Embryo Selection after IVF

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Many of human embryos produced after in vitro fertilization carry abnormal chromosomes. Placing a chromosomally normal embryo (s) into a normal uterus has a very high chance of achieving a pregnancy. Your eggs have been retrieved and the mature eggs were fertilized. Now You and your reproductive endocrinologist are faced with the critical task of how many and which embryo to transfer to the uterus or which ones to freeze.

Why do we Need Embryo Selection?

Selection of the most appropriate embryo(s) for transfer aim at i. Maximizing the chance for pregnancy and ii. Minimizing the risk of twins and other multiple pregnancies. Casual inspection of the embryo does not yield accurate information about its chromosome makeup. One can follow an indiscriminate approach where all embryos are transferred. The problem is this approach yields high unacceptable multiple pregnancy rates. On the other hand one can transfer one embryo at a time. This is a much safer approach in terms of markedly minimizing twin rates but may lower the chance for getting pregnant. In addition it also require a robust freezing program so that frozen embryos can survive thawing. Right now in The US the survival of frozen embryos exceed 95% and the chance for pregnancy with a thawed embryo is approximately equal to a fresh embryo.

Measure of Success: time to conceive or cumulative chance for pregnancy?

One major issue related to fertility treatment especially IVF is how to measure success? specifically consider this question: if you have three embryos and decided to transfer

them one at a time and got pregnant after the third transfer with a singleton, how does that compare to transferring all embryos in the fresh cycle and getting pregnant in twins? before answering it is important to know that twin gestation is associated with higher risk for pre-term delivery, ICU admissions and long term consequences for the babies.

In other words should you consider success as pregnancy taking place after one retrieval (cumulative chance from fresh and frozen embryos) or pregnancy taking place in the fresh cycle only (fresh embryos)? In other words would you like to shorten the time to conceive at the expense of higher risk for multiple pregnancy? Within *reason*, this is a question for you and your reproductive endocrinologist to answer based on your preferences and his practice

You have a Voice: How should you use your embryos after IVF?

You need to have a voice in the number of embryos transferred to your uterus. Although your fertility specialist can discuss numbers and chances and other technical details as well as long term risks for multiple pregnancy, there are questions that cannot be answered by anyone but you.

- How do you feel about twins? triplets and quads?
- Would you accept fetal reduction (removal of one or more sacs from the uterus and leaving only one or two)?
- Do you have the social support system to take care of twins?

For these and many other reasons your input in the number of embryos to transfer is paramount.

Methods of Embryo Selection after IVF

Embryo Morphology and Female Age

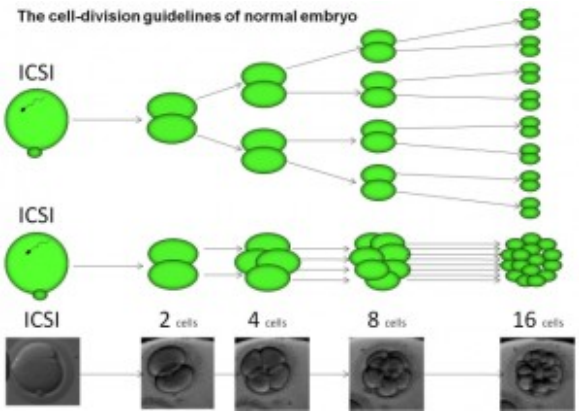
Age is, by far, the strongest predictor of the health of the

embryos. Younger women produce more chromosomally normal embryos than older women. An embryo from a woman at age 30 commonly implants 40% of the time as opposed to 5% or less in a woman age 40. For any given cohort, embryos are graded based on specific morphological criteria from the best looking to the worst. These criteria are technical and followed by all embryologists. Embryos are prioritized for transfer based on their shape. Morphology, however is may be 50 to 60% predictive of pregnancy, far from ideal. The combined use of morphology of embryos, stage of development (day 3 or blastocyst) and age is the standard selection method for which embryo is transferred first and how many. This method has the advantage of being sheep, quick and non-invasive. All other methods must prove superior to morphology + age before adoption.

Extended Culture to Blastocyst Stage (Day 5 Embryo)

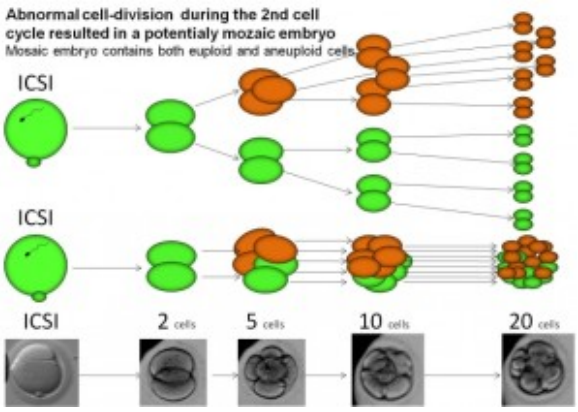
Keeping day 3 embryos in culture may give these embryos may time to develop to blastocysts. Presumably, the better embryos progress to blastocysts or do so faster than less healthy embryos, thus they are preferentially selected for transfer.

