More women than ever before have problems related to fertility. An estimated 6.2 million women in the United States are unable to conceive, an increase of roughly 26% over published data from a decade ago. Although the absolute number of infertile couples has risen dramatically, there has only been a modest increase in infertility rates during this same period of time. The demographics of the reproductive aged female population, however, has changed. Today, women are more career oriented and enter the workforce at a younger age, and a trend toward later marriage and child bearing has emerged. Baby boomers (born between 1945 and 1965) represent a large cohort of women entering their latter reproductive years. Undoubtedly, the higher prevalence of female infertility is related to reproductive aging, combined with an increasing demand by older, less fecund women to bear children.

The rising number of infertile couples has resulted in an escalating demand for fertility services. Approximately 2.7 million women in the United States sought medical help for infertility in 1995. This represents nearly a 2-fold increase since 1987. Many women are of advanced reproductive age, and pose a therapeutic challenge. The medical establishment, reacting to the growing demands and fertility concerns of the older population, has devoted substantial effort and resources to study the effects of aging on female reproduction. The following review summarizes the literature on female fertility, hormonal patterns, and reproductive tract changes associated with advancing age, and the factors known to regulate these changes. Included is a synopsis of the current means of assessing reproductive potential or ovarian reserve, and the clinical relevance of these measurements. Ultimately, an understanding of these issues will enable clinicians to better inform and counsel the older infertile patient.

### Effect of aging on fertility

Although rare, women have achieved spontaneous pregnancies beyond the fifth and even into the sixth decade of life. This is exemplified by the recent report of a 54-year-old Washington woman delivering a spontaneous triplet gestation. How then does one define old with regard to natural fertility? Population-based data indicate that the decline in reproductive potential that accompanies advancing age is a continuum. In the United States, approximately 10% of women between the ages of 20 and 29 years report some difficulty achieving a pregnancy. This proportion increases to 25% between the ages of 30 and 39 years, and to more than 50% in women deferring child bearing beyond age 40.

The task of quantifying the effect of aging on fertility
A difficult one. Confounding variables include the widespread acceptance and use of contraception and the significant social and economic constraints that limit family size. Defining infertility as a failure to conceive within 12 months may also overestimate any age-related effect on fertility, as a significant number of women achieve pregnancy beyond this time. Older women may require more time to conceive; that is, their fecundability is affected, while fertility rates may not be altered to the same degree.

These problems are circumvented in the classic study of fertility rates in the Hutterite population. The Hutterites are a religious sect living in the Dakotas, Montana, and adjacent portions of Canada in whom contraceptive practices are strictly prohibited. The communal nature of their settlements removes any economic burden associated with a growing family size. Historical data derived from 209 Hutterite women, all of whom were married by age 25 and still married by age 45 to 50 (Table I), illustrates 2 points. The first is the remarkably high overall fertility rates achieved in such a population: the 7% infertility rate in women under age 30 years is nearly half that observed in the general population. More important is the overall trend in fertility rates, with a gradual decline noted as early as age 25, accelerating markedly beyond ages 35 to 40. This is represented graphically (Fig 1), along with data from 9 other “natural populations” practicing little or no contraception. Although absolute fertility rates vary between populations, remarkably consistent age-related trends are noted, with a slow decline in fertility rates through the middle of the fourth decade of life followed by a much steeper decline thereafter. Cross-sectional, population-based therefore indicate that a reasonable definition of advanced reproductive age, as pertains to fecundity and fertility, is age 35 and above (the point at which reproductive potential declines at an accelerated rate).

Studies of “natural populations” are imperfect, however, and prone to overestimate the effect of aging on reproductive potential. This is because a number of conditions associated with impaired fertility—such as pelvic inflammatory disease, tubal disease, fibroids, and endometriosis—also increase in frequency with age. Additionally, there may be a decreased frequency of coitus in older couples. To control for male infertility or coital frequency, French investigators analyzed cumulative pregnancy rates after 12 cycles of artificial insemination with donor sperm. There were minimal differences in success

Table I. Estimated percentage of sterile couples at specified ages in the Hutterite population

<table>
<thead>
<tr>
<th>Age of wife (y)</th>
<th>Percent sterile</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>3.5</td>
</tr>
<tr>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>35</td>
<td>11</td>
</tr>
<tr>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>45</td>
<td>87</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

(Reprinted by permission from the American Society for Reproductive Medicine.)

