

# Beating Your Biological Clock

*Antral Follicle Counts & Egg Freezing*



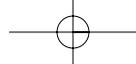
*Since the fertility of all women declines over time, you need to know how many years of fertility you have left so that you can plan for your future.*

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# Table of Contents

**Age-Related Decline in a Woman’s Fertility .....3**

*Most Infertile Women Were Once Fertile.....3*

*Where Are You on Your Biological Clock.....3*

**Age-Related Decline in Fertility Is Due to Loss of Your Egg Supply .....3**

*Evidence That It Is Your Ovary, Not You .....3*

*What IVF Pregnancy Rates Tell Us About How the Ovary Ages.....4*

**You Can Freeze Your Eggs (and Even Your Ovary) for Later.....5**

**Tests for Ovarian Reserve .....7**

*Antral Follicle Count .....7*

**How Does the Biological Clock Work.....8**

*What You Were Born With.....8*

*Antral Follicles and Your Ovarian Reserve .....9*

*Emergence of the Dominant Single Follicle During a  
Normal Ovulatory Monthly Cycle .....10*

**Antral Follicle Count Studies .....12**

*Antral Follicle Count and Your Age.....13*

*Antral Follicle Count and Your Remaining Years of Fertility.....14*

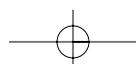
**Is There Anything That Can Preserve the Declining Follicle Pool in Your Ovaries? .....14**

**Antral Follicle Count and Older Women .....15**

*What If I Have Already Had a Child but Want More? .....15*

**You Can Save Your Eggs for Later: Egg Freezing and Banking.....16**

**Summary Of How You Can Preserve Your Fertility .....17**



## **Age-Related Decline in a Woman's Fertility**

### ***Most Infertile Women Were Once Fertile***

Most infertile women were fertile when they were younger. In their early twenties, less than 2 percent of women are infertile. But by their late twenties, 16 percent are infertile, and by their mid thirties (when, in the modern era, most women will first begin to think about having a child), almost 30 percent are infertile. Nonetheless, some women remain fertile into their forties, while others lose their fertility early in their twenties. At some point in time, as the biological clock ages, no matter how much you spend on sophisticated treatment (other than donor eggs), you will no longer be able to get pregnant and have a baby. You need to figure out at what age this will occur for you, and how you should plan your life. Whether you are a young woman in her early twenties who has just finished college and wants to pursue a career, or whether you just want to learn more about relationships and grow further emotionally before you finally settle down, this inexorable biological clock, which you have heard so much about, will become perhaps your major conflict in life.

Nobody wants their biological clock to tick out, and most women (even those who currently are not ready to have a family) want to bear children eventually. Since the majority of women are still fertile even in their mid thirties, many are caught in the dilemma of whether to give up their career plans, or to constantly live with the fear that when they are ready to have children, they won't be able to do so. In fact, at some point in a woman's declining fertility, even the most advanced and the most expensive infertility treatment (short of using donor eggs) will not help her.

### ***Where Are You on Your Biological Clock?***

Although we know that the biological clock is inexorable, the dilemma that most women face is not knowing just where they personally happen to be on that time scale. Women in their late twenties can be infertile, and women in their early forties can be fertile. Although all women undergo an eventual decline in fertility, the big question for every individual woman is just when that will be. If the 44 year-old woman could have known twenty years earlier that she would retain her fertility into her mid forties she might have avoided two decades of worry and fear. If the twenty-six-year-old woman had known she would run out of eggs so early in life, she and her partner might have planned their lives differently as well.

Thankfully, there is now a simple test that allows you and your doctor to take measure of your biological clock, to objectively assess when you should or should not worry about having children, and even to predict how old you will be when you go through menopause. This simple test can be administered at the time of the yearly Pap smear to all young women who need counseling about their future. This pamphlet will give you a firm basis for understanding how your future fertility potential can be determined, and preserved.

## **Age-Related Decline in Fertility Is Due to Loss of Your Egg Supply**

### ***Evidence That It Is Your Ovary, Not You***

Awareness of the natural and normal age-related decline in a woman's fertility was first made startlingly clear to medical professionals in an article in 1982 in the New England Journal of Medicine, resulting from a huge study conducted by a large federation of infertility physicians from France. In this study, 2,193 women who were married to husbands who were azoospermic (had no sperm whatsoever in their ejaculate) had chosen to undergo artificial insemination with donor sperm on a monthly basis until they became pregnant (or gave up). The significance of this study is that there was no male-related contribution to the

infertility problem, because normal donor sperm was being used to inseminate all the women. Furthermore, there would be no reason to suspect any infertility problem in any of these women, as all of their preliminary testing for classical, conventional diagnoses of infertility had been either treated or ruled out. Moreover, there was no possibility that insemination might have occurred sporadically during a time of month when the woman was not fertile, as could be the case with random intercourse. Thus, it was the first controlled, reliable study to demonstrate a clear, age-related decline in the fertility of apparently normal, fertile women unrelated to the sperm count or fertility of their husband. After a full year of monthly insemination with high-quality fertile donor sperm, only 40 percent of women over thirtyfive years of age were able to become pregnant, whereas twice that many women in their twenties were able to conceive.

It has become apparent that technology can only go so far in correcting the inexorable age-related decline in a woman's fertility, and that 25 percent of women in their thirties (and 60 percent by the time they reach forty) will not be able to get pregnant without expensive treatment, even though ten or twenty years earlier they might have been quite fertile. What can a woman do to plan her life as it fits her own particular biological clock?

### ***What IVF Pregnancy Rates Tell Us About How the Ovary Ages***

All certified IVF programs are required to report their pregnancy rate (as well as live delivery rate) results to a government agency. The purpose of this section is simply to point out that good IVF results are obtained in young women with a high ovarian reserve, while poorer IVF results are obtained in older women with a low ovarian reserve. Younger women with many eggs have better results than older women with only a few eggs.

In good IVF programs in the United States, the overall delivered pregnancy rate per treatment cycle for women under thirty-five is about 60 percent. For women ages thirty-five to thirty-seven it is less than 36 percent, for women ages thirty-eight to forty it is only about 25 percent, and for women over forty it is less than 15 percent. However, these results also depend on the woman's ovarian reserve as determined by how many follicles develop (seen on ultrasound) when she is undergoing stimulation with hormones. The ovarian response, i.e., the number of follicles developing from hormonal stimulation, is related to the total number of eggs in the woman's ovaries.

We have broken down the pregnancy rates per treatment cycle in our IVF program, not only according to the age of the woman, but also according to the number of eggs that can be harvested at the time of her IVF procedure. The overall pregnancy rate is well over 50 percent per cycle. But a closer look shows that pregnancy rates depend upon the age of the woman and the number of eggs that are retrieved. Women under thirty who had fewer than ten eggs had a 44 percent delivery rate per cycle, and women who were under thirty who had more than ten eggs had a 66 percent delivery rate per cycle. The pregnancy rate and delivery rate went down in women over age thirty when fewer than ten eggs were retrievable because of a low ovarian reserve. However, the pregnancy rate remained very favorable in women who had more than ten eggs, despite advancing age. Despite advancing age, the pregnancy rate per monthly cycle with IVF was consistently better in women with more eggs. Thus, the deleterious effect of age in the woman is markedly attenuated if she started out with a large storehouse of eggs.