Time Lapse Imaging of Embryos



time lapse embryo imaging-normal embryo division

Embryos are placed in a specific incubator in a specific plate and is observed at predetermined time



time lapse embryo imaging- abnormal embryo division

points using time lapse microscopy / photography. Photos are analyzed manually or through a computer and embryos are graded based on timely division of blastmeres (component cells). There is no evidence so far that pregnancy rate is improved above using morphology. There is extra cost associated with the use of the special plate and is also limited by the number of special incubators available.

PGS (Embryo Chromosome testing)

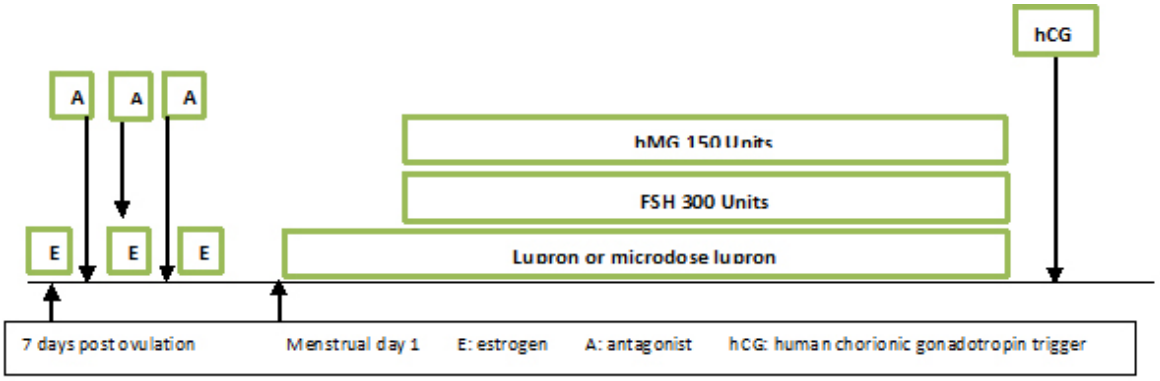
New forms of PGS (performing biopsy at the blastocyst stage) and more accurate platforms for analyzing the biopsied cells are available. However, the concept that better selection will lead to improved IVF results is far from certain.

It success of an IVF cycle is measured after transfer of fresh then frozen embryos till pregnancy ensues (cumulative success) ad patients are will to be patient for 1-2 more months, then any form of embryo selection, PGS or otherwise, will not improve the live birth rates. Moreover, PGS can be harmeful as it may misdiagnose the health of the embryos (see this article on PGS for details). PGS increases the expense of treatment $4000 to 6000

Embryo selection is maybe be able to improve the time to pregnancy, if embryos with the highest implantation potential are transferred first.

> *Based on the available evidence, judicious selection of embryos based on patient age, morphology and the use of extended culture to blastocysts are the standard of care in embryo selection after IVF. Two additional factors to consider is how robust is the freezing program of that specific lab (generally excellent all over the US) and the acceptability of fetal reduction by the couple. Liberal use of single embryo transfer when appropriate should be strongly considered. 'New' ideas should be subjected to rigorous scientific evaluations 'fertility clinical trials' before they are ready for routine use. Thus far, based on published evidence, embryo time lapse imaging and PGS should remain investigational.*

Ovarian stimulation protocols for Low Responders prior to IVF



Flare lupron protocol with luteal priming (synchronization) for Low Responders prior to IVF

Ovarian Stimulation Protocols for Low Responders prior to IVF

Low response to controlled **ovarian stimulation** represent a significant fraction of IVF population presenting for fertility treatment. Low responders may represent 30% or more of women seeking IVF. The proportion may be larger in some areas due to delay in childbearing as a lifestyle choice. Low response to ovarian stimulation is commonly defined as producing 5 eggs or less after stimulation. While may factors may contribute to low response e.g smoking, prior surgery of the ovary, exposure to chemotherapy, the vast majority of are age related. Sometimes low response happens in younger women e.g 30 year old. Young low responders has a better chance of conceiving because their eggs, though few, are healthier (chromosomally normal) than older e.g >38 low responders.

Few strategies can increase egg yield and possibly egg quality in low responders, usually employing one or a combination of

i. increasing the dose of gonadotropins,

ii. avoiding long lupron suppression before start of

stimulation,

iii. adding an oral agent (clomid or letrozole),

iv. synchronizing follicles prior to start injections,

v. using androgen prior to cycle start and sometimes

vi. adding growth hormone.

There is no clear evidence to one protocol over the other. Increasing the dose above a total of 450 units per day does not seem to further increase egg yield in low responders. Some patients respond to one ovarian stimulation protocol over another. One example of low responder protocol is illustrated above. Estradiol and antagonist are used to synchronize the follicles before menses so that they are uniform in growth when stimulation starts. Short lupron is used (flare or microflare) to induce the release of internal gonadotropins. This is followed two days later by high dose of fertility medication (total 450 units per day).

