes must capture ovulated ova and effectively transport sperm and embryos (tubal factor).
- The uterus must be receptive to embryo implantation and capable of supporting subsequent normal growth and development (uterine factor).

The infertility evaluation is designed to isolate and test the integrity of each component, insofar as that is possible, and to identify any abnormalities that might impair or prevent conception. The pace and extent of evaluation should be based on the couple's age, duration of infertility, medical history, physical examination, and preferences.

Some infertility problems once considered insurmountable are now amenable to modern treatments. IVF can effectively bypass irreparable tubal occlusive disease, and intracytoplasmic sperm injection (ICSI) can overcome even severe abnormalities of semen quality. Treatments aimed at increasing gamete density—bringing together more than the usual numbers of oocytes and sperm in the right place at the right time—can increase cycle fecundability for couples with age-related or otherwise unexplained infertility and include ovarian stimulation with IUI or IVF. In women with POI, women beyond normal reproductive age, and women without ovaries, IVF using donor oocytes is highly successful.

A substantial proportion of subfertile couples suffer from unexplained infertility (ie, have normal semen analysis, at least one patent tube, macroscopically normal-appearing uterus and endometrium, and regular menstrual cycle); similarly, a rational treatment does not exist for some common causes of infertility (ie, advanced female age, severe tubal damage, idiopathic oligoasthenospermia).[7] **The scope and sequence of the modern infertility evaluation have shifted focus, from always making a specific diagnosis to using the most efficient and cost-effective tests. Likewise, the focus of treatment for infertility has also shifted, from**

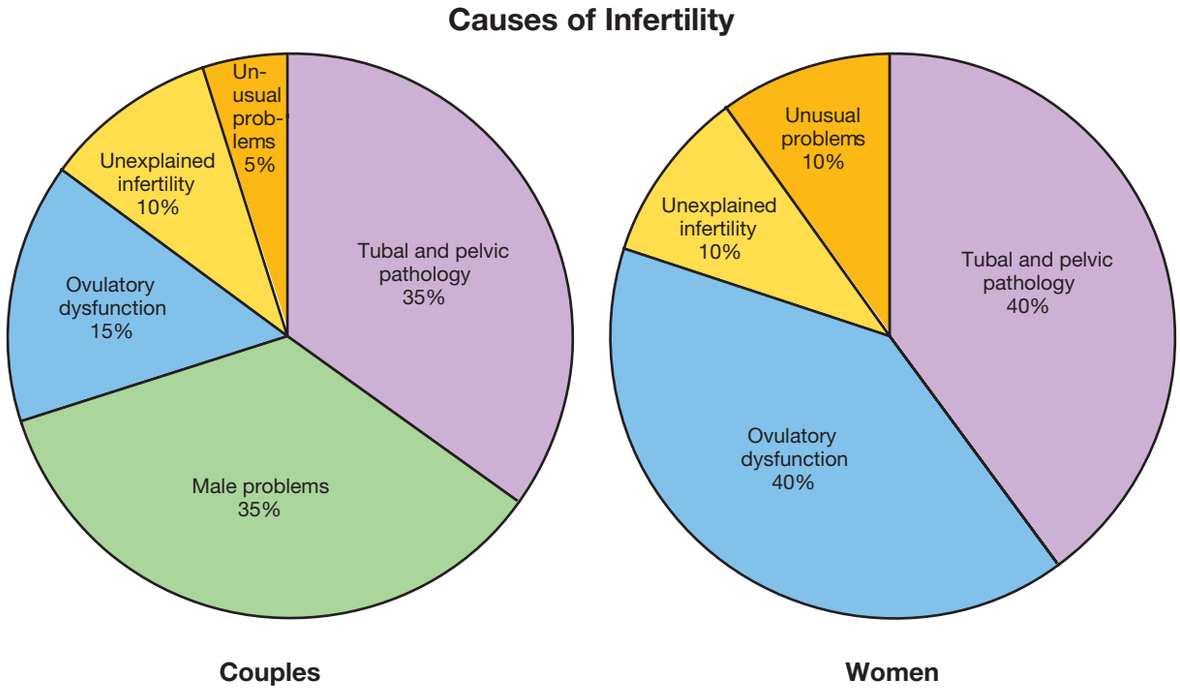


FIGURE 28.3

systematic correction of each identified factor to applying the most efficient and cost-effective therapy, which is often ART.

Indications for Evaluation

When should a formal evaluation for infertility begin? After all, most infertile couples are only subfertile, not truly sterile, and many will conceive, eventually, without treatment. Infertility has a significant spontaneous cure rate that varies with female partner age, duration, past conception history, and the cause(s). **The probability of achieving a live birth without treatment decreases with increasing age and duration of infertility.**[402–407] Overall, the likelihood of pregnancy without treatment declines by about 5% for each additional year of female partner age and by 15% to 25% for each added year of infertility.[404] **The largest majority of spontaneous pregnancies occur within 3 years; thereafter, the prognosis for success without treatment is relatively poor.** Couples that have conceived before generally have a better prognosis than those who have never achieved pregnancy. The cause of infertility also affects the prognosis for success without treatment but, of course, cannot be determined without evaluation. Predictably, the diagnoses of anovulation and unexplained infertility have the best prognosis. The likelihood for success without treatment for couples with male factors, tubal disease, and endometriosis varies widely with the severity of disease; the prognosis is reasonably good for mild oligospermia, tubal adhesions, and mild endometriosis and quite poor for severe male factors, tubal obstruction, and severe endometriosis.

Evaluation should be offered to all couples who have failed to conceive after a year or more of regular unprotected intercourse, but a year of infertility is not a prerequisite for evaluation. Earlier evaluation is justified in the presence of obvious risk factors, such as irregular or infrequent menses, history of pelvic infection, surgery or endometriosis, or having a male partner with known or suspected poor semen quality, and is also warranted after 6 months of unsuccessful effort for women over the age of 35 years. Women older than 40 years can benefit from more immediate evaluation.[1,408]

Education should be offered to any couple who seeks it, regardless of whether they have made any active effort to conceive. It is always helpful to explain the reproductive process, to inform couples that normal cycle fecundability is approximately 20% (far lower than most realize), and to discuss the relationship between age and fertility, when it is relevant. In concerned couples who have not yet truly tested their fertility and have no obvious problems, some basic preliminary evaluation is reasonable to perform, if requested. A complete medical history helps in identifying or ruling out obvious risk factors, and tests to confirm ovulation and semen analysis are easy, inexpensive, and minimally invasive and can quickly identify some of the most common reproductive problems. In women at risk for DOR, an ovarian reserve test is also reasonable because results may help to determine when and how further evaluation and treatment should be recommended.

PRELIMINARY EVALUATION OF THE INFERTILE COUPLE

Any evaluation of infertility must begin with a careful history and physical examination, which will often identify symptoms or signs that suggest a specific cause and help to focus evaluation on the factor(s) most likely responsible. In the female partner, relevant medical history and physical findings are noted in Table 28.2.[408]

Irregular or infrequent menses indicate ovulatory dysfunction. Previous treatment for cervical intraepithelial neoplasia or observation of a mucopurulent cervicitis or cervical stenosis helps to identify unusual women in whom the cervix may present an obstacle. A history of previous hysteroscopic or reconstructive uterine surgery or recently developing symptoms of menorrhagia suggests an abnormality of the uterine cavity; previous uncomplicated first- and second-trimester pregnancy terminations generally do not adversely affect subsequent fertility.[409,410] Worsening dysmenorrhea, new onset of dyspareunia, or physical findings of focal tenderness or cul-de-sac nodularity suggest endometriosis. A history of pelvic infection, septic abortion, ruptured appendix, ectopic pregnancy, abdominal myomectomy, or adnexal surgery should raise suspicion for tubal or peritoneal disease.

TABLE 28.2 History and Examination of the Female Partner

History

- Obstetric history, including gravidity, parity, pregnancy outcomes, and associated complications.
- Menstrual history, including cycle length and characteristics as well as onset and severity of dysmenorrhea.
- Coital frequency and sexual dysfunction.
- Duration of infertility and results of any previous evaluation and treatment.
- Medical and surgical history, including episodes of pelvic inflammatory disease or exposure to sexually transmitted infections.
- Previous abnormal cervical cancer screening results and subsequent treatment.
- Current medications and allergies.
- Occupation and use of tobacco, alcohol, and other drugs.
- Family history of birth defects, mental retardation, early menopause, or reproductive failure.
- Symptoms of thyroid disease, pelvic or abdominal pain, galactorrhea, hirsutism, or dyspareunia.

Physical Examination

- Weight and BMI.
- Thyroid enlargement, nodule, or tenderness.
- Breast secretions and their characteristics.
- Signs of androgen excess.
- Pelvic or abdominal tenderness, organ enlargement, or mass.
- Uterine size, contour, position, and mobility.
- Vaginal or cervical abnormality, secretions, or discharge.Mass, tenderness, or nodularity in the adnexa or cul-de-sac.

Screening Tests

Cervical cancer screening is recommended for all sexually active women above the age of 21 years and who have a cervix. The date and results of the most recent screening should be documented and a new one performed, if needed. The optimal method of screening depends on previous history and age.[411] A blood type, Rh factor, and antibody screening (in Rh-negative women) is also recommended, if not already known.

The American College of Obstetricians and Gynecologists recommend that all women considering pregnancy should be offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies.[412] Women with a family history of fragile X–related disorders or intellectual disability suggestive of fragile X syndrome or women with POI should be offered fragile X permutation carrier screening.[412] Additional screening may also be indicated based on family history or specific ethnicity; an example is the Ashkenazi Jewish population, where an expanded carrier screening for a number of recessive disorders is indicated. Couples with consanguinity should be offered genetic counseling to discuss the increased risk of recessive conditions being expressed in their offspring and the limitations and benefits of carrier screening. More recently, the American College of Medical Genetics has recommended an ethnic and population neutral paradigm and a tiered approach to carrier screening, which is supported by the American College of Obstetrics and Gynecology (ACOG). This implies screening for conditions with a career frequency >1/200 in the population in all women planning a pregnancy.[413] Indeed, expanded carrier screening is increasingly offered, particularly to patients starting ART cycles. When a woman is found to be a carrier for a specific condition, her reproductive partner should be offered screening to provide accurate genetic counseling regarding the risk of an affected child if he tests positive and reproductive options (eg, donor gametes, preimplantation genetic diagnosis, prenatal diagnosis).[412]

All women attempting pregnancy with undocumented previous *rubella* infection or vaccination should be tested for immunity and vaccinated if seronegative.[414] As there has never been a documented case of congenital rubella syndrome attributed to vaccine, the CDC has determined that women need not avoid pregnancy for more than 1 month after vaccination.[415] The CDC also recommends that all women without a history of previous infection or evidence of immunity or vaccination against *varicella* (chicken pox) receive two doses of vaccine and avoid pregnancy for 1 month after each dose.[414] COVID-19 vaccination according

to the most recent CDC guidance as well as annual influenza vaccination should be up-to-date, and a TdaP vaccine should be offered to all women planning pregnancy if they have not received one before. Human papillomavirus (HPV) vaccination is also recommended to all women through age 26.[414]

Screening for **sexually transmitted infections** (STIs) is recommended for all women at moderate to high risk for infection. Decisions regarding STI screening should consider that current recommendations from the CDC include screening all pregnant women for syphilis (rapid plasma reagin, RPR), hepatitis B (hepatitis B surface antigen, HBSAg), hepatitis C, and human immunodeficiency virus type 1 (HIV-1), unless the woman declines the latter, at the first prenatal visit.[416] Chlamydia and gonorrhea screening (nucleic acid–based tests) is recommended for all pregnant women younger than 25 years or older women at increased risk for infection.[416] For patients receiving donor sperm, oocytes, or embryos, the American Society for Reproductive Medicine (ASRM) recommends serologic testing for syphilis, hepatitis B surface antigen, hepatitis C antibody, HIV, chlamydia, gonorrhea, and cytomegalovirus (CMV) IgG and leaves screening for human T-cell lymphotropic virus (HTLV) types I and II at the discretion of the physician.[417] For sexually intimate partners of individuals receiving donor sperm, oocytes, or embryos, the ASRM recommends testing for syphilis, hepatitis B surface antigen, hepatitis C antibody, HIV, chlamydia, and gonorrhea, and leaves screening for HTLV types I and II, and CMV IgM and IgG at the discretion of the physician.[417] Any additional screening laboratory tests should be directed by the medical history and clinical judgment.

MALE FACTOR: ABNORMALITIES OF SEMEN QUALITY

The evaluation and treatment of male infertility are the focus of Chapter 29 but must be addressed briefly here because male factors explain or contribute significantly to infertility in up to 35% of couples. Semen analysis is therefore always an appropriate and important initial step in the evaluation of the infertile couple. In the absence of any known genital abnormality, trauma, surgery, or sexual dysfunction, physical examination of the male partner can be deferred pending the results of the initial semen analysis.

When semen analysis yields equivocal results, additional analyses are required to better define a suspected abnormality. A frankly abnormal semen analysis is an indication for additional evaluation that may be conducted by a gynecologist having the necessary training and experience but is most often performed by a urologist or other specialist in male reproduction.[418] Invasive diagnostic procedures, including tubal patency testing, in the female partner can generally be deferred until evaluation of the male is completed. The range of effective treatment options for couples with severe male factor infertility is limited and will often direct or even dictate what additional evaluation may be relevant in the female partner. When semen quality is normal, attention naturally turns to the female partner.

OVARIAN FACTOR: OVULATORY DYSFUNCTION

Overall, disorders of ovulation account for approximately 15% of the problems identified in infertile couples. Ovulatory dysfunction can be severe enough to prevent conception (anovulation) or only a contributing factor (oligo-ovulation). However, because cycle fecundability averages only approximately 20% even in normally fertile couples, the distinction is moot.

A number of methods can be used to determine whether and when ovulation occurs. Directly or indirectly, all are based on one or another of the hormonal events that characterize the normal ovulatory cycle (Chapter 5). Each of the available tests is useful, and no one test is necessarily best. Some are simple, noninvasive, and inexpensive, and others are more complicated, invasive, and costly. A few can predict when ovulation is likely, with varying accuracy. However, no test, regardless of how sophisticated, can prove that ovulation has actually occurred; the only positive proof that assures the occurrence of ovulation is pregnancy. The most appropriate test to use varies with the information required. The same tests used to diagnose anovulation can be used to assess the effectiveness of treatment.

Menstrual History

Menstrual history alone is often sufficient to establish a diagnosis of oligo-/anovulation. Ovulatory women generally have regular, predictable menstrual cycles between 21 and 35 days, with menstrual flow that is consistent in volume and duration and typically accompanied by a recognizable pattern of premenstrual and menstrual symptoms. Conversely, menstrual cycles in anovulatory women are generally irregular, unpredictable, or infrequent; vary in flow characteristics; and exhibit no consistent pattern of molimina. **Women with regular menses are almost always ovulatory. Women with irregular or infrequent menses may ovulate, but not consistently, and do not require specific diagnostic tests to prove what is already obvious.**

Basal Body Temperature

BBT is body temperature under basal conditions, at rest. For practical purposes, BBT is measured each morning, on awakening and before arising. Traditionally, BBT is measured with an oral glass-mercury thermometer having an expanded scale, typically ranging from 96.0°F to 100.0°F and marked in tenths of 1°; modern electronic thermometers

are a suitable alternative but only if they have the necessary accuracy and precision. As a test of ovulation, daily BBT recordings are based on the thermogenic properties of progesterone; as levels rise after ovulation, BBT also increases. The effects are more qualitative than quantitative, are subtle but nonetheless distinct, and generally easy to detect when daily BBT recordings are plotted on a graph paper.[419] **Synthetic progestins commonly used to induce menses in amenorrheic women (medroxyprogesterone acetate, norethindrone acetate) have similar thermogenic properties and also raise BBT.** A BBT rise in a woman taking a progestin should not be confused with ovulation.

BBT is typically low and fluctuates between 97.0° and 98.0° during the follicular phase of the cycle, modestly but distinctly higher (0.4°–0.8°) during the luteal phase, and falls again to baseline levels just before or after the onset of menses. In ovulatory women, a "biphasic" pattern is usually readily evident. **The ideal BBT recording is distinctly biphasic and reveals a cycle between 25 and 35 days in length, with menses beginning 12 days or more after the rise in temperature.** When pregnancy occurs in a monitored cycle, the onset of menses is delayed and BBT remains elevated, reflecting the sustained production of progesterone by the corpus luteum stimulated by hCG.

BBT recordings provide objective evidence of ovulation and also reveal the approximate time of ovulation. Unfortunately, the temporal relationship between the thermogenic shift in BBT and ovulation frequently is misunderstood. BBT generally falls to its lowest level on the day before or day of ovulation, but the nadir in BBT cannot be reliably identified until after the temperature rises and remains elevated.[420] The shift in BBT occurs when progesterone concentrations rise above approximately 3 to 5 ng/mL, 1 to 5 days *after* the midcycle LH surge and up to 4 days *after* ovulation.[421] The temperature rise is usually somewhat abrupt, but may be gradual and difficult to define, and once apparent (2 or more days of temperature elevation), the most fertile interval has passed. **In cycles monitored with BBT, the interval of highest fertility spans the 7-day interval immediately before the midcycle rise in BBT.** Much of the uncertainty in predicting the time of ovulation can be avoided by reviewing a series of recordings, noting the earliest and latest days of the cycle on which the temperature shift occurred. **Coital timing can be optimized by suggesting intercourse on alternate days beginning 7 days before the earliest observed rise in BBT and ending on the latest day it has been observed.**

The principal advantage that BBT has over other tests of ovulation is low cost. However, some find it stressful, serving as a daily reminder of unsuccessful efforts to conceive, each day beginning with thoughts of a family not yet realized. Since BBT cannot reliably define the time of ovulation and can become tedious, it is not the method of choice for evaluating ovulatory function for most infertile women.[408]

Serum Progesterone Concentration

A serum progesterone measurement is the simplest, most common, objective, and reliable test of ovulatory function, as long as it is appropriately timed. Progesterone levels generally remain below 1 ng/mL during the follicular phase, rise slightly on the day of the LH surge (1–2 ng/mL) and steadily thereafter, peak 7 to 8 days after ovulation, and decline again over the days preceding menses. **A progesterone concentration of less than 3 ng/mL implies anovulation, except when drawn immediately after ovulation or just before the onset of menses, when lower levels might naturally be expected.**[422,423]

When is the best time to measure the serum progesterone concentration to document ovulation? **Ideally, the serum progesterone level should be drawn approximately 1 week before the expected onset of menses, when the concentration is at or near its peak. Contrary to popular belief and practice, cycle day 21 is *not* always the best time to measure the serum progesterone concentration.** Cycle day 21 is a good choice for women with cycles lasting approximately 28 days but a poor choice for women with 35-day cycles. The normal ovulatory cycle is 25 to 35 days long and exhibits a 13- to 15-day luteal phase. At the extremes of normal, ovulation may occur as early as cycle day 10 (in a 25-day cycle) and as late as day 22 (in a 35-day cycle). If ovulation occurs on cycle day 10, day 21 falls 11 days after ovulation, well after progesterone concentrations peak and when they are again nearing basal levels. If ovulation occurs on cycle day 22, day 21 falls 1 day **before** ovulation, when progesterone levels have not yet started to rise. **The best time to test will vary with the overall length of the menstrual cycle, aiming for approximately 1 week before the expected menses. If the menses does not commence 7 days after a serum progesterone reading of less than 3 ng/mL, it may be reasonable to repeat the test.**

Serum progesterone levels have also been used to evaluate the quality of luteal function. Whereas the amount and duration of progesterone production certainly do reflect the functional capacity of the corpus luteum, a truly accurate measure requires frequent serum progesterone determinations that are costly and impractical.[424–426] Judgments based on limited sampling, regardless of how well timed, have numerous pitfalls and cannot define the quality of luteal function reliably.[424,427–431] **There is no consensus on minimum serum progesterone concentration that defines normal luteal function.** A midluteal serum progesterone level greater than 10 ng/mL is a popular standard,[432] but the concentrations observed in normal and abnormal cycles and in conception and nonconception cycles in both fertile and infertile women vary widely and overlap greatly.[433] One reason is that progesterone is secreted by the corpus luteum in distinct pulses, temporally linked to pulsatile LH secretion[434,435]; levels ranging from as low as 4 ng/mL to as high as 40 ng/mL can be observed within brief intervals of time.[435]

A midluteal serum progesterone concentration cannot define the quality of luteal function and has little value beyond documenting ovulation.

Urinary LH Excretion

A wide variety of commercial products allow women to determine not only whether they ovulate, but when, in advance of the actual event. Generally known as "ovulation prediction kits" or "LH kits," the products are all designed to detect the midcycle LH surge in urine.

The midcycle LH surge is a relatively brief event, typically lasting between 48 and 50 hours from start to finish. LH has a short half-life and is rapidly cleared via the urine. Ovulation predictor kits turn positive when the urinary LH concentration exceeds a threshold level normally seen only during the LH surge. In most cycles, the test is positive on a single day, occasionally on 2 consecutive days. To detect the LH surge reliably, testing must be done on a daily basis, generally beginning 2 or 3 days before the surge is expected, based on the overall length of the cycle. The first positive test provides all relevant information; there is no value in continued testing.

Test results are sensitive to both the volume of fluid intake and time of day. There is no need to restrict fluid intake, but patients should be advised to avoid drinking large volumes of fluid a short time before they plan to test. Logically, the first morning void would seem an ideal specimen to test because it is usually the most concentrated. However, results correlate best with the serum LH peak when testing is performed in the late afternoon or early evening hours (4:00–10:00 PM),[421] probably because LH surges often begin in the early morning hours and are not detected in the urine for several hours. Twice-daily testing decreases the frequency of false-negative results (failure to detect the LH surge in an ovulatory cycle), but is generally unnecessary. When performed daily and properly timed, testing will detect the LH surge in most ovulatory cycles. True false-positive tests (detection of an LH surge in an anovulatory cycle) occur in approximately 7% of cycles[436]; equivocal or "borderline" results are also common and can be both confusing and frustrating.

The accuracy of ovulation predictor kits varies. All are useful and reasonably reliable, but some are better and easier to use than others.[397,437,438] The best products predict ovulation within the subsequent 24 to 48 hours, with greater than 90% probability.[396,397] **Ovulation generally occurs 14 to 26 hours after detection of the LH surge and almost always within 48 hours.[396] Consequently, the interval of greatest fertility includes the day the surge is detected and the following 2 days. The day after the first positive test is generally the one best day for timed intercourse or insemination.**[389,396,439,440] Ovulation predictor kits are noninvasive, are widely available, require relatively little time and effort, and invite women to become actively involved in their care without increasing their stress levels.[441,442] Their greatest advantage over other methods is their ability to predict when ovulation will occur. Accurate identification of the midcycle LH surge also defines the length of the follicular and luteal phases, which may reveal subtle and otherwise unrecognized cycle abnormalities warranting treatment. Women who cannot detect an LH surge using the ovulation predictor kits are often anovulatory but may assume the kit is not functioning properly; patients should be advised that failure to detect an LH surge may reveal an important diagnostic component of their infertility. After establishing ovulation, repeated urinary LH monitoring is perhaps best reserved for women who ovulate (based on menstrual history, LH monitoring, or an appropriately timed serum progesterone concentration) but have infrequent intercourse or require insemination (**Figure 28.4**).

Endometrial Biopsy to Assess Luteal Phase Deficiency and Endometrial Receptivity

Endometrial biopsy can be used as a test of ovulation, based on the characteristic histologic changes induced by progesterone. During the follicular phase of the cycle, the endometrium exhibits a proliferative pattern, reflecting the growth stimulated by rising levels of estrogen derived primarily from the dominant ovarian follicle. During the luteal phase, progesterone secreted by the corpus luteum induces the "secretory" transformation of the endometrium. Anovulatory women are always in the follicular phase; their endometrium is always proliferative and may even become hyperplastic with extended exposure to a constant estrogen growth stimulus. **In the absence of treatment with exogenous progesterone or a synthetic progestin, a secretory endometrium implies recent ovulation.**

Endometrial biopsy is a relatively simple office procedure, usually performed with a disposable plastic aspiration cannula, and complications are few. Pretreatment with a nonsteroidal anti-inflammatory drug (NSAID) helps to reduce pain or cramping associated with the procedure. Sedation or anesthetic (paracervical block) is helpful in technically difficult biopsies and in women who are very anxious. When properly timed, in the same way and for the same reasons as a serum progesterone concentration, endometrial biopsy is an effective test of ovulation. However, it is also invasive, uncomfortable, and costly and provides little information beyond what can be obtained from BBT recordings, a serum progesterone concentration, or monitoring urine LH excretion. Therefore, endometrial biopsy has rather limited and specific indications in the evaluation of infertile women. For women with chronic anovulation of long duration, biopsy can identify or exclude endometrial hyperplasia that requires specific treatment. In those few individuals suspected of harboring a chronic endometritis, biopsy is diagnostic. **In the past, endometrial biopsy for diagnosis of luteal phase deficiency (LPD) was considered a basic element of the infertility evaluation but no longer.**

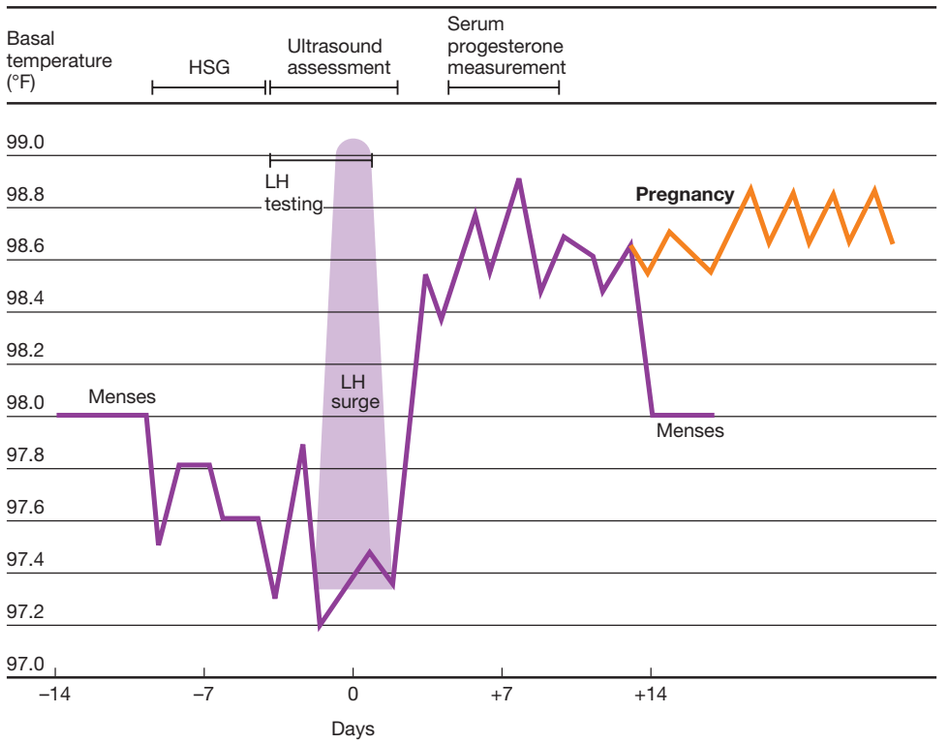


FIGURE 28.4

Inadequate corpus luteum progesterone production or "luteal phase deficiency" (LPD) was long considered an important cause of both infertility and early pregnancy loss.[443,444] The proposed mechanisms were different but related, representing only different points on a pathophysiologic continuum. In theory, because the human implantation window is relatively narrow (spanning the interval from approximately 6 to 10 days after ovulation),[445–447] low circulating progesterone levels could be expected to result in delayed endometrial maturation, causing a shift in the implantation window and failed or late implantation. A long delay would threaten embryo viability or prevent implantation. A shorter delay would allow implantation but result in a tardy or low-amplitude hCG rescue signal that could not stimulate normal amounts of progesterone from an already regressing corpus luteum, or maintain production for the requisite duration,[448–450] with either causing a premature luteal–placental shift and pregnancy loss.[451] In this context, endometrial biopsy was viewed as a bioassay of luteal function because it would reflect both the functional capacity of the corpus luteum and the end-organ response.

The classic histologic features of secretory endometrial development were described by Noyes, Hertig, and Rock, in the lead article of the inaugural issue of **Fertility and Sterility**.[452] The pattern was considered sufficiently predictable to allow experienced pathologists to "date" the endometrium, assigning a histologic day that could be compared to the actual day of sampling, estimated by counting backward from the onset of the next menstrual period (assuming menses began on the 14th postovulatory day), or defined by the number of days elapsed since detection of the LH surge or observation of follicular collapse by serial ultrasonography.[453] Historically, histologic and sampling dates that agreed, within a 2-day interval, were considered normal, whereas a date more than 2 days "out of phase" was the gold standard criterion for the diagnosis of LPD.[454–456] Traditionally, diagnosis of LPD required abnormal results in two (preferably consecutive) cycles, reasoning that reproductive failure could be attributed to LPD only if it was consistent or recurring and acknowledging that LPD could also occur in normal fertile women, at least occasionally.[457–463] Endometrial dating was accepted widely by clinicians and pathologists, and the practice endured, despite numerous challenges to its validity.

The first and most fundamental criticism of the traditional histologic dating criteria was that the normal standard was based on analysis of tissue specimens obtained from infertile women[452]; the reference population was abnormal, by definition, and also likely heterogeneous because infertility has many different causes. Second, the sampling date was estimated retrospectively, after the onset of menses, assuming a uniform 14-day luteal phase, despite numerous studies demonstrating that luteal phase duration varied significantly, even in normal women.[104,109,112,464,465] Moreover, retrospective estimates of the sampling date correlated poorly with

the time of ovulation as defined by the LH surge or observations of follicular collapse[432,453,466] and ignored any effect that biopsy might have on the onset of menses, or when it was perceived to start.[453,462,467] Third, the traditional histologic dating criteria were inherently subjective, and numerous studies had observed significant intraobserver and interobserver variations in histologic interpretation that were great enough to affect diagnosis and management in 20% to 40% of individual women.[456,468–471]

The standard practice of endometrial biopsy and histologic dating for diagnosis of LPD was proven invalid in 2004, for all intents and purposes. A systematic reanalysis of the histologic features used for endometrial dating confirmed the classically described sequence but revealed that the patterns were much less temporally discrete than originally described and demonstrated that normal variations among individuals, between cycles in individuals, and among different observers were too great to reliably define any specific luteal day or even a narrow interval of days.[472] Soon thereafter, a large multicenter trial demonstrated conclusively that abnormal histologic dating could not discriminate infertile from proven fertile women.[473] The second study invalidated the practice of endometrial dating, and the first explained why the method failed.

Recent evidence challenges even the basic premise on which the concept of LPD is founded: that abnormally low circulating progesterone concentrations result in delayed endometrial maturation. In normal women treated with a fixed physiologic dose of estrogen after downregulation with a GnRH agonist and then randomized to receive physiologic (mean progesterone level 19 ng/mL) or grossly low levels of exogenous progesterone treatment (mean progesterone level 5.5 ng/mL), there was no discernible difference in endometrial histology.[474] These observations suggest that the histologic features of secretory endometrium relate more to the duration of progesterone exposure than to the concentration. Studies using a similar design have demonstrated that widely ranging concentrations of estradiol also have no discernible impact on secretory endometrial maturation.[475,476] Altogether, these data indicate that secretory endometrial development can progress normally despite widely varying concentrations of estradiol and progesterone, challenging the traditional paradigm, and serve to further invalidate the use of endometrial histologic dating as a diagnostic tool. **In sum, histologic endometrial dating cannot guide the clinical management of women with reproductive failure and has no place in the diagnostic evaluation of infertility.**

The lack of any valid method for diagnosis of LPD does not refute its existence or its potential importance in the pathophysiology of reproductive failure. The pathogenic mechanisms outlined previously are still viable. Evidence supports the notion of a finite implantation window[445–447] that progesterone is essential for embryo implantation[477] and that delayed implantation might adversely affect corpus luteum function,[448–450] predisposing to reproductive failure.[451] It is entirely possible, if not likely, that abnormally low levels of progesterone might have important functional consequences with no morphologic correlate. Biochemical or molecular markers of endometrial function provide the means to further explore the possibility. The pattern of endometrial gene expression defines distinct functional phases of the cycle.[478] A number of endometrial proteins exhibit patterns of expression or gene regulation during the putative implantation window, suggesting they might serve as markers of endometrial receptivity, including cytokines (leukemia inhibitory factor, colony-stimulating factor-1, and interleukin-1), cell adhesion molecules (the avb3 integrin), transcription factors (HOXA10 and HOXA11),[479–482] glycodelin, and the polymorphic mucin 1,[483,484] osteopontin,[485–487] *N*-acetylglucosamine-6-*O*-sulfotransferase (important in the synthesis of L-selectin ligands),[488] and the L-selectin ligand itself.[489]

Based on the premise that hormonal changes drive specific gene expression cascades, which in turn dictate the histologic and functional changes in the endometrium to prepare for implantation, an analysis of the transcriptomic signature of 134 genes in endometrial biopsies demonstrated a high specificity for endometrial dating. A model developed from these findings has been clinically applied as a commercially available test aimed at improving pregnancy outcomes in women experiencing recurrent implantation failure during assisted reproduction.[490,491] Subsequently, several groups developed similar tests to diagnose asynchronous endometrial response to progesterone based on endometrial gene expression profiles,[492,493] while others proposed models to identify a disrupted rather than an asynchronous pattern based on altered expression of genes involved in cell cycle regulation, cell division, and cytoskeleton and cilia formation.[494] Unfortunately, none of these tests have been adequately validated as a reliable measure of endometrial function or receptivity, and subsequent studies did not support clinical benefit in patients with or without implantation failure undergoing embryo transfer.[495,496] **At the present time, there is no reliable molecular diagnostic biomarker for endometrial receptivity, and available evidence argues against the use of commercialized tests for the purpose of improving outcomes in patients undergoing infertility treatment.**

Transvaginal Ultrasonography

The last and most complicated test of ovulation involves serial TVUS, which permits direct observation of events in the ovary just before and immediately after ovum release. Although still not providing positive proof that ovulation actually occurred, serial TVUS provides detailed information about the size and number of preovulatory follicles and the most accurate estimate of when ovulation occurs.

In its final stages of development, the preovulatory follicle grows at a predictable pace, approximately 2 mm/d (range: 1–3 mm/d). After ovulation, the follicle collapses, margins become less distinct, the density of internal echoes increases,

and the volume of cul-de-sac fluid increases.[497,498] Abnormal patterns of follicle development can also be observed. The follicle may grow at an abnormal pace, collapse when still relatively small, or continue to grow but fail to rupture and persist as a cyst for days after the LH surge—the luteinized unruptured follicle.[499,500] Such subtle forms of ovulatory dysfunction cannot be detected otherwise but are also rare. Because treatment with prostaglandin synthase inhibitors (NSAIDs) can disrupt the ovulatory process and predispose to a luteinized unruptured follicle,[501,502] their use is best limited to the menstrual phase of the cycle in women attempting to conceive.

The use of serial TVUS to monitor the size and number of developing follicles is essential to the safety and effectiveness of ovulation induction with exogenous gonadotropins (Chapter 30), but the costs and logistical demands involved are otherwise difficult to justify. Consequently, the method should generally be reserved for the few in whom the safety or effectiveness of treatment truly hinges on the detailed information it offers.

Summary: Assessment of Ovulation

The evaluation of ovulation is a core component of the evaluation for infertility. All of the different methods are useful, and no single method is necessarily the best. Whereas some are very simple, noninvasive, and inexpensive, others are more complicated, invasive, and costly. A few provide the means to determine not only if ovulation occurs, but also when, with varying accuracy. The best choice among methods varies with the information required. In women with oligomenorrhea or amenorrhea, no formal evaluation is needed to establish a diagnosis of ovulatory dysfunction, but endometrial biopsy to exclude hyperplasia may be prudent, depending on duration. When the only objective is to confirm ovulatory function, as in those with regular monthly menses, a properly timed serum progesterone concentration is the simplest and most reliable method. When circumstances require accurate prediction of ovulation, as in couples having infrequent intercourse or those requiring insemination, urinary LH monitoring is generally the most cost-effective and appropriate choice. In the few who require insemination but consistently fail to detect a midcycle LH surge, serial TVUS can provide the necessary information; however, an ovulatory defect should be suspected. Ultimately, the method chosen should be tailored to the needs of the individual patient.