Furthermore, IVF studies over the last dozen years have proved beyond a doubt that the decreased fertility of older women compared to younger women, except in a few instances, is strictly related to her aging ovaries. Although it is true that the obstetrical management of older women in their forties and fifties must be more assiduous because of the somewhat increased risk of high blood pressure, diabetes, and early labor, these are all manageable problems and have nothing to do with the receptivity of the aging uterus. When older women use eggs donated from younger women, the pregnancy rate is not any lower than for younger women undergoing IVF. Thus, we know from IVF studies that the declining fertility of a woman as she becomes older is strictly related to the aging of her ovaries, and not to the rest of her system.

## You Can Freeze Your Eggs (and Even Your Ovary) for Later

There are two reasons why you should know just where you are on your biological clock. The first is that you can plan your life better if you know when you need to worry. The second is that if you find you do not have much time left, you can actually have your eggs (or even one of your ovaries) retrieved and frozen for later use. Thus, despite the otherwise inexorable ticking of your biological clock (the major cause of infertility in the modern era), you can now preserve your fertility for when you are truly ready to have a baby.

Although embryo freezing has been around for decades, up until recently egg freezing has been very problematic. Now, however, if tests show that you will lose your fertility early in life, you can actually do something about it other than just having a baby before you really wish to have one. You can now successfully have your eggs frozen, or even one of your ovaries.

This is briefly how it works: 70 percent of the human body is water. The only reason we cannot freeze living organisms (like us) and hold them in suspended animation indefinitely is that the water in our cells would become ice, which crystallizes and expands and would destroy the integrity of every cell (just like a bottle of milk in a freezer would break). That is how freezing to death occurs. But if we can avoid that formation of ice crystals inside the cells, then lowering the temperature to  $-196$  degrees Celsius just suspends all metabolic processes, and can do so indefinitely without harm.

Individual cells (like white blood cells and embryos) for decades could be frozen quite successfully on a routine basis by a process called “slow freeze,” in which the water content of the cell is removed and antifreeze solution (like in your car’s radiator) replaces it. There are three components of this slow-freeze process. A cryoprotectant antifreeze solution contains sucrose, a sugar that osmotically pulls water out of the cell. At the same time the antifreeze solution itself (either dimethylsulfoxide [DMSO] or propylene glycol) penetrates into the cell just like in your car’s radiator. Finally, this antifreeze solution, which contains the embryos, is placed in a computer-controlled freezing machine that lowers the temperature slowly ( $-0.3$  degrees Celsius per minute). That way, as ice crystals form preferentially outside of the cell, the concentration of solutes increases on the outside, gradually drawing more and more water out of the cell by increasing osmotic pressure. Thus, by using these three separate processes, the embryo can be more or less freeze-dried, usually without harm.

However, eggs have rarely survived this slow-freeze technique that worked so well for embryos in IVF patients and for stem cells in cancer patients. The reason is that mature eggs have their chromosomes lined up very precisely on a delicate platelike structure called the spindle, and are therefore much more sensitive to temperature drops. A cell that has completed cell division (with a discernible nucleus) has its DNA in a much more stable arrangement than either a mature egg or any cell in the process of dividing. Therefore, the slightest residual intracellular ice formation can kill the mature egg, even though it does not kill an embryo.

A new technique called “vitrification” (perfected by our partners at the Kato Clinic in Japan) avoids this damage not by trying to pull every molecule of water out of the cell, but by using such a super-high concentration of antifreeze that the water inside the cell never becomes ice no matter how low the temperature. This is a rapid technique that completely avoids the use of a slow-freezing machine. Most important, because the temperature drops so quickly and no intracellular ice at all is allowed to form, this technique finally makes freezing of eggs a viable way to preserve fertility. The specific procedure was developed in Japan, and thus far is yielding remarkably good results. So we can now readily freeze your biological clock and save your eggs for when you are finally ready to have children. However, there is another approach which some women may prefer to egg freezing.

An entire ovary can also be preserved, an approach that may even surpass egg freezing in efficacy. The reason for the poor success of egg freezing in the past is that the eggs retrieved through normal IVF-type processes are undergoing chromosomal division. The chromosomes of retrieved eggs are highly organized on a complex spindle, which is very susceptible to minor crystal damage from freezing. However, “rest-

ing” eggs in primordial follicles within an unstimulated ovary are undergoing minimal cellular activity and have no such complex spindle formation. Therefore, these immature, resting eggs are not easily damaged during an appropriately administered freezing procedure. Furthermore, all these resting eggs, called “primordial follicles,” are located in the thin outer one-millimeter crust of the ovary. Thus, we can remove an entire ovary, perform bench microsurgery to remove the inside (to allow successful diffusion of the cryo protectant into the outer crust), and then successfully freeze this ovarian tissue in the same way we have been freezing embryos for decades (see fig. 1). The frozen ovary can be stored safely for many years, and then can be transplanted back and function normally.

In 2004, in St. Louis, we performed the first whole-ovary transplant using this technique, and a young woman who was prematurely menopausal then began to have normal periods, and conceived several months later. She is now able to have her own genetic offspring. This landmark case proved that an ovary can be transplanted through a minimally invasive outpatient procedure, completely restoring normal fertility, as well as hormone production, to an otherwise sterile, menopausal woman. This means that women can now put off childbearing until later years, keeping their ovary young until they decide later to have it transplanted back. Thus, menopause can be delayed and reproductive life span lengthened. The actual procedure to remove the ovary, as well as the procedure for transplanting it back, has been refined to a simple one-day outpatient approach using a very minimal invasive incision.



**FIGURE 1:** *An ovary being prepared for freezing and preservation from a young woman about to undergo radiation and chemotherapy for cancer.*

This technology can be used not only to preserve a woman’s declining fertility until she is finally ready to have a baby, but also to prevent young cancer patients from becoming sterile as a result of chemotherapy and radiation. We can actually take out a cancer patient’s entire ovary and freeze it for grafting back later, or we can retrieve and freeze her individual eggs, before she receives otherwise sterilizing chemotherapy or radiation.

Until recently it was feared that breast cancer, the most common malignancy in women, would not be amenable to IVF and freezing of eggs or embryos, because of the increased estrogen level resulting from ovarian stimulation. The fear was that the breast cancer would be accelerated by that brief elevation in estrogen. However, by taking tamoxifen during the ovarian stimulation and IVF cycle, the breast can be protected from this brief estrogen surge. Then the eggs (or embryos derived from fertilizing those eggs with the partner’s sperm) can be frozen. Immediately thereafter, the woman can undergo her chemotherapy and radiation, which has at least a 50 percent chance of rendering her sterile. But she will now have frozen eggs or embryos that can be returned to her once she is cured so that she can still have her own genetic child. Alternatively, the embryos could be transferred to a surrogate who could carry her baby for her.