There is some evidence that pre-treatment with androgens (testosterone) may improve egg yield. The evidence for the use of DHEA (dehydroepiandrosterone) is limited. There is also week evidence that the use of growth hormone may improve egg quality.

Embryological procedures are also sometimes suggested as ICSI of all available eggs to maximize fertilization and assisted hatching of the egg shell (zona pellucida). Pre-implantation genetic screening is unlikely to be helpful as few embryos are available for testing.

Pre-implantation Genetic Screening (PGS): What are we really talking about?

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The tenant behind **pre-implantation genetic screening (PGS)** is to biopsy one or few cells from each embryo after creation, analyze the chromosomes for each embryo and transfer the ones that has normal chromosomes back into the uterus to boost IVF success and increase the live birth rate.

Central to this idea is that abnormal chromosomes in the embryos is the main reason why an embryo does not yield a newborn. It is logic then that **PGS** should allow the selection for the best embryo (preferably one only) for transfer into the uterus ending into one singleton newborn.

If this premise is accepted then the following assumptions should also be generally accepted

a. All or the majority of embryos reached the appropriate stage of development and expansion to allow biopsy.

b. Biopsy of the embryo does not harm its ability to implant

c. The cell or few cells obtained represent the rest of the embryo (has identical chromosomes to all the other cells in the embryo)

d. The platform used to analyze the embryo chromosomes is close to 100% accurate (otherwise some embryos will be wasted

because they are abnormal according to the test, while they are actually normal). The platform reports only the chromosomes of the embryo and is not accountable for other elements of implantation i.e. the endometrium.

e. The delay (one or more days) needed to finish the testing does not affect embryo implantation

f. Freezing and then thawing of a biopsied embryo does not affect its implantation potential

g. Patients and physicians have agreed on how to calculate success: how many live births one would obtain from all embryos resulting from a single IVF cycle (all fresh and frozen embryos) i.e. total potential of one IVF cycle versus fresh embryo transfer only.

h. The added cost of biopsy and testing of embryos, potential increases the delivery rate and reduces the incidence of multiple pregnancy and miscarriage is cost-effective from the viewpoint of individual and a modern society.

The initial attempt to perform **Preimplantation genetic diagnosis** using an old technology called FISH that tested 7 to 9 chromosomes proved harmful few years ago and that its wide adoption at that time was a form of medical illiteracy : because it depends on logic not actual well conducted study. When the studies were conducted, they all showed that women universally achieved lower pregnancy rates after PGS.

New platforms are now available to test for all the chromosomes (array cGH and SNP array) and using cells (trophoectoderm) obtained from more advanced stages of the embryo (blastocyst). The question in hand is should we adopt these techniques, not as a research tool, but as the standard of care that should be offered to the majority of women undergoing IVF?

How Effective is PGS? The case for Logic

Applying logical thinking to modern pre-implantation genetic screening (PGS) methods indicates:

a. Not all embryos will reach the blastocyst stage (day 5) to be suitable for biopsy. Not all physicians and patients push their embryos to the blastocyst stage especially if few embryos exist in culture on day 3. Moreover, some normal embryos may not survive extended culture to blastocyst.

b. There are no conclusive evidence that biopsy of the trophoectoderm (the part that makes the placenta) of an embryo does not harm the embryo.

c. Mosaicism ; when one or few cells are different in chromosomes than the rest of the cells, is known to take place in embryos. The cells in the trophoectoderm maybe abnormal while the cells in the embryo maybe normal. Interestingly the embryo can later get rid of the abnormal cells in the trophoectoderm. This can lead in misdiagnosis of the embryo as abnormal while the embryo itself has the potential to implant and yield a healthy baby.

d. The platform used to analyze the embryo chromosomes is not 100% accurate either because of the accuracy of the test itself or because of mosaicism. The accuracy reported by labs administering the test is 97%. This means some normal embryos will be discarded and some abnormal embryos will be transferred. Actually the accuracy was not validated by many labs, only very few worldwide. Clinically some physicians have experienced much lower accuracy (80 or 90%). The platform reports only the chromosomes of the embryo and is not accountable for other elements of implantation i.e. the endometrium. So it is possible that the lower accuracy is due to other elements on embryo gentics (other than the number of

chromosomes) or the lining of the uterus.

e. Currently the transfer of embryos into the uterus has to be delayed for one day (day 6) or several weeks (embryo has to be frozen then thawed back after results are obtained). This delay may reduce implantation of the embryo because it will not match the window of implantation in the lining of the uterus. This is a controversial point as some researchers found no difference in implantation between day 5 and day 6. This research, however, is not widely replicated.

f. After PGS some 'normal' embryos will be frozen. The survival of thawed and biopsied embryos is maybe reduced, potentially leading to loss of normal embryos. No large studies on survival of biopsied embryos after thaw exist.

g. Patients and physicians have agreed on how to calculate success: if success is calculated based on how many live births one would obtain from all embryos resulting from a single IVF cycle (fresh and frozen) i.e. total potential of one IVF cycle, then PGD has no value as it will not make an abnormal embryo normal or vice versa. If the success is based on what happens in the fresh cycle only with no regard to frozen embryos then PGS may improve the success rate of IVF. All assuming an excellent embryo freezing program.