Infertile women with ovulatory dysfunction are obvious candidates for ovulation induction. In general, only limited additional evaluation is needed to define the initial treatment of choice, and most women will respond promptly to one of the simpler treatment strategies (Chapter 30). In the majority of cases, it is reasonable and appropriate to begin treatment immediately, even before other potential causes of infertility have been investigated. If anovulation is the only obstacle to overcome, most couples will conceive promptly without further interventions. Women with amenorrhea or hyperandrogenic anovulation deserve additional preliminary evaluation, applying the principles described in Chapters 10 to 12.

CERVICAL FACTOR: ABNORMALITIES OF SPERM–MUCUS INTERACTION

The cervix participates in the reproductive process in several ways. Cervical mucus accepts or captures sperm from the ejaculate and the vagina, excludes the seminal plasma and morphologically abnormal sperm,[503] nurtures sperm biochemically, and serves as a reservoir, thereby prolonging sperm survival and the fertile interval between intercourse and ovulation. Mucus is a glycoprotein gel with solid and liquid phases and has a mosaic ultrastructure with interstitial channels between mucin strands that expand and contract in response to cyclic changes in the steroid hormone environment across the menstrual cycle to facilitate or inhibit the passage of sperm.[504–508] Estrogen stimulates cervical mucus production, and as levels rise during the follicular phase, mucus becomes more abundant and watery, less cellular, and more easily penetrated by sperm.[509] Progesterone inhibits cervical mucus production and renders it opaque, viscid, and impenetrable. The cyclic changes in cervical mucus characteristics help to explain why the cycle day–specific probability of conception rises steadily as ovulation nears and plummets immediately thereafter.

For most of the past century, the postcoital test for diagnosis of cervical factor infertility was considered a basic element of the infertility evaluation. The test involved collection of cervical mucus (by aspiration or with nasal polyp forceps) shortly before the expected time of ovulation (as determined by BBT or urinary LH monitoring in previous cycles) a few to several hours (typically 2–12 hours) after intercourse.[510] The mucus specimen was evaluated for pH, clarity, cellularity, viscosity (the length to which a column of mucus can be stretched in centimeters, known as "spinnbarkeit"), and salinity (evaluated according to the complexity of the network of crystals that forms when mucus is dried on a glass slide, also known as "ferning"), and for the number and motility of surviving sperm. The presence of motile sperm confirmed effective coital technique and sperm survival, and the number of sperm (per high-power field) was used to predict semen quality (sperm density and motility) and cycle fecundability (inverse correlation with time to conception or cumulative conception rates).[511–516] Most considered even a single motile sperm in most fields a normal test result.[516–518]

Abnormal postcoital test results were common, usually due to improper timing, either too early in the cycle when mucus was relatively scant or after ovulation when mucus quality was poor.[519] Timing was optimized by performing the test within 2 days before the LH surge or when TVUS demonstrated a preovulatory follicle.[520] Other explanations for poor-quality mucus were cervicitis, previous treatment

for cervical intraepithelial neoplasia (eg, cryotherapy), and treatment with clomiphene citrate. Potential explanations for the absence of motile sperm in good-quality mucus included ineffective intercourse, failed ejaculation (frequently resulting from performance anxiety), poor semen quality, and use of spermicidal coital lubricants. Observations of degenerating, immotile, "shaking," or agglutinated sperm were considered reason for antisperm antibody testing.[514] An abnormal result was confirmed by repeat testing to establish the diagnosis of cervical factor infertility,[510,516,521] prompting further evaluation with a nucleic acid test for chlamydia and cultures for ureaplasma and mycoplasma (or empirical treatment with antibiotics),[522–524] and semen analysis. Normal semen quality and absence of sperm in good-quality mucus were regarded as evidence of "hostile" cervical mucus or a sperm function abnormality, differentiated by comparisons of partner and donor sperm survival and motility in bovine cervical mucus in vitro and antisperm antibody testing.[525–527] Strategies for correcting or overcoming cervical factor infertility included treatment with exogenous estrogens (to stimulate mucus production)[528] or mucolytic agents (guaifenesin),[529] precoital douching with a sodium bicarbonate solution,[530] and IUI.[514,531–533]

Advocates of routine postcoital testing argued that the postcoital test could identify couples who might benefit from a simple treatment and had prognostic value for predicting the probability of pregnancy without treatment.[406,515] Critics reasoned that results achieved even with IUI suggested only a modest benefit at best,[534] that any prognostic value the test might have was limited to young couples with unexplained infertility of short duration because the test surely had no predictive value in women with infertility due to anovulation or tubal occlusive disease, and that male infertility amenable to treatment with IUI could be more accurately defined by the results of semen analysis. The argument for expectant management in couples with unexplained infertility and a normal postcoital test was dismissed as moot, because few couples seeking evaluation and treatment accepted the recommendation.

The postcoital test for diagnosis of cervical factor is no longer recommended.[408] Abnormalities of cervical mucus production or sperm/mucus interaction are rarely, if ever, the sole or principal cause of infertility. Chronic cervicitis or cervical stenosis resulting from conization or other treatment for cervical disease that might impair sperm–mucus interaction can be identified by speculum examination, and in the absence of such findings, the likelihood that cervical mucus represents an important obstacle is remote. Semen analysis identifies couples with significant male factor infertility. The test has no standard methodology or interpretation[510,518] and has poor reproducibility even among trained observers.[535] The only randomized trial comparing outcomes in women with normal and abnormal postcoital tests found the test invalid because neither test results nor treatment for abnormal tests affected the outcome.[536–538] Office examination after scheduled intercourse is an inconvenient and unwelcome intrusion for most couples, adding further to their burden of stress. Finally, postcoital test results seldom change clinical management, because contemporary treatments for unexplained infertility include IUI (with ovarian stimulation) or IVF, both of which negate any contributing cervical factor.

UTERINE FACTOR: ANATOMIC AND FUNCTIONAL ABNORMALITIES

Abnormalities of the uterus are a relatively uncommon cause of infertility but should always be considered. If for no other reason, they may adversely affect the outcome of pregnancies achieved by successful treatment of more common male, ovarian, and tubal factors. The anatomic uterine abnormalities that can adversely affect fertility include congenital malformations, leiomyomas, and intrauterine adhesions; endometrial polyps have also been implicated, but their reproductive implications are less clear. The only functional uterine abnormality of specific interest in the evaluation of infertility is chronic endometritis. Whereas abnormalities of endometrial receptivity (including LPD) might be viewed as another, they can have no practical significance until there is conclusive evidence that infertility can result from intrinsic endometrial dysfunction that impairs or prevents implantation and a method for diagnosis has been validated.

Anatomic and functional uterine abnormalities that can impair fertility can also adversely affect pregnancy outcome. They are discussed here as a cause of infertility and elsewhere (Chapter 33) as a cause of recurrent pregnancy loss. The embryology or pathogenesis and obstetric consequences of uterine malformations and of leiomyomas are considered at length in Chapter 3. Discussion here is focused on their diagnosis, their impact on fertility, and how they influence evaluation and treatment.

There are three basic methods for evaluation of the uterine cavity: TVUS or saline sonohysterography, hysterosalpingogram (HSG), and hysteroscopy. Each of these has advantages and disadvantages, and the choice among them should be tailored to the needs of the individual patient.

In women with no risk factors for tubal disease and those whose tubal status is already known (from earlier surgery for other indications) or is largely irrelevant (as in women who require IVF for severe male factor infertility), ultrasonography offers a simpler and better tolerated alternative that may also reveal unsuspected ovarian pathology (cyst, endometrioma), with no radiation exposure. When symptoms suggest an anatomic lesion of the uterine cavity (menorrhagia, intermenstrual spotting), sonohysterography is the most sensitive and logical diagnostic test. HSG allows evaluation of tubal patency. Hysteroscopy is definitive but has few diagnostic advantages over sonohysterography and can generally be safely reserved for treatment of abnormalities already identified by less invasive and costly methods.

Transvaginal Ultrasonography and Saline Sonohysterography

TVUS is the most effective and convenient method for the evaluation of uterine factors in infertile women. Saline sonohysterography, involving TVUS during or after the introduction of sterile saline through a catheter designed for the purpose, crisply defines cavity contours and readily demonstrates even small, but potentially important, intrauterine lesions.[539]

In all phases of the cycle, the interface between the endometrium and the myometrium is well defined. The interface between the two layers of the endometrium itself (bordering the uterine cavity) can be difficult to identify very early in the cycle and during the secretory phase but is visible during the latter half of the proliferative phase. Together, the two layers of the endometrium comprise the "endometrial stripe," which changes in appearance and thickness across the cycle. During the proliferative phase, the endometrium is relatively hypoechoic and grows in thickness to yield a prominent "triple line" or trilaminar pattern. During the secretory phase, the endometrium grows little more, or not at all, and increases in echodensity, possibly because the developing network of coiled basilar vessels presents a great many more reflective surfaces. Cycle-dependent changes in uterine artery blood flow parameters (velocity and pulsatility index) measured using color and pulsed Doppler ultrasonography have also been described,[540,541] but diurnal variations and differences between the two uterine arteries (ipsilateral or contralateral to the dominant ovarian follicle) complicate interpretation. In efforts to define a receptive endometrium, several studies have examined the correlation between endometrial stripe thickness and pattern or uterine artery blood flow parameters with implantation or pregnancy rates in IVF cycles,[542–547] but results are conflicting. Whereas some have found correlations between one or more parameters and treatment outcomes, others have not. The few studies examining the endometrium in unstimulated cycles in infertile women have not demonstrated any important correlation between endometrial thickness, pattern, or blood flow and the cause of infertility or prognosis.[548–550] **In the diagnostic evaluation of infertile women, TVUS can identify important uterine pathology but provides no useful measure of endometrial function or receptivity.**

For the identification of congenital malformations, standard two-dimensional (2D) TVUS improves diagnostic accuracy for differentiating septate and bicornuate uteri by revealing the shape of the fundal contour. The septate uterus presents a single unified fundus that is often somewhat broader than normal and sometimes slightly concave; the bicornuate uterus has two entirely separate fundi divided by a distinct midline cleft of varying depth.[551,552] The accuracy of saline sonohysterography exceeds that of HSG, by revealing both the double uterine cavity and the shape of the fundal contour. Three-dimensional (3D) TVUS has the advantage of obtaining a coronal view and providing accurate and reproducible information about external and internal contours of the uterus, ideally when the endometrium is 5 mm or thicker.[552–555] 3D TVUS showed 100% specificity and sensitivity for diagnosing congenital uterine anomalies in two studies, and its concordance with specificity and sensitivity of laparoscopy and hysteroscopy was reported to be 100% and 96%, respectively.[556,557] Another study reported 87% sensitivity and 97% specificity for 3D TVUS after evaluating 214 women with infertility who underwent laparoscopy and hysteroscopy.[558]

Results of studies evaluating the accuracy of TVUS for detection of submucous myomas and endometrial polyps have varied, but, in general, both 2D and 3D TVUS are more sensitive than HSG and approach the accuracy of hysteroscopy.[559–562] In a study of 3,850 infertile women with 74 histologically diagnosed endometrial polyps, 3D TVUS had a sensitivity, specificity, PPV, and NPV of 100%, 99.7%, 98.6%, and 100%, respectively.[563] Other studies yielded similar results for the diagnostic accuracy of 3D TVUS, comparable to that of hysteroscopy.[559,564] 3D TVUS can successfully illustrate the endometrial cavity and map the location of the fibroids, even when they are multiple.[564] Nevertheless, it should be kept in mind that MRI may produce more accurate results than 3D TVUS in some extreme cases. Accuracy for diagnosing fibroids dropped from 98% to 89% for 3D TVUS and from 100% to 94% for MRI, when five or more fibroids were present.[565] It is noteworthy that whereas the detection of an overall or focal increase in endometrial thickness or asymmetry between the two layers in 2D or 3D TVUS suggests a polyp or myoma, saline sonohysterography reveals a polypoid projection into the fluid-filled cavity.

For the diagnosis of intrauterine adhesions, standard TVUS is reasonably specific but rather insensitive[566,567]; a focally narrowed or discontinuous endometrial stripe suggests the diagnosis. Saline sonohysterography compares with HSG, having a relatively high sensitivity (75%) and specificity (over 90%), a modest PPV (approximately 50%), and an excellent NPV (over 95%) for detection of adhesions.[566,568] Women with mild disease exhibit mobile thin, echogenic bands bridging a normally distensible endometrial cavity. Those with severe disease have more broadly based bands or no cavity at all.[569]

Hysterosalpingography

HSG accurately defines the size and shape of the uterine cavity, provides clear images of most uterine developmental anomalies (unicornuate, septate, bicornuate, and didelphys), and, with exceptions, also identifies submucous myomas and intrauterine adhesions that can have important reproductive implications. Although HSG may also reveal endometrial polyps, sonohysterography is a more sensitive method for their detection. A slow injection of contrast medium helps to minimize the risk that a cavitary lesion will be obscured and will go undetected.

The normal uterine cavity is symmetric, roughly triangular in shape, widest at the level of the cornual orifices near the fundus, and relatively smooth in its contours. The various developmental uterine anomalies generally have a fairly characteristic appearance on HSG. A unicornuate uterus is typically somewhat tubular, deviates to the left or right, and has one fallopian tube. Both septate and bicornuate uteri typically exhibit a common lower segment that divides into two distinct horns to yield a Y-shaped configuration with varying distance between the upper arms.[551,553] The two anomalies cannot be differentiated by HSG alone; additional evaluation is required to establish an accurate diagnosis (standard or 3D TVUS, sonohysterography, magnetic resonance imaging [MRI], or laparoscopy).[552] Either anomaly can also be confused with a unicornuate uterus if only one of the two horns is imaged because they divide near or below the tip of the cannula or catheter inserted into the cervix or uterus. To properly study a uterus didelphys or complete septate uterus, the two hemiuteri must be imaged via their separate cervical openings, often found on opposite sides of the two hemivaginas of varying lengths. Myomas and larger polyps generally produce curvilinear filling defects of various sizes and shapes. HSG in women with intrauterine adhesions usually reveals grossly irregular cavity contours and filling defects and, in many with severe disease, no cavity at all.

The accuracy of HSG for detecting intrauterine pathology in infertile women varies with the nature of the abnormality. A large study involving over 300 women comparing HSG to hysteroscopy (the gold standard) observed that HSG had, overall, a 98% sensitivity, 35% specificity, 70% PPV, and 92% NPV, with a 30% false-positive rate and 8% false-negative rate; misdiagnoses are almost entirely related to distinguishing submucous myomas from polyps and were, therefore, relatively unimportant[570]; either requires surgical intervention for correction. In another study of similar design, HSG had a 75% sensitivity for detection of intrauterine adhesions and only a 50% sensitivity for detection of endometrial polyps.[566]

Specific issues concerning the scheduling and preparation for HSG and details regarding technique and interpretation as they relate to the evaluation of tubal factor infertility are addressed in the following section (see Section "Tubal Factor," further on).

Hysteroscopy

Hysteroscopy is the gold standard method for both diagnosis and treatment of intrauterine pathology that may adversely affect fertility. Traditionally, hysteroscopy was reserved for treatment of disease identified by other less invasive methods, but modern operative hysteroscopes with an outer diameter measuring 2 to 3 mm now permit diagnostic and minor operative procedures to be performed safely in the office setting.[571] Major intrauterine pathology generally requires more traditional operative hysteroscopy using instruments having larger caliber and greater capabilities.

Congenital Uterine Anomalies

Developmental uterine anomalies have long been associated with pregnancy loss and obstetric complications, but affected women are generally not infertile. The prevalence of uterine anomalies in infertile women and fertile women with normal reproductive outcomes is similar, approximately 2% to 4%.[572–577] The prevalence is higher among women with poor pregnancy outcomes, such as pregnancy loss (13%), and in those with a history of infertility and recurrent pregnancy loss (24.5%).[578] Consequently, when discovered during an infertility evaluation, anomalies cannot be regarded as the likely cause or even as an important contributing cause of infertility but only as another obstacle that must be considered when planning treatment after evaluation is completed. For example, treatments associated with a substantial risk of multifetal gestation (ovarian stimulation/IUI, IVF) present even greater risks to women with uterine malformations.

In a recent systematic review, septate uterus was the most common anomaly among infertile women (3%), followed by arcuate (2%), bicornuate (1.1%), unicornuate (0.5%), and didelphys (0.3%).[577,578] However, arcuate uterus is considered a variant of the normal without any reproductive or obstetric consequences.[579] The prevalence of only unicornuate and bicornuate uteri was found to be significantly higher in infertile women than in an unselected population of women.[578]

Septate uterus is the anomaly most highly associated with reproductive failure and obstetric complications, including first- and second-trimester pregnancy loss, preterm delivery, fetal malpresentation, and intrauterine growth restriction.[552,577,579,580] Currently available evidence is inconclusive regarding an association between uterine septum and infertility.[579] The mechanisms responsible are poorly understood, but poor septal blood supply, resulting in poor implantation efficiency and embryo growth, and cervical incompetence are the usual suspects.[581–584] Although diagnosis of septate uterus is not an automatic indication for metroplasty, the overall reproductive performance of women with a septum in situ (at least those who are recognized) is rather poor, with term delivery rates of approximately 40%. Most losses occur in the first trimester (approximately 60%). A 2022 systematic review and meta-analysis compared the effect of hysteroscopic septum resection and expectant management.[585] One thousand five hundred eighty-nine participants in 11 studies, 10 of which were observational, were included, and hysteroscopic septum resection was found to be associated with a statistically significant reduction in the rate of pregnancy loss (OR = 0.45, 95% CI = 0.22–0.90), the risk of fetal malpresentation (OR = 0.32, 95% CI = 0.16– 0.65), and the frequency of preterm birth (OR = 0.30, 95% CI = 0.11–0.79). Notably, the likelihood of clinical pregnancy, term live birth, or risk of cesarean delivery were similar between septum resection and expectant management arms.[585] Similarly, in a recent multicenter RCT with a limited sample size of 80

patients with a history of infertility, pregnancy loss, or preterm birth, septum resection was not associated with an increase in live birth rates.[586]

Inevitably, systematic infertility evaluations will identify nulligravid women with a uterine septum who present a management dilemma. The patient should be counseled that the association between a uterine septum and infertility remains controversial. However, a uterine septum is linked to an increased risk of pregnancy loss and obstetric complications. The decision to proceed with septum resection should be made collaboratively with the patient, taking into account their history of pregnancy loss and obstetric complications, if applicable. It is important to acknowledge that the available data are limited, and firm recommendations cannot be made.[579]

Uterine Myomas

Myomas can be identified up to 70% of all reproductive-aged women and in 5% to 10% of infertile women[185,587]; myomas are the only abnormal finding in 1% to 2% of women with infertility. Although they are an established cause of abnormal bleeding, pain, and symptoms relating to pressure on adjacent organs, the impact of myomas on fertility has been more difficult to define, with the bulk of evidence coming from observational studies comparing the prevalence of myomas in fertile and infertile women or the reproductive performance of women with otherwise unexplained infertility before and after myomectomy.[187,188] Most studies are methodologically limited, such as small heterogeneous populations, with inconsistent definitions of myomas with regard to their size, location, and number, and often failing to report relevant outcome measures.[588] **Infertility relating to myomas has been attributed to all of the following mechanisms[589,590]:**

- **Displacement of the cervix, decreasing exposure to sperm**
- **Enlargement or deformity of the uterine cavity, interfering with sperm transport**
- **Obstruction of the interstitial segment of the fallopian tubes**
- **Distorted adnexal anatomy, interfering with ovum capture**
- **Distortion of the uterine cavity or increased or abnormal myometrial contractions, inhibiting sperm or embryo transport**
- **Impaired uterine blood flow, chronic endometritis, or decreased endometrial receptivity, interfering with implantation**

Whereas there is relatively little evidence to support the majority of these mechanisms, a number of observations lend credence to the notion that myomas may impair fertility by interfering with implantation. Glandular atrophy is commonly observed in the endometrium overlying myomas, depending on their proximity, and can also be seen in the opposing endometrium, suggesting that it results from mechanical pressure.[591–593] Molecular studies indicate that submucous and intramural myomas may induce a local decrease in *HOX* gene expression, which has been implicated in the cascade of molecular events involved in implantation.[594,595]

The effects of myomas on fertility are best assessed by studies comparing IVF outcomes in infertile women with and without myomas, because IVF effectively controls for the confounding effects of other fertility factors. Numerous studies have examined the effects of myomas of varying size and location.[596–602] Altogether, these observations permit some conclusions regarding the effects of myomas on IVF outcomes and, by inference, on overall fertility.

There is a clear consensus that submucous myomas have a significant adverse effect on clinical pregnancy rates (OR = 0.3, CI = 0.1–0.7) and delivery rates (OR = 0.3, CI = 0.1–0.8).[188,589,603–607] Available data also support the conclusion that submucous myomas increase risk for pregnancy loss by more than 3-fold,[606,607] while the effect of intramural myomas that do not distort the cavity on the risk of pregnancy loss is less clear.[608] Results of early studies examining the effect of intramural myomas on IVF outcomes were inconsistent, with some finding adverse effects[597–600,609] and others not.[596,606,610–614] Large-scale prospective studies report significantly lower implantation, pregnancy, ongoing pregnancy, and live birth rates.[599,600] A 2010 systematic review of the effect of intramural myomas without uterine cavity involvement on IVF outcome included 19 observational studies, comprising 6,087 IVF cycles.[615] Live birth rate was significantly decreased (RR = 0.79, 95% CI = 0.70–0.88). It is noteworthy that when results from the only two prospective studies reporting live birth rate were pooled, the relative reduction in live birth was 40% (RR = 0.60, 95% CI = 0.41–0.87). Likewise, clinical pregnancy rate was decreased by 21% (RR = 0.79, 95% CI = 0.70–0.88), and implantation rate was decreased by a relative 13% (RR = 0.87, 95% CI = 0.73–1.03).[615] A 2023 systematic review on the effect of noncavity-distorting intramural fibroids (6 cm or smaller in diameter) on IVF outcomes included five studies in which women with fibroids were age-matched with women without fibroids. The study reported significantly decreased live birth rates, clinical pregnancy rates, and increased pregnancy loss rates in the 2 to 6 cm fibroid group.[616] The differences were short of statistical significance in subgroup analyses for fibroids ≤2 cm, which included only one study.[617] All of the evidence concerning the effects of subserosal myomas is consistent in finding no evidence of adverse effects on IVF outcomes. **In sum, the accumulated body of evidence indicates that submucous myomas reduce IVF success rates by approximately 70% and intramural myomas by approximately 20% to 40% and that subserosal myomas have no adverse impact on outcomes. Submucous myomas increase the risk of pregnancy loss after successful IVF at least 3-fold and intramural myomas by more than half.**

Logically, decisions regarding the management of infertile women with myomas should be guided by the evidence concerning their likely importance and the outcomes of surgical intervention. It seems clear that submucous myomas (distorting the uterine cavity) have important adverse effects on fertility and pregnancy outcomes and that myomectomy improves both. A 2009 systematic review of studies examining outcomes after submucous myomectomy concluded that clinical pregnancy rates achieved with IVF were 2-fold higher after surgery than in women with submucous myomas in situ and comparable to those observed in women without myomas.[607] A randomized trial comparing the effects of myomectomy and expectant management on fertility in 181 women with a combination of submucous, intramural, and subserosal myomas observed that myomectomy significantly improved pregnancy rates among women with submucous myomas (43% vs 27%) and those with both submucous and intramural myomas (26% vs 15%), without other interventions.[618] Younger women having a single small submucous myoma and otherwise unexplained infertility have the best prognosis. Results are less encouraging for older women and those with multiple or large submucous myomas. Although complications of hysteroscopic myomectomy are relatively few, the risk of postoperative intrauterine adhesions increases with the size, number, and extent of intramural extension of submucous myomas.

Evidence for the benefits of myomectomy in women with intramural myomas (not distorting the uterine cavity) is less compelling, probably because their impact on fertility is not as great. A randomized trial observed a clinically significant trend toward improved fertility in women with intramural myomas after myomectomy (56% vs 41%).[618] A prospective, nonrandomized cohort study involved 318 women with recurrent pregnancy loss or unexplained infertility and compared women undergoing laparoscopic myomectomy, those with conservatively followed myomas, and a control group of unexplained infertility without myomas.[619] Women undergoing laparoscopic myomectomy had higher live birth rates compared to those with in situ myomas (42% [44/106] vs 11% [12/106], respectively) and those with unexplained infertility without myomas (25% [27/106]). The observations were limited by differences in age between groups, and selection bias is a potential threat to the validity of nonrandomized studies, that is, women who underwent surgery could have different types of myomas than women who did not have surgery.

It is noteworthy that the single existing randomized controlled trial (RCT) found no significant effect of myomectomy on clinical pregnancy rates based on the type of fibroid removed (intramural, odds ratio [OR] = 1.88, confidence interval [CI] = 0.57–6.14, 45 women; submucosal, OR = 2.04, CI = 0.62–6.66, 52 women; intramural-subserosal, OR = 2.0, CI = 0.40–10.09, 31 women; intramural-submucosal, OR = 3.24, CI = 0.72–14.57, 42 women).[618] Similarly, a cohort study involved 63 infertile women with intramural myomas and 100 age-matched controls without myomas undergoing ART with the same stimulation protocol. Nineteen of the 63 women with myomas underwent myomectomy prior to ART.[620] Clinical pregnancy rates were similar between groups (36% myomectomy vs 29% in situ intramural myoma vs 36% controls). Similar results were found in a retrospective cohort study of 58 women undergoing ART with a history of prior myomectomy or myomas in situ.[621]

In contrast to these studies, a trial of 168 women with non–cavity-distorting intramural myomas compared ART outcomes between those who underwent laparoscopy before ART and no surgery prior to ART.[622] Cumulative pregnancy rate (34% [28/84] vs 15% [13/84]) and live birth rate (25% [21/84] vs 12% [10/84]) were significantly higher in the laparoscopy group compared with the nonsurgical group.[622] Benefit of myomectomy was observed in the surgical group of women who had at least one fibroid with a diameter greater than 5 cm. However, many of these studies are underpowered, and the inclusion/exclusion criteria of the size and location of myomas in study subjects are inconsistent. Age differences between groups were not reported or taken into consideration during analyses; fibroid size and number between groups and potential selection bias limit the validity of these findings. A 2024 systematic review reported that pooled analysis of five studies with 1,272 patients showed similar clinical pregnancy rates between women who underwent surgical removal of noncavity distorting intramural fibroids and those with in situ fibroids (OR = 1.34, 95% CI = 0.98–1.82).[623] Thus, these data do not clearly demonstrate an improvement or deterioration in outcomes after myomectomy compared with no surgery.

Decisions regarding the management of infertile women with asymptomatic intramural myomas are among the most difficult clinical judgments. They must consider not only the size, number, and location of myomas and the risks and benefits of the procedure but also age, duration of infertility, ovarian reserve, other infertility factors, and the treatments they require. In most cases, the benefits of myomectomy are modest or uncertain, and the procedure is not without significant potential risks. Myomectomy commonly results in postoperative pelvic and adnexal adhesions, which can decrease fertility if severe[624,625] but are less concerning in women who require IVF for other reasons. Myomectomy generally commits the patient to cesarean delivery to avoid the risk of uterine rupture during labor, which has been reported after myomectomy.[626–629] Whereas excision of large, deep intramural myomas that abut or displace the uterine cavity might reasonably be expected to improve fertility, removal of smaller myomas having no direct anatomic relationship with the cavity will probably not. Whereas excision of anterior and fundal myomas is not likely to result in serious adnexal adhesions, posterior uterine incisions invite the complication. Arguably, excision of any intramural myomas large enough or deep enough to warrant myomectomy also likely warrants recommendation for cesarean delivery.

Whereas myomectomy offers limited, if any, benefits to young women with infertility of short duration and other infertility factors amenable to nonsurgical treatments, it is less difficult to justify in older women with unexplained infertility of long-duration planning to pursue IVF.[630]

Adherence to basic microsurgical principles—gentle tissue handling, meticulous hemostasis, and minimal exposed suture—helps to ensure best surgical results. Adjuvants such as local injection of aqueous Pitressin, tourniquets to compress the uterine arteries, and surgical adhesion barriers aim at those goals. Laparoscopic and robotic myomectomy, performed by those having the requisite training and experience, may offer the same benefits as traditional open or minilaparotomy myomectomy for infertile women with intramural myomas and have the added advantage of lower morbidity (decreased blood loss and shorter recovery time).[631–635] A multicenter, randomized trial comparing reproductive outcomes after laparoscopic and minilaparotomy myomectomy in women with unexplained infertility observed no differences in cumulative pregnancy, live birth, and pregnancy loss rates between the two procedures.[632] There are concerns regarding myoma morcellation and the risk of dissemination of malignant tissue, if the tumor turns out to be a sarcoma. The U.S. Food and Drug Administration (FDA) has provided guidance that limits the use of power morcellation of uterine tissue. These risks should be reviewed with women who consider laparoscopic myomectomy with morcellation.

The careful selection of patients most likely to benefit from myomectomy is far more important than the choice of surgical technique. If the procedure has little or no likely benefit, the choice of technique is irrelevant.

Intrauterine Adhesions (Asherman Syndrome)

Intrauterine adhesions develop as a result of trauma.[636–639] Any insult severe enough to remove or destroy the endometrium can cause adhesions. The gravid uterus is particularly susceptible to injury, especially between the second and fourth weeks postpartum.[640] Inflammation or infection may also predispose to adhesions.[641–643] In approximately 90% of cases, intrauterine adhesions relate to curettage for pregnancy complications, such as missed or incomplete abortion or retained products of conception.[644] Adhesions can also develop after abdominal or hysteroscopic myomectomy, septum resection, or other uterine surgery. In the developing world, genital tuberculosis is an important cause of intrauterine adhesions; although rare in the United States, the possibility must be considered in women who have emigrated from regions where the disease is prevalent.[645]

Intrauterine adhesions can be asymptomatic or cause menstrual disorders (hypomenorrhea, amenorrhea, dysmenorrhea), pain, recurrent pregnancy loss, or infertility.[638,639] The overall incidence of intrauterine adhesions is uncertain but may be increasing.[644,646] The risk of intrauterine adhesions associated with elective termination of pregnancy is generally low, but the prevalence and severity of adhesions may increase with the number of procedures.[647] A temporal relationship between symptoms and a predisposing event; the inability to pass a uterine sound; a negative progestin challenge in amenorrheic women; and irregular, thin, or interrupted endometrial stripe in ultrasound examination suggest the diagnosis. A 3D TVUS can facilitate the diagnosis of intrauterine adhesions. Saline sonohysterography with or without 3D TVUS has high sensitivity and specificity as does HSG. Compared to hysteroscopy (the gold standard), HSG has an approximately 80% sensitivity and specificity for diagnosis of adhesions.[648] A 2015 systematic review of 20 studies compared saline sonohysterography with hysteroscopy for the detection of intrauterine adhesions and reported a 0.82 (95% CI = 0.65–0.93) sensitivity and a 0.99 (95% CI = 0.98–1.00) specificity of saline sonohysterography.[649] A study comparing HSG and sonohysterography with hysteroscopy concluded that the two methods of imaging were equally sensitive for the detection of adhesions[566] but that hysteroscopy is required to define the location and extent of disease.

Hysteroscopy can reveal a variety of findings.[636,643,650] Central adhesive bands can appear as columns or bridges between the opposing walls of the cavity, dividing it into smaller irregular chambers of varying sizes and shapes. Adhesions at the margins of the cavity often appear as half-drawn curtains that may obscure one or both cornual orifices. Depending on their composition (mucosal, fibromuscular, connective tissue), adhesions may or may not have a surface of the endometrium; dense connective tissue adhesions typically do not. Whereas mucosal adhesions generally appear similar to the surrounding normal tissue and are easy to lyse, fibromuscular and connective tissue adhesions are thicker, are typically pale, and must be mechanically divided or dissected. Numerous classification systems have been proposed, but no system has gained wide acceptance or has prognostic value validated by prospective studies.[638,639] Consequently, outcome studies are difficult to interpret and compare.

Hysteroscopy is the method of choice for treatment of intrauterine adhesions and is both safer and more effective than blind curettage. Sometimes, the pressure provided by continuous infusion of distension media can lyse mild adhesions, or pushing the tip of the hysteroscope may suffice. When needed, an assortment of mechanical, electrosurgical, and laser-based instruments allow adhesions to be lysed or cut under direct vision. Indirect evidence suggests cold scissors are safer than electrosurgery regarding the risk of further endometrial damage.[651] In the case of severe adhesions, an HSG can serve as a road map for planning surgery. When possible, identification of anatomic landmarks as the tubal ostia helps proper dissection. Transabdominal ultrasound-guided office hysteroscopy enables orientation in an endometrial cavity obliterated by severe adhesions. Even though simultaneous laparoscopy is suggested as an aid to guide hysteroscopic adhesiolysis, it has several limitations. First

and foremost, laparoscope does not allow the surgeon to understand the location of the hysteroscope in the orthogonal planes until the myometrium is too thinned to allow visualization of the light from the hysteroscope through uterine serosa with the laparoscope. A retrospective study suggests uterine perforation rates are lower with transabdominal ultrasound guidance than with laparoscopic guidance.[652] Increased cost and potential morbidity associated with laparoscopy should also be considered in the absence of another indication for laparoscopy.

Various methods have been used to facilitate hysteroscopic surgery or to improve outcomes. When available, office hysteroscope could be preferred for adhesiolysis, since the smaller instruments can allow fine movements, and does not require cervical dilation for the majority of women. Vaginal administration of misoprostol (200–400 μg) for cervical softening before operative hysteroscopy can reduce or eliminate the need for mechanical dilation and the incidence of operative complications.[653] However, routine use of vaginal misoprostol before office hysteroscopy can lead to leakage of distention medium through a softened cervix and render the procedure more difficult. Various physical barriers, including both unmedicated intrauterine device (IUDs) and balloon catheters, are commonly used as a means to maintain separation between the opposing layers of the endometrium during the immediate postoperative interval. Despite the absence of data on their effect on fertility, solid barriers reduce the rate of adhesion reformation. Balloon catheters shaped to mimic the uterine cavity are available specifically for this purpose. An IUD with a large surface area, such as the Lippes loop, which does not contain copper or progestin, should be preferred. Semisolid barriers such as hyaluronic acid gel also reduce adhesion formation. The absence of the need for removal and better conforming the contours of endometrial cavity can be regarded as advantages of hyaluronic acid gel over solid barriers; however, there are no comparative studies reporting pregnancy rates following the use of solid or semisolid barriers.[651] Postoperative treatment with exogenous estrogens to promote rapid re-epithelialization and reduce risks of recurrent adhesions is frequently used, but its efficacy has not been established[651]; a typical regimen involves treatment with 6 to 8 mg estradiol daily for 4 weeks, adding a progestin (eg, medroxyprogesterone acetate 5–10 mg daily) during the last week.

Complications of hysteroscopic adhesiolysis are the same as with any operative hysteroscopic procedure and are relatively uncommon. Acute complications include uterine perforation, fluid overload and electrolyte imbalance, hemorrhage, and infection; late complications include recurrent adhesions and uterine rupture in a subsequent pregnancy.[654]

Surgical results should be evaluated by HSG or saline sonohysterography after menses.[655] A second operation to lyse persistent or recurrent adhesions may be required when disease is severe. Alternatively, pressure lavage with normal saline under guidance of TVUS can be used to hydrodissect recurrent adhesions that are not particularly dense or extensive.[656] Lysis using a balloon catheter under fluoroscopic control and local anesthesia or intravenous sedation has also been described.[657] Normal cyclic menses can be restored in 70% to 90% of women with intrauterine adhesions, depending on severity.[636] Conception and term delivery rates after successful hysteroscopic lysis of intrauterine adhesions have ranged between 25% and 75%[636,643,658–664]; predictably, the prognosis is better for younger women with mild disease.