If there is not sufficient time (four to six weeks) available to stimulate the ovary and retrieve eggs before going on chemotherapy or having radiation, the woman can have her ovary removed and frozen safely without delaying her treatment. In fact, since 1996 we have been freezing and saving ovaries for young women who have undergone potentially sterilizing chemotherapy or radiation for a variety of different cancers. If careful examination of sample tissue from the ovary reveals no cancer cells, then at some time in the future the ovary can be transplanted back to restore their fertility.

## Tests for Ovarian Reserve

Since the decline in fertility as a woman passes into each decade of life is strictly related to the aging of her ovaries (and consequently her eggs), considerable effort has been made in the past to try to develop tests to determine ovarian reserve. "Ovarian reserve" simply means the number of eggs your ovary has in reserve. The greater your ovarian reserve, the more time is left on your biological clock. In the past, tests to measure ovarian reserve were not really useful. These included day three FSH and estradiol, day three inhibin, the Clomid challenge test, and even ultrasound evaluation of ovarian size. None of these tests were predictive at all.

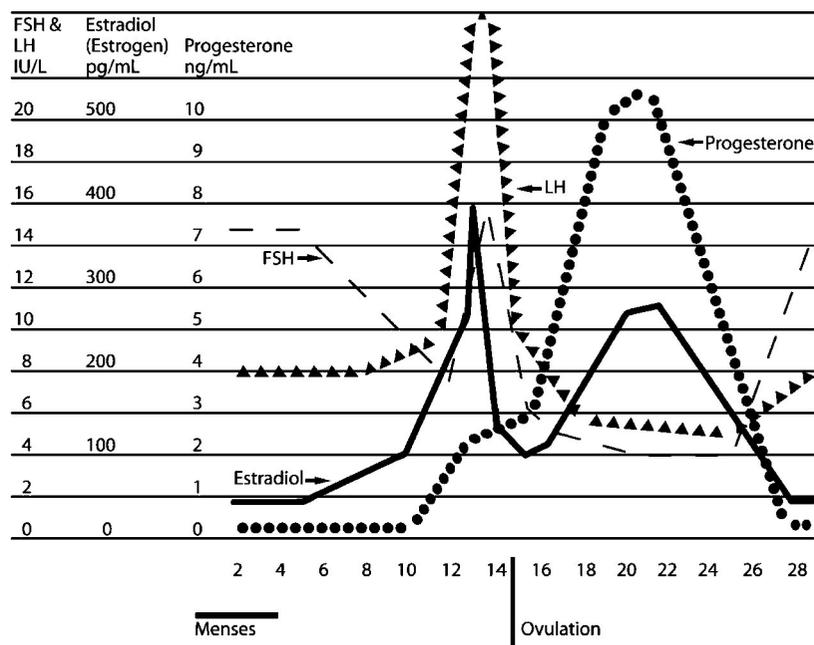


FIGURE 2: *Hormonal Control of a normal menstrual cycle.*

## Antral Follicle Count

None of the previously described tests achieve the accuracy, simplicity, and reproducibility that we have found with ultrasound-performed antral follicle counts for ascertaining where you are on your biological calendar. The ultrasound determination of antral follicle count is the same at any time during the cycle, is independent of birth control pill usage or other hormone administration, and is very easy to have performed by any radiology center or gynecologist with a relatively modern ultrasound machine. The best preparation for this test is to have a very good understanding of just how your ovary works, how your eggs are formed, how they die and diminish over your reproductive life span, and just what it is that the radiologist, or the technician, or the gynecologist, will be looking at when he or she performs, during a routine ultrasound examination, your antral follicle count.

The procedure is extraordinarily simple for any doctor or technician to perform, but only if they are aware of its significance — most are not. Any woman can obtain an easy estimate of when she should or

should not begin to be concerned about her biological clock. A new (and expensive) blood test, called “anti-mullerian” hormone, or AMH level, also measures antral follicle count, but in a different way than simple ultrasound.

## How Does the Biological Clock Work?

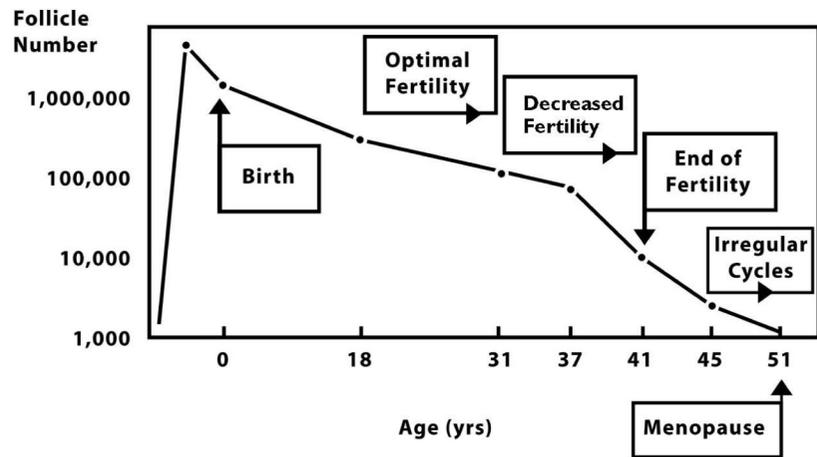
### *What You Were Born With*

Remember, women are born with all the eggs they are ever going to have, and they don't make any new eggs during their lifetime. Women are born with approximately two million eggs in their ovaries, but about eleven thousand of them die every month prior to puberty. As a teenager, a woman has only three hundred thousand to four hundred thousand remaining eggs, and from that point on, approximately one thousand eggs are destined to die each month. This phenomenon is completely independent of any hormone production, birth control pills, pregnancies, nutritional supplements, or even health or lifestyle. Nothing stops this inexorable death of approximately one thousand eggs every month regardless of ovulation, ovarian inhibition, or stimulation. Whenever the woman runs out of her supply of eggs, the ovaries cease to make estrogen, and she goes through menopause. Despite a lot of journalistic hype, there is no similar phenomenon in men. Men continue to make sperm and testosterone at virtually the same rates, with only a very modest diminution as they age.

Many population studies have demonstrated over several decades that the average fertile woman becomes infertile by age forty or earlier, and undergoes menopause by age fifty. The mean age of the end of female fertility (according to all the early population studies of fertile women) precedes menopause by about ten to thirteen years. The end of fertility for an otherwise normal, fertile woman, and the age of the onset of menopause, correlates strictly with the decline in the number of eggs remaining in her ovary.

The average female life expectancy in the Western world is currently about eighty-four, whereas in 1900, the average life expectancy was fifty, and in 1850, it was only forty-two years of age. Meanwhile, the average age at which young girls start menstruating in the modern world has decreased from age thirteen or fourteen to age ten or eleven. Neither the overall life expectancy, nor the age of menarche (the beginning of menstruation) has any effect on the average age of menopause. In fact, the average age of menopause in almost every population studied over any period of time and in any era has remained constant at around fifty. Although some women go through menopause in their twenties (because of POF, i.e., premature ovarian failure) and some go into menopause in their late fifties, the timing does not appear to depend upon any specific element in their lives other than the number of eggs with which they were endowed at birth.