Fig 1. Fertility rates in 10 “natural” populations: Hutterites, marriages 1921-1930 (closed triangles); Geneva bourgeoisie, 17th century (closed squares); Canada, 18th century (closed circles); Normandy, 18th century (open circles); Hutterites, marriages before 1921 (open squares); Tunis, 19th century (open triangles); Normandy, marriages 1674-1742 (closed circles); Norway, marriages 1874-1876 (open squares); Iran, 20th century (closed triangles); Geneva bourgeoisie, 16th century (open circles). (Reprinted with permission from Science. Copyright 1986 American Association for the Advancement of Science.)
rates noted among women under age 30. In contrast, a marked decline in fertility was noted among women older than age 35 (Fig 2). A second study of women enrolled in a donor insemination program confirmed this finding, while subjecting all patients to a comprehensive infertility evaluation, including a laparoscopy to exclude pelvic factors associated with decreased fertility.8 The data thus supports a decline in female reproductive potential directly attributable to advancing chronologic age, with the most prominent effect occurring beyond age 35.

What accounts for the age-related decline in fertility?

Menstrual cycle irregularities and ovulatory dysfunction often precede menopause and typically begin around the latter half of the fifth decade of life. However, there is a period of approximately 10 years in which fertility is markedly diminished despite a continuance of monthly, ovulatory cycles. This period of reproductive and endocrinologic transition is termed perimenopause. It has been mentioned that an age-related decline in pregnancy rates occurs independent of any male7,8 or pelvic8 fertility factors. Success rates with in vitro fertilization (IVF) also decrease with advancing age in a fashion similar to the decline noted in natural cycles, even after controlling for the diminished ovarian response to stimulation often seen in older patients.9 This suggests that factors independent of tubal architecture and motility play a prominent role in the age-related decline in fertility. The lower conception rates noted in older, ovulatory women are likely a result of either diminished oocyte-embryo quality, uterine receptiveness, or both.

Aging of the uterus versus aging of the oocyte. There has been much debate as to whether ovarian or uterine factors are primarily responsible for the age-related decline in fertility. Much of the early data derived from animal studies suggest the uterus plays a predominant role in limiting reproductive efficiency. Markedly lower clinical pregnancy rates have been documented in older animals receiving transplanted ova from young adult donors compared with donor-matched, young recipients.10-12 A number of age-related uterine and endometrial changes have also been described in various animal species (Table II).

<table>
<thead>
<tr>
<th>Table II. Age-related uterine changes in various animal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decline in decidual response (golden hamster)100</td>
</tr>
<tr>
<td>Increase in collagen content (rat)101</td>
</tr>
<tr>
<td>Decrease in endometrial mitotic activity (mouse)102</td>
</tr>
<tr>
<td>Increase in stromal mitotic activity (mouse)102</td>
</tr>
<tr>
<td>Decrease in “attachment reaction” [ultrastructural alteration of uterine epithelial microvilli in response to estrogenic stimuli] (mouse)105</td>
</tr>
<tr>
<td>Decreased concentration of estrogen receptors (rat)104</td>
</tr>
<tr>
<td>Intimal thickening of the endometrial arteries (pig)105</td>
</tr>
<tr>
<td>Glandular cyst formation in deep layer of the endometrium (pig)105</td>
</tr>
<tr>
<td>Decrease in density of endometrial stromal cells (rat)106</td>
</tr>
</tbody>
</table>

![Fig 2](image availability note)
normal endometrial histology in women beyond age 35.\textsuperscript{13} This study was based on a relatively small sample size and has yet to be substantiated by others. Another group failed to demonstrate any histologic difference in endometrial biopsy specimens from older and younger functionally agonadal women on hormone replacement therapy.\textsuperscript{14} On the basis of this finding, it seems reasonable to speculate the endometrium of the older ovulatory woman, with an endogenous supply of estradiol and progesterone, should compare favorably with the endometrial lining of the younger ovulatory patient.

Age-related changes in the temporal pattern of hormonal stimulation, however, may affect endometrial receptivity to the implanting blastocyst. For example, Lenton et al\textsuperscript{15} found a higher incidence of luteal phase deficiency at the extremes of reproductive age. The link between luteal phase deficiency and infertility remains a subject of debate, and no well-controlled studies have been conducted to determine the efficacy of treatment on pregnancy rates in older women with this disorder. A work-up should nevertheless be considered in women with otherwise unexplained infertility. Alternatively, empiric progesterone supplementation, particularly in women of advanced maternal age, may be instituted. Progesterone supplementation and other treatments for luteal phase deficiency in older patients have not resulted in a dramatic improvement in pregnancy rates, implying factors other than an insufficiently primed endometrium must explain the observed decline in fertility with advancing age.