Endometrial Polyps

Endometrial polyps are hyperplastic endometrial growths with a vascular center and a sessile or pedunculated shape extending into the uterine cavity. They are generally rare in young women and increase in incidence with age. The true prevalence of polyps among infertile women is unknown, and varying figures are reported.[665] While one study reported a prevalence of 15.6% among women with unexplained infertility, the figure ranged between 1.4% and 8% among women undergoing ART.[666,667] A number of molecular mechanisms have been implicated in their pathogenesis, including endometrial hyperplasia,[668] overexpression of endometrial aromatase,[636,669] and gene mutations.[670] While a TVUS examination can suggest the diagnosis, sensitivity and specificity vary widely. 3D TVUS can improve diagnostic accuracy, but saline sonohysterography is the most useful method of imaging for detection of endometrial polyps,[561,671,672] although false-positive results due to blood clots, mucus, and shearing of normal endometrium are not uncommon.

Careful, systematic evaluation will inevitably identify polypoid cavitary lesions in some infertile women. Differentiation of small submucous myomas and endometrial polyps can be difficult by any means other than hysteroscopy.[570] Whereas symptomatic women (abnormal bleeding) certainly merit hysteroscopic evaluation and treatment, whether surgery has benefits for asymptomatic infertile women with polyps is less clear. The observation that polyps are resistant to the actions of progesterone suggests they might interfere with implantation[673]; local inflammatory changes and distortion of the uterine cavity have also been implicated.[674]

Evidence from studies examining reproductive performance after hysteroscopic polypectomy is rather weak and conflicting.[189,190,674,675] A study of infertile women with documented but unresected endometrial polyps (<2 cm) found that IVF outcomes in treated (preliminary hysteroscopic polypectomy) and untreated women were not different.[200] In two studies examining outcomes in women with polyps (<1.5–2 cm) identified by ultrasonography during ovarian stimulation for IVF, pregnancy rates in women who proceeded to oocyte retrieval and embryo transfer or had hysteroscopic polypectomy after retrieval and later frozen embryo transfer were not different from those in women without polyps having fresh or frozen embryo transfers.[666,676] The evidence indicating that polyps adversely affect fertility derives from

two randomized controlled trials comparing outcomes after up to four cycles of IUI in infertile women with polyps who underwent preliminary polypectomy or no treatment.[675,677] Two hundred thirty and 120 women were randomized in these trials, and both showed significantly higher pregnancy rates following polypectomy (63.4% vs 28.2% and 41.7% vs 20%).[675,677] Overall, hysteroscopic polypectomy results in a greater than 2-fold increase in clinical pregnancy among subfertile or infertile women who subsequently undergo IUI.[678] Similarly, a retrospective study reported higher natural conception rates among infertile women who had hysteroscopic polypectomy compared with those who had hysteroscopy with a normal cavity (78% vs 42.1%).[189] A recent US cost analysis study suggests that pretreatment office or operative hysteroscopic polypectomy is cost-effective when performed prior to both IUI and IVF over a range of plausible pregnancy rates and procedural costs.[679]

Taken together, the available evidence suggests that polypectomy may improve reproductive performance in infertile women. Treatment must be individualized, depending on the size of a polyp, associated symptoms, and circumstances leading to its discovery.[680,681]

Chronic Endometritis

Chronic endometritis has been regarded traditionally as a distinct but uncommon cause of reproductive failure, but its true prevalence in infertile women is unknown.[682] Available evidence suggests that chronic subclinical endometritis is relatively common in women with symptomatic lower genital tract infections, including cervicitis and recurrent bacterial vaginosis.[683–686] Mucopurulent cervicitis is highly associated with chlamydia (*Chlamydia trachomatis*) and mycoplasma (*Mycoplasma genitalium*) infection, and both organisms, in turn, are associated with chronic endometritis, which likely plays a role in the pathogenesis of tubal factor infertility.[524,686–690] However, the data regarding the true prevalence and importance of chronic endometritis in asymptomatic infertile women are contradictory. While some retrospective studies suggest that up to 57% to 66% of women with unexplained infertility or unexplained recurrent implantation failure are diagnosed with chronic endometritis, in a prospective study involving 678 asymptomatic women who would undergo their first ART cycle, the prevalence of chronic endometritis diagnosed by hysteroscopic biopsy was 2.8%.[691–693] In addition, women with and without chronic endometritis had similar cumulative live birth rates (76% vs 54%, in women with and without chronic endometritis, respectively), and clinical pregnancy rate per embryo transferred was similar between the groups. In a prospective study involving 80 women undergoing single euploid blastocyst transfers, the number of plasma cells identified by immunostaining for CD138 per 10 high power field was not found to be associated with implantation, clinical pregnancy, clinical pregnancy loss, or live birth rates.[694] Therefore, routine serologic testing for past chlamydia exposure, cervical cultures, and endometrial biopsy may be difficult to justify. However, further evaluation and treatment may be appropriate and prudent in infertile women with clinical cervicitis, chronic or recurrent bacterial vaginosis, or other symptoms that suggest pelvic infection.

TUBAL FACTOR: TUBAL OCCLUSION AND ADNEXAL ADHESIONS

Tubal and peritoneal pathology is among the most common causes of infertility, and tubal pathology accounts for 25% to 35% of female infertility.[695] **A history of pelvic inflammatory disease (PID), septic abortion, ruptured appendix, tubal surgery, or ectopic pregnancy strongly suggests the possibility of tubal damage.** Unquestionably, PID is the major cause of tubal factor infertility and ectopic pregnancies. Classic studies in women with PID diagnosed by laparoscopy revealed that the risk of subsequent tubal infertility increases with the number and severity of pelvic infections; overall, the incidence is approximately 10% to 12% after one episode, 23% to 35% after two, and 54% to 75% after three episodes of acute PID.[696–700] The risk of ectopic pregnancy is increased 6- to 7-fold after pelvic infection. Although many women with tubal disease or pelvic adhesions have no known history of previous infection, evidence strongly suggests that "silent" ascending infection is the most likely cause.[686,690] Many such women will have detectable chlamydia antibodies suggesting prior infection (discussed further on). Other causes of tubal factor infertility include inflammation related to endometriosis, inflammatory bowel disease, or surgical trauma. Endometriosis is considered at length in Chapter 35; discussion here is focused on intrinsic tubal disease.

The mechanism responsible for tubal factor infertility obviously involves anatomic abnormalities that prevent the union of sperm and ovum. Proximal tubal obstructions prevent sperm from reaching the distal fallopian tube where fertilization normally occurs. Distal tubal occlusions prevent ovum capture from the adjacent ovary. Whereas proximal tubal obstruction is essentially an all-or-none phenomenon, distal tubal occlusive disease exhibits a spectrum ranging from mild (fimbrial agglutination) to moderate (varying degrees of fimbrial phimosis) to severe (complete obstruction). The likelihood or efficiency of ovum capture is probably inversely related to the severity of disease. Inflammatory damage to internal tubal mucosal architecture cannot be detected easily but may nonetheless impair sperm or embryo transport functions.

HSG and laparoscopy are the two classic methods for evaluation of tubal patency in infertile women and are complementary rather than mutually exclusive; each provides information the other does not, and each has advantages and disadvantages. HSG images the uterine cavity and reveals the internal architecture of the tubal lumen, neither of which can

be evaluated by laparoscopy. Laparoscopy provides detailed information about the pelvic anatomy that HSG cannot, including adhesions, endometriosis, and ovarian pathology. HSG is performed in an outpatient setting, is far less costly than laparoscopy, and may have some therapeutic value[701]; it is also often uncomfortable or painful, involves some radiation exposure, and has risk of infectious complications that can further impair fertility.[702] Rare cases of shock and pulmonary and cerebral embolus and a case of hyperthyroidism due to iodine absorption have been reported following the use of oil-soluble media.[703,704] Laparoscopy is more invasive, usually requires general anesthesia, provides no information regarding the uterine cavity (unless hysteroscopy is also performed), and involves the usual risks of surgery. Sonohysterosalpingography (sonoHSG) is similar to HSG, using ultrasonography and sterile saline instead of fluoroscopy and contrast media, and is another, but less common, method for evaluating tubal factor. Hysterosalpingo-contrast sonography (HyCoSy) is similar to sonoHSG, but either saline–air contrast media or a more recently developed gel comprised of hydroxyethyl cellulose and glycerol is used for demonstrating fallopian tubes. Chlamydia antibody tests represent a fifth, albeit indirect, method for evaluating tubal factor that is relatively inexpensive and minimally invasive.[705–708] Chlamydia antibody tests have been used primarily for screening infertile women to identify those at high risk for having tubal disease who merit evaluation with laparoscopy.

Hysterosalpingography

HSG is best scheduled during the 2- to 5-day interval immediately following the end of menses to minimize the risk for infection, to avoid interference from intrauterine blood and clot, and to prevent any possibility that the procedure might be performed after conception. Even the most sensitive assays for hCG cannot exclude the possibility of pregnancy when HSG is performed during the early luteal phase of the cycle. HSG does not require any specific preparation, although pretreatment with a NSAID (30–60 minutes before) is helpful to decrease discomfort associated with the procedure; more potent analgesics and sedatives are generally not required. For those who are seeking alternative pain control measures, effectiveness of other oral analgesics is unclear, while improved pain relief with a single rectal dose of 50-mg indomethacin 30 minutes before HSG has been reported.[709,710] Infectious complications from HSG are relatively uncommon, even in high-risk women (1–3%).[702–704,711] Even though prophylactic antibiotic treatment is not absolutely necessary for everyone, it can be justified considering the potential consequences of a postprocedure infection. **Treatment with antibiotics (doxycycline 100 mg twice daily for 5 days, beginning 1–2 days before HSG) is prudent when tubal disease is highly suspected and specifically indicated when HSG reveals distal tubal obstruction, because risk for acute salpingitis is increased (~10%) and treatment can prevent clinical infection.**[702,712] To minimize the risk of infection, HSG is best avoided altogether for at least several weeks following any episode of acute PID.

The technique for performing an HSG is quite standard. The study should be performed using image intensification fluoroscopy with a limited number of radiographs. The average HSG requires only 20 to 30 seconds of fluoroscopic time with minimal radiation exposure and has very low risk. Usually, only three basic films are required (a scout, one film to document the uterine contours and tubal patency, and a postevaluation film to detect any areas of contrast loculation). Additional oblique films may be needed when the uterus obscures the tubes or the uterine cavity appears abnormal. Otherwise, they provide little or no more useful information and increase radiation exposure unnecessarily.[713] Contrast can be introduced using a common metal "acorn" cannula, a cervical vacuum cup device, or a balloon catheter. In general, the latter techniques require less fluoroscopic time and smaller volumes of contrast, produce less pain, and are easier to perform.[714–716] Slow injection of contrast (typically 3–10 mL) helps to minimize the discomfort associated with HSG.

Debate regarding the relative advantages and disadvantages of oil- and water-soluble contrast media has raged for years. Advocates of water-soluble contrast media emphasize that oil-soluble medium is too viscous to reveal internal tubal architecture (having prognostic significance),[717] disperses poorly in the pelvis (and therefore cannot detect adnexal adhesions), and has significant risks (granulomatous reactions, intravasation, and embolism).[718,719] Those favoring oil-soluble contrast media argue that granulomatous reactions are rare and that intravasation and embolization are uncommon and almost uniformly benign[720] and cite evidence suggesting that oil-soluble media increase fertility in the months immediately following HSG in women with patent tubes.[701] A methodologically sound trial randomized 1,119 women to HSG with oil or water contrast.[721] Despite identification of a similar number of women with tubal pathology, live birth rates with a combination of expectant management and IUI were significantly higher following the use of oil-based media (rate ratio [RR] = 1.38, 95% CI = 1.17–1.64; $P < 0.001$). A 5-year follow-up report of this trial reported that the oil contrast group had higher rates of live birth (RR = 1.11, 95% CI = 1.03–1.20) and spontaneous pregnancy (RR = 1.18, 95% CI = 1.02–1.38) as well as shorter time to pregnancy (HR = 1.25, 95% CI = 1.09–1.43).[722] A 2020 systematic review supports these findings.[723]

HSG may reveal bilateral tubal patency (60–75%) or unilateral (15–25%) or bilateral (15–25%) tubal occlusion.[724,725] Both false-positive (obstructions that are not real) and false-negative results (patency that is not real) occur, the former being much more common than the latter. Injection of contrast may cause "cornual spasm" (uterine contractions that transiently close the interstitial segment and prevent distal perfusion) that can be misinterpreted as proximal tubal

occlusion. HSG may reveal unilateral tubal patency and contralateral proximal occlusion. Although the observation may represent a true unilateral proximal obstruction, which is rare, catheter placement allowing contrast to take the path of least resistance is the more common cause; most often, the nonvisualizing tube is normal. A false-negative HSG may occur when the contrast entering a widely dilated hydrosalpinx is diluted to yield a blush that is misinterpreted as evidence of tubal patency. Peritubular adhesions surrounding an otherwise normal and patent tube can sequester contrast as it escapes from the tube, resulting in a focal loculation that can be misinterpreted as distal obstruction.

Compared to laparoscopy (the gold standard method) as a test of tubal patency, HSG has an overall sensitivity (ability to detect obstruction when the tubes appear obstructed at laparoscopy) of 94% (95% CI = 74–99%) and specificity (confirmation at laparoscopy when obstruction is detected by HSG) of 92% (95% CI = 87–95%).[726] Thus, if HSG shows patent tubes, tubal blockage is highly unlikely.[727] However, after an HSG that showed proximal tubal blockage, 60% of such women had tubal patency with a repeat HSG 1 month later.[728] Subsequent laparoscopy revealed a similar percentage of women having tubal patency following proximal tubal occlusion on HSG.[727] The PPV and NPV of HSG for tubal occlusion are 38% and 94%, respectively.[727,729,730] **The clinical implications are that when HSG reveals obstruction, there is still a relatively high probability (~60%) that the tube is open, but when HSG demonstrates patency, there is little chance that the tube is actually occluded (~5%).** However, interpretation of HSG results can vary significantly among different observers.[731,732] Consequently, when the treating clinician has not performed the HSG, a personal review of the films is prudent before making recommendations for additional evaluation or treatment. The probability of treatment-independent pregnancy is highest when HSG reveals bilateral tubal patency, substantially lower when neither tube is open, and reduced only slightly when one tube is patent.[724,725] These observations help in deciding whether laparoscopy is needed before starting treatment.

Laparoscopy

Laparoscopy is regarded generally as the definitive test for the evaluation of tubal factors. Issues concerning the scheduling, the use of antibiotics, and the risks of infectious complications are the same as for HSG. Diagnostic laparoscopy is usually performed under general anesthesia but may require only deep sedation and local anesthetic; operative laparoscopy for treatment of disease typically requires general anesthesia. With few exceptions, a systematic and thorough inspection of the pelvis will accurately define the location and extent of any disease. Examination should include the uterus, the anterior and posterior cul-de-sacs, the ovarian surfaces and fossae, and the fallopian tubes. Injection of a dilute blue dye through a cannula attached to the cervix or an intrauterine manipulator permits evaluation of tubal patency ("chromotubation"). Indigo carmine is preferred over methylene blue, which may rarely induce acute methemoglobinemia, particularly in individuals with glucose-6-phosphate dehydrogenase deficiency.[733,734] As with HSG, slow injection of fluid helps to reduce the incidence of false-positive results.

Laparoscopy provides both a panoramic view of the pelvic reproductive anatomy and a magnified view of the uterine, ovarian, tubal, and peritoneal surfaces. Consequently, it can identify milder degrees of distal tubal occlusive disease (fimbrial agglutination, phimosis), pelvic or adnexal adhesions, and endometriosis that adversely affect fertility but escape detection by HyCoSy or HSG. Most importantly, laparoscopy offers the opportunity to treat disease at the time of diagnosis. Lysis of filmy or focal adhesions and excision or ablation of superficial endometriosis are relatively simple procedures well within the capabilities of most surgeons. Excision of ovarian endometriomas, lysis of dense or extensive adhesions involving the cul-de-sac or bowel, excision or ablation of widely disseminated or deeply invasive endometriosis, and fimbrioplasty or salpingoneostomy procedures require greater technical skill and experience.

Although laparoscopy is a better predictor of future fertility than HSG, it is not a perfect test for diagnosis of tubal pathology. Intraoperative chromotubation is subject to the same pitfalls causing false-positive results with HSG. False-negative results with laparoscopy are uncommon but do occur, particularly in cases where the fallopian tubes are obscured by adhesions. Whereas tubal obstructions detected by HSG are frequently not confirmed at laparoscopy, patency almost always is. Laparoscopy is also a better predictor of future treatment-independent pregnancy than HSG because the information gained is more accurate. Again, the prognosis is best when both fallopian tubes are patent, poor when both are blocked, and intermediate when only one tube is open.[725,735] Because many obstructions detected by HSG are not real and all but a few of those identified by laparoscopy are, the prognoses associated with unilateral and bilateral tubal occlusion diagnosed by laparoscopy are significantly worse than when the same diagnosis is made by HSG.

Sonohysterosalpingography and Hysterosalpingo-Contrast Sonography

Sonohysterography is recognized as having a greater sensitivity than HSG for detection of intrauterine pathology. A natural extension of that technique, sonohysterosalpingography (sonoHSG), has been viewed as a means to evaluate tubal patency at the same time, much like HSG. As originally described, sonoHSG relied on observations of fluid accumulation in the cul-de-sac as an indication of tubal patency. However, the technique provided no information regarding tubal anatomy and could not determine whether one or both

tubes were patent. Introduction of a sonographic contrast medium consisting of a surfactant that produces microbubbles when stimulated by ultrasound improved sensitivity for detecting tubal patency, but the standard 2D imaging in the sagittal and transverse planes was still inadequate to visualize the 3D tubal anatomy.

Technologic advances in ultrasonography and introduction of new contrast media further expanded the capabilities of sonoHSG and HyCoSy. 3D TVUS provides the means to generate coronal images. Color-coded Doppler techniques have improved the visualization of fluid movement through the fallopian tubes. A 2014 meta-analysis showed that pooled estimates of sensitivity and specificity of sonoHSG/HyCoSy in comparison to laparoscopy were 92% (95% CI = 82–96%) and 95% (95% CI = 90–97%), respectively. There was no significant difference between the diagnostic performance of sonoHSG compared to HyCoSy.[726] Additional advantages of sonoHSG/HyCoSy are avoiding the risk of ionizing radiation, higher sensitivity and specificity for the detection of intrauterine pathologies, concomitant visualization of the ovaries and myometrium, and completion of assessment at the office without referral to radiology.

Tubal patency assessment with the foam as the sonographic contrast is called hysterosalpingo foam sonography (HyFoSy). The foam contrast is based on hydroxyethylcellulose and glycerol. Similarly to HyCoSy, the foam contrast is introduced through the cervix, and it provides bright echogenicity for about 5 minutes. Based on a 2024 systematic review, HyFoSy has similar performance to HSG in identifying tubal patency and performs better than HyCoSy with saline–air bubble contrast. Moreover, HyFoSy was found to have better agreement with laparoscopy than HyCoSy (RR = 1.15, 95% CI = 1.07–1.25). In 94% (95% CI = 0.91–0.96) of all patients, HyFoSy had the same outcome as laparoscopy, despite being less painful than HSG.[736] The foam contrast has received FDA approval in 2019.

Chlamydia Antibody Tests

A number of studies have suggested that chlamydia antibody tests can be as accurate as HSG or even laparoscopy for detection of tubal pathology, including tubal occlusion, hydrosalpinx, and pelvic adhesions.[705,706,737] The performance of the different tests varies widely with the assay method. Commercial assays differ in detection method (immunofluorescence, microimmunofluorescence, ELISA, immunoperoxidase) and in the source of antigen they use (general or genus-specific major outer membrane proteins, an inactivated organism, whole-cell inclusion). Some methods are highly specific for the chlamydia species of interest (*C. trachomatis*), and others do not distinguish antibodies to *C. trachomatis* from those directed against other chlamydia species (*C. pneumoniae*, *C. psittaci*). As expected, tests having the greatest specificity for *C. trachomatis* perform best for detection of tubal pathology.[706,738,739] Practical considerations suggest that a rapid, highly sensitive but less specific assay is the most suitable test for screening, using a more specific test to confirm the antibody specificity of sera selected by the screening assay.

The predictive value of any diagnostic test depends on the prevalence of the disease of interest in the population tested. If the prevalence of disease in the population is very low or very high, diagnostic testing has little or no value because the outcome rarely affects management, and false-positive (when the prevalence is very high) or false-negative test results (when the prevalence is very low) are common. Diagnostic tests tend to have greatest utility when the prevalence of disease is somewhere in between the extremes.[727] Some have suggested that chlamydia antibody tests might be used to select patients likely to benefit most from laparoscopy, but the predictive value of even some of the more specific chlamydia antibody tests may not be sufficient to justify that approach.[740]

The role for chlamydia antibody tests in the evaluation of infertile women has not been sufficiently defined. Chlamydia antibody tests could prove useful as a pretest to select women who warrant earlier or more detailed evaluation.[741] If applied as a screening tool early in evaluation, a positive test might alert one to the possibility of tubal factors relating to previous chlamydia infection not otherwise suspected. Although selective laparoscopy based on chlamydia antibody tests may be unjustified for all infertile women,[740] it might be effective if limited to women with unexplained infertility (including a normal HSG), identifying those most likely to have undetected tubal factors best addressed before starting aggressive and costly empirical treatments. The utility of chlamydia antibody tests in these or other clinical contexts is uncertain but warrants further investigation. **In summary, chlamydia antibody tests can provide useful information but also have pitfalls that limit their clinical utility. Currently, diagnostic performance of chlamydia antibody testing is limited, and HSG or other imaging modalities remain the standard for assessment of tubal patency, particularly for women at high risk for tubal abnormality.**[742–744]

Tubal Surgery in the Era of ART

For women with tubal factor infertility, treatment options are reconstructive surgery and IVF. Over the last two decades, IVF success rates have increased steadily and now frequently exceed those achieved with surgery. Consequently, IVF has become the treatment of choice for much or most tubal factor infertility, particularly for couples with other infertility factors or severe tubal disease. However, surgery remains an appropriate option in select circumstances and for couples with ethical or religious objections or financial restrictions that preclude IVF. **The decision between surgery and IVF should be based on the following**[742]**:**

- **The age of woman**
- **Ovarian reserve**
- **Semen analysis results**

- **Number of children desired**
- **Site and extent of tubal disease**
- **Presence or absence of other infertility factors**
- **Risk of ectopic pregnancy and other complications**
- **Surgeon's experience**
- **Success rate of IVF program**
- **Patient preference, that is, religious belief, cost, and insurance coverage for each option**

Younger women, women with normal/high ovarian reserve, proven fertility, desiring multiple children will comprise more appropriate candidates for surgical repair. A semen analysis of the male partner should precede the decision. It is difficult to directly compare success rates of surgery and IVF, since the former is usually reported per patient, whereas the latter is per cycle. Advantages of surgery are relatively lower cost, especially if multiple children are desired or several IVF cycles are likely to be undertaken to achieve a live birth. In addition to the cost issues, risk of multiple pregnancy, possible complications, and adverse obstetric and perinatal outcomes as compared with spontaneous pregnancies are other disadvantages of IVF.[745] It should be noted that current practice with single, often frozen, embryo transfers and highly successful strategies to prevent ovarian hyperstimulation syndrome minimize these risks.[742] The indications, preliminary evaluation, techniques, risks, and outcomes for IVF and other forms of ART are the focus of a separate chapter (Chapter 31); discussion here is limited to surgical treatments for tubal factor infertility and the choice between surgery and IVF.

Sterilization Reversal

Approximately 1 million US women have an elective tubal sterilization procedure each year; up to 30% regret the decision, and about 1% later request its reversal.[25,746–748] The most commonly cited reasons for sterilization reversal requests include new relationships, changes in family planning goals, and death of a child. Regrets are more common in younger women, those who were unaware of the spectrum of contraceptive options, women whose decision for sterilization was influenced by a third party (partner, other family member, friend, or physician), and those sterilized postpartum or after an abortion.[746,747,749,750] Women 30 years old or younger are twice as likely as older women to express regret, 3.5 to 18 times more likely to request information about reversal of the procedure, and approximately 8 times more likely to actually have a sterilization reversal or IVF.[746,747,751] For women who want to conceive again, tubal anastomosis is a legitimate option. A preoperative HSG can be useful to assess the proximal segments and to confirm the type of sterilization performed. Laparoscopy may occasionally be necessary to assess the feasibility of surgical repair when the type of procedure is unknown and when destruction or removal of large segments of tube or other pelvic pathology is suspected; otherwise, less than 5% of women will have irreparable tubes.[752]

The most important prognostic factor for achieving a live birth after microsurgical sterilization reversal is age. The type and location of procedure and the final length of the repaired fallopian tubes are also thought to play a role. Younger women, those whose sterilization was performed using rings and clips, and women having no other infertility factors have the best prognosis; success rates are lower for older women, those who were sterilized by cautery (particularly multiple-burn techniques), and women with other infertility factors.[753–760] Cumulative pregnancy rates are similar when one or both tubes are repaired, although the time to conception is longer after unilateral anastomosis.[759] **In properly selected candidates, overall conception rates are generally quite good (45–82%) after microsurgical sterilization reversal.** The risk of ectopic pregnancy ranges between 2% and 10% and is higher after isthmic–ampullary than after isthmic–isthmic anastomoses.[742,761,762] Among all surgical treatments for tubal factor infertility, sterilization reversal has the highest postoperative fecundability. The **best candidates for the procedure are young women desiring more than one additional pregnancy and having no other infertility factors.** Laparoscopic tubal anastomosis abiding microsurgical principles by highly skilled surgeons experienced in the technique takes longer operation time but yields comparable pregnancy rates (25–53%).[763–765] While the use of a robot can facilitate suturing for less experienced surgeons, early studies indicate that operating time is modestly greater, but hospital stay and recovery time are shorter, compared to open microsurgical procedures[766,767]; pregnancy rates are comparable, but the risk of ectopic pregnancy may be increased.[767]

Distal Tubal Obstruction

Distal tubal occlusive disease exhibits a wide spectrum of severity ranging from adherent fimbrial folds to varying degrees of phimosis, to complete obstruction with hydrosalpinges. HSG will generally reveal complete distal tubal obstructions but cannot reliably detect or accurately define lesser degrees of disease when the tubes are still patent. Laparoscopy is the definitive method for diagnosis of distal tubal occlusive disease and also provides the means for treatment. Fimbriolysis refers to the separation of adherent fimbria, fimbrioplasty describes the correction of phimotic but patent fimbria, and neosalpingostomy involves the reopening of a completely obstructed tube. Predictably, surgical success is inversely related to the severity of disease. The extent and character of associated tubo-ovarian adhesions, the tubal thickness, and the condition of the internal ampullary mucosal architecture are all variables that affect prognosis.[768,769] For the milder forms of distal tubal disease, postoperative live birth rates can exceed 50%.[770–772] Results achieved with surgery for more severe disease have varied widely, but success rates are lower (10–35%), and the risk for ectopic pregnancy is higher (5–20%).[769,773–775] Postoperative tubal patency rates

far exceed pregnancy rates; patency is more easily restored than function because mucosal regeneration is slow and often fails altogether.[776,777]

The majority of pregnancies occur within the first 2 years after surgical treatment of distal tubal obstruction. In general, the results achieved by experienced surgeons using traditional microsurgical techniques or laparoscopic methods have been similar. In a case series of 35 women with distal tubal occlusion treated by laparoscopic fimbrioplasty followed for at least 2 years after surgery, the global conception rate was 74%, the intrauterine pregnancy rate was 51%, the live birth rate was 37%, and the ectopic pregnancy rate was 23%.[778] **In younger women with mild distal tubal occlusive disease, laparoscopic surgery may be viewed as an alternative to IVF, but when disease is severe or pregnancy does not occur during the first postoperative year, IVF is the logical choice. For older women with any significant degree of distal tubal disease, IVF is generally the first and best option because cycle fecundability after distal tubal surgery is low (1–2%), time is limited, and IVF is both more efficient and more effective**.[779]

As success rates with IVF have improved steadily, the indications for reconstructive surgery in women with distal tubal occlusive disease have further declined. However, women with severe distal tubal disease still benefit from surgery because hydrosalpinges adversely affect IVF outcomes. Pregnancy, implantation, and delivery rates are decreased by approximately 50%, and pregnancy loss rates are increased in the presence of hydrosalpinges.[780,781] Several mechanisms have been implicated to explain the observation, including mechanical interference with implantation and toxic effects on the embryo or endometrium.[782–784] A 2020 systematic review including four randomized controlled trials involving 455 women observed that the probability of a clinical pregnancy was twice as high after laparoscopic salpingectomy for hydrosalpinges before IVF (RR = 2.02, 95% CI = 1.44–2.82).[785] Laparoscopic occlusion of the fallopian tubes increased the probability of clinical pregnancy, compared to no intervention (RR = 3.21, CI = 1.72–5.99), and neither surgical procedure was superior.[785] **These data demonstrate clearly that laparoscopic salpingectomy or tubal occlusion improves IVF pregnancy rates in women with hydrosalpinges.** This holds true even when only one tube is affected. Moreover, removal or proximal occlusion of unilateral hydrosalpinx seems to improve chances of spontaneous pregnancy. A retrospective series including 25 women with unilateral hydrosalpinx reported 88% pregnancy rate without IVF with an average time to pregnancy of 5.6 months.[786] Similar observations are reported in other series.[787,788]

One concern with salpingectomy or even tubal occlusion or ligation is its impact on ovarian reserve. Despite contradictory results in early reports, salpingectomy does not significantly decrease serum AMH concentration, ovarian response to stimulation, or clinical pregnancy rates with IVF.[789,790] Ovarian stimulation parameters and implantation and clinical pregnancy rates are similar in women who undergo salpingectomy or laparoscopic proximal tubal occlusion.[791]

Ultrasound-guided aspiration of hydrosalpingeal fluid at the time of oocyte retrieval has been suggested as an alternative treatment.[792] The procedure is effective in improving IVF outcome as compared to no intervention, simpler, and less costly than surgical options. However, salpingectomy and proximal tubal occlusion fare better than fluid aspiration with regard to ongoing pregnancy, clinical pregnancy, ectopic pregnancy, and pregnancy loss rates.[793] Moreover, the fluid reaccumulates rapidly.[794] Sclerotherapy with ethanol injection into the hydrosalpinx to decrease secretions and fluid reaccumulation has been tried to improve the success of hydrosalpinx aspiration; however, based on a meta-analysis of 10 studies, fluid reaccumulation rates within 2 weeks of the procedure were similar with and without sclerotherapy (ranging between 21% and 32%).[795] While a recent network meta-analysis of hydrosalpinx treatment before IVF failed to determine the superiority of laparoscopic salpingectomy or tubal occlusion over ultrasound-guided aspiration, the quality of original trials and differences in reported outcomes undermine the authority of this study.[796] Currently, salpingectomy remains the gold standard, and aspiration of hydrosalpingeal fluid can be an option reserved for women who are likely to have severe intra-abdominal adhesions prone to complications with pelvic surgery.

Proximal Tubal Obstruction

Proximal tubal occlusions represent 10% to 25% of all tubal obstructions observed with HSG, many of which are not real (20–40%).[695] Mucus plugs, cellular debris, or uterotubal spasm can cause pseudo proximal obstruction. **Efforts to establish a certain diagnosis of true proximal tubal occlusion are justified; otherwise, many women may needlessly undergo major surgery or IVF.** Repeated HSG can decrease the number of false-positive tests of tubal patency; in a case series including 98 infertile women with a diagnosis of proximal tubal occlusion based on an HSG, repeating the procedure revealed bilateral tubal patency in 14 patients (14%), patency of 1 tube in 12 others (12%), and confirmed bilateral occlusion in 72 patients (74%).[797] Another series of 40 women, 18 with unilateral and 22 with bilateral proximal tubal occlusion, reported that 24 (60%) women achieved bilateral and five others achieved unilateral patency after a second HSG 1 month later.[728]

The pathogenesis of proximal tubal occlusive disease is not well understood; It is presumed to result mostly from infection or chronic inflammation. Histologic studies suggest that obliterative luminal fibrosis is most common, followed by salpingitis isthmica nodosa (SIN), chronic inflammation, and intratubal endometriosis.[695,798] If proximal occlusion is not due to SIN, tubal cannulation using hysteroscopic or fluoroscopic methods is a proven alternative to traditional

microsurgical repair. The specialized catheter systems involved require some training and experience but allow selective tubal perfusion for accurate diagnosis (true occlusion or not) and provide the means for treatment when needed.

A 2017 systematic review and meta-analysis pooled 27 studies involving women with proximal tubal occlusion.[799] Clinical pregnancy rate following tubal catheterization for unilateral or bilateral proximal tubal occlusion was 27% (95% CI = 25–30%). The pooled live birth and ectopic pregnancy rates were 22% (95% CI = 18–26%) and 4% (95% CI 3–5%), respectively. Pregnancy rates were not significantly different between tubal catheterization with hysteroscopic, laparoscopic, or fluoroscopic guidance (31% vs 26%, respectively).[799] However, the reocclusion rate of the opened tubes was approximately 30%,[695] and another study reported similar pregnancy rates following ovarian stimulation and IUI in women with untreated unilateral PTO and in those with unexplained infertility.[800] Nevertheless, selective salpingography with catheterization immediately after HSG shows proximal occlusion can be an appropriate next step since it saves time for both physician and patient. Alternative treatments can be initiated 6 to 12 months after proximal tubal cannulation if pregnancy has not yet occurred.[742]

Microsurgical segmental tubal resection and anastomosis are a proven treatment for true proximal tubal obstruction. Experienced surgeons can achieve pregnancy rates ranging between 50% and 60%,[695,801–803] but the number of surgeons having the necessary expertise is fast declining. Outcomes vary with the cause of the obstruction; reocclusion rates are relatively high with causes other than SIN.

Bipolar tubal disease involves both proximal and distal tubal obstructions. In general, success rates achieved with surgery have been extremely poor, and IVF represents the best treatment option.[802,804,805] When cannulation fails, microsurgery may be considered if IVF is not an option.[742]

Summary: Tubal Surgery in the Era of Art

Since only the best surgeons generally publish their results, the available estimates from surgical series also very likely represent the best possible outcomes. Even so, steady advances in ART have improved IVF outcomes to the extent that they now exceed what can be achieved with tubal reconstructive surgery. Accordingly, surgical treatments for tubal factor infertility are generally in an era of decline; laparoscopic surgery has replaced simple open procedures, and ART has replaced more complicated ones. Tubal surgery remains a legitimate treatment option for women seeking pregnancy after a previous tubal sterilization, for those with mild distal tubal disease (particularly when they are young), and for some women with proximal tubal occlusion. Under virtually all other circumstances, IVF is the best choice. Laparoscopic salpingectomy or proximal tubal occlusion increases IVF success rates 2-fold and should be recommended to all women with hydrosalpinges planning IVF.

UNEXPLAINED INFERTILITY

Unexplained infertility is a diagnosis of exclusion, after systematic evaluation fails to identify a cause. The incidence of unexplained infertility ranges from 10% to as high as 30% among infertile populations, depending on diagnostic criteria.[806–808] **At a minimum, the diagnosis of unexplained infertility implies normal semen analysis, ovulatory function, a normal uterine cavity, and at least unilateral tubal patency.** Ruling out cervical factor by a postcoital test and LPD by endometrial biopsy and performing a diagnostic laparoscopy are no longer required for diagnosis of unexplained infertility.[408,744] Consequently, much of infertility previously attributed to cervical factors, LPD, and mild endometriosis or adhesions is now classified as "unexplained," and couples with unexplained infertility comprise a very heterogeneous group. As such, management is not aimed at correcting an underlying disorder; rather, the focus is on achieving pregnancy.

Excluding false-negative results of standard diagnostic tests, which do occur but are uncommon, there are two potential explanations for unexplained infertility: (1) there truly is no abnormality, and the couple's natural fertility is at the extreme lower end of the normal range, possibly due to female partner age or advanced reproductive aging, and (2) there is a specific cause but not one that can be identified with existing diagnostic tests.