It is this wide variation in endowment of eggs from woman to woman that will determine whether you will lose your fertility early (late twenties or early thirties), or whether you'll be one of the lucky women who is able to have children into her mid- or even late forties. To recap, the average woman will have three hundred thousand to four hundred thousand eggs at the time of puberty. An average of one thousand will die every month, and only one of those thousand every month is destined to ovulate. By age thirty-seven, the average woman will be down to only about twenty-five thousand remaining eggs. When only twenty-five thousand eggs remain in the ovaries, menopause will occur in approximately thirteen years. Thus, the average woman begins to become infertile by age thirty-seven or earlier, when her ovarian reserve goes down to about twenty-five thousand eggs, and at age fifty, she will go through menopause. But there are wide variations from this average. What you need to know, in order to plan your entire life, is where you fit on that curve (see fig. 3).



E.R. TE VELDE ET AL., 1998

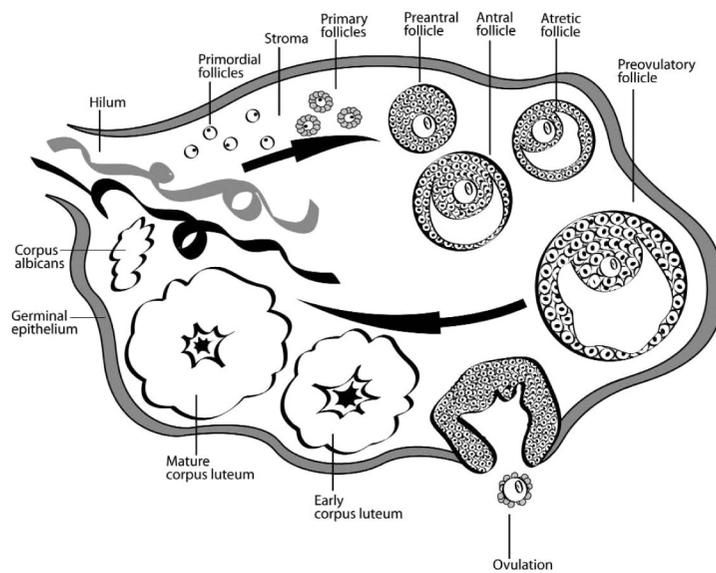
**FIGURE 3:** The decreasing follicle pool and age-related decline in female fertility.

### Antral Follicles and Your Ovarian Reserve

To understand how an antral follicle count ultrasound can tell you where you are on your biological clock, remember that approximately thirty to thirty-five eggs die every day. That is where the number of one thousand per month comes from. They die only because they have initiated their emergence from the resting pool of eggs and have begun their long, three-month development toward becoming an egg that is capable of ovulation. Only one every month, out of the one thousand that tried, will ever make it. In other words, every day thirty or so eggs that are otherwise safely resting in your ovary, protected from the ravages of age by being in a quiescent phase, emerge by some signal that scientists still don't understand into a very long (approximately three-month) developmental process that is completely dissociated from your menstrual cycle or your ovulatory cycle. Once that three-month growth has reached the antral stage, when the follicles finally become sensitive to the hormones of your monthly menstrual cycle, they will rapidly die and disappear if they are not rescued by FSH. Here is how it happens:

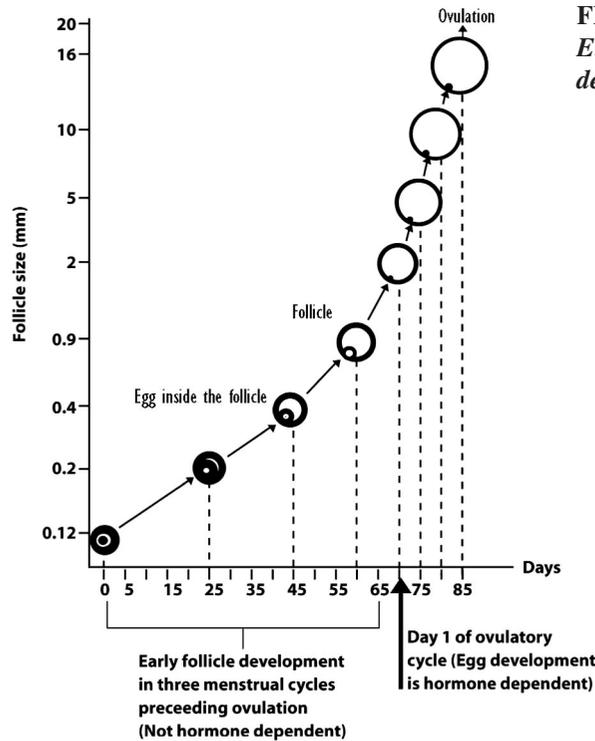
Each egg in your ovaries is enclosed within a resting follicle. Every day, thirty to thirty-five of these resting follicles begin their eighty-five days of development toward eventually trying to ovulate. At any time, a view into your ovary reveals follicles (with their enclosed eggs) in every stage of resting or growing (see fig. 4). There are early primordial, or resting, follicles; there are somewhat larger primary follicles; there are larger pre-antral follicles (which are beginning to form a fluid-filled space); and there are antral follicles, which are just becoming visible under ultrasound at a size of approximately one to two millimeters in diameter. In addition, at midcycle, on day fourteen, there is normally a dominant pre-ovulatory follicle. After ovulation, that follicle becomes a corpus luteum, which begins to secrete progesterone.

It is often erroneously thought that just one follicle develops every month, during the first two weeks of



**FIGURE 4:** Various stages of follicles in the human ovary.

the cycle, ultimately culminating in a large, twenty-millimeter follicle from which the egg is ovulated at approximately day fourteen (in a typical twenty-eight-day ovulatory menstrual cycle). Development of this single, dominant follicle every month with its increasing production of estrogen, and the entire regulation of the monthly cycle via the pituitary hormones of FSH and LH, only gives a tiny part of the picture; it only shows what is happening to one egg in an ovary that contains, in a fertile young woman, as many as 200,000 eggs. That one egg that was destined to ovulate, developed as the single dominant follicle out of the thirty or so much smaller pre-antral and antral follicles, which had been developing in the ovary for as long as seventy days prior to the beginning of the current twenty-eight day menstrual cycle (see fig. 5).