The advent of modern IVF and donor egg programs has provided a more direct means of investigating the relative contribution of the uterus and oocyte to human reproductive senescence. Young women undergoing standard IVF with their own eggs, compared with older women (\textgreater age 40) undergoing IVF with eggs donated by this younger subset of women, demonstrated similar success rates.\textsuperscript{16} This implies egg donation overcomes age-related changes in fertility. The slightly higher success rate in the older recipient group underscores the belief that ovarian hyperstimulation might have an adverse effect on IVF outcome (a hypothesis that has since received much attention in the medical literature \textsuperscript{17,21}). To control for this potentially confounding variable, implantation and pregnancy rates in older (\textgreater age 40) and younger women with premature ovarian failure undergoing IVF with donor eggs were compared (Table III).\textsuperscript{22} Neither group was subjected to ovarian hyperstimulation, and comparable success rates were achieved. Although a different cohort of young donor eggs were used in each patient, a more controlled study comparing older and younger recipient cycles transferred with oocytes from the same donor would later yield similar results.\textsuperscript{25} These findings strongly implicate egg quality as the major factor responsible for waning reproductive function with aging.

The debate in the literature, however, remains ongoing. Investigators in London and Israel have reported higher clinical pregnancy rates in younger versus older women undergoing egg donation.\textsuperscript{24, 25} In a third study, a lower mean age was noted among egg donor recipients who conceived, controlling for age of the donor and number of embryos transferred per cycle.\textsuperscript{26} In this report, the small number of embryos transferred per cycle (median of 2) may account for the lower overall conception rates seen with advancing maternal age. This would be consistent with an analysis of 24 patients undergoing IVF described in a recent review article in which implantation rates among older recipients were significantly lower than those seen in young egg donors, despite similar ongoing pregnancy rates.\textsuperscript{27} The average number of embryos transferred per recipient cycle was 3.6 and suggests a critical number of transferred embryos may be necessary to overcome a decrease in implantation efficiency in older women.

Differing regimens for endometrial priming and luteal phase support in donor egg cycles may further account for the conflicting reports in the literature. A study comparing 3 groups of women undergoing IVF with donor egg (women > 40 years of age receiving 50 mg supplemental progesterone, women > 40 years of age receiving

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**Table III.** Results of transfer of fertilized donor ova in women aged 40 years and above with ovarian failure compared with women under 40 years with ovarian failure and women 40 years and above undergoing standard in vitro fertilization and embryo transfer

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Ongoing Pregnancy Rate</th>
<th>Implantation Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 40</td>
<td>30%</td>
<td>3.2%</td>
</tr>
<tr>
<td>40+</td>
<td>25%</td>
<td>2.8%</td>
</tr>
<tr>
<td>40+</td>
<td>20%</td>
<td>2.4%</td>
</tr>
</tbody>
</table>

Table available in print only
100 mg supplemental progesterone, and women < 40 years of age receiving 50 mg supplemental progesterone (Table IV) noted women over age 40 receiving the lower (physiologic) dose of supplemental progesterone had markedly lower pregnancy rates compared with both the older cohort receiving higher (pharmacologic) doses of progesterone and the younger-aged women receiving 50 mg supplemental progesterone. This finding suggests a detrimental effect on uterine receptivity with aging, which is overcome in most egg donation studies by the administration of supraphysiologic doses of progesterone. In the literature, progesterone supplements in both 50 and 100 mg doses have been used in recipient cycles. What is often unclear, however, is the duration of exposure to exogenous progesterone, making direct comparisons difficult. In addition, 1 group using the lower 50-mg dose of progesterone omits all women who did not have endometrial biopsies in mock cycles preceding administration of supraphysiologic doses of progesterone. Of note, progesterone levels have been shown to remain stable in older cycling women (described later), although a progesterone receptor defect or deficiency may exist at the level of the endometrium. The aging oocyte in perspective. Clearly, the aging oocyte is a primary factor contributing to the decrease in reproductive potential. The changes responsible for this effect, however, are poorly understood. No consistent morphologic differences have been reported among mature oocytes and early (2- to 3-day-old) embryos from younger and older women undergoing IVF. Furthermore, IVF data has historically demonstrated minimal differences in fertilization and embryo cleavage rates with advancing age. The mechanism whereby these older embryos fail to implant and develop normally has yet to be fully elucidated.

Embryonal maldevelopment may, at least partially, be related to the increase in chromosomal abnormalities seen in abortuses and offspring of older women. The spontaneous abortion rate in patients undergoing egg donation has been shown to correlate with the age of the donor, implicating the oocyte as the primary factor responsible for the high rate of aneuploidy (the most common cause of early pregnancy loss) in women of advanced maternal age. An age-dependent increase in chromosomal nondisjunction has also been demonstrated in oocytes from both stimulated and unstimulated ovaries. This likely contributes to the observed increase in embryonal aneuploidy with advancing maternal age.