Undoubtedly, much of unexplained infertility relates to the natural decline in fertility with increasing age. Unexplained infertility is more common in women over age 35; in a study involving over 7,000 infertile women, those over the age of 35 years were nearly twice as likely to have unexplained infertility (OR = 1.8, CI = 1.4–2.7).[401] Yet it is important to differentiate age-related infertility and unexplained infertility. Despite the natural decline in fertility with age, diagnostic criteria for infertility do not include age. A mathematical model of human conception according to a woman's age suggests that the rate of false-positive diagnosis of unexplained infertility, in women who are infertile due to natural age-related decline in fertility, rises from 10% for women trying for a pregnancy before the age of 35 to 80% for women who start trying to conceive at age 40.[808,809] Older reproductive-age women may benefit from different management strategies for unexplained infertility compared to younger women. For instance, younger women with unexplained infertility could be more likely benefit from IVF because they could be expected to have occult ovulation defects, occult endometriosis, subtle disorders of tubal function that cannot be noticed with tests of tubal patency, or a male factor, which wouldn't show by a standard semen analysis. In contrast, older women with age related infertility would be less likely to benefit from IVF since it cannot improve oocyte quality, or even substantially increase the number of oocytes available for fertilization if the ovarian reserve is also decreased with age.[808,809]

Logically, the most likely occult causes of infertility relate to abnormalities in gametes or implantation, for which there is no valid diagnostic test. Genetic or functional abnormalities in zona pellucida proteins could interfere with sperm penetration and cause fertilization failure.[810] Abnormalities in the centrosome could interfere with normal spindle formation and function, preventing fertilization or resulting in arrested early embryonic development.[811] Although failed fertilization occurs in less than 5% of IVF cycles and does not always reoccur in subsequent cycles,[812,813] a marked decrease in fertilization efficiency could easily result in unexplained infertility. A higher incidence of fertilization failure has been observed in several, but not all, studies of IVF outcomes in couples with unexplained infertility.[814,817] Evidence that up to 75% of human pregnancies fail soon after conception implicates early embryopathy and implantation failure as likely causes of unexplained infertility.[39,818,819] Although aneuploidy is common in early human embryos,[820,821] a recurring nonrandom genetic defect in the embryo or trophectoderm could cause early loss. Intrinsic genetic abnormalities in endometrial function and receptivity could interfere with apposition, adhesion, attachment, or invasion of the embryo, causing implantation failure.[822–824] **It is important to emphasize that all of the potential causes of unexplained infertility could coexist with known causes for infertility, helping to explain why many couples with identified ovarian, male, uterine, or tubal infertility factors fail to achieve a successful pregnancy despite receiving proven effective treatments.**[825]

Unexplained infertility likely represents either the lower extreme of the normal distribution of reproductive efficiency or abnormalities of sperm or oocyte function, fertilization, implantation, or embryo development that cannot be detected reliably by standard methods of evaluation. Although many couples with unexplained infertility may be expected to conceive without treatment, their already low and steadily declining cycle fecundity provides ample justification for offering treatment to those concerned enough to seek evaluation. The goal of treatment is to increase monthly fecundability to a level more closely approximating that observed in normally fertile couples.

The prognosis for untreated couples with unexplained infertility is similar to that for couples with minor infertility factors, such as mild oligospermia or endometriosis; age of the female partner and duration of infertility are the primary variables that affect pregnancy rates.[404,826,827] **In studies evaluating treatments for unexplained infertility, untreated patients have a cycle fecundability ranging typically between 2% and 4%,[828] or about 80% to 90% lower than in normal fertile couples (20–25%). The likelihood of pregnancy without treatment decreases progressively with increasing age of the female partner and increasing duration of infertility.**[404,829] After 3 years of infertility, the likelihood of pregnancy without treatment falls to approximately 40%, and after 5 years to about 20%, of what it was when efforts to conceive first began.[393] Only approximately 14% of couples with unexplained infertility managed expectantly for up to 7 years achieve a pregnancy resulting in a live birth; the prognosis is better when the female partner is under age 30.[404,827] **The effect of duration of infertility is important to understand. Because spontaneous pregnancy rates are highest among couples with a relatively short duration of infertility and success rates achieved with all forms of treatment for unexplained infertility other than IVF are similar, treatments can appear more effective in couples with a longer duration of infertility having a lower probability for conceiving without treatment.**

By definition, the cause of unexplained infertility is unknown. Consequently, all treatments for unexplained infertility are empiric. Although methods differ, the basic strategy is the same for all—to bring together more than the usual numbers of oocytes and sperm in the right place at the right time. To this end, the most common treatments include IUI, ovarian stimulation with oral agents or gonadotropins and IUI, and IVF.

Intrauterine Insemination

Although several studies have examined the effectiveness of IUI as treatment for unexplained infertility in natural cycles,[514,532,828,830,831] a 2006 meta-analysis concluded that none provided reliable data because of problems with design, such as crossover trials that do not include data from the first phase of the study or populations not limited to couples with unexplained infertility.[832] The two most informative studies were published subsequently and included only couples with unexplained infertility or an abnormal postcoital test, with expectant management as the control treatment.[833,834] In the first trial (average age 32 years, average duration of infertility 2.5 years), 43 live births were observed among 191 couples receiving IUI (23%) over 6 months, compared to 32 in 193 couples (17%) managed expectantly.[833] Although the effect difference (6% over 6 months) was not significant (OR = 1.46, CI = 0.88–2.43), more women randomized to IUI judged their treatment acceptable. In the second trial (average age 30 years, average duration of infertility 1.7 years), 11 ongoing pregnancies were observed among 51 couples receiving IUI (22%), compared to 9 in 48 couples (19%) managed expectantly.[834] **The best available evidence suggests that treatment with IUI in natural cycles has no clinically important effects.**

Clomiphene Citrate, Letrozole, or Gonadotropins With Timed Intercourse

Numerous studies have examined the effectiveness of clomiphene therapy without IUI as treatment for unexplained infertility.[835–838] However, only two are truly informative trials, including only patients with unexplained infertility, using placebo or expectant management as the control treatment.[833,839] In one trial (average age 30 years, average duration of infertility 4.3 years), 10 pregnancies were observed among 76 couples (13%) receiving clomiphene treatment over 290 cycles (3% per cycle), compared to 4 in 72 couples (6%) receiving placebo over 274 cycles (1% per cycle).[839] In the other (average age 32 years,

average duration of infertility 2.5 years), 26 pregnancies were observed among 192 couples receiving clomiphene (14%), compared to 32 in 193 couples (17%) managed expectantly.[833] The differences between treatment and control pregnancy rates (per couple or per cycle) were not significant in either trial. **Although clomiphene with timed intercourse is commonly used as a treatment for unexplained infertility, the best available evidence indicates it has no significant benefit.** While letrozole is associated with higher live birth rates than clomiphene in ovulation induction for polycystic ovary syndrome, this is not the case when it is used for the management of unexplained infertility. A 2019 systematic review reported similar outcomes with letrozole and clomiphene; however, some of the studies included in this review were later retracted or are subject to investigation by the publisher.[840] The American Society of Reproductive Medicine (ASRM) does not recommend letrozole and timed intercourse as a treatment for unexplained infertility.[841] While no studies directly compare gonadotropins and timed intercourse with expectant management for unexplained infertility, indirect evidence does not support such practice. Gonadotropins are associated with higher risk of multiple pregnancy than oral agents and are costlier and less convenient. ASRM recommends against gonadotropin and timed intercourse as a treatment for unexplained infertility.

Clomiphene Citrate and IUI

Combined treatment with clomiphene and IUI is commonly recommended for couples with unexplained infertility, but evidence for its effectiveness has been limited. In a review of eight studies involving 932 treatment cycles, the estimated cycle fecundity was 5.6% with clomiphene and 8.3% with clomiphene and IUI.[828] The one trial (average age 33 years, average duration of infertility 3.5 years), including an untreated control group (timed intercourse), included patients with unexplained infertility or treated endometriosis.[842] Limiting analysis to cycles observed before crossover, eight pregnancies were observed in 23 couples (35%) receiving clomiphene and IUI over 73 treatment cycles (11% per cycle), compared to 4 in 28 couples (14%) over 103 cycles (4% per cycle). The 7.1% absolute difference (CI = −1.0 to 15.2) in cycle fecundability was not significant, and even if it were, the treatment effect was quite modest; the calculated number needed to treat was 15, implying that one additional pregnancy might be expected for every 15 treatment cycles. However, a more recent trial compared cumulative live birth rates between 201 couples with unexplained infertility who were randomized to expectant management or IUI with either clomiphene or letrozole (only seven women) ovarian stimulation for three cycles.[843] Average female age was 34, and average duration of infertility was 4.5 years. Women in the clomiphene/letrozole IUI group had a significantly higher cumulative live birth rate than expectantly managed women (31 [31%] live births among 101 women vs 9 [9%] live births among 100 women; risk ratio [RR] = 3.41, 95% CI = 1.71–6.79). Per-protocol analyses yielded similar results. Based on these figures, the number needed to treat is five; one additional live birth can be expected for every five women undergoing up to three cycles of clomiphene IUI.

Results of three other crossover trials involving control groups receiving an active treatment (instead of placebo or no treatment) are difficult to interpret confidently because no data were provided for the first phase of the study.[844–846] A fourth management trial (the fast track and standard treatment [FASTT] trial) compared outcomes in two groups, one randomly assigned to receive three cycles of treatment with clomiphene and IUI followed by up to six cycles of IVF and the other assigned to receive three cycles of clomiphene and IUI, followed by three cycles of treatment with gonadotropins and IUI, followed by up to six cycles of IVF.[847] Notably, 55 pregnancies were observed among 233 couples over 646 treatment cycles (8.5% per cycle) in the first group and 68 in 242 couples over 648 treatment cycles (10.5% per cycle) in the second; overall, 123 pregnancies were observed in 475 couples (26%) over 1,294 cycles (9.5% per cycle). Per-cycle pregnancy rates for CC/IUI, FSH/IUI, and IVF were 7.6%, 9.8%, and 30.7%, respectively. The overall pregnancy rate achieved with CC/IUI in the FASTT trial compares favorably with the expected 2% to 4% cycle fecundability among couples with unexplained infertility, which supports the use of clomiphene and IUI in the treatment of unexplained infertility. In two large retrospective studies involving a total of more than 8,000 cycles of treatment with clomiphene and IUI, cycle fecundability ranged between 5% and 10% per cycle after four to six cycles for women aged 40 years and younger and was under 5% for those over age 40.[848,849]

In sum, considering the relatively modest cost and complexity (compared to the alternatives, discussed further on), treatment with clomiphene and IUI can be justified because the cycle fecundability observed in large prospective and retrospective studies is significantly higher than can be expected in couples with unexplained infertility receiving no treatment.

Letrozole and IUI

Increasing use of aromatase inhibitors led to letrozole being employed in IUI cycles. Effectiveness of letrozole and IUI has been compared with clomiphene IUI cycles in multiple studies. A 2014 systematic review included four randomized controlled trials comparing letrozole IUI with clomiphene IUI[850]; however, one was later retracted by the journal.[851] While comiphene was used at a dose of 100 mg/d for 5 days in the remaining three trials, one used letrozole at 2.5 mg/d dose for 5 days, one 2.5 mg/d for 9 days, and the other 7.5 mg/d for 5 days prior to IUI.[852–854] When these three randomized controlled trials are pooled with a large US trial,[855] live birth rates (RR = 0.80, 95% CI = 0.59–1.10), clinical pregnancy rates (RR = 1.21, 95% CI = 0.74–1.96), pregnancy loss rates (RR = 0.52, 95% CI = 0.20–1.38), and

multiple pregnancy rates (RR = 1.09, 95% CI = 0.53–2.23) were similar between letrozole and clomiphene IUI cycles. These findings are consistent with the majority of studies on the subject. Letrozole combined with IUI appears to be as effective as clomiphene combined with IUI for the treatment of unexplained infertility, with the choice between the two often depending on individual preferences. However, it is important to note that letrozole is not FDA-approved for this specific indication. Therefore, the decision to use letrozole should be made only after a thorough discussion with the patient, outlining the risks, benefits, and alternatives. Given that letrozole with IUI does not demonstrate superior efficacy over clomiphene with IUI, the latter may be considered a safer and more established option.

Gonadotropins and IUI

Gonadotropin therapy without IUI for treatment of unexplained infertility has been evaluated in only a few clinical trials. In the largest of these trials, pregnancy rates resulting from treatment with gonadotropins and intracervical insemination were higher than was achieved with insemination alone, but the difference was small (3.6%).[856] **Although treatment with gonadotropins alone can increase cycle fecundability, compared with no treatment, the effect is quite modest and no better than can be achieved by treatment with clomiphene or letrozole and IUI.**

More commonly, gonadotropin treatment is combined with IUI for the treatment of unexplained infertility. Among four trials comparing gonadotropins and IUI with no treatment, two were crossover trials providing no results for the first phase of treatment.[857] In a US trial (average age 32 years, average duration of infertility 3.6 years), 77 pregnancies were observed among 231 couples (33%) receiving treatment with gonadotropins and IUI over 618 cycles (12% per cycle), compared to 23 pregnancies in 233 couples (10%) receiving intracervical insemination over 706 cycles (3% per cycle).[856] A Dutch trial (average age 33 years, average duration of infertility 2 years) observed 29 pregnancies among 127 couples (23%) receiving gonadotropins and IUI over 676 cycles (4% per cycle), compared to 34 in 126 couples (27%) managed expectantly over 737 cycles (5% per cycle).[858]

The differing results of the two trials emphasize again the influence of the duration of infertility on outcomes achieved with treatment for unexplained infertility. In the US trial, involving couples infertile for an average of 3.6 years, fecundability in those receiving treatment with gonadotropins and IUI (12% per cycle) was 9% higher than in couples receiving intracervical insemination (3% per cycle), and only 10% of couples in the latter group conceived. In the Dutch trial, involving couples with an average of 2 years of infertility and a better prognosis for achieving pregnancy without treatment,[827] fecundability of those receiving gonadotropins and IUI (4% per cycle) was no better than in couples managed expectantly (5% per cycle) and 27% of couples receiving no treatment conceived. Together, the results of the two trials indicate that treatment with gonadotropins and IUI has little benefit when the prognosis is reasonably good and modest benefit when the prognosis is poor (one additional pregnancy for every 11 treatment cycles).

In a recent multicenter US trial, 900 women with unexplained infertility (average age 32.2 years, average duration of infertility 35 months) were randomized to ovarian stimulation and IUI up to four cycles with gonadotropin, clomiphene, or letrozole.[855] Clinical pregnancies occurred in 35.5%, 28.3%, and 22.4% of women and live birth in 32.2%, 23.3%, and 18.7%, respectively; pregnancy rates with gonadotropin were significantly higher than the rates with letrozole (absolute difference = −13.1, 95% CI = −20.3 to −6.0, $P = 0.001$) but not with clomiphene (−7.2, 95% CI = −14.7 to 0.2). Per-cycle pregnancy rates were 12.3%, 7.9%, and 6.2% with gonadotropin, clomiphene, and letrozole, respectively. Despite the favorable trend in favor of gonadotropin over clomiphene, the former was associated with significantly higher multiple pregnancy rate, 31.8% versus 9.4% (22.4%, 95% CI = 11.6–33.2%). Of note, while there were only twin pregnancies in the clomiphene and letrozole groups, 10 out of 34 multiple pregnancies were triplets in the gonadotropin group. Regarding the comparison between gonadotropin and clomiphene, a Belgian trial randomized 330 couples (average female age 32 years, average duration of fertility 25.5 months) with unexplained infertility to ovarian stimulation IUI with low-dose gonadotropin or clomiphene at cycle level.[859] Gonadotropin starting dosage was 37.5 to 75 IU/d. Both clinical pregnancy (14.4% vs 9%) and live birth rates (13.8% vs 8.7%) were significantly higher in gonadotropin-stimulated cycles. Multiple live birth rates were similar between the groups (6.5% vs 3.6%), while cycle cancellation rate was higher in the clomiphene group.[859]

The results of treatment with gonadotropins and IUI for unexplained infertility raise two clinically relevant questions. The first concerns what benefits treatment with gonadotropins and IUI might have in couples first treated with clomiphene and IUI and failing to conceive. The only data addressing the question directly come from the "FASTT" trial described previously, in which 50 pregnancies were observed among 169 couples (30%) receiving treatment with gonadotropins and IUI over 439 cycles (11% per cycle) after failing to conceive over three cycles of treatment with clomiphene and IUI.[847] Although cycle fecundability (11% per cycle) was slightly higher than was achieved with clomiphene and IUI in the same population (9.5% per cycle), the difference is not clinically important, especially when considering the greater costs, complexity, and risks associated with the use of gonadotropins. Moreover, a 2022 systematic review and individual patient data meta-analysis suggests that gonadotropin IUI is not associated with higher live birth rates than oral antiestrogen (clomiphene or letrozole) IUI when strict cancellation criteria (cancelling IUI in the presence of >3 dominant follicles), consistent with contemporary practice, are applied (RR = 1.15, 95% CI = 0.94–1.41).[860] **Considering the totality of available evidence, gonadotropin IUI does not seem**

to improve pregnancy or live birth rates without bringing about a higher risk of multiple pregnancy. Given the cost, inconvenience, and risks, gonadotropins are not recommended, alone or in combination with oral antiestrogens, for ovarian stimulation IUI cycles in patients with unexplained infertility.[841]

Assisted Reproductive Technology

Observations in ART cycles frequently provide insight into the possible causes of a couple's unexplained infertility because the procedures involved address or eliminate many of the unknown variables. Sperm and oocytes will be combined effectively. Fertilization and early embryonic development can be observed directly, and embryo transfer ensures that embryos will reach the endometrial cavity. Although the chromosomal composition of embryos and endometrial receptivity may seem like the only factors remaining, the list of unknowns is, in truth, much longer.

Few trials have compared ART with no treatment or a different treatment such as gonadotropins and IUI,[861–865] and only some have been limited to couples with unexplained infertility. When compared on a per-cycle basis, IVF is clearly superior to ovarian stimulation with IUI. However, other factors in addition to effectiveness are considered in decision-making. Given the more invasive nature, higher cost, and per-cycle success rate of IVF, trials have compared IVF with three to six cycles of ovarian stimulation cycles.[863–865] Three randomized controlled trials, all including women with an average age of 32 to 34 years and average duration of infertility about 2.5 years, compared IVF with ovarian stimulation IUI. The first multicenter Dutch trial randomized 116 couples to one cycle of IVF elective single embryo transfer or three cycles of ovarian stimulation IUI and reported similar ongoing pregnancy rates (24% vs 21%).[863] Another multicenter Dutch trial randomized 602 women to three cycles of IVF with elective single embryo transfer, six cycles of natural cycle IVF, or six cycles of ovarian simulation IUI and reported similar cumulative live birth rates of 52%, 43%, and 47%.[864] The most recent British trial randomized 207 women to one cycle of IVF or three cycles of ovarian stimulation IUI and reported similar cumulative live birth rates of 33.9% and 28.7%, respectively.[865] Based on published studies, the ASRM recommends 3 to 4 cycles of ovarian stimulation with IUI before considering IVF for couples with unexplained infertility.[841] However, it is important to note that the aforementioned trials do not reflect the current success rates of ART, which have been steadily improving. According to 2021 US data, cumulative live birth rates per intended retrieval cycle exceeded 50% for women under age 35 and 40% for women aged 35 to 37 years.[33] As such, **IVF is the most effective treatment for couples with unexplained infertility**. Consequently, the decision regarding when to proceed to IVF in cases of unexplained infertility remains complex and should be made collaboratively with the patient, considering medical, psychological, social, and financial factors.

Summary: Treatment of Unexplained Infertility

Overall, the effects of treatments for unexplained infertility other than IVF are relatively small. In many cases, treatment options involving ovarian stimulation with IUI may only hasten pregnancy for couples who would ultimately conceive on their own, given time. IVF is the most effective treatment for unexplained infertility. Careful counseling is essential and must take into account the couple's age, the duration of infertility, and the outcome of any previous pregnancies; before treatment is recommended, an ovarian reserve test is also prudent. Couples who choose treatment should be informed thoroughly about the relative costs, risks, prognoses, and logistical challenges associated with different treatments so that they may select the one that best meets their needs and preferences. Partners can have differing levels of concern about their infertility and tolerance for risk and uncertainty.[866] Together, the medical evidence and shared decision making determine the choice of management.[867]

ADOPTION

With proper evaluation and treatment, the majority of couples evaluated for infertility will achieve pregnancy. For those who fail treatments, ART with donor eggs and/or a gestational surrogate and adoption are realistic options. Couples considering adoption have a wide range of choices, including social agency adoptions, private adoptions, and international adoptions. In some states, private adoption is not legal, but where it is, private adoption can be an effective, more rapid alternative to adoption through a social agency. In most cases, the biologic mother has the opportunity to know the adopting parents and may reconsider her decision and reclaim her child for a time before the adoption is finalized. Those who prefer anonymity or who wish to avoid such potentially devastating disappointments will likely make a different choice. Couples interested in adoption should be referred to those knowledgeable about adoption laws in individual states and all of the available options.

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril*. 2020;113(3):533–535.
2. Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertil Steril*. 2003;79:577.
3. Gnoth C, Godehardt D, Godehardt E, Frank-Herrmann P, Freundl G. Time to pregnancy: results of the German prospective study and impact on the management of infertility. *Hum Reprod*. 2003;18:1959.
4. World Health Organization. *Infertility Prevalence Estimates, 1990–2021*. World Health Organization; 2023.
5. Thoma ME, McLain AC, Louis JF, et al. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. *Fertil Steril*. 2013;99(5):1324.e1.
6. Centers for Disease Control and Prevention. National survey of family growth. Accessed January 16, 2018. https://www.cdc.gov/nchs/nsfg/key_statistics/i.htm#infertility

7. Osterman MJK, Hamilton BE, Martin JA, Driscoll AK, Valenzuela CP. Births: final data for 2022. *Natl Vital Stat Rep*. 2024;73(2):1–56.
8. Ventura SJ, Martin JA, Curtin SC, Mathews TJ. Report of final natality statistics, 1996. *Mon Vital Stat Rep*. 1998;46:1.
9. Ventura SJ, Hamilton BE, Sutton PD. Revised birth and fertility rates for the United States, 2000 and 2001. *Natl Vital Stat Rep*. 2003;51:1.
10. U.S. Census Bureau. Educational attainment in the United States: 2022. U.S. Census Bureau. Accessed August 13, 2024. https://www.census.gov/data/tables/2022/demo/educational-attainment/cps-detailed-tables.html
11. Esencan E, Simsek B, Seli E. Analysis of female demographics in the United States: life expectancy. education, employment, family building decisions, and fertility service utilization. *Curr Opin Obstet Gynecol*. 2021;33(3):170–177.
12. Bureau of Labor Statistics, Employment Projections. Civilian labor force participation rate by age, sex, race, and ethnicity. September 6, 2023. https://www.bls.gov/emp/tables/civilian-labor-force-participation-rate.htm
13. Norton AJ, Miller L; United States Bureau of the Census.Marriage, divorce, and remarriage in the 1990's. U.S. Census Bureau; 1992. Accessed August 7, 2025. https://www2.census.gov/library/publications/1992/demographics/p23-180.pdf
14. Mayol-Garcia Y, Gurrentz B, Kreider RM. Number, timing, and duration of marriages and divorces: 2016: current population reports. U.S. Census Bureau; 2021:70–167. Accessed August 10, 2025. https://www.census.gov/library/publications/2021/demo/p70-167.html
15. Kreider R, Fields J. *Number, Timing, and Duration of Marriages and Divorces, 1996*. U.S. Department of Commerce Economics, Statistics Administration, U.S. Census Bureau; 2002.
16. National Center for Health Statistics. Vital statistics of the United States: marriage and divorce for 1989 to 1995. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 1997.
17. National Center for Health Statistics. Advance report of final marriage statistics, 1989 and 1990. *Mon Vital Stat Rep*. 1995;43(12):1–24.
18. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. Births: final data for 2002. *Natl Vital Stat Rep*. 2003;52:1.
19. Martinez GM, Daniels K. Fertility of men and women aged 15–49 in the United States: National Survey of Family Growth, 2015–2019. *Natl Health Stat Report*. 2023;(179):1–22.
20. The World Factbook. Country comparison—total fertility rate. https://www.cia.gov/the-world-factbook/field/total-fertility-rate/country-comparison/
21. Stephen EH, Chandra A. Declining estimates of infertility in the United States: 1982–2002. *Fertil Steril*. 2006;86:516.
22. Nugent CN, Chandra A. Infertility and impaired fecundity in women and men in the United States, 2015–2019. *Natl Health Stat Report*. 2024;(202):1–19.
23. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med*. 2012;9(12):e1001356.
24. National Center for Health Statistics. Key statistics from the national survey of family growth 2015–2019. https://www.cdc.gov/nchs/nsfg/key_statistics/i-keystat.htm#infertilityservices
25. Stephen EH, Chandra A. Use of infertility services in the United States: 1995. *Fam Plann Perspect*. 2000;32:132.
26. Menken J, Trussell J, Larsen U. Age and infertility. *Science*. 1986;233:1389.
27. Tietze C. Reproductive span and rate of reproduction among Hutterite women. *Fertil Steril*. 1957;8:89.
28. Maroulis GB. Effect of aging on fertility and pregnancy. *Semin Reprod Endocrinol*. 1991;9:165.
29. van Noord-Zaadstra BM, Looman CW, Alsbach H, Habbena JDF, te Velde ER, Karbaat J. Delaying child-bearing: effect of age on fecundity and outcome of pregnancy. *Br Med J*. 1991;302:1361.
30. Schwartz D, Mayaux MJ; Federation CECOS. Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. *N Engl J Med*. 1982;306:404.
31. Virro MS, Shewchuk AB. Pregnancy outcome in 242 conceptions after artificial insemination with donor sperm and effects of maternal age on the prognosis for successful pregnancy. *Am J Obstet Gynecol*. 1984;148:518.
32. Shenfield F, Doyle P, Valentine A, Steele SJ, Tan S-L. Effects of age, gravidity and male infertility status on cumulative conception rates following artificial insemination with cryopreserved donor semen: analysis of 2998 cycles of treatment in one centre over 10 years. *Hum Reprod*. 1993;8:60.
33. Centers for Disease Control and Prevention. *2021 Assisted Reproductive Technology Fertility Clinic and National Summary Report*. U.S. Dept of Health and Human Services; 2023.
34. Stein ZA. A woman's age: childbearing and child rearing. *Am J Epidemiol*. 1985;121:327.
35. Hassold T, Chiu D. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum Genet*. 1985;70:11.
36. Warburton D, Kline J, Stein Z, Strobino B. Cytogenetic abnormalities in spontaneous abortions of recognized conceptions. In: Porter IH, Hatcher N, Willey AM, eds. *Perinatal Genetics: Diagnosis and Treatment*. Academic Press; 1986:23–40.
37. Centers for Disease Control and Prevention. *2016 Assisted Reproductive Technology Fertility Clinic and National Summary Report*. U.S. Dept of Health and Human Services; 2018.
38. Gosden RG. Maternal age: a major factor affecting the prospects and outcome of pregnancy. *Ann N Y Acad Sci*. 1985;442:45.
39. Wilcox AJ, Weiberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med*. 1988;319:189.
40. Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril*. 1996;65:503.
41. Warburton D. Reproductive loss: how much is preventable? *N Engl J Med*. 1987;316:158.
42. Block E. Quantitative morphological investigations of the follicular system in women. *Acta Anat (Basel)*. 1952;14:108.
43. Block E. A quantitative morphological investigation of the follicular system in newborn female infants. *Acta Anat (Basel)*. 1953;17:201.
44. Baker TG. A quantitative and cytological study of germ cells in human ovaries. *Proc R Soc Lond B Biol Sci*. 1963;158:417.
45. Vaskivuo TE, Anttonen M, Herva R, et al. Survival of human ovarian follicles from fetal to adult life: apoptosis, apoptosis-related proteins, and transcription factor GATA-4. *J Clin Endocrinol Metab*. 2001;86:3421.
46. Markstrom E, Svensson E, Shao R, Svanberg B, Billig H. Survival factors regulating ovarian apoptosis—dependence on follicle differentiation. *Reproduction*. 2002;123:23.
47. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update*. 2002;8:141.
48. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J Clin Endocrinol Metab*. 1987;65:1231.
49. Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. *Hum Reprod*. 1996;11:1484.
50. Battaglia DE, Goodwin P, Klein NA, Soules MR. Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. *Hum Reprod*. 1996;11:2217.
51. Gougeon A, Echochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. *Biol Reprod*. 1994;50:653.
52. Coxworth JE, Hawkes K. Ovarian follicle loss in humans and mice: lessons from statistical model comparison. *Hum Reprod*. 2010;25(7):1796.
53. Gougeon A, Chainy GB. Morphometric studies of small follicles in ovaries of women at different ages. *J Reprod Fertil*. 1987;81:433.
54. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum Reprod*. 1992;7:1342.
55. Kirkwood TB. Ovarian ageing and the general biology of senescence. *Maturitas*. 1998;30:105.
56. Leidy LE, Godfrey LR, Sutherland MR. Is follicular atresia biphasic? *Fertil Steril*. 1998;70:851.
57. Cant MA, Johnstone RA. Reproductive conflict and the separation of reproductive generations in humans. *Proc Natl Acad Sci U S A*. 2008;105:5332.
58. Tilly JL, Telfer EE. Purification of germline stem cells from adult mammalian ovaries: a step closer towards control of the female biological clock? *Mol Hum Reprod*. 2009;15:393.
59. Faddy MJ. Follicle dynamics during ovarian ageing. *Mol Cell Endocrinol*. 2000;163:43.
60. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. *Hum Reprod*. 2008;23:699.
61. Da Silva-Buttkus P, Marcelli G, Franks S, Stark J, Hardy K. Inferring biological mechanisms from spatial analysis: prediction of a local inhibitor in the ovary. *Proc Natl Acad Sci U S A*. 2009;106:456.
62. Nilsson E, Rogers N, Skinner MK. Actions of anti-Müllerian hormone on the ovarian transcriptome to inhibit primordial to primary follicle transition. *Reproduction*. 2007;134:209.
63. Adhikari D, Liu K. Molecular mechanisms underlying the activation of mammalian primordial follicles. *Endocr Rev*. 2009;30:438.
64. Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremmer WJ, Soules MR. Reproductive aging: accelerated ovarian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. *J Clin Endocrinol Metab*. 1996;81:1038.
65. Burger HG, Dudley EC, Hopper JL, et al. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. *J Clin Endocrinol Metab*. 1999;84:4025.
66. Wise PM, Smith MJ, Dubal DB, et al. Neuroendocrine modulation and repercussions of female reproductive aging. *Recent Prog Horm Res*. 2002;57:235.
67. Bellino FL, Wise PM. Nonhuman primate models of menopause workshop. *Biol Reprod*. 2003;68:10.
68. Rehman HU, Masson EA. Neuroendocrinology of female aging. *Gend Med*. 2005;2:41.
69. Yin W, Gore AC. Neuroendocrine control of reproductive aging: roles of GnRH neurons. *Reproduction*. 2006;131:403.
70. Nozaki M, Mitsunaga F, Shimizu K. Reproductive senescence in female Japanese monkeys (*Macaca fuscata*): age- and season-related changes in hypothalamic-pituitary-ovarian functions and fecundity rates. *Biol Reprod*. 1995;52:1250.
71. van Look PF, Lothian H, Hunter WM, Michie EA, Baird DT. Hypothalamic-pituitary-ovarian function in perimenopausal women. *Clin Endocrinol (Oxf)*. 1977;7:13.
72. Weiss G, Skurnick JH, Goldsmith LT, Santoro NF, Park SJ. Menopause and hypothalamic-pituitary sensitivity to estrogen. *JAMA*. 2004;292:2991.
73. Gill S, Sharpless JL, Rado K, Hall JE. Evidence that GnRH decreases with gonadal steroid feedback but increases with age in postmenopausal women. *J Clin Endocrinol Metab*. 2002;87:2290.