For exampe If you are a young woman <38, with a good number of available embryo on day 5, say 4 blastocysts that are suitable for biopsy, you may elect to

i. transfer one embryo in the fresh cycle and freeze 3 embryos. If you are not pregnant, then transfer one embryo in each subsequent frozen cycle. If you are destined to get pregnant you will do that within a maximum of 3 months after your initial IVF and the risk for multiple pregnancy is minimized to 1% or less. If you were not destined to get pregnant no testing would have helped you or

ii. Alternatively, you may elect to test all your embryos in

the fresh cycle, transfer one normal embryo, if any and freeze any normal embryo remaining. The potential benefit is getting pregnant in the fresh cycle instead of getting pregnant 1-3 months later. Also you will reduce the risk of miscarriage because abnormal embryos will likely be eliminated. The potential risks are misdiagnosis by PGS (not 100% accurate), loss of a thawed embryo (did not survive biopsy and freeze) and lower implantation potential of a normal embryo due to biopsy and delayed transfer.

h. A cost-effective analysis for PGS is not available at this time. The added costs are biopsy and testing of embryos. The potential benefits are increase in the delivery rate and reduction in multiple pregnancy and miscarriage. In the scenario above you either pay for i. frozen embryo transfer(s) if you do not get pregnant in the fresh cycle or ii. pay for ICSI (required for PGS by the majority of programs), biopsy and testing in the fresh cycle and frozen embryo transfer(s) if you do not get pregnant in the fresh cycle. In terms of multiple pregnancy, it can be minimized in either pathways if your physician is transfers one embryo anyway, tested or not. Things are not that simple, the payer will also make a difference: PGS is completely out of a patient pocket as it is not covered by any insurance while frozen embryo transfer may or may not be covered.

How Effective is PGS? The case for Published Studies

In general decision making in biological sciences is not amenable to logic, but determined by well designed ad well conducted studies. So far, three studies were published using the new platforms for embryo chromosome analysis, aiming at increasing IVF success. The studies were criticized because of

1. Restricted to young women (median age 31 to 32) so results cannot be generalized to the general IVF population: 2 studies

2. Did not account for frozen embryos: all studies

3. The studies did not demonstrate superiority of PGS to transfer best embryos based on morphology (shape): one study. Specifically a transfer of a tested embryo in the fresh cycle was not inferior to transfer of two untested embryos. Non inferiority does not mean superiority. Noninferiority study design is not suitable for a PGS study as patients and physicians are only interested in such an expensive treatment that can harm their embryos only if it promises superior results for their infertility treatment. Moreover, treatment could actually be inferior because a limit is placed that will make the outcome non inferior, in that study 20%. So if the difference is less than 20% PGS is considered not inferior.

4. End point should be live birth or ongoing pregnancy. Surrogate or intermediate endpoints as pregnancy, implantation (short of a baby in hand or at least pregnancy beyond 20 weeks) are not ideal outcomes.

Randomized studies related to **pre-implantation genetic testing** using newer platforms were independently analyzed. So far no study showed that PGS is superior to the strategy of transferring the best embryo based on morphology (the standard of care). Moreover due to factors related to the biology of reproduction and that the accuracy of the test is unlikely to reach 100% accuracy soon, it is unlikely that PGS will prove beneficial to women undergoing IVF for fertility treatment. PGS may only shorten the time to pregnancy but will not be able to improve the pregnancy rate and due to inaccuracies may even reduce it.

Alternatives to PGS are being studied. One alternative is time lapse photography of the embryos to observe the cell division of the embryo cells and select the best embryo for transfer. It is noninvasive but further studies are required before its ready for general use. Another alternative is polar body biopsy of oocytes but results of ongoing studies are not

available yet.

It is possible that factors in this article could be interpreted differently in a specific situation by patients and their physicians, in conjunction with the number of mature eggs produced, but it does not appear that PGS is ready for generalized application in the majority of IVF population.

Fertility Treatment Options

Fertility Treatment Options: What Are Infertility Treatments?

Following detailed fertility investigation of the male tubal and ovarian factors, patient and her reproductive endocrinologist decide together on the optimal **fertility treatment options**.

Factors to consider in selecting the best **fertility treatment options** include:

Sperm source

1. Is there a male partner: if so what is the ejaculate volume, sperm concentration, motility and shape? if >10 million moving sperm then pregnancy through intercourse or IUI is possible. Lower numbers indicates **IVF** or ICSI. If azospermia (no sperm in the ejaculate) then surgical sperm retrieval may be needed (TESE) or donor sperm can be used.
2. If there is no male partner: anonymous or known donor sperm is used

Tubal Factor

1. Open fallopian tubes allow for natural conception or IUI.
2. Blocked fallopian tubes require IVF. Sometimes tubes can be fixed using tubal surgery.
3. Blocked and dilated fallopian tubes (Hydrosalpinx) require surgical removal of the dilated tubes followed by IVF. Dilated tubes are very difficult to fix and can leak fluid into the uterine cavity and prevent implantation of the embryo.