An increased rate of aneuploidy, however, does not fully account for the dramatic decline in fertility with advancing age. The percentage of chromosomally abnormal embryos diagnosed by fluorescence in situ hybridization of cells obtained by blastomere biopsy is on the order of 60% to 70% in women of advanced reproductive age. With repeated attempts at conception, particularly with the retrieval of multiple oocytes during stimulated cycles, adequate numbers of euploid embryos should be achieved, negating at least in part the potential adverse impact of chromosomal aneuploidy on age-related pregnancy rates. Moreover, while the proportion of aneuploid abortuses increases in older women, the frequency of both aneuploid and euploid abortuses is higher in older women. This suggests that factors other than aberrations in chromosome complement must contribute to the increased pregnancy wastage with advancing age. Lim and Tsakok found a statistically significant, age-dependent increase in chromosomal degeneration (but not aneuploidy) among oocytes analyzed within 2 days of retrieval. They hypothesize that oocytes with mild degenerative changes (defined as the fragmentation of no more than 2 chromosomes at metaphase) may still fer-

---

**Table IV.** Clinical characteristics and pregnancy outcome of women older and women younger than age 40 years having oocyte donation with 50 mg supplemental progesterone and women older than age 40 years receiving 100 mg supplemental progesterone

<table>
<thead>
<tr>
<th></th>
<th>&gt; 40 y (50 mg P)</th>
<th>&gt; 40 y (100 mg P)</th>
<th>&lt; 40 y (50 mg P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>24</td>
<td>35</td>
<td>28</td>
</tr>
<tr>
<td>Age (y ± SE)</td>
<td>41.9 ± 0.3</td>
<td>42.0 ± 0.3</td>
<td>35.4 ± 0.6</td>
</tr>
<tr>
<td>Infertility (y)</td>
<td>5.3 ± 0.7</td>
<td>6.2 ± 0.8</td>
<td>6.7 ± 0.7</td>
</tr>
<tr>
<td>Age donor (y)</td>
<td>28.3 ± 0.8</td>
<td>27.6 ± 0.7</td>
<td>29.6 ± 0.7</td>
</tr>
<tr>
<td>Embryos transferred (No.)</td>
<td>3.7 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Ongoing-delivered (%)</td>
<td>5 (21)</td>
<td>19 (54)</td>
<td>13 (46)</td>
</tr>
</tbody>
</table>

*P, Progesterone. (Reprinted by permission from the American Society for Reproductive Medicine.)*
utilize and undergo normal cleavage. These embryos would thus appear morphologically no different from healthy embryos, despite an impaired ability to implant and develop normally. This provides an alternative, though unproven explanation for the lower fertility and ongoing pregnancy rates seen in older women undergoing otherwise normal IVF cycles.

It is interesting to speculate as to the relative contribution of nuclear and cytoplasmic changes within the aging oocyte to the decline in reproductive efficiency in older women. Successful IVF and early human embryonic development by using an enucleated immature oocyte from a young donor followed by germinal vesicle transfer from an older patient have been reported. To date, no pregnancies have been described using this technique. Another procedure with donor cytoplasm injected into a recipient oocyte has resulted in a successful pregnancy in a patient with a history of multiple prior IVF failures. The benefit of these approaches to women otherwise requiring egg donation to achieve a pregnancy is obvious; the ability to confer one’s own genetic profile to offspring, excluding mitochondrial deoxyribonucleic acid contained within the donor ooplasm. Unfortunately, the pathophysiology of the aging human oocyte remains poorly understood, and the significance of an extranuclear factor in female infertility has not been established. Chromosome fragility, and hence nuclear factors, are likely of paramount importance in mediating the oocyte factors responsible for decreased fertility with advancing age. The interplay between cytoplasmic and nuclear factors during gametogenesis, however, is complex. There is a higher frequency of abnormal meiotic spindle formation in oocytes from older versus younger women. The meiotic spindle, composed of microtubules and associated cytoskeletal proteins, assembles initially outside the cell nucleus. This may not only explain the higher frequency of chromosomal nondisjunctive events observed in aging oocytes, but lends support to the notion that donor cytoplasm may play a role in at least part of the age-related decline in both fertility and euploid offspring. At present, this issue is subject to pure conjecture pending the outcome of any future investigative efforts.

The application of newer techniques for extended in vitro culturing of embryos has for the first time identified significant age-related differences in embryo morphology (ie, arrest at morula stage, diminished blastocyst expansion rate). This is theoretically of clinical relevance because blastocyst culture has been purported to promote optimal embryo selection in women of all ages. A 50% per transfer pregnancy rate has recently been reported in women above age 39 years undergoing blastocyst transfer. Although encouraging, an unusually large number of oocytes were retrieved (mean 22) for this patient population, resulting in a large number of available blastocysts for transfer (mean, 2.9 per patient). The higher attrition rate associated with extended culture techniques may actually make this treatment modality less feasible for the older poor responder with fewer oocytes and embryos. Blastocyst transfer has only recently been adopted into practice, and the consequences of such a treatment strategy have yet to be clinically tested.