74. Hall JE, Lavoie HB, Marsh EE, Martin KA. Decrease in gonadotropin-releasing hormone (GnRH) pulse frequency with aging in postmenopausal women. *J Clin Endocrinol Metab*. 2000;85:1794.
75. Rossmanith WG. Gonadotropin secretion during aging in women: review article. *Exp Gerontol*. 1995;30:369.
76. Kwekkeboom DJ, de Jong FH, van Hemert AM, Vandenbroucke JP, Valkenburg HA, Lamberts SW. Serum gonadotropins and alpha-subunit decline in aging normal postmenopausal women. *J Clin Endocrinol Metab*. 1990;70:944.
77. de Koning CH, Popp-Snijders C, Schoemaker J, Lambalk CB. Elevated FSH concentrations in imminent ovarian failure are associated with higher FSH and LH pulse amplitude and response to GnRH. *Hum Reprod*. 2000;15:1452.
78. Matt DW, Kauma SW, Pincus SM, Veldhuis JD, Evans WS. Characteristics of luteinizing hormone secretion in younger versus older premenopausal women. *Am J Obstet Gynecol*. 1998;178:504.
79. Klein NA, Battaglia DE, Clifton DK, Bremner WJ, Soules MR. The gonadotropin secretion pattern in normal women of advanced reproductive age in relation to the monotropic FSH rise. *J Soc Gynecol Investig*. 1996;3:27.
80. Reame NE, Kelche RP, Beitins IZ, Yu MY, Zawacki CM, Padmanabhan V. Age effects of follicle-stimulating hormone and pulsatile luteinizing hormone secretion across the menstrual cycle of premenopausal women. *J Clin Endocrinol Metab*. 1996;81:1512.
81. Randolph JF, Ginsburg KA, Leach RE, et al. Elevated early follicular gonadotropin levels in women with unexplained infertility do not provide evidence for disordered gonadotropin-releasing hormone secretion as assessed by luteinizing hormone pulse characteristics. *Fertil Steril*. 2003;80:320.
82. Eshkol A, Lunenfeld B, Insler V. The effect of sex steroids on pituitary responsiveness to gonadotropin releasing hormone. *J Steroid Biochem*. 1975;6:1061.
83. Fujimoto VY, Klein NA, Battaglia DE, Bremner WJ, Soules MR. The anterior pituitary response to a gonadotropin-releasing hormone challenge test in normal older reproductive-age women. *Fertil Steril*. 1996;65:539.
84. Klein NA, Battaglia DE, Miller PB, Branigan EF, Giudice LC, Soules MR. Ovarian follicular development and the follicular fluid hormones and growth factors in normal women of advanced reproductive age. *J Clin Endocrinol Metab*. 1996;81:1946.
85. Hofmann GE, Danforth DR, Seifer DB. Inhibin-B: the physiologic basis of the clomiphene citrate challenge test for ovarian reserve screening. *Fertil Steril*. 1998;69:474.
86. Welt CK, McNicholl DJ, Taylor AE, Hall JE. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J Clin Endocrinol Metab*. 1999;84:105.
87. Seifer DB, Scott RT Jr, Bergh PA, et al. Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. *Fertil Steril*. 1999;72:63.
88. Klein NA, Houmard BS, Hansen KR, et al. Age-related analysis of inhibin A, inhibin B, and activin a relative to the intercycle monotropic follicle-stimulating hormone rise in normal ovulatory women. *J Clin Endocrinol Metab*. 2004;89:2977.
89. Hale GE, Zhao X, Hughes CL, Burger HG, Robertson DM, Fraser IS. Endocrine features of menstrual cycles in middle and late reproductive age and the menopausal transition classified according to the Staging of Reproductive Aging Workshop (STRAW) staging system. *J Clin Endocrinol Metab*. 2007;92:3060.
90. Knauff EA, Eijkemans MJ, Lambalk CB, et al. Anti- Müllerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. *J Clin Endocrinol Metab*. 2009;94:786.
91. Burger HG, Hale GE, Dennerstein L, Robertson DM. Cycle and hormone changes during perimenopause: the key role of ovarian function. *Menopause*. 2008;15:603.
92. Welt CK, Smith ZA, Pauler DK, Hall JE. Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. *J Clin Endocrinol Metab*. 2001;86:2531.
93. Burger HG, Dudley EC, Hopper JL, et al. The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample. *J Clin Endocrinol Metab*. 1995;80:3537.
94. Burger HG, Groome NP, Robertson DM. Both inhibin A and B respond to exogenous follicle-stimulating hormone in the follicular phase of the human menstrual cycle. *J Clin Endocrinol Metab*. 1998;83:4167.
95. Landgren BM, Collins A, Csemiczky G, Burger HG, Baksheev L, Robertson DM. Menopause transition: annual changes in serum hormonal patterns over the menstrual cycle in women during a nine-year period prior to menopause. *J Clin Endocrinol Metab*. 2004;89:2763.
96. Seifer DB, Gardiner AC, Ferreira KA, Peluso JJ. Apoptosis as a function of ovarian reserve in women undergoing in vitro fertilization. *Fertil Steril*. 1996; 66:593.
97. Santoro N, Brown JR, Adel T, Skurnick JH. Characterization of reproductive hormonal dynamics in the perimenopause. *J Clin Endocrinol Metab*. 1996;81:1495.
98. Shideler SE, DeVane GW, Kalra PS, Benirschke K, Lasley BL. Ovarian-pituitary hormone interactions during the perimenopause. *Maturitas*. 1989;11:331.
99. de Koning CH, McDonnell J, Themmen AP, de Jong FH, Homburg R, Lambalk CB. The endocrine and follicular growth dynamics throughout the menstrual cycle in women with consistently or variably elevated early follicular phase FSH compared with controls. *Hum Reprod*. 2008;23:1416.
100. Miro F, Parker SW, Aspinall LJ, Coley J, Perry PW, Ellis JE. Sequential classification of endocrine stages during reproductive aging in women: the FREEDOM study. *Menopause*. 2005;12:281.
101. Mersereau JE, Evans ML, Moore DH, et al. Luteal phase estrogen is decreased in regularly menstruating older women compared with a reference population of younger women. *Menopause*. 2008;15:482.
102. Pal L, Zhang K, Zeitlian G, Santoro N. Characterizing the reproductive hormone milieu in infertile women with diminished ovarian reserve. *Fertil Steril*. 2010;93:1074.
103. Burger H. The menopausal transition—endocrinology. *J Sex Med*. 2008;5:2266.
104. Treloar AE, Boynton RE, Borghild GB, Brown BW. Variation of the human menstrual cycle through reproductive life. *Int J Fertil*. 1967;12:77.
105. Klein NA, Harper AJ, Houmard BS, Sluss PM, Soules MR. Is the short follicular phase in older women secondary to advanced or accelerated dominant follicle development? *J Clin Endocrinol Metab*. 2002;87:5746.
106. Hansen KR, Thyer AC, Sluss PM, Bremner WJ, Soules MR, Klein NA. Reproductive ageing and ovarian function: is the early follicular phase FSH rise necessary to maintain adequate secretory function in older ovulatory women? *Hum Reprod*. 2005;20:89.
107. van Zonneveld P, Scheffer GJ, Broekmans FJ, et al. Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. *Hum Reprod*. 2003;18:495.
108. Beemsterboer SN, Homburg R, Gorter NA, Schats R, Hompes PG, Lambalk CB. The paradox of declining fertility but increasing twinning rates with advancing maternal age. *Hum Reprod*. 2006;21:1531.
109. Lenton EA, Landgren BM, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: identification of the short luteal phase. *Br J Obstet Gynaecol*. 1984;91:685.
110. Miro F, Aspinall LJ. The onset of the initial rise in follicle-stimulating hormone during the human menstrual cycle. *Hum Reprod*. 2005;20:96.
111. Hoekstra C, Zhao ZZ, Lambalk CB, et al. Dizygotic twinning. *Hum Reprod Update*. 2008;14:37.
112. Vollman RF. The menstrual cycle. In: Friedman E, ed. *Major Problems in Obstetrics and Gynecology*. W.B. Saunders; 1977:11–193.
113. Soules MR, Sherman S, Parrott E, et al. Executive summary: stages of reproductive aging workshop (STRAW). *Fertil Steril*. 2001;76:874.
114. Treloar AE. Menstrual cyclicity and the pre-menopause. *Maturitas*. 1981;3:249.
115. Broekmans FJ, Faddy MJ, Scheffer G, te Velde ER. Antral follicle counts are related to age at natural fertility loss and age at menopause. *Menopause*. 2004;11:607.
116. Magursky V, Mesko M, Sokolik L. Age at the menopause and onset of the climacteric in women of Martin District, Czechoslovakia: statistical survey and some biological and social correlations. *Int J Fertil*. 1975;20:17.
117. Bengtsson C, Lindquist O, Redvall L. Menstrual status and menopausal age of middle-aged Swedish women: a population study of women in Goteborg 1968–69 and 1974–75. *Acta Obstet Gynecol Scand*. 1981;60:269.
118. Hagstad A, Johansson S, Wilhelmsson C, Janson PO. Gynaecology of middle-aged women—menstrual and reproductive histories. *Maturitas*. 1985;7:99.
119. Luoto R, Kaprio J, Uutela A. Age at natural menopause and sociodemographic status in Finland. *Am J Epidemiol*. 1994;139:64.
120. van Noord PA, Dubas JS, Dorland M, Boersma H, te Velde E. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. *Fertil Steril*. 1997;68:95.
121. de Vries E, den Tonkelaar I, van Noord PA, van der Schouw YT, te Velde ER, Peeters PH. Oral contraceptive use in relation to age at menopause in the DOM cohort. *Hum Reprod*. 2001;16:1657.
122. Lawlor DA, Ebrahim S, Smith GD. The association of socio-economic position across the life course and age at menopause: the British Women's Heart and Health Study. *Br J Obstet Gynaecol*. 2003;110:1078.
123. Cramer DW, Xu H, Harlow BL. Family history as a predictor of early menopause. *Fertil Steril*. 1995;64:740.
124. Torgerson DJ, Avenell A, Russell IT, Reid DM. Factors associated with onset of menopause in women aged 45–49. *Maturitas*. 1994;19:83.
125. Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. *J Clin Endocrinol Metab*. 1998;83:1875.
126. Treloar SA, Do KA, Martin NG. Genetic influences on the age at menopause. *Lancet*. 1998;352:1084.
127. de Bruin JP, Bovenhuis H, van Noord PA, et al. The role of genetic factors in age at natural menopause. *Hum Reprod*. 2001;16:2014.
128. Thomas F, Renaud F, Benefice E, de Meeus T, Guegan JF. International variability of ages at menarche and menopause: patterns and main determinants. *Hum Biol*. 2001;73:271.
129. Parazzini F. Determinants of age at menopause in women attending menopause clinics in Italy. *Maturitas*. 2007;56:280.
130. Soares SR, Melo MA. Cigarette smoking and reproductive function. *Curr Opin Obstet Gynecol*. 2008;20:281.
131. van Asselt KM, Kok HS, Pearson PL, et al. Heritability of menopausal age in mothers and daughters. *Fertil Steril*. 2004;82:1348.
132. Murabito JM, Yang Q, Fox C, Wilson PW, Cupples LA. Heritability of age at natural menopause in the Framingham Heart Study. *J Clin Endocrinol Metab*. 2005;90:3427.
133. Torgerson DJ, Thomas RE, Reid DM. Mothers and daughters menopausal ages: is there a link? *Eur J Obstet Gynecol Reprod Biol*. 1997;74:63.
134. Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, et al; European Society for Human Reproduction and Embryology Guideline Group on POI. ESHRE guideline: management of women with premature ovarian insufficiency. *Hum Reprod*. 2016;31:926–937.
135. Panay N, Anderson RA, Bennie A, et al; ESHRE, ASRM, CREWHIRL and IMS Guideline Group on POI. Evidence-based guideline: premature ovarian insufficiency. *Fertil Steril*. 2025;123:221–236.

136. Tibiletti MG, Testa G, Vegetti W, et al. The idiopathic forms of premature menopause and early menopause show the same genetic pattern. *Hum Reprod*. 1999;14:2731.
137. Vegetti W, Marozzi A, Manfredini E, et al. Premature ovarian failure. *Mol Cell Endocrinol*. 2000;161:53.
138. Nikolaou D, Templeton A. Early ovarian ageing: a hypothesis: detection and clinical relevance. *Hum Reprod*. 2003;18:1137.
139. Farhi J, Homburg R, Ferber A, Orvieto R, Ben Rafael Z. Non-response to ovarian stimulation in normogonadotrophic, normogonadal women: a clinical sign of impending onset of ovarian failure pre-empting the rise in basal follicle stimulating hormone levels. *Hum Reprod*. 1997;12:241.
140. de Boer EJ, den Tonkelaar I, te Velde ER, Burger CW, Klip H, van Leeuwen FE. A low number of retrieved oocytes at in vitro fertilization treatment is predictive of early menopause. *Fertil Steril*. 2002;77:978.
141. Lawson R, El-Toukhy T, Kassab A, et al. Poor response to ovulation induction is a stronger predictor of early menopause than elevated basal FSH: a life table analysis. *Hum Reprod*. 2003;18:527.
142. Kok HS, van Asselt KM, van der Schouw YT, Peeters PH, Wijmenga C. Genetic studies to identify genes underlying menopausal age. *Hum Reprod Update*. 2005;11:483.
143. Skillern A, Rajkovic A. Recent developments in identifying genetic determinants of premature ovarian failure. *Sex Dev*. 2008;2:228.
144. Guzeloglu-Kayisli O, Lalioti MD, Sasson I, Aydiner F, Sakkas D, Seli E. Embryonic poly(A) binding protein (EPAB) is required for oocyte maturation and female fertility in mouse. *Biochem J*. 2012;446:47.
145. Esencan E, Kallen A, Zhang M, Seli E. Translational activation of maternally-derived mRNAs in oocytes and early embryos and the role of embryonic poly(A) binding protein (EPAB). *Biol Reprod*. 2019;100(5):1147–1157.
146. Wang T, Babayev E, Jiang Z, et al. Mitochondrial unfolded protein response gene *Clpp* is required to maintain ovarian follicular reserve during aging, for oocyte competence, and development of pre-implantation embryos. *Aging Cell*. 2018;17:e12784.
147. Zhang M, Bener MB, Jiang Z, et al. Mitofusin 1 is required for female fertility and to maintain ovarian follicular reserve. *Cell Death Dis*. 2019;10:560.
148. Zhang M, Bener MB, Jiang Z, et al. Mitofusin 2 plays a role in oocyte and follicle development and is required to maintain ovarian follicular reserve during reproductive aging. *Aging (Albany NY)*. 2019;11:3919–3938.
149. Knauff EA, Franke L, van Es MA, et al. Genome-wide association study in premature ovarian failure patients suggests ADAMTS19 as a possible candidate gene. *Hum Reprod*. 2009;24:2372.
150. Laissue P, Lakhal B, Benayoun BA, et al. Functional evidence implicating FOXL2 in non-syndromic premature ovarian failure and in the regulation of the transcription factor OSR2. *J Med Genet*. 2009;46:455.
151. Kevenaar ME, Themmen AP, Rivadeneira F, et al. A polymorphism in the AMH type II receptor gene is associated with age at menopause in interaction with parity. *Hum Reprod*. 2007;22:2382.
152. He C, Kraft P, Chen C, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. *Nat Genet*. 2009;41:724.
153. Stolk L, Zhai G, van Meurs JB, et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. *Nat Genet*. 2009;41:645.
154. Chen CT, Liu CT, Chen GK, et al. Meta-analysis of loci associated with age at natural menopause in African-American women. *Hum Mol Genet*. 2014; 23:3327.
155. Shen C, Delahanty RJ, Gao YT, et al. Evaluating GWAS-identified SNPs for age at natural menopause among Chinese women. *PLoS One*. 2013;8:e58766.
156. Murray A, Bennett CE, Perry JR, et al; ReproGen Consortium. Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study. *Hum Mol Genet*. 2011;20:186.
157. Stolk L, Perry JR, Chasman DI, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet*. 2012;44:260.
158. AlAsiri S, Basit S, Wood-Trageser MA, et al. Exome sequencing reveals MCM8 mutation underlies ovarian failure and chromosomal instability. *J Clin Invest*. 2015;125(1):258.
159. Desai S, Wood-Trageser M, Matic J, et al. MCM8 and MCM9 nucleotide variants in women with primary ovarian insufficiency. *J Clin Endocrinol Metab*. 2017;102:576.
160. Jacobs SL, Metzger DA, Dodson WC, Haney AF. Effect of age on response to human menopausal gonadotropin stimulation. *J Clin Endocrinol Metab*. 1990;71:1525.
161. Piltonen T, Koivunen R, Ruokonen A, Tapanainen JS. Ovarian age-related responsiveness to human chorionic gonadotropin. *J Clin Endocrinol Metab*. 2003;88:3327.
162. Kuliev A, Cieslak J, Verlinsky Y. Frequency and distribution of chromosome abnormalities in human oocytes. *Cytogenet Genome Res*. 2005;111:193.
163. Hunt PA, Hassold TJ. Human female meiosis: what makes a good egg go bad? *Trends Genet*. 2008;24:86.
164. Pellestor F, Anahory T, Hamamah S. Effect of maternal age on the frequency of cytogenetic abnormalities in human oocytes. *Cytogenet Genome Res*. 2005;111:206.
165. Pellestor F, Andreo B, Arnal F, Humeau C, Demaille J. Maternal aging and chromosomal abnormalities: new data drawn from in vitro unfertilized human oocytes. *Hum Genet*. 2003;112:195.
166. Pellestor F, Andreo B, Anahory T, Hamamah S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. *Eur J Med Genet*. 2006;49:103.
167. Angell R. First-meiotic-division nondisjunction in human oocytes. *Am J Hum Genet*. 1997;61:23.
168. Angell R. Mechanism of chromosome nondisjunction in human oocytes. *Prog Clin Biol Res*. 1995;393:13.
169. Kuliev A, Cieslak J, Ilkevitch Y, Verlinsky Y. Chromosomal abnormalities in a series of 6,733 human oocytes in preimplantation diagnosis for age-related aneuploidies. *Reprod Biomed Online*. 2003;6:54.
170. Geraedts J, Montag M, Magli MC, et al. Polar body array CGH for prediction of the status of the corresponding oocyte. Part I: clinical results. *Hum Reprod*. 2011;26:3173.
171. Hong KH, Franasiak JM, Werner MM, et al. Embryonic aneuploidy rates are equivalent in natural cycles and gonadotropin-stimulated cycles. *Fertil Steril*. 2019;112(4):670-676.
172. Ata B. Why ovarian stimulation should be aimed to maximize oocyte yield. *Reprod Biomed Online*. 2023;46(4):655-658.
173. Franasiak JM, Forman EJ, Hong KH, et al. Aneuploidy across individual chromosomes at the embryonic level in trophectoderm biopsies: changes with patient age and chromosome structure. *J Assist Reprod Genet*. 2014;31:1501.
174. Lamb NE, Feingold E, Savage A, et al. Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. *Hum Mol Genet*. 1997;6:1391.
175. Lamb NE, Freeman SB, Savage-Austin A, et al. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. *Nat Genet*. 1996;14:400.
176. Nasmyth K. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu Rev Genet*. 2001;35:673.
177. Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell*. 1997;91:35.
178. Nicklas BJ, Toth MJ, Goldberg AP, Poehlman ET. Racial differences in plasma leptin concentrations in obese postmenopausal women. *J Clin Endocrinol Metab*. 1997;82:315.
179. Nicklas RB. How cells get the right chromosomes. *Science*. 1997;275:632.
180. Nasmyth K, Peters JM, Uhlmann F. Splitting the chromosome: cutting the ties that bind sister chromatids. *Science*. 2000;288:1379.
181. Mahmood R, Brierley CH, Faed MJ, Mills JA, Delhanty JD. Mechanisms of maternal aneuploidy: FISH analysis of oocytes and polar bodies in patients undergoing assisted conception. *Hum Genet*. 2000;106:620.
182. Sandalinas M, Marquez C, Munne S. Spectral karyotyping of fresh, non-inseminated oocytes. *Mol Hum Reprod*. 2002;8:580.
183. Volarcik K, Sheean L, Goldfarb J, Woods L, Abdul-Karim FW, Hunt P. The meiotic competence of in-vitro matured human oocytes is influenced by donor age: evidence that folliculogenesis is compromised in the reproductively aged ovary. *Hum Reprod*. 1998;13:154.
184. Nagele F, O'Connor H, Davies A, Badawy A, Mohamed H, Magos A. 2500 Outpatient diagnostic hysteroscopies. *Obstet Gynecol*. 1996;88:87.
185. Day Baird D, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol*. 2003;188:100.
186. DeWaay DJ, Syrop CH, Nygaard IE, Davis WA, Van Voorhis BJ. Natural history of uterine polyps and leiomyomata. *Obstet Gynecol*. 2002;100:3.
187. Donnez J, Jadoul P. What are the implications of myomas on fertility? A need for a debate? *Hum Reprod*. 2002;17:1424.
188. Olive DL, Pritts EA. Fibroids and reproduction. *Semin Reprod Med*. 2010;28(3):218.
189. Varasteh NN, Neuwirth RS, Levin B, Keltz MD. Pregnancy rates after hysteroscopic polypectomy and myomectomy in infertile women. *Obstet Gynecol*. 1999;94:168.
190. Mastrominas M, Pistofidis GA, Dimitropoulos K. Fertility outcome after outpatient hysteroscopic removal of endometrial polyps and submucous fibroids. *J Am Assoc Gynecol Laparosc*. 1996;3:S29.
191. Noci I, Borri P, Chieffi O, et al. Aging of the human endometrium: a basic morphological and immunohistochemical study. *Eur J Obstet Gynecol Reprod Biol*. 1995;63:181.
192. Yaron Y, Botchan A, Amit A, Kogosowski A, Yovel I, Lessing JB. Endometrial receptivity: the age-related decline in pregnancy rates and the effect of ovarian function. *Fertil Steril*. 1993;60:314.
193. Meldrum DR. Female reproductive aging—ovarian and uterine factors. *Fertil Steril*. 1993;59:1.
194. Pellicer A, Simon C, Remohi J. Effects of aging on the female reproductive system. *Hum Reprod*. 1995;10(suppl 2):77.
195. Centers for Disease Control and Prevention. *2007 Assisted Reproductive Technology Success Rates*. National Summary and Fertility Clinic Reports; 2009.
196. Sauer MV, Paulson RJ, Lobo RA. Pregnancy in women 50 or more years of age: outcomes of 22 consecutively established pregnancies from oocyte donation. *Fertil Steril*. 1995;64:11.
197. Abdalla HI, Burton G, Kirkland A, et al. Age, pregnancy and miscarriage: uterine versus ovarian factors. *Hum Reprod*. 1993;8:1512.
198. Taylor HS. Role of the uterus in fertility, pregnancy, and developmental programming. *Fertil Steril*. 2018;110(5):849.
199. Segal TR, Kim K, Mumford SL, Goldfarb JM, Weinerman RS. How much does the uterus matter? Perinatal outcomes are improved when donor oocyte embryos are transferred to gestational carriers compared to intended parent recipients. *Fertil Steril*. 2018;110:888.

200. Reig A, Franasiak J, Scott RT Jr, Seli E. The impact of age beyond ploidy: outcome data from 8,175 euploid single embryo transfers. *J Assist Reprod Genet*. 2020;37:595–602.
201. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril*. 2001;75:237.
202. Eskenazi B, Wyrobek AJ, Sloter E, et al. The association of age and semen quality in healthy men. *Hum Reprod*. 2003;18(2):447.
203. Kumar N, Singh AK, Choudhari AR. Impact of age on semen parameters in male partners of infertile couples in a rural tertiary care center of central India: a cross-sectional study. *Int J Reprod Biomed*. 2017;15(8):497.
204. Girsh E, Katz N, Genkin L, et al. Male age influences oocyte-donor program results. *J Assist Reprod Genet*. 2008;25(4):137.
205. Nankin HR. Fertility in aging men. *Maturitas*. 1985;7:259.
206. Bujan L, Mieusset R, Mondinat C, Mansat A, Pontonnier F. Sperm morphology in fertile men and its age related variation. *Andrologia*. 1988;20:121.
207. Rolf C, Behre HM, Nieschlag E. Reproductive parameters of older compared to younger men of infertile couples. *Int J Androl*. 1996;19:135.
208. Chia SE, Lim ST, Tay SK. Factors associated with male infertility: a case-control study of 218 infertile and 240 fertile men. *Br J Obstet Gynaecol*. 2000;107:55.
209. Tennekoon KH, Karunanayake EH. Serum FSH, LH, and testosterone concentrations in presumably fertile men: effect of age. *Int J Fertil*. 1993;38:108.
210. Vermeulen A, Kaufman JM. Ageing of the hypothalamo-pituitary-testicular axis in men. *Horm Res*. 1995;43:25.
211. Hassan MA, Killick SR. Effect of male age on fertility: evidence for the decline in male fertility with increasing age. *Fertil Steril*. 2003;79(suppl 3):1520.
212. Faber BM, Mercan R, Hamacher P, Muasher SJ, Toner JP. The impact of an egg donor's age and her prior fertility on recipient pregnancy outcome. *Fertil Steril*. 1997;68:370.
213. Dunson DB, Colombo B, Baird DD. Changes with age in the level and duration of fertility in the menstrual cycle. *Hum Reprod*. 2002;17:1399.
214. Begon E, Lefebvre T, Arbo E, et al. Does paternal age affect the live birth rate in donor oocyte cycles? A systematic review and meta-analysis. *J Assist Reprod Genet*. 2023;40(3):617-626.
215. Marsidi AM, Kipling LM, Kawwass JF, Mehta A. Influence of paternal age on assisted reproductive technology cycles and perinatal outcomes. *Fertil Steril*. 2021;116(2):380–387.
216. du Fossé NA, van der Hoorn MP, van Lith JMM, le Cessie S, Lashley EELO. Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. *Hum Reprod Update*. 2020;26(5):650–669.
217. Egozcue J, Blanco J, Anton E, Egozcue S, Sarrate Z, Vidal F. Genetic analysis of sperm and implications of severe male infertility—a review. *Placenta*. 2003;24(suppl 2):S62.
218. Jenkins TG, Aston KI, Pflueger C, Cairns BR, Carrell DT. Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. *PLoS Genet*. 2014;10:e1004458.
219. Kaltsas A, Zikopoulos A, Vrachnis D, et al. Advanced paternal age in focus: unraveling its influence on assisted reproductive technology outcomes. *J Clin Med*. 2024;13(10):2731.
220. Santiago J, Silva JV, Santos MAS, Fardilha M. Age-dependent alterations in semen parameters and human sperm MicroRNA profile. *Biomedicines*. 2023;11(11):2923.
221. Zumoff B, Strain GW, Kream J, et al. Age variation of the 24-hour mean plasma concentrations of androgens, estrogens, and gonadotropins in normal adult men. *J Clin Endocrinol Metab*. 1982;54:534.
222. Veldhuis JD. Recent insights into neuroendocrine mechanisms of aging of the human male hypothalamic-pituitary-gonadal axis. *J Androl*. 1999;20:1.
223. Hermann M, Untergasser G, Rumpold H, Berger P. Aging of the male reproductive system. *Exp Gerontol*. 2000;35:1267.
224. Steiner AZ, Pritchard D, Stanczyk FZ, et al. Association between biomarkers of ovarian reserve and infertility among older women of reproductive age. *JAMA*. 2017;318(14):1367–1376.
225. Harris BS, Jukic AM, Truong T, Nagle CT, Erkanli A, Steiner AZ. Markers of ovarian reserve as predictors of future fertility. *Fertil Steril*. 2023;119(1):99–106.
226. Korsholm AS, Petersen KB, Bentzen JG, Hilsted LM, Andersen AN, Hvidman HW. Investigation of anti-Müllerian hormone concentrations in relation to natural conception rate and time to pregnancy. *Reprod Biomed Online*. 2018;36(5):568–575.
227. Hagen CP, Vestergaard S, Juul A, et al. Low concentration of circulating antimüllerian hormone is not predictive of reduced fecundability in young healthy women: a prospective cohort study. *Fertil Steril*. 2012;98(6):1602–1608.e2.
228. Zarek SM, Mitchell EM, Sjaarda LA, et al. Is anti-Müllerian hormone associated with fecundability? Findings from the EAGeR trial. *J Clin Endocrinol Metab*. 2015;100(11):4215–4221.
229. Ferraretti AP, La Marca A, Fauser BC, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod*. 2011;26(7):1616–1624.
230. Reig A, Garcia-Velasco JA, Seli E. Bologna vs. POSEIDON criteria as predictors of the likelihood of obtaining at least one euploid embryo in poor ovarian response: an analysis of 6,889 cycles. *Fertil Steril*. 2023;120(3 Pt 2):605–614.
231. Poseidon G, Alviggi C, Andersen CY, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil Steril*. 2016;105(6):1452–1453.
232. Muasher SJ, Oehninger S, Simonetti S, et al. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. *Fertil Steril*. 1988;50:298.
233. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome. *Fertil Steril*. 1989;51:651.
234. 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.
235. Pearlstone AC, Fournet N, Gambone JC, Pang SC, Buyalos RP. Ovulation induction in women age 40 and older: the importance of basal follicle-stimulating hormone level and chronological age. *Fertil Steril*. 1992;58:674.
236. Scott RT Jr, Hofmann GE. Prognostic assessment of ovarian reserve. *Fertil Steril*. 1995;63:1.
237. Bukman A, Heineman MJ. Ovarian reserve testing and the use of prognostic models in patients with subfertility. *Hum Reprod Update*. 2001;7:581.
238. Barroso G, Oehninger S, Monzo A, Kolm P, Gibbons WE, Muasher SJ. High FSH:LH ratio and low LH levels in basal cycle day 3: impact on follicular development and IVF outcome. *J Assist Reprod Genet*. 2001;18:499.
239. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update*. 2006;12:685.
240. Roberts JE, Spandorfer S, Fasouliotis SJ, Kashyap S, Rosenwaks Z. Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization. *Fertil Steril*. 2005;83:37.
241. Abdalla H, Thum MY. Repeated testing of basal FSH levels has no predictive value for IVF outcome in women with elevated basal FSH. *Hum Reprod*. 2006;21:171.
242. Scott RT Jr, Hofmann GE, Oehninger S, Muasher SJ. Intercycle variability of day 3 follicle-stimulating hormone levels and its effect on stimulation quality in in vitro fertilization. *Fertil Steril*. 1990;54:297.
243. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. Serum antimüllerian hormone/müllerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. *Fertil Steril*. 2004;82:1323.
244. Eldar-Geva T, Ben-Chetrit A, Spitz IM, et al. Dynamic assays of inhibin B, anti-Müllerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. *Hum Reprod*. 2005;20:3178.
245. McIlveen M, Skull JD, Ledger WL. Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population. *Hum Reprod*. 2007;22:778.
246. Phophong P, Ranieri DM, Khadum I, Meo F, Serhal P. Basal 17β-estradiol did not correlate with ovarian response and in vitro fertilization treatment outcome. *Fertil Steril*. 2000;74:1133.
247. Evers JL, Slaats P, Land JA, Dumoulin JC, Dunselman GA. Elevated levels of basal estradiol-17beta predict poor response in patients with normal basal levels of follicle-stimulating hormone undergoing in vitro fertilization. *Fertil Steril*. 1998;69:1010.
248. Smotrich DB, Widra EA, Gindoff PR, Levy MJ, Hall JL, Stillman RJ. Prognostic value of day 3 estradiol on in vitro fertilization outcome. *Fertil Steril*. 1995;64:1136.
249. Licciardi FL, Liu HC, Rosenwaks Z. Day 3 estradiol serum concentrations as prognosticators of ovarian stimulation response and pregnancy outcome in patients undergoing in vitro fertilization. *Fertil Steril*. 1995;64:991.
250. Buyalos RP, Daneshmand S, Brzechffa PR. Basal estradiol and follicle-stimulating hormone predict fecundity in women of advanced reproductive age undergoing ovulation induction therapy. *Fertil Steril*. 1997;68:272.
251. Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. *Lancet*. 1987;330:645.
252. Yong PY, Baird DT, Thong KJ, McNeilly AS, Anderson RA. Prospective analysis of the relationships between the ovarian follicle cohort and basal FSH concentration, the inhibin response to exogenous FSH and ovarian follicle number at different stages of the normal menstrual cycle and after pituitary down-regulation. *Hum Reprod*. 2003;18:35.
253. Yanushpolsky EH, Hurwitz S, Tikh E, Racowsky C. Predictive usefulness of cycle day 10 follicle-stimulating hormone level in a clomiphene citrate challenge test for in vitro fertilization outcome in women younger than 40 years of age. *Fertil Steril*. 2003;80:111.
254. Csemiczky G, Harlin J, Fried G. Predictive power of clomiphene citrate challenge test for failure of in vitro fertilization treatment. *Acta Obstet Gynecol Scand*. 2002;81:954.
255. Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. Intercycle variability of ovarian reserve tests: results of a prospective randomized study. *Hum Reprod*. 2004;19:590.
256. Hannoun A, Abu Musa A, Awwad J, Kaspar H, Khalil A. Clomiphene citrate challenge test: cycle to cycle variability of cycle day 10 follicle stimulating hormone level. *Clin Exp Obstet Gynecol*. 1998;25:155.
257. Hendriks DJ, Broekmans FJ, Bancsi LF, de Jong FH, Looman CW, Te Velde ER. Repeated clomiphene citrate challenge testing in the prediction of outcome in IVF: a comparison with basal markers for ovarian reserve. *Hum Reprod*. 2005;20:163.
258. Hendriks DJ, Mol BW, Bancsi LF, te Velde ER, Broekmans FJ. The clomiphene citrate challenge test for the prediction of poor ovarian response and nonpregnancy in patients undergoing in vitro fertilization: a systematic review. *Fertil Steril*. 2006;86:807.
259. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. Antral follicle count, anti-müllerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? *Br J Obstet Gynaecol*. 2005;112:1384.

260. Muttukrishna S, Suharjono H, McGarrigle H, Sathanandan M. Inhibin B and anti-Müllerian hormone: markers of ovarian response in IVF/ICSI patients? *Br J Obstet Gynaecol*. 2004;111:1248.
261. Balasch J, Creus M, Fabregues F, et al. Inhibin, follicle-stimulating hormone, and age as predictors of ovarian response in in vitro fertilization cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment. *Am J Obstet Gynecol*. 1996;175:1226.
262. Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum Müllerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril*. 2002; 77:468.
263. Hall JE, Welt CK, Cramer DW. Inhibin A and inhibin B reflect ovarian function in assisted reproduction but are less useful at predicting outcome. *Hum Reprod*. 1999;14:409.
264. Creus M, Penarrubia J, Fabregues F, et al. Day 3 serum inhibin B and FSH and age as predictors of assisted reproduction treatment outcome. *Hum Reprod*. 2000;15:2341.
265. Smeenk JM, Sweep FC, Zielhuis GA, Kremer JA, Thomas CM, Braat DD. Antimüllerian hormone predicts ovarian responsiveness, but not embryo quality or pregnancy, after in vitro fertilization or intracytoplasmic sperm injection. *Fertil Steril*. 2007;87:223.
266. Vigier B, Tran D, Legeai L, Bezard J, Josso N. Origin of anti-Müllerian hormone in bovine freemartin fetuses. *J Reprod Fertil*. 1984;70:473.
267. Durlinger AL, Kramer P, Karels B, et al. Control of primordial follicle recruitment by anti-Müllerian hormone in the mouse ovary. *Endocrinology*. 1999;140:5789.
268. Durlinger AL, Gruijters MJ, Kramer P, et al. Anti-Müllerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. *Endocrinology*. 2001;142:4891.
269. Weenen C, Laven JS, Von Bergh AR, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod*. 2004;10:77.
270. Durlinger AL, Visser JA, Themmen APN. Regulation of ovarian function: the role of anti-Müllerian hormone. *Reproduction*. 2002;124:601.
271. Fanchin R, Schonauer LM, Righini C, Frydman N, Frydman R, Taieb J. Serum anti-Müllerian hormone dynamics during controlled ovarian hyperstimulation. *Hum Reprod*. 2003;18:328.
272. Catteau-Jonard S, Pigny P, Reyss AC, Decanter C, Poncelet E, Dewailly D. Changes in serum anti-müllerian hormone level during low-dose recombinant follicular-stimulating hormone therapy for anovulation in polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2007;92:4138.
273. Kevenaar ME, Meerasahib MF, Kramer P, et al. Serum anti-müllerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinology*. 2006;147:3228.
274. Sowers MR, Eyvazzadeh AD, McConnell D, et al. Anti-müllerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. *J Clin Endocrinol Metab*. 2008;93:3478.
275. van Rooij IA, Tonkelaar I, Broekmans FJ, et al. Anti-müllerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause*. 2004;11:601.
276. van Rooij IA, Broekmans FJ, Scheffer GJ, et al. Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril*. 2005;83:979.
277. 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.
278. Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-Müllerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod*. 2005;20:923.
279. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. *Hum Reprod*. 2007;22:1837.
280. 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.
281. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Müllerian hormone: ovarian reserve testing and its potential implications. *Hum Reprod Update*. 2014;20:688.
282. Freour T, Mirallie S, Bach-Ngohou K, Denis M, Barriere P, Masson D. Measurement of serum anti-Müllerian hormone by Beckman Coulter ELISA and DSL ELISA: comparison and relevance in assisted reproduction technology (ART). *Clin Chim Acta*. 2007;375:162.
283. Dorgan JF, Spittle CS, Egleston BL, Shaw CM, Kahle LL, Brinton LA. Assay reproducibility and within-person variation of Müllerian inhibiting substance. *Fertil Steril*. 2010;94:301.
284. van Rooij IA, Broekmans FJ, te Velde ER, et al. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod*. 2002;17:3065.
285. Silberstein T, MacLaughlin DT, Shai I, et al. Müllerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology. *Hum Reprod*. 2006;21:159.
286. Ebner T, Sommergruber M, Moser M, Shebl O, Schreier-Lechner E, Tews G. Basal level of anti-Müllerian hormone is associated with oocyte quality in stimulated cycles. *Hum Reprod*. 2006;21:2022.
287. Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT Jr, Seli E. Diminished ovarian reserve and poor response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in performance. *Hum Reprod*. 2018;33:1489.
288. Pacheco A, Cruz M, Iglesias C, García-Velasco JA. Very low anti-müllerian hormone concentrations are not an independent predictor of embryo quality and pregnancy rate. *Reprod Biomed Online*. 2018;37(1):113.
289. Ficicioglu C, Kutlu T, Baglam E, Bakacak Z. Early follicular antimüllerian hormone as an indicator of ovarian reserve. *Fertil Steril*. 2006;85:592.
290. Gnoth C, Schuring AN, Friol K, Tigges J, Mallmann P, Godehardt E. Relevance of anti-Müllerian hormone measurement in a routine IVF program. *Hum Reprod*. 2008;23:1359.
291. Penarrubia J, Fabregues F, Manau D, et al. Basal and stimulation day 5 anti-Müllerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist–gonadotropin treatment. *Hum Reprod*. 2005;20:915.
292. Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev*. 1996;17:121.
293. Pache TD, Wladimiroff JW, de Jong FH, Hop WC, Fauser BC. Growth patterns of nondominant ovarian follicles during the normal menstrual cycle. *Fertil Steril*. 1990;54:638.
294. Ruess ML, Kline J, Santos R, Levin B, Timor-Tritsch I. Age and the ovarian follicle pool assessed with transvaginal ultrasonography. *Am J Obstet Gynecol*. 1996;174:624.
295. Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril*. 1999;72:845.
296. Meldrum DR, Chetkowski RJ, Steingold KA, Randle D. Transvaginal ultrasound scanning of ovarian follicles. *Fertil Steril*. 1984;42:803.
297. Bancsi LF, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Impact of repeated antral follicle counts on the prediction of poor ovarian response in women undergoing in vitro fertilization. *Fertil Steril*. 2004;81:35.
298. Hansen KR, Morris JL, Thyer AC, Soules MR. Reproductive aging and variability in the ovarian antral follicle count: application in the clinical setting. *Fertil Steril*. 2003;80:577.
299. Scheffer GJ, Broekmans FJ, Bancsi LF, Habbema JD, Looman CW, Te Velde ER. Quantitative transvaginal two- and three-dimensional sonography of the ovaries: reproducibility of antral follicle counts. *Ultrasound Obstet Gynecol*. 2002;20:270.
300. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev*. 2009;30:465.
301. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril*. 2005;83:291.
302. Hirshfield AN. Rescue of atretic follicles in vitro and in vivo. *Biol Reprod*. 1989;40:181.
303. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil Steril*. 2002;77:328.
304. Frattarelli JL, Lauria-Costab DF, Miller BT, Bergh PA, Scott RT. Basal antral follicle number and mean ovarian diameter predict cycle cancellation and ovarian responsiveness in assisted reproductive technology cycles. *Fertil Steril*. 2000;74:512.
305. Frattarelli JL, Levi AJ, Miller BT, Segars JH. A prospective assessment of the predictive value of basal antral follicles in in vitro fertilization cycles. *Fertil Steril*. 2003;80:350.
306. Chang MY, Chiang CH, Hsieh TT, Soong YK, Hsu KH. Use of the antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril*. 1998;69:505.
307. Ng EH, Tang OS, Ho PC. The significance of the number of antral follicles prior to stimulation in predicting ovarian responses in an IVF programme. *Hum Reprod*. 2000;15:1937.
308. Kupesic S, Kurjak A, Bjelos D, Vujisic S. Three-dimensional ultrasonographic ovarian measurements and in vitro fertilization outcome are related to age. *Fertil Steril*. 2003;79:190.
309. Lass A, Silye R, Abrams D-C, et al. Follicular density in ovarian biopsy of infertile women: a novel method to assess ovarian reserve. *Hum Reprod*. 1997;12:1028.
310. Lass A, Skull J, McVeigh E, Margara R, Winston RM. Measurement of ovarian volume by transvaginal sonography before human menopausal gonadotrophin superovulation for in-vitro fertilization can predict poor response. *Hum Reprod*. 1997;12:294.
311. Jayaprakasan K, Campbell B, Hopkisson J, Clewes J, Johnson I, Raine-Fenning N. Establishing the intercycle variability of three-dimensional ultrasonographic predictors of ovarian reserve. *Fertil Steril*. 2008;90:2126.
312. Elter K, Sismanoglu A, Durmusoglu F. Intercycle variabilities of basal antral follicle count and ovarian volume in subfertile women and their relationship to reproductive aging: a prospective study. *Gynecol Endocrinol*. 2005;20:137.
313. Merce LT, Gomez B, Engels V, Bau S, Bajo JM. Intraobserver and interobserver reproducibility of ovarian volume, antral follicle count, and vascularity indices obtained with transvaginal 3-dimensional ultrasonography, power Doppler angiography, and the virtual organ computer-aided analysis imaging program. *J Ultrasound Med*. 2005;24:1279.
314. Jarvela IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Quantification of ovarian power Doppler signal with three-dimensional ultrasonography to predict response during in vitro fertilization. *Obstet Gynecol*. 2003;102:816.