Ovarian Factor

1. Women who do not ovulate due to polycystic ovary syndrome (PCOS): ovulation can be induced using oral medications (clomid or letrozole) or injection medications (gonadotropins). This is usually combined with IUI.
2. Women who do not ovulate due to defect in the master gland in the brain (Hypothalamic amenorrhea): ovulation can be induced using injection medications (gonadotropins). This is usually combined with IUI.
3. Women diminished ovarian reserve and unexplained (idiopathic) infertility commonly have lower quality eggs and may benefit from inducing multiple ovulation followed by IUI or IVF, to increase the chance that one of the eggs is healthy (chromosomally normal).

Donor Eggs

1. Donor eggs are needed in women with low egg reserve that fail multiple IVF cycles after menopause or those who carry some genetic abnormalities.
2. Donor eggs can enable same sex male couples parent a child (together with a gestational carrier).

Gestational carriers

1. Gestational carriers enable women to parent a child if the uterus is absent or was removed due to a disease e.g endometrial cancer or if the lining of the uterus is damaged e.g intrauterine scarring due to prior scrapping.
2. Gestational carrier enable women who cannot get pregnant to parent a child e.g history of breast cancer
3. Gestational carriers enable same sex male couples to parent a child.

Genetic analysis of the eggs or embryos (PGD)

1. Women and men with risk of conceiving a child with a specific genetic disorder e.g cystic fibrosis, sickle cell anemia should consider testing their embryos before transfer into the uterus (PGD)
2. PGD can also be used for selecting the sex of the baby for family balancing.
3. PGD can be used to test the chromosomes of the embryo to increase the chance for pregnancy in women select women but its efficacy for that purpose is still being investigated.

Fertility Preservation

1. Women at risk for diminished fertility due to a medical problem or treatment e.g breast cancer can freeze their eggs or embryos to use later
2. Men at risk for azospermia due to genetic factors, cancer and cancer treatment can freeze sperm for use later
3. Many other techniques for fertility preservation can also be applied to adults and children to preserve reproductive organs and tissue.

Many **fertility treatment choices** exist to help women and men conceive a child. One or more of these methods can be tailored

to each

i. individual circumstances:

singles women or men,

heterosexual couples or

same sex couples.

ii. reproductive aim:

wants to get pregnant now versus later,

wants one child only or accepts twins,

wants to conceive a child of certain sex,

will use own uterus or a gestational carrier,

will use own gametes- sperm or egg or donor gametes.

To learn more about fertility treatment options please visit nycivf.org

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Fertility Treatments You Should Avoid

Which Fertility Treatments You Should Avoid?

Infertility is defined as inability to conceive after one year

(6 months in women >35 years) of regular unprotected intercourse (no contraception) and in the absence of any known cause for infertility. Earlier referral is recommended in

1. older women 35 years or more,
2. unable to have intercourse (e.g erectile dysfunction..),
3. genetic (e.g cystic fibrosis carrier), medical or pregnancy related risk factor (e.g systemic lupus, hepatitis C, HIV, hepatitis B…),
4. if a fertility factor is suspected (no ovulation,PCOS, hypothalamic amenorrhea, male factor, endometriosis, tubal disease..) or
5. if fertility preservation is desired following cancer diagnosis.

Evidence is accumulating of the most effective **fertility treatments** after fertility assessment. Many fertility treatments are offered indiscriminately, they have little chance of succeeding or are risky (ovarian hyperstimulation syndrome, multiple pregnancy). In general simple logic does not determine if a treatment is effective or not. It is only through well conducted studies we can prove the efficacy of such a treatment. Moreover, considering the final outcome- a live healthy newborn- should be the one to look for in such a study.

The following is not a medical advice, but a review of recent evidence related to fertility treatment options. You should discuss treatment with your fertility specialist. It is possible that sometimes these treatments are indicated for fertility treatment in special circumstances. Fertility treatments you should avoid may include:

You should not time your ovulation

If you have access to intercourse with a male partner every other day, timing ovulation using any method, does not increase your chance for natural conception. If you have

intercourse twice or more a week you have excellent chance of conceiving within one year. Studies evaluating timed intercourse using basal body temperature charts, urine LH kits, cervical mucus, failed to show improvement in pregnancy rate beyond intercourse every other day. No evidence that fertility apps improve the chance for conception.