### Regulation of reproductive senescence

A significant number of women in their latter reproductive years achieve pregnancy quite readily, while others are unable to conceive despite the continuance of monthly ovulatory cycles. What accounts for the disparity and variance among individuals? The transition to a less fertile state may be governed primarily by the rate at which a woman depletes her follicular reserves. Women are born with a finite number of oocytes, which actually begin to decline before birth and throughout postnatal life until menopause. It has been suggested a spectrum exists, such that when the follicular pool is depleted below a certain threshold, fertility is compromised. Further reductions in follicular reserves result in oligo-ovulation, perimenopause, and ultimately menopause.

A threshold of follicle numbers is necessary for normal reproductive function in the rat. In human beings, support for this theory is based on more indirect, circumstantial evidence. Cigarette smoking, for instance, is known to be associated with an earlier age of onset of menopause, presumably as a result of toxic effects on the ovary. Whether or not the decline in fertility associated with cigarette use is a direct result of a depletion of ovarian reserves, or primarily related to other tobacco-related effects, however, is unclear. Dwindling follicular reserves also coincide with a rising output of pituitary gonadotropins, perhaps related to the subtle, but clinically significant decline in serum inhibins in older, premenopausal women. Elevated follicle-stimulating hormone (FSH) levels are in turn associated with a diminished reproductive capacity (described later), lending further credence to this hypothesis. The prevailing view has thus been that an exhaustion of ovarian follicles is the most important determinant in declining reproductive function and ultimately the transition to menopause.

Although a steady rate of follicular decline occurs in the rodent, this does not appear to be the case in human beings. Faddy et al have shown the loss of ovarian follicles with advancing age is biexponential, with an accelerated rate of decline beginning (on average) in the latter half of the fourth decade of life (Fig 3). At this stage of a woman’s life, fertility is known to decrease at an accelerated rate. Whether a common link exists between these 2 events or it is a matter of pure coincidence is unknown. Interestingly, if the initial rate of follicular atresia were to be sustained (ie, no acceleration phase), it is estimated menopause would not be reached until the mid-70s, coinciding with aging of other organ systems.

What is the “trigger” for this phenomenon? The accel-
eration may be driven by elevated serum levels of FSH (a change known to coincide in timing with accelerated follicular loss). FSH may recruit a larger cohort of follicles ultimately destined to undergo atresia, resulting in a greater depletion of oocytes per cycle. In vitro data, however, indicate FSH may spare ovarian follicles from the atretic process. There is evidence to suggest follicular atresia occurs through a genetically mediated pathway of programmed cell death or apoptosis.\(^\text{50}\) A number of hormonal and other factors have been shown to modulate this process (Table \(V\)), with FSH having a suppressive effect on ovarian cell loss in cultures of rat granulosa cells. Extrapolating this finding in vivo to humans is premature, but it is tempting to speculate that elevated levels of FSH (and perhaps estrogen) in older premenopausal women may alternatively represent an adaptive response intended to limit the accelerated loss of ovarian follicles as women age, rather than serve as the primary impetus for this process.

Despite their heterogeneity, most steroids and peptides known to influence ovarian cell apoptosis either originate centrally (gonadotropin-releasing hormone, FSH, luteinizing hormone, growth hormone) or are under the control of factors produced in higher cortical centers (estrogens, androgens, insulin-like growth factors). The hypothalamic-pituitary changes in premenopausal, middle-aged women (increase in FSH,\(^\text{51-55}\) increase in interpulse interval, and duration of luteinizing hormone pulses\(^\text{56}\)) may represent an intrinsic effect of aging on neuroendocrine tissues rather than a secondary event mediated by follicular decline. A growing body of evidence has emerged to lend credence to this hypothesis.\(^\text{57, 58}\) Unfortunately, much of this data is derived from studies performed in rodents, and relatively little is known regarding

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**Table V.** Steroids, peptides, and other factors with a documented in vitro or in vivo influence on ovarian cell apoptosis in the rat model

<table>
<thead>
<tr>
<th>Factors associated with induction of ovarian cell apoptosis</th>
<th>Factors associated with suppression of ovarian cell apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH agonist(^\text{107})</td>
<td>Gonadotropins (LH and FSH)(^\text{107-109})</td>
</tr>
<tr>
<td>Estrogen withdrawal(^\text{107, 112})</td>
<td>Growth hormone(^\text{109})</td>
</tr>
<tr>
<td>Androgens(^\text{107, 112})</td>
<td>Insulin-like growth factor-1(^\text{109})</td>
</tr>
<tr>
<td>Interleukin-6(^\text{112})</td>
<td>Interleukin-1(^\text{110})</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein-3(^\text{11})</td>
<td>Nitric oxide(^\text{110})</td>
</tr>
<tr>
<td>Tumor necrosis factor alpha(^\text{108})</td>
<td>Growth factors (epidermal, basic fibroblast, keratinocyte)(^\text{109, 111})</td>
</tr>
</tbody>
</table>

GnRH, Gonadotropin-releasing hormone.
the extraovarian mechanisms (if any) regulating ovarian senescence in humans.