315. Schild RL, Knobloch C, Dorn C, Fimmers R, van der Ven H, Hansmann M. The role of ovarian volume in an in vitro fertilization programme as assessed by 3D ultrasound. *Arch Gynecol Obstet*. 2001;265:67.
316. Kwee J, Elting ME, Schats R, McDonnell J, Lambalk CB. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. *Reprod Biol Endocrinol*. 2007;5:9.
317. Merce LT, Barco MJ, Bau S, Troyano JM. Prediction of ovarian response and IVF/ICSI outcome by three-dimensional ultrasonography and power Doppler angiography. *Eur J Obstet Gynecol Reprod Biol*. 2007;132:93.
318. Fanchin R, de Ziegler D, Olivennes F, Taieb J, Dzik A, Frydman R. Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a simple and reliable screening test for detecting 'poor responders' in in-vitro fertilization. *Hum Reprod*. 1994;9:1607.
319. Seifer DB, Lambert-Messerlian G, Hogan JW, Gardiner AC, Blazar AS, Berk CA. Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertil Steril*. 1997;67:110.
320. Corson SL, Gutmann J, Batzer FR, Wallace H, Klein N, Soules MR. Inhibin-B as a test of ovarian reserve for infertile women. *Hum Reprod*. 1999;14:2818.
321. Dzik A, Lambert-Messerlian G, Izzo VM, Soares JB, Pinotti JA, Seifer DB. Inhibin B response to EFORT is associated with the outcome of oocyte retrieval in the subsequent in vitro fertilization cycle. *Fertil Steril*. 2000;74:1114.
322. Kwee J, Elting MW, Schats R, Bezemer PD, Lambalk CB, Schoemaker J. Comparison of endocrine tests with respect to their predictive value on the outcome of ovarian hyperstimulation in IVF treatment: results of a prospective randomized study. *Hum Reprod*. 2003;18:1422.
323. Winslow KL, Toner JP, Brzyski RG, Oehninger SC, Acosta AA, Muasher SJ. The gonadotropin-releasing hormone agonist stimulation test—a sensitive predictor of performance in the flare-up in vitro fertilization cycle. *Fertil Steril*. 1991;56:711.
324. Galtier-Dereure F, De Bouard V, Picto MC, et al. Ovarian reserve test with the gonadotrophin-releasing hormone agonist buserelin: correlation with in-vitro fertilization outcome. *Hum Reprod*. 1996;11:1393.
325. Ranieri DM, Phophong P, Khadum I, Meo F, Davis C, Serhal P. Simultaneous evaluation of basal FSH and oestradiol response to GnRH analogue (F-G-test) allows effective drug regimen selection for IVF. *Hum Reprod*. 2001;16:673.
326. Verhagen TE, Hendriks DJ, Bancsi LF, Mol BW, Broekmans FJ. The accuracy of multivariate models predicting ovarian reserve and pregnancy after in vitro fertilization: a meta-analysis. *Hum Reprod Update*. 2008;14:95.
327. van Tilborg TC, Oudshoorn SC, Eijkemans MJC, et al. Individualized FSH dosing based on ovarian reserve testing in women starting IVF/ICSI: a multicentre trial and cost-effectiveness analysis. *Hum Reprod*. 2017;32:2485.
328. van Tilborg TC, Torrance HL, Oudshoorn SC, et al; OPTIMIST study group. Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 1: the predicted poor responder. *Hum Reprod*. 2017;32:2496.
329. Oudshoorn SC, van Tilborg TC, Eijkemans MJC, et al; OPTIMIST Study Group. Individualized versus standard FSH dosing in women starting IVF/ICSI: an RCT. Part 2: the predicted hyper responder. *Hum Reprod*. 2017;32:2506.
330. Harrison RF, Jacob S, Spillane H, Mallon E, Hennelly B. A prospective randomized clinical trial of differing starter doses of recombinant follicle-stimulating hormone (follitropin-beta) for first time in vitro fertilization and intracytoplasmic sperm injection treatment cycles. *Fertil Steril*. 2001;75:23.
331. Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. *Hum Reprod*. 2005;20:611.
332. Popovic-Todorovic B, Loft A, Ziebe S, Andersen AN. Impact of recombinant FSH dose adjustments on ovarian response in the second treatment cycle with IVF or ICSI in "standard" patients treated with 150 IU/day during the first cycle. *Acta Obstet Gynecol Scand*. 2004;83:842.
333. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. *Hum Reprod Update*. 2003;9:61.
334. Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. *Fertil Steril*. 2009;91:749.
335. Barrenetxea G, Agirregoikoa JA, Jimenez MR, de Larruzea AL, Ganzabal T, Carbonero K. Ovarian response and pregnancy outcome in poor-responder women: a randomized controlled trial on the effect of luteinizing hormone supplementation on in vitro fertilization cycles. *Fertil Steril*. 2008;89:546.
336. Kahn JL, Wu H, Gerber R, et al. Protocol change improves live birth and recurrent cycle cancellation rates after a previous IVF cycle cancellation: an analysis of 13 000 autologous cycles reported to SART CORS. *Hum Reprod*. 2024;39:1504–1511.
337. Scott RT Jr, Elkind-Hirsch KE, Styne-Gross A, Miller KA, Frattarelli JL. 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.
338. van Rooij IA, de Jong E, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. High follicle-stimulating hormone levels should not necessarily lead to the exclusion of subfertile patients from treatment. *Fertil Steril*. 2004; 81:1478.
339. Lashen H, Ledger W, Lopez-Bernal A, Barlow D. Poor responders to ovulation induction: is proceeding to in-vitro fertilization worthwhile? *Hum Reprod*. 1999;14:964.
340. Zhen XM, Qiao J, Li R, Wang LN, Liu P. The clinical analysis of poor ovarian response in in-vitro-fertilization embryo-transfer among Chinese couples. *J Assist Reprod Genet*. 2008;25:17.
341. Scott RT Jr, Leonardi MR, Hofmann GE, Illions EH, Neal GS, Navot D. A prospective evaluation of clomiphene citrate challenge test screening of the general infertility population. *Obstet Gynecol*. 1993;82:539.
342. Scott RT, Opsahl MS, Leonardi MR, Neall GS, Illions EH, Navot D. Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Hum Reprod*. 1995;10:1706.
343. Leach RE, Moghissi KS, Randolph JF, et al. Intensive hormone monitoring in women with unexplained infertility: evidence for subtle abnormalities suggestive of diminished ovarian reserve. *Fertil Steril*. 1997;68:413.
344. Hofmann GE, Sosnowski J, Scott RT, Thie J. Efficacy of selection criteria for ovarian reserve screening using the clomiphene citrate challenge test in a tertiary fertility center population. *Fertil Steril*. 1996;66:49.
345. National Center for Health Statistics. Prevalence of obesity and Severe obesity among adults: United States, 2017–2018. https://www.cdc.gov/nchs/products/databriefs/db360.htm
346. Practice Committee of the American Society for Reproductive Medicine. Obesity and reproduction. *Fertil Steril*. 2021;116(5):1266.
347. Practice Committee of the American Society for Reproductive Medicine. Tobacco or marijuana use and infertility: a committee opinion. *Fertil Steril*. 2024;121(4):589–603.
348. Hull MG, North K, Taylor H, Farrow A, Ford WC; Avon Longitudinal Study of Pregnancy and Childhood Study Team. Delayed conception and active and passive smoking. *Fertil Steril*. 2000;74:725.
349. Laurent SL, Thompson SJ, Addy C, Garrison CZ, Moore EE, An epidemiologic study of smoking and primary infertility in women. *Fertil Steril*. 1992;57:565.
350. Baird DD, Wilcox AJ. Cigarette smoking associated with delayed conception. *JAMA*. 1985;253:2979.
351. Suonio S, Saarikoski S, Kauhanen O, Metsapelto A, Terho J, Vohlonen I. Smoking does affect fecundity. *Eur J Obstet Gynecol Reprod Biol*. 1990;34:89.
352. Howe G, Westhoff C, Vessey M, Yeates D. Effects of age, cigarette smoking, and other factors on fertility: findings in a large prospective study. *Br Med J (Clin Res Ed)*. 1985;290:1697.
353. Cooper GS, Baird DD, Hulka BS, Weinberg CR, Savitz DA, Hughes CL Jr. Follicle-stimulating hormone concentrations in relation to active and passive smoking. *Obstet Gynecol*. 1995;85:407.
354. El-Nemr A, Al-Shawaf T, Sabatini L, Wilson C, Lower AM, Grudzinskas JG. Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. *Hum Reprod*. 1998;13:2192.
355. Sharara FI, Beatse SN, Leonardi MR, Navot D, Scott RT Jr. Cigarette smoking accelerates the development of diminished ovarian reserve as evidenced by the clomiphene citrate challenge test. *Fertil Steril*. 1994;62:257.
356. Rowland AS, Baird DD, Long S, et al. Influence of medical conditions and lifestyle factors on the menstrual cycle. *Epidemiology*. 2002;13:668.
357. Zenzes MT, Wang P, Casper RF. Cigarette smoking may affect meiotic maturation of human oocytes. *Hum Reprod*. 1995;10:3213.
358. Zenzes MT, Reed TE, Wang P, Klein J. Cotinine, a major metabolite of nicotine, is detectable in follicular fluids of passive smokers in in vitro fertilization therapy. *Fertil Steril*. 1996;66:614.
359. Zenzes MT, Krishnan S, Krishnan B, Zhang H, Casper RF. Cadmium accumulation in follicular fluid of women in in vitro fertilization-embryo transfer is higher in smokers. *Fertil Steril*. 1995;64:599.
360. Zenzes MT, Bielecki R, Reed TE. Detection of benzo(a)pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. *Fertil Steril*. 1999;72:330.
361. Zenzes MT, Puy LA, Bielecki R, Reed TE. Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. *Mol Hum Reprod*. 1999;5:125.
362. Liu Y, Chen S, Pang D, et al. Effects of paternal expo- sure to cigarette smoke on sperm DNA methylation and long-term meta- bolic syndrome in offspring. *Epigenetics Chromatin*. 2022;15:3.
363. Augood C, Duckitt K, Templeton AA. Smoking and female infertility: a systematic review and meta-analysis. *Hum Reprod*. 1998;13:1532.
364. Centers for Disease Control and Prevention. Preconception and interconception health status of women who recently gave birth to a live-born infant—Pregnancy Risk Assessment Monitoring System (PRAMS)—United States, 26 reporting areas, 2004. *MMWR Morb Mortal Wkly Rep*. 2007;56(10):1.
365. Centers for Disease Control and Prevention.Current cigarette smoking among adults in the United States. Accessed August 16, 2024. https://www.cdc.gov/tobacco/data_statistics/fact_sheets/adult_data/cig_smoking/index.htm
366. Fiore MC. US public health service clinical practice guideline: treating tobacco use and dependence. *Respir Care*. 2000;45:1200.
367. Kasman AM, Thoma ME, McLain AC, Eisenberg ML. Association between use of marijuana and time to pregnancy in men and women: findings from the National Survey of Family Growth. *Fertil Steril*. 2018;109(5):866–871.
368. Wise LA, Wesselink AK, Hatch EE, et al. Marijuana use and fecundability in a North American preconception cohort study. *J Epidemiol Community Health*. 2018;72(3):208–215.
369. Braillon A, Bewley S. Committee Opinion No. 722: marijuana use during pregnancy and lactation. *Obstet Gynecol*. 2017;130(4):e205–e209.
370. Smith CG, Asch RH. Drug abuse and reproduction. *Fertil Steril*. 1987;48:355.
371. Bracken MB, Eskenazi B, Sachse K, McSharry J-E, Hellenbrand K, Leon-Summers L. Association of cocaine use with sperm concentration, motility, and morphology. *Fertil Steril*. 1990;53:315.

372. Mueller BA, Daling JR, Weiss NS, Moore DE. Recreational drug use and the risk of primary infertility. *Epidemiology*. 1990;1:195.
373. Grodstein F, Goldman MB, Cramer DW. Infertility in women and moderate alcohol use. *Am J Public Health*. 1994;84:1429.
374. Tolstrup JS, Kjaer SK, Holst C, et al. Alcohol use as predictor for infertility in a representative population of Danish women. *Acta Obstet Gynecol Scand*. 2003;82:744.
375. Greenlee AR, Arbuckle TE, Chyou PH. Risk factors for female infertility in an agricultural region. *Epidemiology*. 2003;14:429.
376. Nagy F, Pendergrass PB, Bowen DC, Yeager JC. A comparative study of cytological and physiological parameters of semen obtained from alcoholics and nonalcoholics. *Alcohol*. 1986;21:17.
377. Anwar MY, Marcus M, Taylor KC. The association between alcohol intake and fecundability during menstrual cycle phases. *Hum Reprod*. 2021;36(9):2538–2548.
378. Fan D, Liu L, Xia Q, et al. Female alcohol consumption and fecundability: a systematic review and dose-response meta-analysis. *Sci Rep*. 2017;7(1):13815.
379. Rao W, Li Y, Li N, Yao Q, Li Y. The association between caffeine and alcohol consumption and IVF/ICSI outcomes: a systematic review and dose-response meta-analysis. *Acta Obstet Gynecol Scand*. 2022;101(12):1351–1363.
380. Hakim RB, Gray RH, Zacur H. Alcohol and caffeine consumption and decreased fertility. *Fertil Steril*. 1998;70:632.
381. Bolumar F, Olsen J, Rebagliato M, Bisanti L; European Study Group on Infertility and Subfecundity. Caffeine intake and delayed conception: a European multicenter study on infertility and subfecundity. *Am J Epidemiol*. 1997;145:324.
382. Caan B, Quesenberry CP Jr, Coates AO. Differences in fertility associated with caffeinated beverage consumption. *Am J Public Health*. 1998;88:270.
383. Cnattingius S, Signorello LB, Anneren G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. *N Engl J Med*. 2000;343:1839.
384. American College of Obstetricians and Gynecologists, Committee on Obstetric Practice. Moderate Caffeine Consumption During Pregnancy. Committee Opinion No. 462. Washington, DC: American College of Obstetricians and Gynecologists; 2010. Reaffirmed 2020. Accessed August 10, 2025. https://www.acog.org/-/media/project/acog/acogorg/clinical/files/committee-opinion/articles/2010/08/moderate-caffeine-consumption-during-pregnancy.pdf
385. Hatch EE, Wise LA, Mikkelsen EM, et al. Caffeinated beverage and soda consumption and time to pregnancy. *Epidemiology*. 2012;23(3):393–401.
386. Wesselink AK, Wise LA, Rothman KJ, et al. Caffeine and caffeinated beverage consumption and fecundability in a preconception cohort. *Reprod Toxicol*. 2016;62:39–45.
387. Purdue-Smithe AC, Kim K, Schliep KC, et al. Preconception caffeine metabolites, caffeinated beverage intake, and fecundability. *Am J Clin Nutr*. 2022;115(4):122–-1236.
388. Hruska KS, Furth PA, Seifer DB, Sharara FI, Flaws JA. Environmental factors in infertility. *Clin Obstet Gynecol*. 2000;43:821.
389. Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Reproductive Endocrinology and Infertility. Optimizing natural fertility: a committee opinion. *Fertil Steril*. 2022;117(1):53–63.
390. Stevens VC. Some reproductive studies in the baboon. *Hum Reprod Update*. 1997;3:533.
391. 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.
392. Guttmacher AF. Factors affecting normal expectancy of conception. *JAMA*. 1956;161:855.
393. Evers JL. Female subfertility. *Lancet*. 2002;360:151.
394. Wilcox AJ, Weinberg CR, Baird DD. Post-ovulatory ageing of the human oocyte and embryo failure. *Hum Reprod*. 1998;13:394.
395. Dunson DB, Baird DD, Wilcox AJ, Weinberg CR. Day-specific probabilities of clinical pregnancy based on two studies with imperfect measures of ovulation. *Hum Reprod*. 1999;14:1835.
396. Miller PB, Soules MR. The usefulness of a urinary LH kit for ovulation prediction during menstrual cycles of normal women. *Obstet Gynecol*. 1996;87:13.
397. Nielsen MS, Barton SD, Hatasaka HH, Stanford JB. Comparison of several one-step home urinary luteinizing hormone detection test kits to OvuQuick. *Fertil Steril*. 2001;76:384.
398. Manders M, McLindon L, Schulze B, Beckmann MM, Kremer JA, Farquhar C. Timed intercourse for couples trying to conceive. *Cochrane Database Syst Rev*. 2015;(3):CD011345.
399. Agarwal SK, Haney AF. Does recommending timed intercourse really help the infertile couple? *Obstet Gynecol*. 1994;84:307.
400. Miller JH, Weinberg RK, Canino NL, Klein NA, Soules MR. The pattern of infertility diagnoses in women of advanced reproductive age. *Am J Obstet Gynecol*. 1999;181:952.
401. Maheshwari A, Hamilton M, Bhattacharya S. Effect of female age on the diagnostic categories of infertility. *Hum Reprod*. 2008;23:538.
402. Snick HK, Snick TS, Evers JL, Collins JA. The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. *Hum Reprod*. 1997;12:1582.
403. Hull MG, Glazener CM, Kelly NJ, et al. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)*. 1985;291:1693.
404. Collins JA, Burrows EA, Wilan AR. The prognosis for live birth among untreated infertile couples. *Fertil Steril*. 1995;64:22.
405. Sundstrom I, Ildgruben A, Hogberg U. Treatment-related and treatment-independent deliveries among infertile couples, a long-term follow-up. *Acta Obstet Gynecol Scand*. 1997;76:238.
406. Eimers JM, te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril*. 1994;61:44.
407. Bostofte E, Bagger P, Michael A, Stakemann G. Fertility prognosis for infertile couples. *Fertil Steril*. 1993;59:102.
408. Practice Committee of the American Society for Reproductive Medicine. Fertility evaluation of infertile women: a committee opinion. *Fertil Steril*. 2021;116(5):1255–1265.
409. Stubblefield P, Monson R, Schoenbaum S, Wolfson CE, Cookson DJ, Ryan KJ. Fertility after induced abortion: a prospective follow-up study. *Obstet Gynecol*. 1984;62:186.
410. Frank P, McNamee R, Hannaford PC, Kay CR, Hirsch S. The effect of induced abortion on subsequent fertility. *Br J Obstet Gynaecol*. 1993;100:575.
411. U.S. Preventive Services Task Force. Cervical cancer: screening. Published August 21, 2018. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/cervical-cancer-screening
412. American College of Obstetricians and Gynecologists. Committee Opinion No. 690: carrier screening in the age of genomic medicine. *Obstet Gynecol*. 2017;129(3):e35.
413. Gregg AR, Aarabi M, Klugman S, et al; ACMG Professional Practice and Guidelines Committee. Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG) [published correction appears in *Genet Med*. 2021;23(10):2015]. *Genet Med*. 2021;23(10):1793–1806..
414. Practice Committee of the American Society for Reproductive Medicine. Current recommendations for vaccines for patients planning pregnancy: a committee opinion. *Fertil Steril*. 2024;122(1):62–67.
415. Soares RC, Siqueira MM, Toscano CM, et al. Follow-up study of unknowingly pregnant women vaccinated against rubella in Brazil, 2001–2002. *J Infect Dis*. 2011;204:S729.
416. Workowski KA, Bachmann LH, Chan PA, et al. Sexually transmitted infections treatment guidelines, 2021. *MMWR Recomm Rep*. 2021;70(4):1–187.
417. Practice Committee of the American Society for Reproductive Medicine and the Practice Committee for the Society for Assisted Reproductive Technologies. Gamete and embryo donation guidance. *Fertil Steril*. 2024;122(5):1395–1410.
418. Schlegel PN, Sigman M, Collura B, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. *Fertil Steril*. 2021;115(1):54–61.
419. Bates GW, Garza DE, Garza MM. Clinical manifestations of hormonal changes in the menstrual cycle. *Obstet Gynecol Clin North Am*. 1990;17:299.
420. Quagliarello J, Arny M. Inaccuracy of basal body temperature charts in predicting urinary luteinizing hormone surges. *Fertil Steril*. 1986;45:334.
421. Luciano AA, Peluso J, Koch E, Maier D, Kuslis S, Davison E. Temporal relationship and reliability of the clinical, hormonal, and ultrasonographic indices of ovulation in infertile women. *Obstet Gynecol*. 1990;75:412.
422. Abraham GE, Maroulis GB, Marshall JR. Evaluation of ovulation and corpus luteum function using measurements of plasma progesterone. *Obstet Gynecol*. 1974;44:522.
423. Wathen NC, Perry L, Lilford RJ, Chard T. Interpretation of single progesterone measurement in diagnosis of anovulation and defective luteal phase: observations on analysis of the normal range. *Br Med J*. 1984;288:7.
424. Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremmer WJ. Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. *J Clin Endocrinol Metab*. 1989;69:804.
425. Soules MR, Clifton DK, Cohen NL, Bremner WJ, Steiner RA. Luteal phase deficiency: abnormal gonadotropin and progesterone secretion patterns. *J Clin Endocrinol Metab*. 1989;69:813.
426. Li TC, Lenton EA, Dockery P, Cooke ID. A comparison of some clinical and endocrinological features between cycles with normal and defective luteal phases in women with unexplained infertility. *Hum Reprod*. 1990;5:805.
427. Cooke ID, Morgan CA, Parry TE. Correlation of endometrial biopsy and plasma progesterone levels in infertile women. *J Obstet Gynaecol Br Commonw*. 1972;79:647.
428. Shepard MK, Senturia YD. Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertil Steril*. 1977;28:541.
429. Rosenfeld DL, Chudow S, Bronson RA. Diagnosis of luteal phase inadequacy. *Obstet Gynecol*. 1980;56:193.
430. Cumming DC, Honore LH, Scott JZ, Williams KP. The late luteal phase in infertile women: comparison of simultaneous endometrial biopsy and progesterone levels. *Fertil Steril*. 1985;43:715.
431. Li T-C, Lenton EA, Dockery P, Rogers AW, Cooke ID. The relation between daily salivary progesterone profile and endometrial development in the luteal phase of fertile and infertile women. *Br J Obstet Gynaecol*. 1989;96:445.
432. Jordan J, Craig K, Clifton DK, Soules MJ. Luteal phase defect: the sensitivity and specificity of diagnostic methods in common use. *Fertil Steril*. 1994;62:54.
433. Hull MG, Savage PE, Bromham DR, Ismail AA, Morris AF. The value of a single serum progesterone measurement in the midluteal phase as a criterion of a potentially fertile cycle ("ovulation") derived from treated and untreated conception cycles. *Fertil Steril*. 1982;37:355.
434. Ellinwood WE, Norman RL, Spies HG. Changing frequency of pulsatile luteinizing hormone and progesterone secretion during the luteal phase of the menstrual cycle of rhesus monkeys. *Biol Reprod*. 1984;31:714.

435. Filicori M, Butler JP, Crowley WF. Neuroendocrine regulation of the corpus luteum in the human: evidence for pulsatile progesterone secretion. *J Clin Invest*. 1984;73:1638.
436. McGovern PG, Myers ER, Silva S, et al. Absence of secretory endometrium after false-positive home urine luteinizing hormone testing. *Fertil Steril*. 2004;82:1273.
437. Martinez AR, Bernardus RE, Vermeiden JP, Schoemaker J. Reliability of home urinary LH tests for timing of insemination: a consumer's study. *Hum Reprod*. 1992;7:751.
438. Johnson S, Ellis J, Godbert S, Ali S, Zinaman M. Comparison of a digital ovulation test with three popular line ovulation tests to investigate user accuracy and certainty. *Expert Opin Med Diagn*. 2011;5(6):467.
439. Martinez AR, Bernardus RE, Vermeiden JP, Schoemaker J. Time schedules of intrauterine insemination after urinary luteinizing hormone surge detection and pregnancy results. *Gynecol Endocrinol*. 1994;8:1.
440. Meyer WR, Smith PM, Clark MR, Cusmano LL, Fritz MA. Therapeutic cup insemination with cryopreserved donor sperm: prognostic value of cervical mucus score at insemination and the number of motile sperm in mucus at 24 hours. *Fertil Steril*. 1996;66:435.
441. Tiplady S, Jones G, Campbell M, Johnson S, Ledger W. Home ovulation tests and stress in women trying to conceive: a randomized controlled trial. *Hum Reprod*. 2013;28(1):138.
442. Jones G, Carlton J, Weddell S, Johnson S, Ledger WL. Women's experiences of ovulation testing: a qualitative analysis. *Reprod Health*. 2015;12:116.
443. Jones GS. Some newer aspects of the management of infertility. *JAMA*. 1949; 1411:1123.
444. Jones GS. The luteal phase defect. *Fertil Steril*. 1976;27:351.
445. Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. *Am J Anat*. 1956;98:435.
446. Navot RW, Scott RT, Doresch K, Veeck LL, Liu HC, Rosenwaks Z. The window of embryo transfer and the efficiency of human conception *in vitro*. *Fertil Steril*. 1991;55:114.
447. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. *Fertil Steril*. 1992;58:537.
448. Fritz MA, Hess DL, Patton PE. Influence of corpus luteum age on the steroidogenic response to exogenous human chorionic gonadotropin in normal cycling women. *Am J Obstet Gynecol*. 1992;167:709.
449. Lessey BA, Fritz MA. Defective luteal function. In: Fraser IS, ed. *Estrogen and Progestogens in Clinical Practice*. Churchill Livingstone; 1998:437–454.
450. Tay PY, Lenton EA. The optimum time for exogenous human chorionic gonadotropin to rescue the corpus luteum. *J Assist Reprod Genet*. 1999;16:495.
451. Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med*. 1999;340:1796.
452. Noyes RW, Hertig AW, Rock J. Dating the endometrial biopsy. *Fertil Steril*. 1950;1:3.
453. Shoupe D, Mishell DR Jr, Lacarra M, et al. Correlation of endometrial maturation with four methods of estimating day of ovulation. *Obstet Gynecol*. 1989;73:88.
454. Noyes RW, Haman JO. Accuracy of endometrial dating: correlation of endometrial dating with basal body temperature and menses. *Fertil Steril*. 1953;4:504.
455. Andrews WC. Luteal phase defects. *Fertil Steril*. 1979;32:501.
456. Duggan MA, Brashert P, Ostor A, et al. The accuracy and interobserver reproducibility of endometrial dating. *Pathology*. 2001;33:292.
457. Aksel S. Sporadic and recurrent luteal phase defects in cyclic women: comparison with normal cycles. *Fertil Steril*. 1980;33:372.
458. Wentz AC, Kossoy L, Parker RA. The impact of luteal phase inadequacy in an infertile population. *Am J Obstet Gynecol*. 1990;162:937.
459. Li TC, Dockery P, Cooke ID. Endometrial development in the luteal phase of women with various types of infertility: comparison with women of normal fertility. *Hum Reprod*. 1991;6:325.
460. Balasch J, Fabreques F, Creus M, Vanrell JA. The usefulness of endometrial biopsy for luteal phase evaluation in infertility. *Hum Reprod*. 1992;7:973.
461. Peters AJ, Lloyd RP, Coulam CP. Prevalence of out-of-phase endometrial biopsy specimens. *Am J Obstet Gynecol*. 1992;166:1738.
462. Batista MC, Cartledge TP, Merino MJ, et al. Midluteal phase endometrial biopsy does not accurately predict luteal function. *Fertil Steril*. 1993;59:294.
463. Davis OK, Berkeley AS, Naus GJ, Cholst IN, Freedman KS. The incidence of luteal phase defect in normal, fertile women, determined by serial endometrial biopsies. *Fertil Steril*. 1989;51:582.
464. Johannisson E, Parker RA, Landgren BM, Diczfalusy E. Morphometric analysis of the human endometrium in relation to peripheral hormone levels. *Fertil Steril*. 1982;38:564.
465. Munster K, Schmidt L, Helm P. Length and variation in the menstrual cycle–a cross-sectional study from a Danish county. *Br J Obstet Gynaecol*. 1992;99:422.
466. Guermandi E, Vegetti W, Bianchi MM, Uglietti A, Ragni G, Crosignani P. Reliability of ovulation tests in infertile women. *Obstet Gynecol*. 2001;97:92.
467. Lenton EA, Landgren B, Sexton L, Harper R. Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. *Br J Obstet Gynaecol*. 1984;91:681.
468. Scott RT, Snyder RR, Strickland DM, et al. The effect of interobserver variation in dating endometrial histology on the diagnosis of luteal phase defects. *Fertil Steril*. 1988;50:888.
469. Gibson M, Badger GJ, Byrn F, Lee KR, Korson R, Trainer TD. Error in histologic dating of secretory endometrium: variance component analysis. *Fertil Steril*. 1991;56:242.
470. Scott RT, Snyder RR, Bagnall JW, Reed KD, Adair CF, Hensley SD. Evaluation of the impact of intraobserver variability on endometrial dating and the diagnosis of luteal phase defects. *Fertil Steril*. 1993;60:652.
471. Smith S, Hosid S, Scott L. Endometrial biopsy dating: interobserver variation and its impact on clinical practice. *J Reprod Med*. 1995;40:1.
472. Murray MJ, Meyer WR, Zaino RJ, et al. A critical reanalysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating: a systematic study of the secretory phase in normally cycling, fertile women. *Fertil Steril*. 2004;81:1333.
473. Coutifaris C, Myers ER, Guzick DS, et al. Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertil Steril*. 2004;82:1264.
474. Usadi RS, Groll JM, Lessey BA, et al. Endometrial development and function in experimentally induced luteal phase deficiency. *J Clin Endocrinol Metab*. 2008;93:4058.
475. Groll JM, Usadi RS, Lessey BA, Lininger R, Young SL, Fritz MA. Effects of variations in serum estradiol concentrations on secretory endometrial development and function in experimentally induced cycles in normal women. *Fertil Steril*. 2009;92:2058.
476. Osman EK, Wang T, Zhan Y, et al. Varying levels of serum estradiol do not alter the timing of the early endometrial secretory transformation. *Hum Reprod*. 2020;35(7):1637–1647.
477. Young SL, Lessey BA. Progesterone function in human endometrium: clinical perspectives. *Semin Reprod Med*. 2010;28:5.
478. Talbi S, Hamilton AE, Vo KC, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology*. 2006;147:1097.
479. Eun Kwon H, Taylor HS. The role of HOX genes in human implantation. *Ann N Y Acad Sci*. 2004;1034:1.
480. Taylor HS, Arici A, Olive D, Igarashi P. HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest*. 1998;101(7):1379.
481. Taylor HS. The role of HOX genes in human implantation. *Hum Reprod Update*. 2000;6(1):75.
482. Taylor HS, Igarashi P, Olive DL, Arici A. Sex steroids mediate HOXA11 expression in the human peri-implantation endometrium. *J Clin Endocrinol Metab*. 1999;84(3):1129.
483. Lessey BA. Endometrial integrins and the establishment of uterine receptivity. *Hum Reprod*. 1998;13(suppl 3):247.
484. Lindhard A, Bentin-Ley U, Ravn V, et al. Biochemical evaluation of endometrial function at the time of implantation. *Fertil Steril*. 2002;78:221.
485. Apparao KB, Murray MJ, Fritz MA, et al. Osteopontin and its receptor alphavbeta(3) integrin are coexpressed in the human endometrium during the menstrual cycle but regulated differentially. *J Clin Endocrinol Metab*. 2001;86:4991.
486. Carson DD, Lagow E, Thathiah A, et al. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod*. 2002;8:871.
487. Allan G, Campen C, Hodgen G, et al. Identification of genes with differential regulation in primate endometrium during the proliferative and secretory phases of the cycle. *Endocr Res*. 2003;29:53.
488. Kao LC, Germeyer A, Tulac S, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology*. 2003;144:2870.
489. Genbacev OD, Prakobphol A, Foulk RA, et al. Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. *Science*. 2003;299:405.
490. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, et al. A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature. *Fertil Steril*. 2011;95:50.
491. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, et al. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril*. 2013;100:818.
492. Haouzi D, Dechaud H, Assou S, De Vos J, Hamamah S. Insights into human endometrial receptivity from transcriptomic and proteomic data. *Reprod BioMed Online*. 2012;24(1):23–34.
493. Enciso M, Carrascosa JP, Sarasa J, et al. Development of a new comprehensive and reliable endometrial receptivity map (ER Map/ER Grade) based on RT-qPCR gene expression analysis. *Hum Reprod*. 2018;33:220–228.
494. Koot YE, van Hooff SR, Boomsma CM, et al. An endometrial gene expression signature accurately predicts recurrent implantation failure after IVF. *Sci Rep*. 2016;6:19411.
495. Doyle N, Combs JC, Jahandideh S, Wilkinson V, Devine K, O'Brien JE. Live birth after transfer of a single euploid vitrified-warmed blastocyst according to standard timing vs. timing as recommended by endometrial receptivity analysis. *Fertil Steril*. 2022;118(2):314–321.
496. Mei Y, Wang Y, Ke X, Liang X, Lin Y, Wang F. Does endometrial receptivity array improve reproductive outcomes in euploid embryo transfer cycles? a systematic review. *Front Endocrinol (Lausanne)*. 2023;14:1251699.
497. de Crespigny LC, O'Herlihy C, Robinson HP. Ultrasonic observation of the mechanism of human ovulation. *Am J Obstet Gynecol*. 1981;139:636.
498. Ecochard R, Marret H, Rabilloud M, et al. Sensitivity and specificity of ultrasound indices of ovulation in spontaneous cycles. *Eur J Obstet Gynecol Reprod Biol*. 2000;91:59.
499. Petsos P, Chandler C, Oak M, Ratcliffe WA, Wood R, Anderson DC. The assessment of ovulation by a combination of ultrasound and detailed serial hormone profiles in 35 women with long-standing unexplained infertility. *Clin Endocrinol (Oxf)*. 1985;22:739.