**FIGURE 5:**  
*Eight-five days of follicle development.*

GOUGEON, 1996

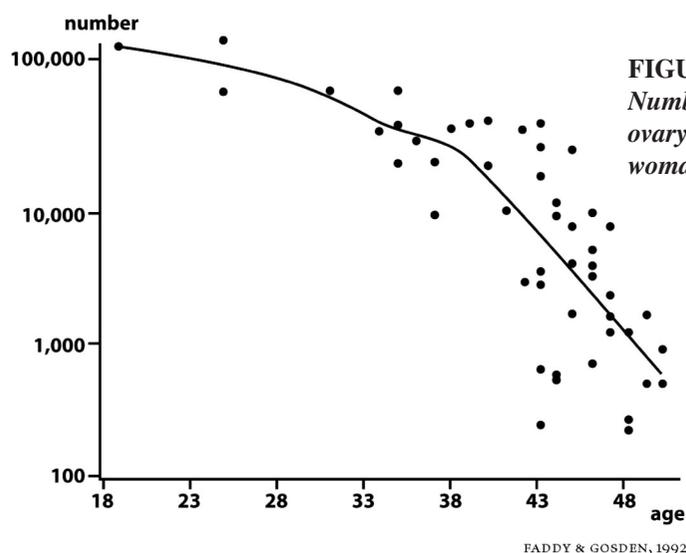
Most of the ovaries' 300,000 to 400,000 follicles are quiescent and doing nothing during any given month, but out of that primordial pool a certain number (an average of thirty to forty) will begin to develop each day. By approximately seventy days of development, these follicles will have grown to approximately two millimeters in size, and at that size they are readily visible with modern, high-quality ultrasound scanning. During the first seventy days of a follicle's development, it is completely independent of any hormonal influence. FSH and the monthly hormonal cycle have no influence yet. Sometime between 0.2 millimeters and 2 millimeters in size, these so-called antral follicles begin to become sensitive to stimulation by FSH from the pituitary gland. Prior to the time when these tiny follicles finally become ready to enter the current menstrual / ovulatory cycle, they are completely unaffected by whatever hormonal events have been taking place in the previous cycles.

As previously stated, the number of follicles leaving the resting pool (destined to become either the lucky egg that is ovulated, or the unlucky ones that undergo atresia, i.e., cell death) may average about thirty per day, or one thousand per month, and that number is related to the age of the woman, and to her declining fertility. Thus, when a woman is only twenty years of age, an average of thirty-seven follicles per day leave the resting stage. When she is thirty-five years of age, an average of ten follicles per day leave the resting stage, and when she is forty-five years of age, an average of two follicles per day leave the resting stage. This means that the number of follicles per day that begin to become antral, and thereby capable of rescue from death by FSH stimulation, is inversely related to the age of the woman. The younger the woman and the larger the total number of eggs in her ovaries, the greater the number of eggs in any given month, or any given day, that will leave the resting phase and develop into antral follicles (of which only

one per month is destined to ovulate; all the others will die).

So the number of egg-containing follicles remaining in the ovary undergoes a steady decline from an average of 400,000 eggs at age eighteen to an average of 25,000 eggs by age thirty-seven. After age thirty-seven or thirty-eight, there is then a very dramatic acceleration of the monthly decline of remaining eggs. Not only is your egg / follicle pool already down because of a steady decline over the previous twenty years, but the rate of the decline after age thirty-seven becomes even steeper than in prior years (see fig. 6). The number of follicles per day that leave this resting pool and begin the three-month developmental path toward being available for future ovulation diminishes dramatically in direct proportion to the number of eggs that are left in the ovary. When the antral follicle first becomes large enough (one to two millimeters) to be visible on ultrasound, it then also becomes susceptible to hormonal stimulation, and the number of visible antral follicles is directly proportional to ovarian reserve. Therefore, the antral follicle count as determined by ultrasound will give you an accurate read on how many eggs are left in your ovaries.

The antral follicle count also tells you the number of eggs that can be retrieved in an ovulatory stimulation cycle for IVF. To understand this, we will quickly review the normal menstrual cycle with the ovulation of a single egg and explain what happens when we give FSH injections to stimulate multiple follicle development for an IVF cycle. Remember that the number of eggs we are able to retrieve in an IVF cycle, regardless of age, is the most important determinant of your likelihood of pregnancy; it is also the most important determinant of any age-related decline in your natural fertility.



### *Emergence of the Dominant Single Follicle During a Normal Ovulatory Monthly Cycle*

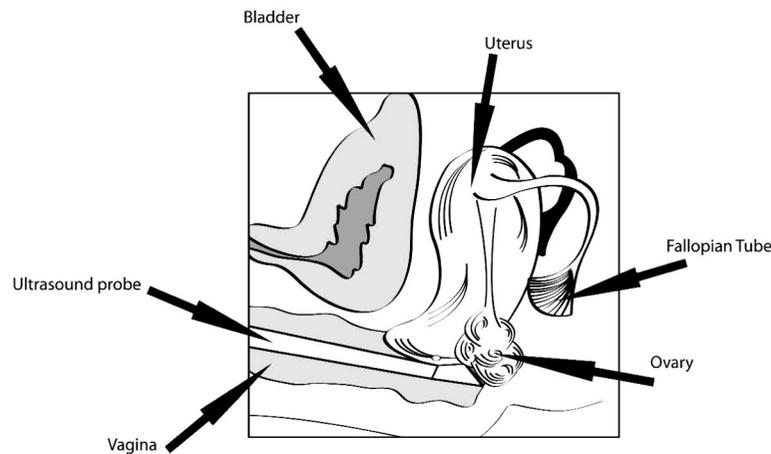
At the time of your menses (menstruation), as a result of the rapid fall in estradiol (estrogen) and progesterone secretion from the ovulated follicle of the previous month, the uterus sheds the lining that had built up during that month in preparation for pregnancy (see fig. 2). This sudden drop in estrogen causes the FSH secreted from the pituitary gland to rise dramatically around day twenty-six of the previous twenty-eight-day cycle. So, two days later, on day one of your menstruation (the beginning of your next cycle), this elevated FSH stimulates only the development of follicles that had left the resting pool 70 days earlier, and that are now antral. As these antral follicles grow in response to FSH, they secrete estrogen and inhibin B, which in turn suppress further the pituitary secretion of FSH. Thus, as the antral follicles become more mature (by day six), the FSH begins to decline. If these antral follicles were not rescued by the increased FSH level on day one of the menstrual cycle, when they have finally reached the antral size, they would die immediately.

A competitive struggle then ensues between all of these approximately thirty antral follicles to see which one will become the “lead follicle” that will ovulate on day fourteen. The antral follicle that is most

sensitive to FSH in the first few days of your cycle becomes even more sensitized to FSH, and thus gains the lead over all the other follicles (which die off because of lower and lower levels of FSH). Once the dominant follicle gains the lead, it will never relinquish it, because it requires less FSH than the others to get the same degree of stimulation. Because FSH continually declines toward the middle of your cycle just prior to your ovulation, all the other antral follicles that month (which have finally become hormone dependent after almost three months of non-hormone-related growth) will die. When they reach this stage of development, the follicles are completely dependent on FSH for survival. Once the estrogen production exponentially peaks, around day twelve or thirteen, it stimulates a dramatic rise in LH from the pituitary gland, and that rise in LH is what prepares the one remaining follicle for ovulation.