While follicular loss may be a final common pathway, whether or not this process is intrinsically regulated within the ovary, is primarily governed by neuroendocrine changes, or is some combination of these 2 factors remains unknown. A better understanding of these issues is requisite to the development of clinical therapies that may someday enable the clinician to retard the process of follicular atresia and prolong the reproductive life span of the fertility patient.

**Hormonal changes in the older, ovulatory female**

Changes in the hormonal profile of the postmenopausal female, including elevations in gonadotropins and a decrease in ovarian steroids and peptides, have been well documented. More recently, efforts have focused on the hormonal milieu of the older ovulatory female, as significant clinical changes (most notably diminished fecundity and fertility) have become appreciated in this population.

An analysis of the changing hormonal environment of the aging woman has scientific and clinical application. Age-related effects on reproductive hormones may indirectly compromise folliculogenesis, maturation of the endometrial lining, and development of the embryo. A number of reproductive hormones have also been shown to modulate ovarian apoptotic processes, which have implications for malignant degeneration and premature failure of the ovary if suppressed or potentiated, respectively. In addition, hormonal discrepancies between young and old cycling women have been used as clinical markers of advanced reproductive age (described later under “Assessing ovarian reserve in the older fertility patient”).

A growing body of data focus on the endocrinology of the perimenopause. Elevations of FSH and estradiol, and decreases in inhibin-B levels represent the most clinically significant hormonal alterations in older reproductive-aged women, with predictive value as measures of reproductive capacity in some studies. Alterations in somatotrophin levels have received increasing attention because there is a growing appreciation for their role in female reproductive physiology. Studies to date, however, seem to indicate minimal, if any changes in serum levels of these proteins across the spectrum of reproductive age.

Age-related changes in the hormonal milieu of the ovarian follicle have recently been examined. An analysis of follicular fluid hormone levels in series of IVF patients has demonstrated declining levels of estradiol (E2) and progesterone (P) per follicle with advancing age. A significant decline in the E2/P ratio was also noted, suggesting an aberrant follicular maturation process (reflected by a shift in follicular production of estrogen to progesterone) in older women. A second group of investigators, in contrast, have intimated a “better” dominant follicle in women of advanced reproductive age on the basis of a trend toward higher E2, increased P, decreased testosterone (T), and an increased E2/T ratio in the follicular fluid of older volunteers during unstimulated, spontaneous cycles. This same group has also published data showing stable follicular fluid levels of inhibins with advancing age—again suggesting a stable follicular apparatus in older women despite an overall decline in the follicular pool. Age-related changes in insulin-like growth factor I and growth hormone binding protein levels have also been found in midcycle follicular fluid samples, although it is unclear whether this is merely a consequence of changing serum levels of these products or a true reflection of an aging follicle.

A comparison of the in vitro biosynthetic capacity of follicular cells in younger and older reproductive-aged women has also revealed inconsistent findings between researchers. One study reported the production of alpha-inhibin and P are lower after culture of a purified granulosa-luteal cell preparation derived from follicular aspirates of older IVF patients at the time of egg retrieval. Another group, however, failed to confirm this finding in cultures produced by cells retrieved during unstimulated cycles. Whether any significant age-related steroidalogenic effects occur in the cellular components of the developing follicle thus remains unclear.

**Assessing “ovarian reserve” in the older fertility patient**

Because fertility diminishes with aging, the aggressive use of fertility drugs and standard assisted reproductive techniques in older patients may be of limited value. A number of these women, however, are able to conceive readily without egg donation or costly, invasive, and emotionally draining reproductive procedures. The ability to identify individuals with diminished reproductive potential or “ovarian reserve” is therefore of practical value, and has received much attention.

**Basal FSH levels.** The rise in basal FSH is the most consistent, reproducible alteration in the hormonal profile of the older cycling female. An analysis of basal FSH levels and pregnancy outcomes, however, was not performed for more than a decade after this relationship was first discovered. Researchers initially described an association between elevated FSH levels and declining ovarian response to stimulation, with a low pregnancy rate noted in women with decreased E2 and follicular response patterns after administration of exogenous gonadotropins. A number of investigators would later prospectively correlate elevated FSH values (> 20-25 IU/L) with extremely low pregnancy rates (0%-5%) in IVF patients, controlling for age of the patient. Advancing age, however, remained an independent determinant of IVF perfor-
mance, albeit a less powerful predictor than basal FSH alone.80 This is also applicable to women with 1 ovary, when similar thresholds for determining normative values are used.81

Questions regarding the reproducibility of the test were subsequently addressed. The mean deviation in basal FSH levels in patients undergoing serial testing is highest in women with an abnormal result, indicating a propensity for subsequent FSH determinations to occasionally cross into the normal range.82 Women with a normal test result after an abnormal screen respond poorly to stimulation with gonadotropins and have poor pregnancy rates after assisted reproduction.82 There is thus no value to serial testing in women who have a prior well-documented elevation of basal FSH (in the hope of selecting a more fecund month).