500. Daly DC, Soto-Albors C, Walters C, Ying YK, Riddick DH. Ultrasonographic assessment of luteinized unruptured follicle syndrome in unexplained infertility. *Fertil Steril*. 1985;43:62.
501. Priddy AR, Killick SR, Elstein M, et al. The effect of prostaglandin synthetase inhibitors on human preovulatory follicular fluid prostaglandin, thromboxane, and leukotriene concentrations. *J Clin Endocrinol Metab*. 1990;71:235.
502. Smith G, Roberts R, Hall C, Nuki G. Reversible ovulatory failure associated with the development of luteinized unruptured follicles in women with inflammatory arthritis. *Br J Rheumatol*. 1996;35:458.
503. Eggert-Kruse W, Reimann-Andersen J, Rohr G, Pohl S, Tilgen W, Runnebaum B. Clinical relevance of sperm morphology assessment using strict criteria and relationship with sperm-mucus interaction in vivo and in vitro. *Fertil Steril*. 1995;63:612.
504. Bonilla-Musoles F. Scanner electron microscopy of the cervical mucus. *Clin Exp Obstet Gynecol*. 1983;10:151.
505. Overstreet JW, Katz DF, Yudin AI. Cervical mucus and sperm transport in reproduction. *Semin Perinatol*. 1991;15:149.
506. Yudin AI, Hanson FW, Katz DF. Human cervical mucus and its interaction with sperm: fine structural view. *Biol Reprod*. 1989;40:661.
507. Katz DF. Human cervical mucus: research update. *Am J Obstet Gynecol*. 1991;165:1984.
508. Chretien FC. Involvement of the glycoproteic meshwork of cervical mucus in the mechanism of sperm orientation. *Acta Obstet Gynecol Scand*. 2003;82:449.
509. Katz DF, Slade DA, Nakajima ST. Analysis of pre-ovulatory changes in cervical mucus hydration and sperm penetrability. *Adv Contracept*. 1997;13:143.
510. Oei SG, Keirse MJ, Bloemenkamp KW, Helmerhorst FM. European postcoital tests: opinions and practice. *Br J Obstet Gynaecol*. 1995;102:621.
511. Franken DR, Pretorius E, Grobler S, De Wet JI. Important semen parameters during postcoital testing. *Arch Androl*. 1985;14:213.
512. Hull MG, Savage PE, Bromham DR. Prognostic value of the postcoital test: prospective study based on time-specific conception rates. *Br J Obstet Gynaecol*. 1982;89:299.
513. Quagliarello J, Arny M. Intracervical versus intrauterine insemination: correlation of outcome with antecedent postcoital testing. *Fertil Steril*. 1986;46:870.
514. Glazener CM, Coulson C, Lambert PA, et al. The value of artificial insemination with husband's semen in infertility due to failure of postcoital sperm-mucus penetration—controlled trial of treatment. *Br J Obstet Gynaecol*. 1987;94:774.
515. Eimers JM, te Velde ER, Gerritse R, van Kooy RJ, Kremer J, Habbema JD. The validity of the postcoital test for estimating the probability of conceiving. *Am J Obstet Gynecol*. 1994;171:65.
516. Glazener CM, Ford WC, Hull MG. The prognostic power of the post-coital test for natural conception depends on duration of infertility. *Hum Reprod*. 2000;15:1953.
517. Collins JA, So Y, Wilson EH, Wrixon W, Casper RF. The postcoital test as a predictor of pregnancy among 355 infertile couples. *Fertil Steril*. 1984;41:703.
518. Oei SG, Helmerhorst FM, Keirse MJ. When is the post-coital test normal? A critical appraisal. *Hum Reprod*. 1995;10:1711.
519. Fehring RJ. Accuracy of the peak day of cervical mucus as a biological marker of fertility. *Contraception*. 2002;66:231.
520. Hamilton CJ, Evers JL, de Haan J. Ultrasound increases the prognostic value of the postcoital test. *Gynecol Obstet Invest*. 1986;21:80.
521. Kovacs GT, Newman GB, Henson GL. The postcoital test: what is normal? *Br Med J*. 1978;1:818.
522. Cimino C, Borruso AR, Napoli P, Cittadini E. Evaluation of the importance of Chlamydia T. and/or Mycoplasma H. and/or Ureaplasma U. genital infections and of antisperm antibodies in couples affected by muco-semen incompatibility and in couples with unexplained infertility *Acta Eur Fertil*. 1993;24:13.
523. Critchlow CW, Wölner-Hanssen P, Eschenbach DA, et al. Determinants of cervical ectopia and of cervicitis: age, oral contraception, specific cervical infection, smoking, and douching. *Am J Obstet Gynecol*. 1995;173:534.
524. Manhart LE, Critchlow CW, Holmes KK, et al. Mucopurulent cervicitis and *Mycoplasma genitalium*. *J Infect Dis*. 2003;187:650.
525. Tredway DR, Wortham JW Jr, Condon-Mahony M, Baker D, Shane JM. Correlation of postcoital evaluation with in vitro sperm cervical mucus determinations and ureaplasma cultures. *Fertil Steril*. 1985;43:286.
526. Farhi J, Valentine A, Bahadur G, Shenfield F, Steele SJ, Jacobs HS. In-vitro cervical mucus-sperm penetration tests and outcome of infertility treatments in couples with repeatedly negative post-coital tests. *Hum Reprod*. 1995;10:85.
527. Keel BA, Schalue TK. Correlation of the bovine cervical mucus penetration test with human sperm characteristics in 1,406 ejaculates. *Arch Androl*. 2000;44:109.
528. Bateman BG, Nunley WC Jr, Kolp LA. Exogenous estrogen therapy for the treatment of clomiphene citrate-induced cervical mucus abnormalities: is it effective? *Fertil Steril*. 1990;54:577.
529. Check JH, Adelson HG, Wu CH. Improvement of cervical factor with guaifenesin. *Fertil Steril*. 1982;37:707.
530. Ansari AH, Gould KG, Ansari VM. Sodium bicarbonate douching for improvement of the postcoital test. *Fertil Steril*. 1980;33:608.
531. Friedman A, Haas S, Kredentser J, Stewart E, Schiff I. A controlled trial of intrauterine insemination for cervical factor and male factor: a preliminary report. *Int J Fertil*. 1989;34:199.
532. Kirby CA, Flaherty SP, Godfrey BM, Warnes GM, Matthews CD. A prospective trial of intrauterine insemination of motile spermatozoa versus timed intercourse. *Fertil Steril*. 1991;56:102.
533. te Velde ER, van Kooy RJ, Waterreus JJ. Intrauterine insemination of washed husband's spermatozoa: a controlled study. *Fertil Steril*. 1989;51:182.
534. Mol BW. Diagnostic potential of the postcoital test. In: Heineman MJ, ed. *Evidence-Based Medicine in Clinical Practice*. American Society for Reproductive Medicine; 2001.
535. Glatstein IZ, Harlow BL, Hornstein MD. Practice patterns among reproductive endocrinologists: the infertility evaluation. *Fertil Steril*. 1997;67:443.
536. Griffith CS, Grimes DA. The validity of the postcoital test. *Am J Obstet Gynecol*. 1990;162:615.
537. Oei SG, Bloemenkamp KW, Helmerhorst FM, Naaktgeboren N, Keirse MJ. Evaluation of the postcoital test for assessment of "cervical factor" infertility. *Eur J Obstet Gynecol Reprod Biol*. 1996;64:217.
538. Oei SG, Helmerhorst FM, Bloemenkamp KW, Hollants FA, Meerpoel DE, Keirse MJ. Effectiveness of the postcoital test: randomised controlled trial. *Br Med J*. 1998;317:502.
539. Breitkopf D, Goldstein SR, Seeds JW. ACOG technology assessment in obstetrics and gynecology number 3, September 2003—saline infusion sonohysterography. *Obstet Gynecol*. 2003;102:659.
540. Sladkevicius P, Valentin L, Marsal K. Blood flow velocity in the uterine and ovarian arteries during the normal menstrual cycle. *Ultrasound Obstet Gynecol*. 1993;3:199.
541. Tan SL, Zaidi J, Campbell S, Doyle P, Collins W. Blood flow changes in the ovarian and uterine arteries during the normal menstrual cycle. *Am J Obstet Gynecol*. 1996;175:625.
542. Gonen Y, Casper RF. Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for in vitro fertilization (IVF). *J In Vitro Fert Embryo Transf*. 1990;7:146.
543. Khalifa E, Brzyski RG, Oehninger S, Acosta AA, Muasher SJ. Sonographic appearance of the endometrium: the predictive value for the outcome of in-vitro fertilization in stimulated cycles. *Hum Reprod*.1992;7:677.
544. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review. *Hum Reprod Update*. 1996;2:323.
545. Rashidi BH, Sadeghi M, Jafarabadi M, Tehrani Nejad ES. Relationships between pregnancy rates following in vitro fertilization or intracytoplasmic sperm injection and endometrial thickness and pattern. *Eur J Obstet Gynecol Reprod Biol*. 2005;120:179.
546. Merce LT, Barco MJ, Bau S, Troyano J. Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to in vitro fertilization/embryo transfer outcome? *Fertil Steril*. 2008;89:111.
547. Chen SL, Wu FR, Luo C, et al. Combined analysis of endometrial thickness and pattern in predicting outcome of in vitro fertilization and embryo transfer: a retrospective cohort study. *Reprod Biol Endocrinol*. 2010;24:30.
548. Steer CV, Tan SL, Mason BA, Campbell S. Midluteal-phase vaginal color Doppler assessment of uterine artery impedance in a subfertile population. *Fertil Steril*. 1994;61:53.
549. Tinkanen H, Kujansuu E, Laippala P. Vascular resistance in uterine and ovarian arteries: its association with infertility and the prognosis of infertility. *Eur J Obstet Gynecol Reprod Biol*. 1994;57:111.
550. Isaksson R, Tiitinen A, Reinikainen LM, Cacciatore B. Comparison of uterine and spiral artery blood flow in women with unexplained and tubal infertility. *Ultrasound Obstet Gynecol*. 2003;21:174.
551. Reuter KL, Daly DC, Cohen SM. Septate versus bicornuate uteri: errors in imaging diagnosis. *Radiology*. 1989;172:749.
552. Homer HA, Li TC, Cooke ID. The septate uterus: a review of management and reproductive outcome. *Fertil Steril*. 2000;73:1.
553. Pellerito JS, McCarthy SM, Doyle MB, Glickman MG, DeCherney AH. Diagnosis of uterine anomalies: relative accuracy of MR imaging, endovaginal sonography, and hysterosalpingography. *Radiology*. 1992;183:795.
554. Kelly SM, Sladkevicius P, Campbell S, Nargund G. Investigation of the infertile couple: a one-stop ultrasound-based approach. *Hum Reprod*. 2001;16:2481.
555. Benacerraf BR, Shipp TD, Bromley B. Which patients benefit from a 3D reconstructed coronal view of the uterus added to standard routine 2D pelvic sonography? *AJR Am J Roentgenol*. 2008;190(3):626.
556. Faivre E, Fernandez H, Deffieux X, Gervaise A, Frydman R, Levaillant JM. Accuracy of three-dimensional ultrasonography in differential diagnosis of septate and bicornuate uterus compared with office hysteroscopy and pelvic magnetic resonance imaging. *J Minim Invasive Gynecol*. 2012;19(1):101.
557. Ghi T, Casadio P, Kuleva M, et al. Accuracy of three-dimensional ultrasound in diagnosis and classification of congenital uterine anomalies. *Fertil Steril*. 2009;92(2):808.
558. Moini A, Mohammadi S, Hosseini R, Eslami B, Ahmadi F. Accuracy of 3-dimensional sonography for diagnosis and classification of congenital uterine anomalies. *J Ultrasound Med*. 2013;32(6):923.
559. Becker E Jr, Lev-Toaff AS, Kaufman EP, Halpern EJ, Edelweiss MI, Kurtz AB. The added value of transvaginal sonohysterography over transvaginal sonography alone in women with known or suspected leiomyoma. *J Ultrasound Med*. 2002;21:237.
560. Leone FP, Lanzani C, Ferrazzi E. Use of strict sonohysterographic methods for preoperative assessment of submucous myomas. *Fertil Steril*. 2003;79:998.
561. Sylvestre C, Child TJ, Tulandi T, Tan SL. A prospective study to evaluate the efficacy of two- and three-dimensional sonohysterography in women with intrauterine lesions. *Fertil Steril*. 2003;79:1222.

562. Makris N, Kalmantis K, Skartados N, Papadimitriou A, Mantzaris G, Antsaklis A. Three-dimensional hysterosonography versus hysteroscopy for the detection of intracavitary uterine abnormalities. *Int J Gynaecol Obstet*. 2007;97:6.
563. Kupesic S, Kurjak A, Skenderovic S, Bjelos D. Screening for uterine abnormalities by three-dimensional ultrasound improves perinatal outcome. *J Perinat Med*. 2002;30(1):9.
564. La Torre R, De Felice C, De Angelis C, Coacci F, Mastrone M, Cosmi EV. Transvaginal sonographic evaluation of endometrial polyps: a comparison with two dimensional and three dimensional contrast sonography. *Clin Exp Obstet Gynecol*. 1999;26(3–4):171.
565. Stadtmauer L, Shah A. Gynecologic surgery: preoperative assessment with ultrasound. *Clin Obstet Gynecol*. 2017;60(1):82.
566. Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. *Fertil Steril*. 2000;73:406.
567. Fedele L, Bianchi S, Dorta M, Vignali M. Intrauterine adhesions: detection with transvaginal US. *Radiology*. 1996;199:757.
568. Salle B, Gaucherand P, de Saint Hilaire P, Rudigoz RC. Transvaginal sonohysterographic evaluation of intrauterine adhesions. *J Clin Ultrasound*. 1999;27:131.
569. Davis PC, O'Neill MJ, Yoder IC, Lee SI, Mueller PR. Sonohysterographic findings of endometrial and subendometrial conditions. *Radiographics*. 2002;22:803.
570. Preutthipan S, Linasmita V. A prospective comparative study between hysterosalpingography and hysteroscopy in the detection of intrauterine pathology in patients with infertility. *J Obstet Gynaecol Res*. 2003;29:33.
571. Karayalcin R, Ozcan S, Moraloglu O, Ozyer S, Mollamahmutoglu L, Batioglu S. Results of 2500 office-based diagnostic hysteroscopies before IVF. *Reprod Biomed Online*. 2010;20:689.
572. Simon C, Martinez L, Pardo F, Tortajada M, Pellicer A. Müllerian defects in women with normal reproductive outcome. *Fertil Steril*. 1991;56:1192.
573. Acién P. Incidence of Müllerian defects in fertile and infertile women. *Hum Reprod*. 1997;12:1372.
574. Ashton D, Amin HK, Richart RM, Neuwirth RS. The incidence of asymptomatic uterine anomalies in women undergoing transcervical tubal sterilization. *Obstet Gynecol*. 1988;72:28.
575. Raga F, Bauset C, Remohi J, Bonilla-Musoles F, Simón C, Pellicer A. Reproductive impact of congenital Müllerian anomalies. *Hum Reprod*. 1997;12:2277.
576. Jurkovic D, Gruboeck K, Tailor A, Nicolaides KH. Ultrasound screening for congenital uterine anomalies. *Br J Obstet Gynaecol*. 1997;104:1320.
577. Grimbizis GF, Camus M, Tarlatzis BC, Bontis JN, Devroey P. Clinical implications of uterine malformations and hysteroscopic treatment results. *Hum Reprod Update*. 2001;7:161.
578. Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning N, Coomarasamy A. The prevalence of congenital uterine anomalies in unselected and high-risk populations: a systematic review. *Hum Reprod Update*. 2011;17:761.
579. Practice Committee of the American Society for Reproductive Medicine. Evidence-based diagnosis and treatment for uterine septum: a guideline. *Fertil Steril*. 2024;122(2):251–265.
580. Venetis CA, Papadopoulos SP, Campo R, Gordts S, Tarlatzis BC, Grimbizis GF. Clinical implications of congenital uterine anomalies: a meta-analysis of comparative studies. *Reprod Biomed Online*. 2014;29:665.
581. Burchell RC, Creed F, Rasoulpour M, Whitcomb M. Vascular anatomy of the human uterus and pregnancy wastage. *Br J Obstet Gynaecol*. 1978;85:698.
582. Candiani GB, Fedele L, Zamberletti D, De Virgiliis D, Carinelli S. Endometrial patterns in malformed uteri. *Acta Eur Fertil*. 1983;14:311.
583. Rock JA, Murphy AA. Anatomic abnormalities. *Clin Obstet Gynecol*. 1986;29:886.
584. Fedele L, Bianchi S, Marchini M, Franchi D, Tozzi L, Dorta M. Ultrastructural aspects of endometrium in infertile women with septate uterus. *Fertil Steril*. 1996;65:750.
585. Carrera M, Perez Millan F, Alcazar JL, et al. Effect of hysteroscopic metroplasty on reproductive outcomes in women with septate uterus: systematic review and meta-analysis. *J Minim Invasive Gynecol*. 2022;29:465–475.
586. Rikken JFW, Kowalik CR, Emanuel MH, et al. Septum resection versus expectant management in women with a septate uterus: an international multicentre open-label randomized controlled trial. *Hum Reprod*. 2021;36:1260–1267.
587. Cramer SF, Patel A. The frequency of uterine leiomyomas. *Am J Clin Pathol*. 1990;94:435.
588. Practice Committee of the American Society of Reproductive Medicine. Removal of myomas in asymptomatic patients to improve fertility and/or reduce miscarriage rate: a guideline. *Fertil Steril*. 2017;108:416.
589. Practice Committee of the American Society for Reproductive Medicine. Myomas and reproductive function. *Fertil Steril*. 2008;90(suppl 5):125.
590. Don EE, Mijatovic V, Huirne JAF. Infertility in patients with uterine fibroids: a debate about the hypothetical mechanisms. *Hum Reprod*. 2023;38(11):2045–2054.
591. Deligdish L, Loewenthal M. Endometrial changes associated with myomata of the uterus. *J Clin Pathol*. 1970;23:676.
592. Sharma SP, Misra SD, Mittal VP. Endometrial changes–a criterion for the diagnosis of submucous uterine leiomyoma. *Indian J Pathol Microbiol*. 1979;22:33.
593. Maguire M, Segars J. Benign uterine disease: leiomyomata and benign polyps. In: Aplin JD, Fazleabas AT, Glasser SR, Giudice LC, eds. *The Endometrium: Molecular, Cellular and Clinical Perspectives*. Informa HealthCare; 2008:797.
594. Rackow BW, Taylor HS. Submucosal uterine leiomyomas have a global effect on molecular determinants of endometrial receptivity. *Fertil Steril*. 2010;93:2027.
595. Horcajadas JA, Goyri E, Higon MA, et al. Endometrial receptivity and implantation are not affected by the presence of uterine intramural leiomyomas: a clinical and functional genomics analysis. *J Clin Endocrinol Metab*. 2008; 93(9):3490.
596. Farhi J, Ashkenazi J, Feldberg D, Dicker D, Orvieto R, Ben Rafael Z. Effect of uterine leiomyomata on the results of in-vitro fertilization treatment. *Hum Reprod*. 1995;10:2576.
597. Eldar-Geva T, Meagher S, Healy DL, MacLachlan V, Breheny S, Wood C. Effect of intramural, subserosal, and submucosal uterine fibroids on the outcome of assisted reproductive technology treatment. *Fertil Steril*. 1998;70:687.
598. Stovall DW, Parrish SB, Van Voorhis BJ, Hahn SJ, Sparks AE, Syrop CH. Uterine leiomyomas reduce the efficacy of assisted reproduction cycles: results of a matched follow-up study. *Hum Reprod*. 1998;13:192.
599. Hart R, Khalaf Y, Yeong CT, Seed P, Taylor A, Braude P. A prospective controlled study of the effect of intramural uterine fibroids on the outcome of assisted conception. *Hum Reprod*. 2001;16:2411.
600. Khalaf Y, Ross C, El-Toukhy T, Hart R, Seed P, Braude P. The effect of small intramural uterine fibroids on the cumulative outcome of assisted conception. *Hum Reprod*. 2006;21:2640.
601. Somigliana E, De Benedictis S, Vercellini P, et al. Fibroids not encroaching the endometrial cavity and IVF success rate: a prospective study. *Hum Reprod*. 2011;26:834.
602. Christopoulos G, Vlismas A, Salim R, Islam R, Trew G, Lavery S. Fibroids that do not distort the uterine cavity and IVF success rates: an observational study using extensive matching criteria. *BJOG*. 2017;124:615.
603. Ezzati M, Norian JM, Segars JH. Management of uterine fibroids in the patient pursuing assisted reproductive technologies. *Womens Health (Lond)*. 2009;5:413.
604. Benecke C, Kruger TF, Siebert TI, Van der Merwe JP, Steyn DW. Effect of fibroids on fertility in patients undergoing assisted reproduction: a structured literature review. *Gynecol Obstet Invest*. 2005;59:225.
605. Somigliana E, Vercellini P, Daguati R, Pasin R, De Giorgi O, Crosignani PG. Fibroids and female reproduction: a critical analysis of the evidence. *Hum Reprod Update*. 2007;13:465.
606. Klatsky PC, Tran ND, Caughey AB, Fujimoto VY. Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. *Am J Obstet Gynecol*. 2008;198:357.
607. Pritts EA, Parker WH, Olive DL. Fibroids and infertility: an updated systematic review of the evidence. *Fertil Steril*. 2009;91:1215.
608. Hartmann KE, Velez Edwards DR, Savitz DA, et al. Prospective cohort study of uterine fibroids and miscarriage risk. *Am J Epidemiol*. 2017;186:1140.
609. Healy DL. Impact of uterine fibroids on ART outcome. *Environ Health Perspect*. 2000;108(suppl 5):845.
610. Dietterich C, Check JH, Choe JK, Nazari A, Fox F. The presence of small uterine fibroids not distorting the endometrial cavity does not adversely affect conception outcome following embryo transfer in older recipients. *Clin Exp Obstet Gynecol*. 2000;27:168.
611. Jun SH, Ginsburg ES, Racowsky C, Wise LA, Hornstein MD. Uterine leiomyomas and their effect on in vitro fertilization outcome: a retrospective study. *J Assist Reprod Genet*. 2001;18:139.
612. Surrey ES, Lietz AK, Schoolcraft WB. Impact of intramural leiomyomata in patients with a normal endometrial cavity on in vitro fertilization-embryo transfer cycle outcome. *Fertil Steril*. 2001;75:405.
613. Check JH, Choe JK, Lee G, Dietterich C. The effect on IVF outcome of small intramural fibroids not compressing the uterine cavity as determined by a prospective matched control study. *Hum Reprod*. 2002;17:1244.
614. Oliveira FG, Abdelmassih VG, Diamond MP, Dozortsev D, Melo NR, Abdelmassih R. Impact of subserosal and intramural uterine fibroids that do not distort the endometrial cavity on the outcome of in vitro fertilization-intracytoplasmic sperm injection. *Fertil Steril*. 2004;81:582.
615. Sunkara SK, Khairy M, El-Toukhy T, Khalaf Y, Coomarassamy A. The effect of intramural fibroids without uterine cavity involvement on the outcome of IVF treatment: a systematic review and meta-analysis. *Hum Reprod*. 2010;25:418.
616. Erden M, Uyanik E, Polat M, Ozbek IY, Yarali H, Mumusoglu S. The effect of ≤6 cm sized noncavity-distorting intramural fibroids on in vitro fertilization outcomes: a systematic review and meta-analysis. *Fertil Steril*. 2023;119(6):996–1007.
617. Yan L, Yu Q, Zhang YN, et al. Effect of type 3 intramural fibroids on in vitro fertilization-intracytoplasmic sperm injection outcomes: a retrospective cohort study. *Fertil Steril*. 2018;109:817–822.e2.
618. Casini ML, Rossi F, Agostini R, Unfer V. Effects of the position of fibroids on fertility. *Gynecol Endocrinol*. 2006;22:106.
619. Bulletti C, De Ziegler D, Polli V, Flamigni C. The role of leiomyomas in infertility. *J Am Assoc Gynecol Laparosc*. 1999;6:441.
620. Aboulghar MM, Al-Inany HG, Aboulghar MA, Serour GI, Mansour RT. The effect of intramural fibroids on the outcome of IVF. *Middle East Fertil Soc J*. 2004;9:263.
621. Seoud M, Patterson R, Musher S, Coddington C. Effects of myoma or prior myomectomy on in vitro fertilization (IVF) performance. *J Assist Reprod Genet*. 1992;9:217.
622. Bulletti C, DE Ziegler D, Levi Setti P, Cicinelli E, Polli V, Stefanetti M. Myomas, pregnancy outcome, and in vitro fertilization. *Ann N Y Acad Sci*. 2004;1034:84.
623. Pritts TL, Ogden M, Parker W, Ratcliffe J, Pritts EA. Intramural leiomyomas and fertility: a systematic review and meta-analysis. *Obstet Gynecol*. 2024;144(2):171–179.

624. Fauconnier A, Dubuisson JB, Ancel PY, Chapron C. Prognostic factors of reproductive outcome after myomectomy in infertile patients. *Hum Reprod*. 2000;15:1751.
625. Dubuisson JB, Fauconnier A, Chapron C, Kreiker G, Norgaard C. Second look after laparoscopic myomectomy. *Hum Reprod*. 1998;13:2102.
626. Lieng M, Istre O, Langebrekke A. Uterine rupture after laparoscopic myomectomy. *J Am Assoc Gynecol Laparosc*. 2004;11:92.
627. Banas T, Klimek M, Fugiel A, Skotniczny K. Spontaneous uterine rupture at 35 weeks' gestation, 3 years after laparoscopic myomectomy, without signs of fetal distress. *J Obstet Gynaecol Res*. 2005;31:527.
628. Grande N, Catalano GF, Ferrari S, Marana R. Spontaneous uterine rupture at 27 weeks of pregnancy after laparoscopic myomectomy. *J Minim Invasive Gynecol*. 2005;12:301.
629. Parker WH, Iacampo K, Long T. Uterine rupture after laparoscopic removal of a pedunculated myoma. *J Minim Invasive Gynecol*. 2007;14:362.
630. Taylor HS. Fibroids: when should they be removed to improve in vitro fertilization success? *Fertil Steril*. 2018;109(6):784.
631. Advincula AP, Song A. The role of robotic surgery in gynecology. *Curr Opin Obstet Gynecol*. 2007;19:331.
632. Palomba S, Zupi E, Falbo A, et al. A multicenter randomized, controlled study comparing laparoscopic versus minilaparotomic myomectomy: reproductive outcomes, *Fertil Steril*. 2007;88:933.
633. Holloway RW, Patel SD, Ahmad S. Robotic surgery in gynecology. *Scand J Surg*. 2009;98:96.
634. Luciano AA. Myomectomy. *Clin Obstet Gynecol*. 2009;52:362.
635. Agdi M, Tulandi T. Minimally invasive approach for myomectomy. *Semin Reprod Med*. 2010;28:228.
636. Al-Inany H. Intrauterine adhesions: an update. *Acta Obstet Gynecol Scand*. 2001;80:986.
637. Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome–one century later. *Fertil Steril*. 2008;89:759.
638. Berman JM. Intrauterine adhesions. *Semin Reprod Med*. 2008;26:349.
639. Thomson AJ, Abbott JA, Deans R, Kingston A, Vancaillie TG. The management of intrauterine synechiae. *Curr Opin Obstet Gynecol*. 2009;21:335.
640. Jensen PA, Stromme WB. Amenorrhea secondary to puerperal curettage (Asherman's syndrome). *Am J Obstet Gynecol*. 1972;113:150.
641. Romer T. Post-abortion-hysteroscopy—a method for early diagnosis of congenital and acquired intrauterine causes of abortions. *Eur J Obstet Gynecol Reprod Biol*. 1994;57:171.
642. Taylor PJ, Cumming DC, Hill PJ. Significance of intrauterine adhesions detected hysteroscopically in eumenorrheic infertile women and role of antecedent curettage in their formation. *Am J Obstet Gynecol*. 1981;139:239.
643. Shaffer W. Role of uterine adhesions in the cause of multiple pregnancy losses. *Clin Obstet Gynecol*. 1986;29:912.
644. Schenker JG. Etiology of and therapeutic approach to synechia uteri. *Eur J Obstet Gynecol Reprod Biol*. 1996;65:109.
645. Tripathy SN. Infertility and pregnancy outcome in female genital tuberculosis. *Int J Gynaecol Obstet*. 2002;76:159.
646. Westendorp IC, Ankum WM, Mol BW, Vonk J. Prevalence of Asherman's syndrome after secondary removal of placental remnants or a repeat curettage for incomplete abortion. *Hum Reprod*. 1998;13:3347.
647. Friedler S, Margalioth EJ, Kafka I, Yaffe H. Incidence of post-abortion intrauterine adhesions evaluated by hysteroscopy—a prospective study. *Hum Reprod*. 1993;8:442.
648. Roma Dalfo A, Ubeda B, Ubeda A, et al. Diagnostic value of hysterosalpingography in the detection of intrauterine abnormalities: a comparison with hysteroscopy. *AJR Am J Roentgenol*. 2004;183:1405.
649. Seshadri S, El-Toukhy T, Douiri A, Jayaprakasan K, Khalaf Y. Diagnostic accuracy of saline infusion sonography in the evaluation of uterine cavity abnormalities prior to assisted reproductive techniques: a systematic review and meta-analyses. *Hum Reprod Update*. 2015;21(2):262–274.
650. Schenker JG, Margalioth EJ. Intrauterine adhesions: an updated appraisal. *Fertil Steril*. 1982;37:593.
651. AAGL Elevating Gynecologic Surgery. AAGL practice report: practice guidelines on intrauterine adhesions developed in collaboration with the European Society of Gynaecological Endoscopy (ESGE). *J Minim Invasive Gynecol*. 2017;24:695.
652. Kresowik JD, Syrop CH, Van Voorhis BJ, Ryan GL. Ultrasound is the optimal choice for guidance in difficult hysteroscopy. *Ultrasound Obstet Gynecol*. 2012;39:715.
653. Tasma ML, Louwerse MD, Hehenkamp WJ, et al. Misoprostol for cervical priming prior to hysteroscopy in postmenopausal and premenopausal nulliparous women; a multicentre randomised placebo controlled trial. *BJOG*. 2018;125:81.
654. Cooper JM, Brady RM. Late complications of operative hysteroscopy. *Obstet Gynecol Clin North Am*. 2000;27:367.
655. American Association of Gynecologic Laparoscopists. Practice guidelines for management of intrauterine synechiae. *J Minim Invasive Gynecol*. 2010;17:1.
656. Coccia ME, Becattini C, Bracco GL, Pampaloni F, Bargelli G, Scarselli G. Pressure lavage under ultrasound guidance: a new approach for outpatient treatment of intrauterine adhesions. *Fertil Steril*. 2001;75:601.
657. Karande V, Levrant S, Hoxsey R, Rinehart J, Gleicher N. Lysis of intrauterine adhesions using gynecoradiologic techniques. *Fertil Steril*. 1997;68:658.
658. March CM. Intrauterine adhesions. *Obstet Gynecol Clin North Am*. 1995;22:491.
659. Pistofidis GA, Dimitropoulos K, Mastrominas M. Comparison of operative and fertility outcome between groups of women with intrauterine adhesions after adhesiolysis. *J Am Assoc Gynecol Laparosc*. 1996;3:S40.
660. Lindheim SR, Kavic S, Shulman SV, Sauer MV. Operative hysteroscopy in the office setting. *J Am Assoc Gynecol Laparosc*. 2000;7:65.
661. Goldenberg M, Sivan E, Sharabi Z, Bider D, Rabinovici J, Seidman DS. Outcome of hysteroscopic resection of submucous myomas for infertility. *Fertil Steril*. 1995;64:714.
662. Katz Z, Ben-Arie A, Lurie S, Manor M, Insler V. Reproductive outcome following hysteroscopic adhesiolysis in Asherman's syndrome. *Int J Fertil Menopausal Stud*. 1996;41:462.
663. Capella-Allouc S, Morsad F, Rongieres-Bertrand C, Taylor S, Fernandez H. Hysteroscopic treatment of severe Asherman's syndrome and subsequent fertility. *Hum Reprod*. 1999;14:1230.
664. Zikopoulos KA, Kolibianakis EM, Platteau P, et al. Live delivery rates in subfertile women with Asherman's syndrome after hysteroscopic adhesiolysis using the resectoscope or the Versapoint system. *Reprod Biomed Online*. 2004;8:720.
665. Kodaman PH. Hysteroscopic polypectomy for women undergoing IVF treatment: when is it necessary? *Curr Opin Gynecol Obstet*. 2016;28:184.
666. Lass A, Williams G, Abusheikha N, Brinsden P. The effect of endometrial polyps on outcomes of in vitro fertilization (IVF) cycles. *J Assist Reprod Genet*. 1999;16:410.
667. Shokeir TA, Shalan HM, El-Shafei MM. Significance of endometrial polyps detected hysteroscopically in eumenorrheic infertile women. *J Obstet Gynaecol Res*. 2004;30:84.
668. Jovanovic AS, Boynton KA, Mutter GL. Uteri of women with endometrial carcinoma contain a histopathological spectrum of monoclonal putative precancers, some with microsatellite instability. *Cancer Res*. 1996;56:1917.
669. Pal L, Niklaus AL, Kim M, Pollack S, Santoro N. Heterogeneity in endometrial expression of aromatase in polyp-bearing uteri. *Hum Reprod*. 2008;23:80.
670. Dal Cin P, Vanni R, Marras S, et al. Four cytogenetic subgroups can be identified in endometrial polyps. *Cancer Res*. 1995;55:1565.
671. Kamel HS, Darwish AM, Mohamed SA. Comparison of transvaginal ultrasonography and vaginal sonohysterography in the detection of endometrial polyps. *Acta Obstet Gynecol Scand*. 2000;79:60.
672. Lindheim SR, Morales AJ. Comparison of sonohysterography and hysteroscopy: lessons learned and avoiding pitfalls. *J Am Assoc Gynecol Laparosc*. 2002;9:223.
673. Mittal K, Schwartz L, Goswami S, Demopoulos R. Estrogen and progesterone receptor expression in endometrial polyps. *Int J Gynecol Pathol*. 1996;15:345.
674. Spiewankiewicz B, Stelmachow J, Sawicki W, Cendrowski K, Wypych P, Swiderska K. The effectiveness of hysteroscopic polypectomy in cases of female infertility. *Clin Exp Obstet Gynecol*. 2003;30:23.
675. Perez-Medina T, Bajo-Arenas J, Salazar F, et al. Endometrial polyps and their implication in the pregnancy rates of patients undergoing intrauterine insemination: a prospective, randomized study. *Hum Reprod*. 2005;20:1632.
676. Isikoglu M, Berkkanoglu M, Senturk Z, Coetzee K, Ozgur K. Endometrial polyps smaller than 1.5 cm do not affect ICSI outcome. *Reprod Biomed Online*. 2006;12:199.
677. Shohayeb A Shaltout A. Persistent endometrial polyps may affect the pregnancy rate in patients undergoing intrauterine insemination. *Middle East Fertil Soc J*. 2011;16:259.
678. Bosteels J, van Wessel S, Weyers S, et al. Hysteroscopy for treating subfertility associated with suspected major uterine cavity abnormalities. *Cochrane Database Syst Rev*. 2018;12(12):CD009461.
679. Mouhayar Y, Yin O, Mumford SL, Segars JH. Hysteroscopic polypectomy prior to infertility treatment: a cost analysis and systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2017;213:107.
680. Afifi K, Anand S, Nallapeta S, Gelbaya TA. Management of endometrial polyps in subfertile women: a systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2010;151:117.
681. Lieng M, Istre O, Qvigstad E. Treatment of endometrial polyps: a systematic review. *Acta Obstet Gynecol Scand*. 2010;89:992.
682. Czernobilsky B. Endometritis and infertility. *Fertil Steril*. 1978;30:119.
683. Paavonen J, Kiviat N, Brunham RC, et al. Prevalence and manifestations of endometritis among women with cervicitis. *Am J Obstet Gynecol*. 1985;152:280.
684. Korn AP, Hessol N, Padian N, et al. Commonly used diagnostic criteria for pelvic inflammatory disease have poor sensitivity for plasma cell endometritis. *Sex Transm Dis*. 1995;22:335.
685. Korn AP, Bolan G, Padian N, Ohm-Smith M, Schachter J, Landers DV. Plasma cell endometritis in women with symptomatic bacterial vaginosis. *Obstet Gynecol*. 1995;85:387.
686. Wiesenfeld HC, Hillier SL, Krohn MA, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet Gynecol*. 2002;100:456.
687. Polisseni F, Bambirra EA, Camargos AF. Detection of chronic endometritis by diagnostic hysteroscopy in asymptomatic infertile patients. *Gynecol Obstet Invest*. 2003;55:205.
688. Paukku M, Puolakkainen M, Paavonen T, Paavonen J. Plasma cell endometritis is associated with *Chlamydia trachomatis* infection. *Am J Clin Pathol*. 1999;112:211.
689. Mount S, Mead P, Cooper K. *Chlamydia trachomatis* in the endometrium: can surgical pathologists identify plasma cells? *Adv Anat Pathol*. 2001;8:327.