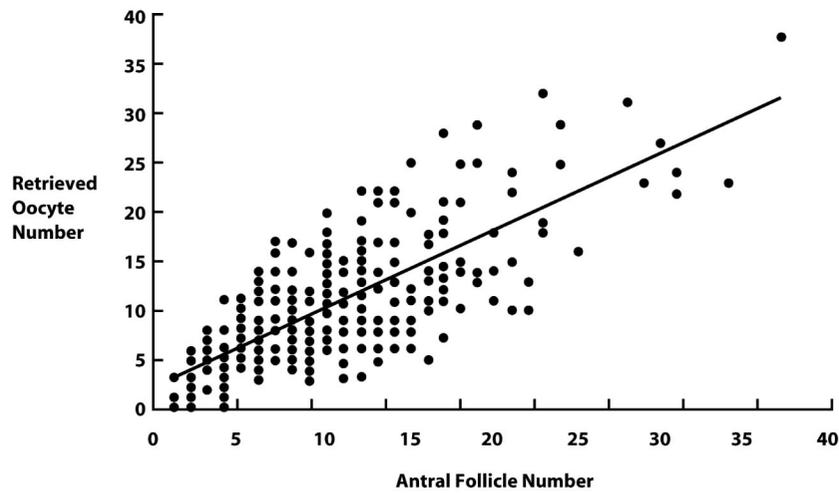
In preparation for IVF, FSH injections are given in the early part of the cycle so that the FSH level never declines, as it would normally. This sustained elevation of FSH, which is all that the administration of ovulatory stimulation hormones amounts to, sustains almost all of the thirty or so antral follicles so that no single follicle can gain dominance over the others. Therefore, the number of eggs retrieved in a hormonal stimulation cycle for IVF is directly reflective of your antral follicle count, and your antral follicle count is directly reflective of your total remaining number of eggs.

## Antral Follicle Count Studies



**FIGURE 7:** *Side view of ultrasound probe in vagina during routine pelvic exam.*

Transvaginal ultrasound is a simple, routine procedure (available everywhere) for viewing the ovaries, utilizing ultra-high-frequency sound waves. A probe (which is smaller than the speculum used for your standard pelvic exam and Pap smear) is placed in the vagina, and a clear image of your ovaries and uterus can be plainly seen (see fig. 7).

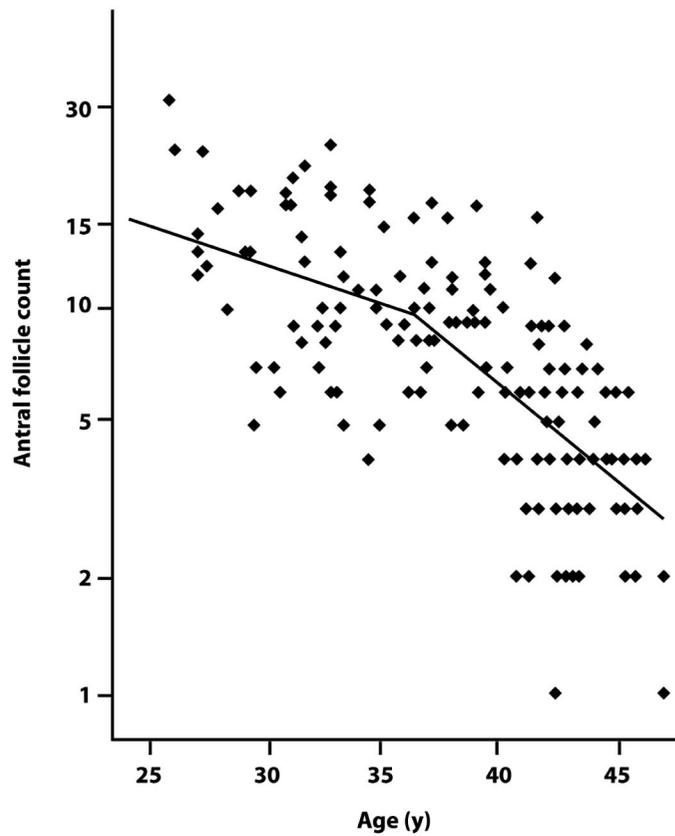


HSIEH, CHANG & TSAI, 2001

FIGURE 8: *Number of eggs retrieved in IVF cycle in relation to antral follicle count.*

### *Antral Follicle Count and Your Age*

The number of primordial follicles in the ovary decreases throughout childhood and adult life, eventually leading to ovaries that are almost devoid of follicles at the age of menopause. The antral follicle count is a simple and easy way to measure that decline in any woman (see fig. 9).



SCHEFFER ET AL., 1999

FIGURE 9: *Decline of antral follicle count with increasing age of a woman.*

Transvaginal ultrasound can thus provide an accurate measurement of the total number of antral follicles at any time in your menstrual cycle, and will indicate readily your ovarian reserve and your reproductive future.

Such information would be of great help to young patients, who could relax somewhat about their biological clock if they knew they had a comparatively large ovarian reserve.

### ***Antral Follicle Count and Your Remaining Years of Fertility***

The reliability of antral follicle count is completely independent of the menstrual cycle. Unlike hormone evaluations, the ultrasound evaluation of antral follicle count can be used on any day of the menstrual cycle to show those follicles that have left the resting state and have reached the antral size. This is a daily event that occurs independently of all the other monthly variations in the menstrual cycle.

Antral follicle count can, of course, be used for counseling infertile women about to undergo IVF so that they will know what their chances are for a successful result. However, it is also extremely useful for all women who are thinking about getting pregnant either sooner or later, and who need to know if it is risky for them to put this decision off. It is even possible to predict at what age menopause will occur. Fertile women who have an antral follicle count of twenty to forty, regardless of age, can anticipate becoming infertile within ten to fifteen years and will likely reach menopause about ten years later. An otherwise fertile woman whose antral follicle count is only ten is likely to become infertile very soon, and to have menopause within about thirteen years. Women who have antral follicle counts of less than five are very unlikely to be able to get pregnant with or without infertility treatment, and they are likely to have menopause begin sometime within the next seven to eight years. Of course, these are average and median figures, and cannot predict exactly for each individual patient. But it can be concluded that even younger women with antral follicle counts of less than ten (total from both ovaries) have no time to waste if they want to have children.

Thus, transvaginal ultrasound, which should be a simple and readily available tool in most gynecologists' offices and certainly in any radiology imaging center, as well as blood AMH levels, can provide an accurate and reproducible measurement of the total number of antral follicles throughout the menstrual cycle, which is indicative of the woman's ovarian reserve and her reproductive future.

### **Is There Anything That Can Preserve the Declining Follicle Pool in Your Ovaries?**

The question that will naturally come to the mind of every young woman who is contemplating putting off childbearing is whether there is some way of slowing down the rate of emergence of eggs from the resting follicle pool into the antral state, thus keeping her eggs quiescent, so that they will not undergo atresia and die. A primordial follicle is safe until it leaves its resting state and becomes destined to enter an ovulatory cycle (whether it is the dominant follicle that ovulates three months later, or whether it is one of the many follicles that will become antral but then decay). The inexorable loss of eggs with age might be prevented if you could somehow keep the eggs in their primitive, resting state, preventing the natural emergence of one thousand or so eggs each month (thirteen thousand per year) into that three months of growth that will eventually lead to the death of all but one.