The significant variance among serial FSH determinations, however, highlights the poor predictability of an isolated normal FSH concentration (given that a number of these patients will ultimately have evidence of diminished ovarian reserve in subsequent testing). Serial determinations are prohibitive in terms of cost and time, and alternative means of assessing a patient’s ability to conceive have been sought.

**Basal estradiol level.** An improvement in the predictive power of an isolated FSH determination was gained by concomitant measurement of day 3 E2 levels.83, 84 Basal E2 levels (> 75-80 pg/mL) were also predictive of IVF outcome independent of FSH levels,83, 85 although clinically significant intercycle variability may exist for an isolated basal estradiol determination.80 While a combination of these 2 biochemical markers (FSH and E2) remains the screening test of choice in many centers, others have turned to provocative testing in an attempt to better delineate patients with diminished reproductive capacity.

**Clomiphene citrate challenge test.** The clomiphene citrate challenge test is a provocative test in which a basal FSH level is determined, and if normal, 100 mg of clomiphene citrate is then administered on cycle days 5 to 9 followed by a second FSH level on day 10. The test is considered abnormal if the FSH value on either day 3 or day 10 is elevated. Patients with diminished ovarian reserve respond to clomiphene citrate with a smaller, less hormonally active cohort of follicles. This in turn leads to lower levels of E2 and inhibin, resulting in an inadequate suppression of FSH. Presumably, higher detection rates would be achieved, as a number of women with a normal day 3, but abnormal day 10, FSH level would now be classified as having diminished ovarian reserve.

A summary of 3 studies examining the predictive power of the clomiphene citrate challenge test, along with 2 of the larger series on basal FSH screening, has recently been compiled (Table VI).88 The predictive value of an abnormal clomiphene citrate challenge test was extremely high in all 3 studies, with an overall cumulative pregnancy rate of only 1.3% (1/75). This is comparable with the 1.5% (2/136) cumulative pregnancy rate among women with abnormal day 3 FSH values. The predictive value of the clomiphene citrate challenge test in the referenced studies, however, is limited to women older than 35 years of age. (Younger women were not tested.) Nevertheless, among older, at-risk patients, the clomiphene citrate challenge test identified a significantly larger proportion of patients with compromised fecundity, with an average detection rate of 29% compared with an age-adjusted (> aged 35) rate of 6%80 for basal FSH screening alone. That is, most patients with an abnormal clomiphene citrate challenge test had a normal day 3 FSH and would have been incorrectly stratified as “normals” had a provocative test not been performed.

**Additional measures of ovarian reserve.** Another approach to measuring ovarian reserve involves the response to the agonist action of leuprolide acetate during a “flare up” protocol for IVF. Four distinct E2 response patterns may be recognized (Fig 4), with an early doubling of the E2 value followed by a prompt decline the following day (“pattern A”) associated with the highest clinical pregnancy rates.89 The leuprolide agonist stimulation test was subsequently described90 and (like the clomiphene citrate challenge test) shown to have improved negative predictive value over a basal FSH alone in assessing ovarian reserve in women undergoing IVF.91 The limited data and added expense associated with this test, however, may preclude practitioners from using it as a general screening tool.

**Table VI. Summary of results from 3 studies of ovarian reserve screening using the clomiphene citrate challenge test and 2 studies of screening using isolated basal follicle-simulating hormone.**

<table>
<thead>
<tr>
<th>Author</th>
<th>N</th>
<th>Test</th>
<th>Normal (%)</th>
<th>Pregnant (%)</th>
<th>Abnormal (%)</th>
<th>Pregnant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Navot et al897</td>
<td>51</td>
<td>CCCT</td>
<td>33 (64.8)</td>
<td>14 (42.4)</td>
<td>18 (35.2)</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>Loumaye et al113</td>
<td>114</td>
<td>CCCT</td>
<td>94 (82.5)</td>
<td>26 (27.7)</td>
<td>20 (17.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tanbo et al114</td>
<td>91</td>
<td>CCCT</td>
<td>54 (59)</td>
<td>6 (12)</td>
<td>37 (41)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Scott et al79</td>
<td>758</td>
<td>FSH</td>
<td>702 (92.6)</td>
<td>152 (25.2)</td>
<td>56 (7.4)</td>
<td>2 (3.6)</td>
</tr>
<tr>
<td>Toner et al80</td>
<td>1478</td>
<td>FSH</td>
<td>1398 (95)</td>
<td>(? &lt; 15)</td>
<td>80 (5)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