Age category (years)	Pregnant after 1 year (12 cycles) (%)	Pregnant after 2 years (24 cycles) (%)
19–26	92	98
27–29	87	95
30–34	86	94
35–39	82	90

Use letrozole instead of clomid for ovulation induction in PCOS

There is high quality evidence that letrozole (aromatase inhibitor) is superior to clomid for induction of ovulation in women with PCOS and yeilds higher pregnancy rates. 750 infertile women with a diagnosis of PCOS, aged of 18-39 years, were enrolled: 376 patients were assigned to receive clomiphene 50 mg/day and 374 were assigned to receive letrozole 2.5 mg/day in doses escalating to 7.5 mg/day for a total of 5 days per cycle for up to five cycles. The drugs were provided in identical capsules over the same schedule. Ovulation rates with letrozole were significantly superior to clomiphene. Monthly chance for pregnancy and for a live birth was 30% higher in the letrozole group.

Avoid undergoing clomid or letrozole cycles without ultrasound monitoring

Although twins and higher order multiple pregnancies are not as common as in gonadotropin (injection medications) use [8%

versus 30%] clomid is probably responsible for more twins than any other treatment because of its widespread use. Do not undergo ovulation induction without ultrasound monitoring to evaluate response and the number of follicles developing. Consider cycle cancellation if many follicles appear in the ovary.

Metformin alone is inferior to clomid in induction of ovulation and improving fertility

There is strong evidence that clomid is superior to metformin in ovulation induction in women diagnosed with PCOS. Letrozole or clomid are the medications of choice for induction of ovulation, not metformin. There is also no strong evidence that metformin reduces the chance for miscarriage.

Do not use oral medications for unexplained infertility

Unexplained (idiopathic) infertility is diagnosed in women who failed to conceive with regular ovulation, patent fallopian tubes and near normal patent sperm analysis. Women with unexplained infertility, mild male factor or minimal endometriosis do not conceive mostly because of chromosomal abnormalities of the egg. Ovarian stimulation using oral medications usually yields one or two eggs (close to natural cycles) while using injection medications can produce more eggs thus increasing the chance that one of them is healthy. There is no evidence that oral medications increase the odds of pregnancy in women with UEI.

Avoid gonadotropins-IUI and proceed directly to IVF

In women receiving oral medications (clomid)-IUI proceeding

directly to IVF or proceeding immediately to IVF as first line treatment and avoiding injection medication-IUI is more successful in achieving pregnancy, is faster and minimizes the risk of multiple pregnancy.

The FASTT trial randomized 247 couples to receive three cycles of clomiphene citrate (CC)/IUI then three cycles of FSH/IUI and then up to six cycles of IVF versus 256 couples to an accelerated treatment, that omitted the three cycles of FSH/IUI. An increased rate of pregnancy was observed in the accelerated arm and pregnancy was achieved 3 months faster. Per cycle pregnancy rates for CC/IUI, FSH/IUI, and IVF were 7.6%, 9.8%, and 30.7%, respectively. The observed incremental difference was a savings of $2,624 per couple for accelerated treatment. The study demonstrated that FSH/IUI treatment was of no added value.

The FORT-T trial randomized couples with ≥6 months of unexplained infertility with female partner aged *38-42 years* to treatment with two cycles of clomiphene citrate (CC) and intrauterine insemination (IUI), follicle stimulating hormone (FSH)/IUI, or immediate IVF, followed by IVF if not pregnant. The cumulative clinical pregnancy rates per couple after the first two cycles of CC-IUI, FSH-IUI, or immediate IVF were 21.6%, 17.3%, and 49.0%, respectively. The majority (84%) of live-born infants resulting from treatment were achieved via IVF. Immediate IVF demonstrated superior pregnancy rates with fewer treatment cycles in the immediate IVF group.

Avoid using DHEA, GH or aspirin as adjuvants to IVF

There is no conclusive evidence that pretreatment, prior to IVF, with dehydroepiandrosterone (DHEA), growth hormone (GH) or other medications improves the pregnancy rate r live birth rates.

Avoid transferring two or more embryos when feasible

Multiple pregnancy carries an higher risk to the mother and to the health and neurological functions of the newborn. Outcomes in twins are definitely inferior to singleton babies. Women <38 years with a good quality embryo in there first or second IVF cycles should consider single embryo transfer. In the third cycle consider double embryo transfer.

Avoid routine use of pre-implantation genetic screening to improve the pregnancy rate after IVF

Chromosome analysis of embryos is available. There is no conclusive evidence that PGD will increase the chance for a live newborn. PGD will definitely not make the embryos healthy. If accurate, it will just enable finding the healthy embryo faster but the total number of healthy embryos, if any, will remain the same per completed IVF cycle. The accuracy of the test is no 100%, it is costly and require taking one or few cells from each embryo. Young women with good ovarian reserve have excellent pregnancy rate even with single embryo transfer. Moreover embryo freeze-thaw cycles yield comparable outcomes to fresh IVF cycles. Older women and women with low egg reserve produce a small number of embryos, which means that testing is not an efficient approach. PGD may have some role in older women e.g.>40 years producing a large number of embryos e.g >6 embryos. These women are the outliers.