690. Taylor-Robinson D. *Mycoplasma genitalium*—an up-date. *Int J STD AIDS*. 2002;13:145.
691. Cicinelli E, Matteo M, Trojano G, et al. Chronic endometritis in patients with unexplained infertility: prevalence and effects of antibiotic treatment on spontaneous conception. *Am J Reprod Immunol*. 2018;79:e12782.
692. Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod*. 2015;30:323.
693. Kasius JC, Fatemi HM, Bourgain C, et al. The impact of chronic endometritis on reproductive outcome. *Fertil Steril*. 2011;96:1451.
694. Herlihy NS, Klimczak AM, Titus S, et al. The role of endometrial staining for CD138 as a marker of chronic endometritis in predicting live birth. *J Assist Reprod Genet*. 2022;39(2):473–479.
695. Honore GM, Holden AE, Schenken RS. Pathophysiology and management of proximal tubal blockage. *Fertil Steril*. 1999;71:785.
696. Westrom L. Effect of acute pelvic inflammatory disease on fertility. *Am J Obstet Gynecol*. 1975;121:707.
697. Westrom I. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. *Am J Obstet Gynecol*. 1980;138:880.
698. Westrom L, Joesoef R, Reynolds G, Hagdu A, Thompson SE. Pelvic inflammatory disease and fertility. *Sex Transm Dis*. 1992;19:185.
699. Westrom LV. Sexually transmitted diseases and infertility. *Sex Transm Dis*. 1994;21:S32.
700. Westrom L. Effect of pelvic inflammatory disease on fertility. *Venereology*. 1995;8:219.
701. Mohiyiddeen L, Hardiman A, Fitzgerald C, et al. Tubal flushing for subfertility. *Cochrane Database Syst Rev*. 2015;(5):CD003718.
702. Forsey JP, Caul EO, Paul ID, Hull MG. *Chlamydia trachomatis*, tubal disease and the incidence of symptomatic and asymptomatic infection following hysterosalpingography. *Hum Reprod*. 1990;5:444.
703. Uzun O, Findik S, Danaci M, Katar D, Erkan L. Pulmonary and cerebral oil embolism after hysterosalpingography with oil soluble contrast medium. *Respirology*. 2004;9:134.
704. Ma G, Mao R, Zhai H. Hyperthyroidism secondary to hysterosalpingography: an extremely rare complication: a case report. *Medicine (Baltimore)*. 2016; 95:e5588.
705. Dabekausen YA, Evers JL, Land JA, Stals FS. *Chlamydia trachomatis* antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. *Fertil Steril*. 1994;61:833.
706. Mol BW, Dijkman B, Wertheim P, Lijmer J, van der Veen F, Bossuyt PM. The accuracy of serum chlamydial antibodies in the diagnosis of tubal pathology: a meta-analysis. *Fertil Steril*. 1997;67:1031.
707. Thomas K, Coughlin L, Mannion PT, Haddad NG. The value of *Chlamydia trachomatis* antibody testing as part of routine infertility investigations. *Hum Reprod*. 2000;15:1079.
708. Veenemans LM, van der Linden PJ. The value of *Chlamydia trachomatis* antibody testing in predicting tubal factor infertility. *Hum Reprod*. 2002;17:695.
709. Hindocha A, Beere L, O'Flynn H, Watson A, Ahmad G. Pain relief in hysterosalpingography. *Cochrane Database Syst Rev*. 2015;(9):CD006106.
710. Karaman E, Cim N, Alkis I, Yildirim A, Yildizhan R. Rectal indomethacin use in pain relief during hysterosalpingography: a randomized placebo controlled trial. *J Obstet Gynaecol Res*. 2016;42:195.
711. Stumpf PG, March CM. Febrile morbidity following hysterosalpingography: identification of risk factors and recommendations for prophylaxis. *Fertil Steril*. 1980;33:487.
712. Pittaway DE, Winfield AC, Maxson W, Daniell J, Herbert C, Wentz AC. Prevention of acute pelvic inflammatory disease after hysterosalpingography: efficacy of doxycycline prophylaxis. *Am J Obstet Gynecol*. 1983;147:623.
713. Perisinakis K, Damilakis J, Grammatikakis J, Theocharopoulos N, Gourtsoyiannis N. Radiogenic risks from hysterosalpingography. *Eur Radiol*. 2003; 13:1522.
714. Tur-Kaspa I, Seidman DS, Soriano D, Greenberg I, Dor J, Bider D. Hysterosalpingography with a balloon catheter versus a metal cannula: a prospective, randomized, blinded comparative study. *Hum Reprod*. 1998;13:75.
715. Ricci G, Guastalla P, Ammar L, Cervi G, Guarnieri S, Sartore A. Balloon catheter vs. cervical vacuum cup for hysterosalpingography: a prospective, randomized, single-blinded study. *Fertil Steril*. 2007;87:1458.
716. Cohen SB, Wattiez A, Seidman DS, et al. Comparison of cervical vacuum cup cannula with metal cannula for hysterosalpingography. *BJOG*. 2001;108:1031.
717. Lindequist S, Justesen P, Larsen C, Rasmussen F. Diagnostic quality and complications of hysterosalpingography: oil- versus water-soluble contrast media—a randomized prospective study. *Radiology*. 1991;179:69.
718. Moore DE, Segars JH Jr, Winfield AC, Page DL, Eisenberg AD, Holburn GE. Effects of contrast agents on the fallopian tube in a rabbit model. *Radiology*. 1990;176:721.
719. Grosskinsky CM, Clark RL, Wilson PA, Novotny DB. Pelvic granulomata mimicking endometriosis following the administration of oil-based contrast media for hysterosalpingography. *Obstet Gynecol*. 1994;83:890.
720. Nunley WC Jr, Bateman BG, Kitchin JD III, Pope TL Jr. Intravasation during hysterosalpingography using oil-base contrast medium—a second look. *Obstet Gynecol*. 1987;70:309.
721. 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;25:2043.
722. van Rijswijk J, van Welie N, Dreyer K, et al. Tubal flushing with oil-based or water-based contrast at hysterosalpingography for infertility: long-term reproductive outcomes of a randomized trial. *Fertil Steril*. 2020;114(1):155–162.
723. Wang R, Watson A, Johnson N, et al. Tubal flushing for subfertility. *Cochrane Database Syst Rev*. 2020;10(10):CD003718.
724. Mol BW, Swart P, Bossuyt PM, van der Veen F. Is hysterosalpingography an important tool in predicting fertility outcome? *Fertil Steril*. 1997;67:663.
725. Mol BW, Collins JA, Burrows EA, van der Veen F, Bossuyt PM. Comparison of hysterosalpingography and laparoscopy in predicting fertility outcome. *Hum Reprod*. 1999;14:1237.
726. Maheux-Lacroix S, Boutin A, Moore L, et al. Hysterosalpingosonography for diagnosing tubal occlusion in subfertile women: a systematic review with meta-analysis. *Hum Reprod*. 2014;29:953.
727. Evers JLH, Land JA, Mol BW. Evidence-based medicine for diagnostic questions. *Semin Reprod Med*. 2003;21:9.
728. Dessole S, Meloni GB, Capobianco G, Manzoni MA, Ambrosini G, Canalis GC. A second hysterosalpingography reduces the use of selective technique for treatment of a proximal tubal obstruction. *Fertil Steril*. 2000;73:1037.
729. Swart P, Mol BW, van der Veen F, van Beurden M, Redekop WK, Bossuyt PM. The accuracy of hysterosalpingography in the diagnosis of tubal pathology: a meta-analysis. *Fertil Steril*. 1995;64:486.
730. Coppus SF, Opmeer BC, Logan S, van der Veen F, Bhattacharya S, Mol BW. The predictive value of medical history taking and Chlamydia IgG ELISA anti- body testing (CAT) in the selection of subfertile women for diagnostic laparoscopy: a clinical prediction model approach. *Hum Reprod*. 2007;22:1353.
731. Mol BW, Swart P, Bossuyt PM, van Beurden M, van der Veen F. Reproducibility of the interpretation of hysterosalpingography in the diagnosis of tubal pathology. *Hum Reprod*. 1996;11:1204.
732. Glatstein IZ, Sleeper LA, Lavy Y, et al. Observer variability in the diagnosis and management of the hysterosalpingogram. *Fertil Steril*. 1997;67:233.
733. Bilgin H, Ozcan B, Bilgin T. Methemoglobinemia induced by methylene blue perturbation during laparoscopy. *Acta Anaesthesiol Scand*. 1998;42:594.
734. Mhaskar R, Mhaskar AM. Methemoglobinemia following chromopertubation in treated pelvic tuberculosis. *Int J Gynaecol Obstet*. 2002;77:41.
735. Maas JW, Evers JL, ter Riet G, Kessels AG. Pregnancy rate following normal versus abnormal hysterosalpingography findings: a meta-analysis. *Gynecol Obstet Invest*. 1997;43:79.
736. Cassiman EG, Harter S, Mougel R, et al. Is HyFosy the new gold standard for assessing tubal patency? A systematic review and meta-analysis. *Reprod BioMed Online*. 2024;50(1):155–162.
737. Land JA, Evers JL, Goossens VJ. How to use Chlamydia antibody testing in subfertility patients. *Hum Reprod*. 1998;13:1094.
738. Jones CS, Maple PA, Andrews NJ, Paul ID, Caul EO. Measurement of IgG antibodies to *Chlamydia trachomatis* by commercial enzyme immunoassays and immunofluorescence in sera from pregnant women and patients with infertility. pelvic inflammatory disease, ectopic pregnancy, and laboratory diagnosed *Chlamydia psittaci*/*Chlamydia pneumoniae* infection. *J Clin Pathol*. 2003;56:225.
739. Land JA, Gijsen AP, Kessels AG, Slobbe ME, Bruggeman CA. Performance of five serological chlamydia antibody tests in subfertile women. *Hum Reprod*. 2003;18:2621.
740. Johnson NP, Taylor K, Nadgir AA, Chinn DJ, Taylor PJ. Can diagnostic laparoscopy be avoided in routine investigation for infertility? *Br J Obstet Gynaecol*. 2000;107:174.
741. Akande VA, Hunt LP, Cahill DJ, Caul EO, Ford WC, Jenkins JM. Tubal damage in infertile women: prediction using chlamydia serology. *Hum Reprod*. 2003;18:1841.
742. The Practice Committee of the American Society for Reproductive Medicine. Role of tubal surgery in the era of assisted reproductive technology: a committee opinion. *Fertil Steril*. 2021;115:1143–1150.
743. Broeze KA, Opmeer BC, Coppus SF, et al. Integration of patient characteristics and the results of Chlamydia antibody testing and hysterosalpingography in the diagnosis of tubal pathology: an individual patient data meta-analysis. *Hum Reprod*. 2012;27:2979.
744. Guideline Group on Unexplained Infertility; Romualdi D, Ata B, Bhattacharya S, et al. Evidence-based guideline: unexplained infertility. *Hum Reprod*. 2023;38(10): 1881–1890.
745. Turkgeldi E, Yagmur H, Seyhan A, Urman B, Ata B. Short and long term outcomes of children conceived with assisted reproductive technology. *Eur J Obstet Gynecol Reprod Biol*. 2016;207:129.
746. Borrero SB, Reeves MF, Schwarz EB, Bost JE, Creinin MD, Ibrahim SA. Race, insurance status, and desire for tubal sterilization reversal. *Fertil Steril*. 2008;90:272.
747. Schmidt JE, Hillis SD, Marchbanks PA, Jeng G, Peterson HB. Requesting information about and obtaining reversal after tubal sterilization: findings from the U.S. Collaborative Review of Sterilization. *Fertil Steril*. 2000;74:892.
748. Chi IC, Jones DB. Incidence, risk factors, and prevention of poststerilization regret in women: an updated international review from an epidemiological perspective. *Obstet Gynecol Surv*. 1994;49:722.
749. Neuhaus W, Bolte A. Prognostic factors for preoperative consultation of women desiring sterilization: findings of a retrospective analysis. *J Psychosom Obstet Gynaecol*. 1995;16:45.

750. Hardy E, Bahamondes L, Osis MJ, Costa RG, Faundes A. Risk factors for tubal sterilization regret, detectable before surgery, *Contraception*. 1996;54:159.
751. Curtis KM, Mohllajee AP, Peterson HB. Regret following female sterilization at a young age: a systematic review. *Contraception*. 2006;73:205.
752. Opsahl MS, Klein TA. The role of laparoscopy in the evaluation of candidates for sterilization reversal. *Fertil Steril*. 1987;48:546.
753. Henderson SR. The reversibility of female sterilization with the use of microsurgery: a report on 102 patients with more than one year of follow-up. *Am J Obstet Gynecol*. 1984;149:57.
754. Rock JA, Chang YS, Limpaphayom K, et al. Microsurgical tubal anastomosis: a controlled trial in four Asian centers. *Microsurgery*. 1984;5:95.
755. Boeckx W, Gordts S, Buysse K, Brosens I. Reversibility after female sterilization. *Br J Obstet Gynaecol*. 1986;93:839.
756. te Velde ER, Boer ME, Looman CW, Habbema JD. Factors influencing success or failure after reversal of sterilization: a multivariate approach. *Fertil Steril*. 1990;54:270.
757. Winston RM. Tubal surgery or in vitro fertilization (IVF)? *J Assist Reprod Genet*. 1992;9:309.
758. Dubuisson JB, Chapron C, Nos C, Morice P, Aubriot FX, Garnier P. Sterilization reversal: fertility results. *Hum Reprod*. 1995;10:1145.
759. Rouzi AA, Mackinnon M, McComb PF. Predictors of success of reversal of sterilization. *Fertil Steril*. 1995;64:29.
760. Glock JL, Kim AH, Hulka JF, Hunt RB, Trad FS, Brumsted JR. Reproductive outcome after tubal reversal in women 40 years of age or older. *Fertil Steril*. 1996;65:863.
761. Penzias AS, DeCherney AH. Is there ever a role for tubal surgery? *Am J Obstet Gynecol*. 1996;174:1218.
762. Van Voorhis BJ. Comparison of tubal ligation reversal procedures. *Clin Obstet Gynecol*. 2000;43:641.
763. Yoon TK, Sung HR, Kang HG, Cha SH, Lee CN, Cha KY. Laparoscopic tubal anastomosis: fertility outcome in 202 cases. *Fertil Steril*. 1999;72:1121.
764. Falcone T, Goldberg JM, Margossian H, Stevens L. Robotic-assisted laparoscopic microsurgical tubal anastomosis: a human pilot study. *Fertil Steril*. 2000; 73:1040.
765. Cha SH, Lee MH, Kim JH, Lee CN, Yoon TK, Cha KY. Fertility outcome after tubal anastomosis by laparoscopy and laparotomy. *J Am Assoc Gynecol Laparosc*. 2001;8:348.
766. Rodgers AK, Goldberg JM, Hammel JP, Falcone T. Tubal anastomosis by robotic compared with outpatient minilaparotomy. *Obstet Gynecol*. 2007;109:1375.
767. Dharia Patel SP, Steinkampf MP, Whitten SJ, Malizia BA. Robotic tubal anastomosis: surgical technique and cost effectiveness. *Fertil Steril*. 2008;90:1175.
768. Boer-Meisel ME, te Velde ER, Habbema JD, Kardaun JW. Predicting the pregnancy outcome in patients treated for hydrosalpinx: a prospective study. *Fertil Steril*. 1986;45:23.
769. Canis M, Mage G, Pouly JL, Manhes H, Wattiez A, Bruhat MA. Laparoscopic distal tuboplasty: report of 87 cases and a 4-year experience. *Fertil Steril*. 1991;56:616.
770. Diamond E. Lysis of postoperative pelvic adhesions in infertility. *Fertil Steril*. 1979;31:287.
771. Gomel V. Salpingo-ovariolysis by laparoscopy in infertility. *Fertil Steril*. 1983;40:607.
772. Donnez J, Casanas-Roux F. Prognostic factors of fimbrial microsurgery. *Fertil Steril*. 1986;46:200.
773. Dubuisson JB, Bouquet de Joliniere J, Aubriot FX, Darai E, Foulot H, Mandelbrot L. Terminal tuboplasties by laparoscopy: 65 consecutive cases. *Fertil Steril*. 1990; 54:401.
774. Dlugi AM, Reddy S, Saleh WA, Mersol-Barg MS, Jacobsen G. Pregnancy rates after operative endoscopic treatment of total (neosalpingostomy) or near total (salpingostomy) distal tubal occlusion. *Fertil Steril*. 1994;62:913.
775. Taylor RC, Berkowitz J, McComb PF. Role of laparoscopic salpingostomy in the treatment of hydrosalpinx. *Fertil Steril*. 2001;75:594.
776. Kitchin JD III, Nunley WC Jr, Bateman BG. Surgical management of distal tubal occlusion. *Am J Obstet Gynecol*. 1986;155:524.
777. Daniell JF, Diamond MP, McLaughlin DS, et al. Clinical results of terminal salpingostomy with the use of the CO_2 laser: report of the Intraabdominal Laser Study Group. *Fertil Steril*. 1986;45:175.
778. Audebert AJ, Pouly JL, Von Theobald P. Laparoscopic fimbrioplasty: an evaluation of 35 cases. *Hum Reprod*. 1998;13:1496.
779. Marana R, Quagliarello J. Distal tubal occlusion: microsurgery versus in vitro fertilization—a review. *Int J Fertil*. 1988;33:107.
780. Zeyneloglu HB, Arici A, Olive D. Adverse effects of hydrosalpinx on pregnancy rates after in vitro fertilization—embryo transfer. *Fertil Steril*. 1998;70:492.
781. Camus E, Poncelet C, Goffinet F, et al. Pregnancy rates after in-vitro fertilization in cases of tubal infertility with and without hydrosalpinx: a meta-analysis of published comparative studies. *Hum Reprod*. 1999;14:1243.
782. Beyler SA, James KP, Fritz MA, Meyer WR. Hydrosalpingeal fluid inhibits in-vitro embryonic development in a murine model. *Hum Reprod*. 1997;12:2724.
783. Meyer WR, Castelbaum AJ, Somkuti S, et al. Hydrosalpinges adversely affect markers of endometrial receptivity. *Hum Reprod*. 1997;12:1393.
784. Strandell A, Lindhard A, Waldenstrom U, Thorburn J. Hydrosalpinx and IVF outcome: cumulative results after salpingectomy in a randomized controlled trial. *Hum Reprod*. 2001;16:2403.
785. Melo P, Georgiou EX, Johnson N, et al. Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. *Cochrane Database Syst Rev*. 2020;10(10):CD002125.
786. Sagoskin AW, Lessey BA, Mottla GL, et al. Salpingectomy or proximal tubal occlusion of unilateral hydrosalpinx increases the potential for spontaneous pregnancy. *Hum Reprod*. 2003;18:2634.
787. Aboulghar MA, Mansour RT, Serour GI. Spontaneous intrauterine pregnancy following salpingectomy for a unilateral hydrosalpinx. *Hum Reprod*. 2002;17:1099.
788. Choe J, Check JH. Salpingectomy for unilateral hydrosalpinx may improve in vivo fecundity. *Gynecol Obstet Invest*. 1999;48:285.
789. Kotlyar A, Gingold J, Shue S, Falcone T. The effect of salpingectomy on ovarian function. *J Minim Invasive Gynecol*. 2017;24:563.
790. Mohamed AA, Yosef AH, James C, Al-Hussaini TK, Bedaiwy MA, Amer SAKS. Ovarian reserve after salpingectomy: a systematic review and meta-analysis. *Acta Obstet Gynecol Scand*. 2017;96:795.
791. Zhang Y, Sun Y, Guo Y, Li TC, Duan H. Salpingectomy and proximal tubal occlusion for hydrosalpinx prior to in vitro fertilization: a meta-analysis of randomized controlled trials. *Obstet Gynecol Surv*. 2015;70:33.
792. Van Voorhis BJ, Sparks AE, Syrop CH, Stovall DW. Ultrasound-guided aspiration of hydrosalpinges is associated with improved pregnancy and implantation rates after in-vitro fertilization cycles. *Hum Reprod*. 1998;13:736.
793. Tsiami A, Chaimani A, Mavridis D, Siskou M, Assimakopoulos E, Sotiriadis A. Surgical treatment for hydrosalpinx prior to in-vitro fertilization embryo transfer: a network meta-analysis. *Ultrasound Obstet Gynecol*. 2016;48:434.
794. Bloechle M, Schreiner T, Lisse K. Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of a serometra. *Hum Reprod*. 1997;12:703.
795. Cohen A, Almog B, Tulandi T. Hydrosalpinx sclerotherapy before in vitro fertilization: systematic review and meta-analysis. *J Minim Invasive Gynecol*. 2018;25(4):600–607.
796. Pérez-Milán F, Caballero-Campo M, Carrera-Roig M, et al. Systematic review and network meta-analysis of hydrosalpinx treatment before in vitro fertilization. *Ultrasound Obstet Gynecol*. 2024:1–13.
797. Al-Jaroudi D, Herba MJ, Tulandi T. Reproductive performance after selective tubal catheterization. *J Minim Invasive Gynecol*. 2005;12:150.
798. Fortier KJ, Haney AF. The pathologic spectrum of uterotubal junction obstruction. *Obstet Gynecol*. 1985;65:93.
799. De Silva PM, Chu JJ, Gallos ID, Vidyasagar AT, Robinson L, Coomarasamy A. Fallopian tube catheterization in the treatment of proximal tubal obstruction: a systematic review and meta-analysis. *Hum Reprod*. 2017;32:836.
800. Farhi J, Ben-Haroush A, Lande Y, Fisch B. Role of treatment with ovarian stimulation and intrauterine insemination in women with unilateral tubal occlusion diagnosed by hysterosalpingography. *Fertil Steril*. 2007;88:396.
801. Patton PE, Williams TJ, Coulam CB. Microsurgical reconstruction of the proximal oviduct. *Fertil Steril*. 1987;47:35.
802. Patton PE, Williams TJ, Coulam CB. Results of microsurgical reconstruction in patients with combined proximal and distal tubal occlusion: double obstruction. *Fertil Steril*. 1987;48:670.
803. Marana R, Quagliarello J. Proximal tubal occlusion: microsurgery versus IVF—a review. *Int J Fertil*. 1988;33:338.
804. Gleicher N, Confino E, Corfman R, et al. The multicentre transcervical balloon tuboplasty study: conclusions and comparison to alternative technologies. *Hum Reprod*. 1993;8:1264.
805. Wiedemann R, Hepp H. Selection of patients for IVF therapy or alternative therapy methods. *Hum Reprod*. 1989;4:23.
806. Dodson WC, Whitesides DB, Hughes CL Jr, Easley HA III, Haney AF. Superovulation with intrauterine insemination in the treatment of infertility: a possible alternative to gamete intrafallopian transfer and in vitro fertilization. *Fertil Steril*. 1987;48:441.
807. Crosignani PG, Collins J, Cooke ID, Diczfalusy E, Rubin B. Recommendations of the ESHRE workshop on 'Unexplained Infertility,' held in Anacapri, August 28–29, 1992. *Hum Reprod*. 1993;8:977.
808. Somigliana E, Paffoni A, Busnelli A, et al. Age-related infertility and unexplained infertility: an intricate clinical dilemma. *Hum Reprod*. 2016;31:1390.
809. ESHRE Capri Workshop Group. A prognosis-based approach to infertility: understanding the role of time *Hum Reprod*. 2017;32:1556.
810. Conner SJ, Lefievre L, Hughes DC, Barratt CL. Cracking the egg: increased complexity in the zona pellucida. *Hum Reprod*. 2005;20:1148.
811. Chatzimeletiou K, Morrison EE, Prapas N, Prapas Y, Handyside AH. The centrosome and early embryogenesis: clinical insights. *Reprod Biomed Online*. 2008;16:485.
812. Moomjy M, Sills ES, Rosenwaks Z, Palermo GD. Implications of complete fertilization failure after intracytoplasmic sperm injection for subsequent fertilization and reproductive outcome. *Hum Reprod*. 1998;13:2212.
813. Liu J, Nagy Z, Joris H, et al. Analysis of 76 total fertilization failure cycles out of 2732 intracytoplasmic sperm injection cycles. *Hum Reprod*. 1995;10:2630.
814. Gurgan T, Urman B, Yarali H, Kisnisci HA. The results of in vitro fertilization-embryo transfer in couples with unexplained infertility failing to conceive with superovulation and intrauterine insemination. *Fertil Steril*. 1995;64:93.
815. Ruiz A, Remohi J, Minguez Y, Guanes PP, Simon C, Pellicer A. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril*. 1997;68:171.

816. Takeuchi S, Minoura H, Shibahara T, Shen X, Futamura N, Toyoda N. In vitro fertilization and intracytoplasmic sperm injection for couples with unexplained infertility after failed direct intraperitoneal insemination. *J Assist Reprod Genet*. 2000;17:515.
817. Martin JS, Nisker JA, Parker JI, Kaplan B, Tummon IS, Yuzpe AA. The pregnancy rates of cohorts of idiopathic infertility couples gives insights into the underlying mechanism of infertility. *Fertil Steril*. 1995;64:98.
818. Boklage CE. Survival probability of human conceptions from fertilization to term. *Int J Fertil*. 1990;35:75.
819. Macklon NS, Geraedts JP, Fauser BC. Conception to ongoing pregnancy: the "black box" of early pregnancy loss. *Hum Reprod Update*. 2002;8:333.
820. Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod*. 2004;19:2849.
821. Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med*. 2007;357:9.
822. Mardon H, Grewal S, Mills K. Experimental models for investigating implantation of the human embryo. *Semin Reprod Med*. 2007;25:410.
823. Fazleabas AT. Physiology and pathology of implantation in the human and nonhuman primate. *Semin Reprod Med*. 2007;25:405.
824. Tapia A, Gangi LM, Zegers-Hochschild F, et al. Differences in the endometrial transcript profile during the receptive period between women who were refractory to implantation and those who achieved pregnancy. *Hum Reprod*. 2008;23:340.
825. Collins JA, Van Steirteghem A. Overall prognosis with current treatment of infertility. *Hum Reprod Update*. 2004;10:309.
826. Lenton EA, Weston GA, Cooke ID. Long-term follow-up of the apparently normal couple with a complaint of infertility. *Fertil Steril*. 1977;28:913.
827. Hunault CC, Habbema JD, Eijkemans MJ, Collins JA, Evers JL, te Velde ER. Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples, based on the synthesis of three previous models. *Hum Reprod*. 2004;19:2019.
828. Guzick DS, Sullivan MW, Adamson GD, et al. Efficacy of treatment for unexplained infertility. *Fertil Steril*. 1998;70:207.
829. Collins J, Rowe T. Age of the female partner is a prognostic factor in prolonged unexplained infertility: a multicenter study. *Fertil Steril*. 1989;52:15.
830. Zikopoulos K, West CP, Thong PW, Kalser EM, Morrison J, Wu FCW. Homologous intra-uterine insemination has no advantage over timed natural intercourse when used in combination with ovulation induction for the treatment of unexplained infertility. *Hum Reprod*. 1993;8:563.
831. Martinez AR, Bernardus RE, Voorhorst FJ, Vermeiden JP, Schoemaker J. Intrauterine insemination does and clomiphene citrate does not improve fecundity in couples with infertility due to male or idiopathic factors: a prospective, randomized, controlled study. *Fertil Steril*. 1990;53:847.
832. Verhulst SM, Cohlen BJ, Hughes E, Te Velde E, Heineman MJ. Intra-uterine insemination for unexplained subfertility. *Cochrane Database Syst Rev*. 2006;(4):CD001838.
833. Bhattacharya S, Harrild K, Mollison J, et al. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. *Br Med J*. 2008;337:a716.
834. Steures P, van der Steeg JW, Hompes PG, et al. Effectiveness of intrauterine insemination in subfertile couples with an isolated cervical factor: a randomized clinical trial. *Fertil Steril*. 2007;88:1692.
835. Randall JM, Templeton A. The effects of clomiphene citrate upon ovulation and endocrinology when administered to patients with unexplained infertility. *Hum Reprod*. 1991;6:659.
836. Glazener CMA, Coulson C, Lambert PA, et al. Clomiphene treatment for women with unexplained infertility: placebo-controlled study of hormonal responses and conception rates. *Gynecol Endocrinol*. 1990;4:75.
837. Harrison RF, O'Moore RR. The use of clomiphene citrate with and without human chorionic gonadotropin. *Ir Med J*. 1983;76:273.
838. Fujii S, Fukui A, Fukushi Y, Kagiya A, Sato S, Saito Y. The effects of clomiphene citrate on normal ovulatory women. *Fertil Steril*. 1997;68:997.
839. Fisch P, Casper RF, Brown SE, et al. Unexplained infertility: evaluation of treatment with clomiphene citrate and human chorionic gonadotropin. *Fertil Steril*. 1989;51:828.
840. Eskew AM, Bronwyn BS, Hardi A, et al. Letrozole compared with clomiphene citrate for unexplained infertility: a systematic review and meta-analysis. *Obstet Gynecol*. 2019;133(3):437–444.
841. Practice Committee of the American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. *Fertil Steril*. 2020;113(2):305–322.
842. Deaton JL, Gibson N, Blackmer KM, Nakajima ST, Badger GJ, Brumsted JR. A randomized, controlled trial of clomiphene citrate and intrauterine insemination in couples with unexplained infertility. *Fertil Steril*. 1990;54:1083.
843. Farquhar CM, Liu E, Armstrong S, Arroll N, Lensen S, Brown J. Intrauterine insemination with ovarian stimulation versus expectant management for unexplained infertility (TUI): a pragmatic, open-label, randomised, controlled, two-centre trial. *Lancet*. 2018;391:441.
844. Arici A, Byrd W, Bradshaw K, Kutteh WH, Marshburn P, Carr BR. Evaluation of clomiphene citrate and human chorionic gonadotropin treatment: a prospective, randomized, crossover study during intrauterine insemination cycles. *Fertil Steril*. 1994;61:314.
845. Karlstrom P-O, Bergh T, Lundkvist O. A prospective randomized trial of artificial insemination versus intercourse in cycles stimulated with human menopausal gonadotropin or clomiphene citrate. *Fertil Steril*. 1993;59:554.
846. Agarwal S, Mittal S. A randomised prospective trial of intrauterine insemination versus timed intercourse in superovulated cycles with clomiphene. *Indian J Med Res*. 2004;120:519.
847. Reindollar RH, Regan MM, Neumann PJ, et al. A randomized clinical trial to evaluate optimal treatment for unexplained infertility; the fast track and standard treatment (FASTT) trial. *Fertil Steril*. 2010;94:888.
848. Custers IM, Steures P, Hompes P, et al. Intrauterine insemination: how many cycles should we perform? *Hum Reprod*. 2008;23:885.
849. Dovey S, Sneeringer RM, Penzias AS. Clomiphene citrate and intrauterine insemination: analysis of more than 4100 cycles. *Fertil Steril*. 2008;90:2281.
850. Liu A, Zheng C, Lang J, Chen W. Letrozole versus clomiphene citrate for unexplained infertility: a systematic review and meta-analysis. *J Obstet Gynaecol Res*. 2014;40(5):1205–1216.
851. Badawy A, Elnashar A, Totongy M. RETRACTED: clomiphene citrate or aromatase inhibitors for superovulation in women with unexplained infertility undergoing intrauterine insemination: a prospective randomized trial [retraction of *Fertil Steril*. 2020;114(3):670]. *Fertil Steril*. 2009;92(4):1355–1359.
852. Al-Fozan H, Al-Khadouri M, Tan SL, Tulandi T. A randomized trial of letrozole versus clomiphene citrate in women undergoing superovulation. *Fertil Steril*. 2004;82(6):1561–1563.
853. Fouda UM, Sayed AM. Extended letrozole regimen versus clomiphene citrate for superovulation in patients with unexplained infertility undergoing intrauterine insemination: a randomized controlled trial. *Reprod Biol Endocrinol*. 2011;9:84.
854. Sammour A, Biljan MM, Tan SL, da Fonseca LM. Prospective randomized trial comparing the effects of letrazole (LE) and clomiphene citrate (CC) on follicular development, endometrial thickness and pregnancy rate in patients undergoing super-ovulation prior to intrauterine insemination (IUI). *Fertil Steril*. 2001;76:S110.
855. Diamond MP, Legro RS, Coutifaris C, et al; NICHD Reproductive Medicine Network. Letrozole, gonadotropin, or clomiphene for unexplained infertility. *N Engl J Med*. 2015;373:1230.
856. Guzick DS, Carson SA, Coutifaris C, et al; National Cooperative Reproductive Medicine Network. Efficacy of superovulation and intrauterine insemination in the treatment of infertility. *N Engl J Med*. 1999;340:177.
857. Gregoriou O, Vitoratos N, Papadias C, Konidaris S, Gargaropoulos A, Louridas C. Controlled ovarian hyperstimulation with or without intrauterine insemination for the treatment of unexplained infertility. *Int J Gynaecol Obstet*. 1995;48:55.
858. Steures P, van der Steeg JW, Hompes PG, et al. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. *Lancet*. 2006;368:216.
859. Peeraer K, Debrock S, De Loecker P, et al. Low-dose human menopausal gonadotrophin versus clomiphene citrate in subfertile couples treated with intrauterine insemination: a randomized controlled trial. *Hum Reprod*. 2015;30(5):1079.
860. Wessel JA, Danhof NA, van Eekelen R, et al. Ovarian stimulation strategies for intrauterine insemination in couples with unexplained infertility: a systematic review and individual participant data meta-analysis. *Hum Reprod Update*. 2022;28(5):733–746.
861. Crosignani PG, Walters DE, Soliani A. The ESHRE multicentre trial on the treatment of unexplained infertility: a preliminary report—European Society of Human Reproduction and Embryology. *Hum Reprod*. 1991;6:953.
862. Goverde AJ, McDonnell J, Vermeiden JP, Schats R, Rutten FF, Schoemaker J. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. *Lancet*. 2000;355:13.
863. Custers IM, König TE, Broekmans FJ, et al. Couples with unexplained subfertility and unfavorable prognosis: a randomized pilot trial comparing the effectiveness of in vitro fertilization with elective single embryo transfer versus intrauterine insemination with controlled ovarian stimulation. *Fertil Steril*. 2011;96:1107.
864. Bensdorp AJ, Tjon-Kon-Fat RI, Bossuyt PM, et al. Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine insemination with controlled ovarian hyperstimulation. *BMJ*. 2015;350:g7771.
865. Nandi A, Bhide P, Hooper R, et al. Intrauterine insemination with gonadotropin stimulation or in vitro fertilization for the treatment of unexplained subfertility: a randomized controlled trial. *Fertil Steril*. 2017;107:1329.
866. van Peperstraten AM, Nelen WL, Hermens RP, et al. Why don't we perform elective single embryo transfer? A qualitative study among IVF patients and professionals. *Hum Reprod*. 2008;23:2036.
867. Elit L, Charles C, Dimitry S, et al. It's a choice to move forward: women's perceptions about treatment decision making in recurrent ovarian cancer. *Psychooncology*. 2010;19:318.