CCCT, Clomiphene citrate challenge test; FSH, follicle-simulating hormone. (Reprinted and modified with permission from the American Society for Reproductive Medicine.88)

*Ovulation induction patients.*
More recently, inhibin has emerged as an alternative measure of the follicular pool. In vitro data first suggested day 3 FSH levels may actually reflect an indirect assessment of granulosa cell inhibin production. Cultured granulosa cells taken from women with normal ovarian reserve produced higher levels of inhibin compared with women with a diminished reserve as assessed by basal FSH testing. In vivo, both basal and stimulated follicular phase inhibin-B levels (day 3 and day 10 values after clomiphene citrate) and luteal phase inhibin-A levels have been shown to correlate inversely with serum FSH testing. One center, using a day 3 inhibin value of 45 pg/mL for defining the lower limit of normal, reported basal inhibin-B levels to be predictive of conception rates in an IVF program independent of age, day 3 FSH, and day 3 E2. Another group failed to substantiate this finding, casting some doubt as to the clinical utility of this marker. Serum inhibin assays are not widely available and have yet to be confirmed as a useful measure of ovarian reserve.

Others have examined the role of transvaginal sonography as a predictor of ovarian response to gonadotropin stimulation. Ovarian volume <3 cm or low antral follicle counts, or both, measured in the early follicular phase of the cycle, have been shown to be associated with a high cycle cancellation rate and decreased numbers of oocytes retrieved during IVF. The predictive value of sonography has not been compared with other measures of ovarian reserve in a randomized controlled fashion and remains investigational.

**Clinical application of ovarian reserve screening.** It seems reasonable to offer some measure of ovarian reserve to all women of advanced reproductive age who are experiencing difficulty conceiving. One must be careful, however, in the interpretation and applicability of these tests to a particular patient. While basal FSH and E2 levels have been validated as useful indicators of success rates in women undergoing assisted reproduction, these tests have never been studied outside this context. Therefore, they may not be useful measures of reproductive potential in an older woman attempting spontaneous conception.

One published study validated the clomiphene citrate challenge test as a significant predictor of reproductive potential in the general infertile population. In this study, the clomiphene citrate challenge test was a stronger predictor of ovarian reserve than age alone, although women above age 40 with a normal test result still experienced low (<10%) cumulative pregnancy rates. Age was thus a significant independent predictor of pregnancy outcome, particularly in the older patient more likely to be offered testing. Furthermore, an abnormal result in all women studied was associated with a reasonable (9%) clinical pregnancy rate (versus a 0%-5% pregnancy rate in earlier studies), limiting the clinical utility of the test in younger women. Until further data are gathered, the clomiphene citrate challenge test should be interpreted with caution in couples attempting spontaneous conception, particularly when faced with an abnormal or normal result in a younger or older patient, respectively.

When used properly, an assessment of ovarian reserve does have important therapeutic implications. All of the more extensively studied tests, when applied to older patients undergoing assisted reproduction, have been found to be predictive in the event of an abnormal result. Such patients often require egg donation to achieve pregnancy and should be counseled accordingly. The predictability of a normal test, on the other hand, is more limited. It seems reasonable to offer standard IVF to patients without laboratory evidence of compromised ovarian reserve, although maternal age remains an important independent predictor of reproductive success in these women.

**Comment**

The impact of aging on the female reproductive tract is significant. Fertility begins to decline as early as the mid-20s and falls dramatically through the late 30s and 40s, preceding the menopause by over a decade. Ovarian senescence seems to play a primary role in declining fertility, although age-related effects on the uterus may contribute to this phenomenon. Fertilization and embryo cleavage rates appear to be unaltered in the aging oocyte, and the factors accounting for impaired implantation and growth of the resulting embryo have yet to be clearly defined. An increase in the aneuploidy rate may in part contribute to impaired conception and early pregnancy wastage but does not fully explain the dramatic decline in fertility observed in women of advanced reproductive age. The use of donor eggs with IVF has prolonged the reproductive life span of women, reversing most if not all of the untoward age-related effects on pregnancy rates.

A number of subtle hormonal changes occur in older, ovulatory women preceding the change to acyclicity and the perimenopause. A monotropic elevation of FSH is the most consistent and significant hormonal change in this subset of women. The FSH elevation has traditionally been thought to result from a declining follicular pool and altered negative feedback signals, although there is some evidence implicating a primary neuroendocrine effect with advancing age. Measurement of basal FSH and E2, and a provocative test of FSH suppression, the clomiphene citrate challenge test, have been applied clinically as prognostic indicators of ovarian reserve. Age, however, remains an important independent predictor of success, and should be emphasized when counseling patients.
REFERENCES


88. Wainsos KL, Toner JP, Bresky RG, Oehninger SC, Acosta AA, Muasher SJ. The gonadotropin-releasing hormone agonist stim-