If the hormone FSH were in some way necessary for the early development of these eggs from primordial to the antral state, then presumably the administration of birth control pills in high enough doses would reduce the FSH level, delay menopause, and lengthen the period of time in which you retain your fertility. On the contrary, if you undergo hormonal stimulation to retrieve many eggs from multiple follicles in a given month, does that in some way reduce your ovarian pool of eggs and hasten the day when you will run out of eggs?

Most authorities who have done extensive research in this area do not believe that FSH has any effect on the follicles leaving the resting pool prior to their becoming larger and entering the pre-antral phase. There are no receptors for FSH in the resting follicles. Furthermore, we have found that young women who use birth control pills for a long period of time have antral follicle counts indistinguishable from those who have not been on the Pill. Likewise, women who have been on strong hormonal suppression (with Lupron) for as long as half a year to suppress endometriosis have similar responses to ovarian stimulation as a matched group of women who have not been on prolonged suppression with birth control pills. Thus, we see no evidence that either antral follicle count or ovarian reserve can be preserved by maintaining low FSH levels by using birth control pills. Furthermore, stimulation with FSH in multiple cycles of IVF in the same woman causes no reduction in antral follicle count or ovarian reserve.

Birth control pills do not suppress antral follicles. Being on birth control pills neither suppresses the antral follicle count nor interferes with the ability to determine a woman's ovarian reserve (which would be a problem with any kind of hormonal testing like day three FSH or estradiol). Birth control pills work by suppressing the pituitary secretion of FSH and LH. This prevents ovulation by halting the further development of follicles beyond the antral stage. However, it is clear that it does not prevent the formation of antral follicles, which, at least until the early pre-antral stage, are not hormone dependent. Thus, we have found that even young women on birth control pills can rely on routine antral follicle count testing to give them a prediction of their ovarian reserve, and birth control pills do not slow down the biological clock.

From the opposite point of view, ovulatory stimulation does not deplete the ovary of eggs. FSH shots administered in a stimulatory cycle for IVF simply allow us to retrieve approximately fifteen to thirty eggs out of the one thousand that will have reached the antral stage and allow them to continue developing and not die. So hormonal stimulation does not cause you to lose more eggs, nor does hormonal suppression (birth control pills) prevent the loss of eggs with age.

## **Antral Follicle Count and Older Women**

### ***What If I Have Already Had a Child but Want More?***

We see countless patients who had no difficulty getting pregnant years earlier, having had one or even two children, but who now find themselves inexplicably unable to get pregnant again. They wonder how they could suddenly have become infertile. This is only confusing if you think there is a specific diagnosis that explains the cause of infertility, as though it were a disease that could be characterized and then treated like other illnesses. The problem is that most cases of infertility (though of course not all) are simply related to the decline in ovarian function, which is an inescapable result of the years going by.

We saw a forty-one-year-old woman who had no difficulty getting pregnant thirteen years earlier, when she was twenty-eight years old. At age thirty-seven she attempted once again to have more children and, indeed, got pregnant but miscarried. She got pregnant again three years later, but miscarried again. She got pregnant again once more later that year and again miscarried. Subsequently, she was not able to get pregnant at all. These recurrent miscarriages in women who were once fertile and are now older (assuming no intervening uterine problem) are almost always related to declining ovarian reserve. The ovary performs its salvaging function of pushing out whatever eggs it can. Many of these eggs are from the bottom of the follicular pool and, therefore, have a higher incidence of chromosomal errors. Her miscarriages were caused by chromosomal errors in the egg maturation process and were just a danger signal, warning her that she had already run extremely low on her follicular reserve several years earlier. Infertility and recurrent miscarriage are different expressions of the same problem of declining ovarian reserve.

You may still be fairly young, with no history of difficulty getting pregnant when you were even younger, and still have age related secondary infertility. A twentynine year old woman had had her first child with her husband four years earlier, when she was twenty-five, and never dreamed she would become infertile. She had perfect, regular, twentyeight day cycles, but a routine infertility evaluation by another clinic found that her husband had a low sperm count (seven million sperm per cc, with only 10 percent

motility). When she was four years younger, she had no problem getting pregnant despite the low sperm count, but now, to have more children, she needed infertility treatment.

The fact that you have had children in the past and therefore assume yourself to be fertile does not mean that you should not continue to have regular antral follicle count monitoring to help you make your decision about when you and your husband should try to have your next child. If your antral follicle count is below ten or fifteen, you may have to consider doing this sooner rather than later.

## **You Can Save Your Eggs for Later: Egg Freezing and Banking**

There are many couples in their twenties and early thirties who are married and committed to each other, but just don't want children yet. But they are afraid to put off having children into their late thirties or early forties for two reasons: (1) They are afraid that with their biological clock ticking, they will not be able to have children if they wait another ten years, and (2) they are afraid that if they do get pregnant later they will be in an age category where this poses a high risk of abnormal embryos and chromosomal defects in their children. These couples can undergo IVF while they are still young and have their embryos successfully frozen and stored. At a later date, the embryos can be thawed, and the wife can get pregnant even when she is older, with no increased risk of Down syndrome.

Although human embryos can be successfully frozen and thawed, and can result in happy, healthy babies, eggs, until very recently, could not be successfully frozen and thawed. The success rate in freezing eggs had always been extremely low, but this has all changed now. For young female cancer patients, whose ovaries would surely be destroyed by heavy chemotherapy and radiation, we can now remove an ovary, freeze it, and save it to be grafted back to the woman after she has been cured of the cancer. We can do the same with her individual eggs, and save them for subsequent IVF.

Both egg and ovary freezing are now also available for women who just feel a need to delay childbearing until their late thirties and forties, by which time their egg supply will very likely have been depleted. These are wonderful options for the woman who wants her own genetic child but does not anticipate starting a family for many years.

For decades we have been able to use cryogenic technology to freeze and store embryos derived from IVF in order that women not have to risk having a dangerous multiple pregnancy. The embryos can be thawed safely at a later date, and the pregnancy rate with these frozen embryos is still very high. That is nothing new. We have been able to do this since 1983, and long-term follow-up shows no deleterious effect on subsequent offspring. In fact, for many years young couples who are happily married, but want to put off childbearing until later, could readily have their embryos (derived from the husband's sperm and the wife's eggs) frozen and saved for later so that they do not sacrifice their chances for later parenthood.

However, freezing embryos for a future date does not solve the problem for unmarried women who want to have children in the future but have not yet met the right man. For these women, freezing their eggs, or even an entire ovary, would be the ideal solution. Until very recently, this holy grail of IVF was unattainable. The reason is that in order for fertilizable eggs to be retrieved, they must be in a mature state of development, with a complex alignment of chromosomes, and this makes them susceptible to even the slightest ice-crystal damage. However, with a new technique of vitrification, recently refined in Japan, ice-crystal formation is avoided completely, and early results indicate that very high pregnancy rates can now be achieved with frozen eggs. Thus, a woman who knows that she is nearing a time when she will lose her fertility because of her biologic clock can now freeze her eggs, or a piece of one ovary, and have her babies later.