Avoid using a physician with no experience in managing fertility problems

This will likely cause delay, reduce success and may increase complications. If you seek a specialist care, avoid any treatment that you do not understand its rationale. The

choices are usually expectant treatment (regular intercourse), ovarian stimulation-IUI or IVF. Know the expected success rate and multiple pregnancy rate for each option offered to you by a reproductive endocrinologist.

Fertility Treatment Men Should Avoid

1. Avoid treating abnormal sperm parameters with oral or injection medications or supplements. No such treatment was demonstrated to improve the chance for a live born in female partner.
2. Avoid surgery for varicocele even if sperm parameters are abnormal. Surgery for varicocele is a **treatment** that was not proven to increase the odds of live born in female partner.

To lean more about fertility treatments please visit nycivf.org

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Should you Test Embryos created after IVF if You had Recurrent Miscarriage?

PGD Recurrent Miscarriage

1. *Early pregnancy loss approximately < 10 weeks*, mainly due to chromosomal abnormalities of the embryo and

2. *Late pregnancy loss ≥ 10 weeks* due to structural uterine abnormalities, hormonal factors, blood clotting abnormalities, immunological factors and chromosomal abnormalities of the embryos (less likely than early loss).

Women with history of recurrent miscarriages should be tested for all these factors before a fertility treatment plan is finalized.

Factors that point to chromosomal abnormalities as a cause for recurrent miscarriage

i. Advanced maternal age,
ii. Diminished ovarian reserve (e.g high FSH, low AMH),
iii. Early pregnancy loss before a fetal heart activity is detected (chemical pregnancy, blighted ovum),
iv. Abnormal chromosomes of the products of conception and
v. Abnormal chromosome configuration of male or female partner e.g chromosome translocation. Less than 5% of couples miscarry due to a translocation in the male or female partner.

Structural abnormalities of the uterus are detected using saline sonography, hysteroscopy or MRI scan. Blood tests can detect hormonal abnormalities, clotting abnormalities and immunological factors.

Should you Test Embryos created after IVF if You had Recurrent Miscarriage? (if chromosomal abnormalities of the embryos are suspected)

Factors to consider before deciding to test embryos:

I. Embryos should probably be tested in women or men that

carry abnormal chromosome configuration e.g. translocation as they produce higher proportion of abnormal embryos than parents of the same age

II. Embryos should be tested if avoiding another miscarriage is a priority, especially if prior miscarriages took place later in the first trimester and required surgery. Repeated scrapping of the uterus can damage the lining that may be difficult to treat (Asherman syndrome).

III. There is no prove that PGD for chromosomes will improve the chance for conceiving a normal child. PGD will only detect what you have and is not a therapeutic procedure. The decision for embryo testing using PGD should be individualized for all other parents. Older women may not have any normal embryos to transfer after testing. Although testing may avoid a pregnancy with an abnormal embryo that implant and survive >10 weeks, the majority of abnormal embryos do not implant or are miscarried extremely early.

Just because it is available, sounds plausible and you have the means to do it, does not mean you should test your embryos prior to IVF.

Gender Selection What you Need to Know

Gender Selection what you need to

know

A fascinating topic that stirs discussions on both sides. Selecting the sex of the baby, boy or girl, is an additional procedure that can be combined with IVF, for specific indications. A male baby is the result of fertilization of an egg (X chromosome) with a Y chromosome bearing sperm. Female baby results from fertilization of the egg with an X bearing sperm.

Indication for Gender (Sex) Selection

The World Health Organization defines the indication for sex selection into

- Medical reasons—such as preventing the birth of children affected or at risk of X-linked disorders.
- Family balancing—where couples choose to have a child of one sex because they already have one or more children of the other sex.
- Gender preference— in favor of one sex often male offspring stemming from cultural, social, and economic bias in favor of male children and as a result of policies requiring couples to limit reproduction to one child. Biases vary among different communities e.g Germans has no preference, American men may prefer biological sons and American women has no preference (Gallup 2011) and both prefer adopted daughters.

Ethics of Gender Selection

Many European countries and Canada and Asian countries have banned sex selection in cases unrelated to any health purpose. The American Society of Reproductive Medicine (ASRM) has allowed pre-fertilization sex selection through sperm sorting and IUI and discouraged the use of IVF and PGD solely for sex selection. Sperm sorting is not available in the US as it is

not approved by FDA.

The bio-ethical concerns related to sex selection include:

1. Healthy fertile couples choosing IVF for the sole reason of sex selection. An alternative argument is that the risk of pregnancy outweighs the risk of IVF and this becomes relevant in women seeking to limit the number of pregnancies and babies to two of opposite sex.
2. Future imbalance of population and changing the sex ratio. This is unlikely through IVF as only 1% of world population are born following assisted reproduction. Sex ratio imbalance does occur through sex-selective abortion.

Criteria to Consider Couples for Sex Selection Procedures

To address some ethical and safety concerns related to **sex (gender) selection** using IVF + PGD, couples requesting sex selection should fulfill minimal criteria

1. IVF indicated for other fertility factors
2. Not in the first cycle for the first baby except for genetic reason
3. In subsequent cycles or selecting for the under-represented gender for family balancing only

Sperm sorting is not available in The US (not FDA approved).