This new technique of freezing, called "vitrification," avoids the damage caused by ice forming inside the cell. With vitrification, you are not trying to pull every last molecule of water out, because it is impossible to do this 100%. In fact, 70% of the cell is water, and at best you can reduce that to 30%. So with the conventional controlled rate slow-freezing technique, there is always going to be some intra-cellular ice crystal formation, causing some damage to embryos, and severely damaging most eggs. Vitrification, on the other hand, uses a super high concentration of antifreeze (DMSO and ethylene glycol), and drops the

temperature so rapidly that the water inside the cell never becomes ice. It just instantaneously super-cools into a solid with no ice crystal formation at all.

We can now freeze and thaw, and even refreeze and rethaw, with impunity, using this new protocol from Dr. Masashige Kuwayama from the Kato Clinic in Tokyo. With conventional “slow freezing,” the temperature of the embryo goes down at precisely 0.3° C per minute. With vitrification (using four times the concentration of antifreeze, or cryoprotectant), the temperature is dropped at 23,000° C per minute, i.e., 70,000 times faster. At that speed of cooling, and at that concentration of antifreeze, ice crystals simply cannot form.

Of course, it is not quite as simple as it might sound. Such high concentrations of antifreeze, in a few minutes, could be toxic to cells. Therefore, the embryos (or eggs) must first be placed in lower concentrations of antifreeze (and sucrose to draw some water out), and then left in high concentrations only for less than a minute before instantaneous freezing. Then when the time comes to thaw the embryo, it must be instantaneously warmed, immediately taken out of the high concentration of antifreeze, and then placed into a solution with lower concentration, in order to avoid antifreeze toxicity. This requires more skill than conventional freezing, but it is faster, cheaper, and most importantly, avoids almost all freezing damage to either eggs or embryos. Such a reliable method of embryo freezing gives the IVF program much greater ability to avoid dangerous multiple pregnancy, and makes scheduling for procedures like egg donation simpler for the patient. Our frozen embryo pregnancy rates are extremely high with this technique, and we can freeze embryos without hurting at all your chance of conception.

Using this vitrification technique for freezing, we can preserve eggs, as well as embryos and sperm. This allows us to preserve the fertility of young women for the future in egg banks if they wish to delay childbearing. In St. Louis, we have demonstrated for the first time that an entire ovary can be removed and then grafted back after freezing and thawing so that even a menopausal woman can gain back her youthful fertility many years later. This new capability will be especially important to women undergoing treatment for cancer, because all the eggs that might have been lost to chemotherapy can be preserved by first removing, freezing, and storing her ovary for use later.

If you are considering freezing your unfertilized eggs, or one of your ovaries, just because you want to put off childbearing until you are older, the best approach is first to determine just where you are on your biological clock so that you can know when it's time to worry. We can now monitor your biological clock from your early twenties on, so you can decide when you ought to try to have a baby naturally. If you find out that your biological clock is more advanced than you feel comfortable with, you have the option of freezing an ovary or eggs and saving them until you are finally ready to have your child.

## Summary Of How You Can Preserve Your Fertility

1) Transvaginal ultrasound examinations are routinely performed by gynecologists, obstetricians, and IVF doctors, as well as in radiology imaging centers, but no one has bothered before to pay close attention to the small one- to two-millimeter follicles present in the ovary because no thought was given to their great significance. Thus, you will probably have to understand the scientific detail that has preceded this summary in order to explain to your physician what you would like to have done. I am sure that in the next five years we will see a revolution in the counseling of young women so that in addition to their yearly Pap smear, they will have this simple ultrasound exam to guide them in their reproductive planning. But it is not just a routine exam that I am suggesting — it is a specific counting of the number of one- to two-millimeter follicles, the so-called antral follicles. This number is constant throughout your menstrual cycle and gives a true measure of your reproductive outlook.

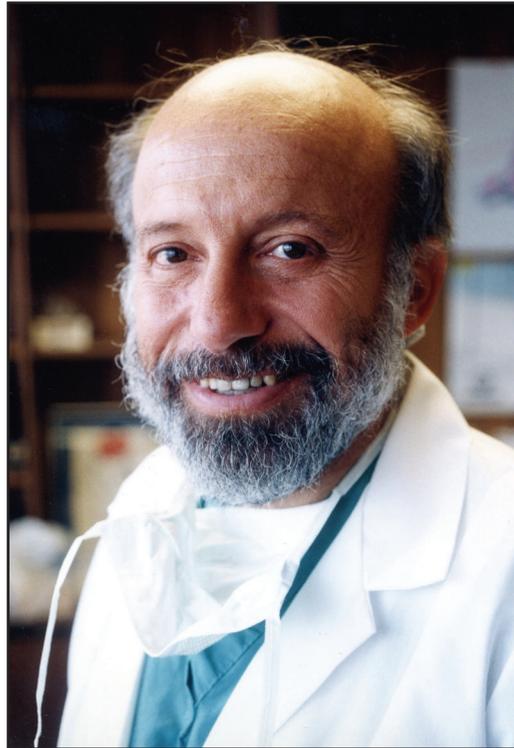
2) The antral follicles (the small one-millimeter to two-millimeter follicles located on the surface of the ovary) should be abundant in number, certainly greater than twenty. Your antral follicle count is the same at any time during your menstrual cycle and is unrelated to any hormone-dependent aspect of your monthly cycle. This makes sense because the follicles are not visible to ultrasound until they reach the size at which they first become hormone dependent. Thus, there is a continuous daily process of antral follicle for-

mation that began nearly three months earlier (seventy days) with the emergence from the resting follicle state.

3) There are problems with all the other methods of trying to determine ovarian reserve. The day three FSH, the day three estradiol, and even the Clomid challenge test have been shown to be very faulty and to reveal a problem only if you are near the extreme edge of the reproductive lifetime. Ovarian volume and overall physical fitness have no relationship to the number of eggs you were endowed with at birth. Normal twenty-eight day cycles and ovulation detection kits are also of no use whatsoever. Even women who are in their forties, with very few follicles left, can have normal twenty-eight-day cycles and ovulate.

4) The antral follicle count can then be used to determine when you will be likely to have your last naturally conceived child, and even when you are likely to go through menopause. Of course, a high antral follicle count does not guarantee that you are fertile. However, the age-related decline in fertility that people are most worried about in making their life decisions can be determined by the antral follicle count. Of course, even with many eggs, older women are less fertile than younger women with the same number of eggs. But if at a young age, you have a low antral follicle count, that surely means you are in trouble and have to take some action.

5) Once you know where you are on your biological clock, you can now do something about it. You can have your eggs frozen, or ovarian tissue biopsied and frozen, and thus preserve your fertility for the future, and thereby expand your reproductive lifetime.



*I hope this information has been of use  
to you. I look forward to meeting you  
personally when we have our  
consultation.*

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**2006**



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