Methods of Sex Selection

The choice of sex selection method is dependent on availability, cost and accuracy. The majority of couples do not merely prefer certain sex they specifically desire a boy or a girl. For couples interested in a certain sex only, methods of **gender selection** should be very accurate, close to 99%.

Pre-Fertilization

MicroSort

Not available for use in the US. Sperm is sorted and used for intrauterine insemination (IUI). Sperm carrying an X chromosome have approximately 2.8% more DNA material than sperm carrying a Y chromosome. This is the basis of separating X and Y sperm. Microsort requires an excellent sperm sample of 140 million sperm and 50% motility for IUI and 70 million sperm and 50% motility for IVF. Studies reported that 91% of those attempting for a girl do conceive a girl, while the success rates for sex selection and boys using MicroSort® is lower at 74%. The pregnancy rate after ovarian stimulation and IUI is approximately 10%. Thus the success rate for getting pregnant with the desired sex using this method is 7.5 to 9% per attempt or less.

Other pre-fertilization methods

The Shettles Method is based on the fact that male and female sperm travel and survive in the reproductive tract for varying amounts of time. So you time intercourse about 12 hours prior to ovulation for a boy and several days before ovulation for a girl. There is no proof that this method is effective.

*Ericsson Albumin Method. S*perm is filtered through albumin and used for IUI. In addition for a girl, Clomid is used since it has been shown to increase the number of girls. There is no scientific proof that this method is effective.

Post-Fertilization (Pre-embryo transfer)

IVF + Pre-Implantation Genetic Diagnosis (PGD). The ovary is stimulated and eggs are harvested using transvaginal ultrasound, under sedation. A sperm is injected in each egg. On day three, when the resulting embryos are about eight cell each, one cell is biopsied and tested to X and Y chromosome. Laser beam is sued to make a hole in the egg shell and one

cell is sucked out. This can also accomplished after biopsy of blastocyst – trophoectoderm biopsy (day 5). The desired embryo(s) are transferred into the uterus. This method is over 99% accurate. *We, in addition sort the sperm used for fertilization and freeze the desired sorted sperm and use it for fertilization of eggs. The aim is to enrich for the desired sperm (X or Y) to increase the chance of getting many embryos of the desired sex.*

PGD can also be performed on frozen thawed embryos for couples that has frozen embryos and coming for frozen embryo transfer and desire sex selection for family balancing.

The biopsy material can also be tested for all the chromosomes or for certain gene. Imposing more criteria on the embryos will certainly make fewer embryos available for transfer.

If IVF cycle pregnancy rate is 35%, then the chance for achieving pregnancy with the desired sex is approximately 35% or less if that sex is found in the embryos

Considerations for sex selection using IVF + PGD

1. Ovarian reserve: In gender selection cycles, the embryos available for transfer are fewer than in IVF cycles without sex selection. Each embryo has to be of good quality in addition to being of the desired sex to be considered for transfer onto the uterus. Women with diminished egg number and quality will have small number of embryos available for testing and are less likely to realize good quality embryos of the desired sex than women with good reserve and good egg quality. Ovarian reserve is directly related to the mother's age.
2. Inconclusive result. Sometimes, the embryo sex cannot be determined due to degeneration of DNA material in the cell. This minimized by appropriate fixation of the biopsy in expert hand.
3. The desired sex could be under-represented in the

embryos , by chance or due to other factors. One of the pre-fertilization methods can be used to enrich the sperm sample for the desired sex

How Do you Know if Gender Selection Worked?

After conception, there are many ways you can confirm success of **gender selection**

1. Non invasive perinatal testing (NIPT): a blood test that reports on chromosomes 21, 18, 13, X and Y using free fetal DNA floating in the mother circulation
2. Ultrasound at 18 weeks or after can detect the external genitalia of the baby.
3. CVS or amniocentesis these are invasive methods to test the chromosomes of the baby. They are the most accurate. Being invasive, they should only be used if indicated, not just to confirm sex.\

To learn more about gender selection please visit nycivf.org

Sex Selection

Sex selection is considered for one of three reasons:

1. Avoiding sex related genetic disorders. These are genes mostly carried on the X chromosomes and affect boys more than girls since they have one X chromosome e.g hemophilia

2. Family balancing: couples that have children of one sex and

desire a child of the opposite sex

Suppose you could only have one child. Would you prefer that it be a boy or a girl?

	Boy	Girl	Either/ Doesn't matter (vol.)	Not sure	No opinion
	%	%	%	%	%
2011 Jun 9-12	40	28	26	3	3
2007 Jun 11-14	37	28	26	5	4
2003 Jul 18-20	38	28	27	5	2
2000 Dec 2-4	42	27	25	4	2
1997 May 6-7	36	23	38	2	1
1997 Feb 24-26 ^	41	29	25	--	5
1996 Feb 23-25 ^	41	31	25	--	3
1990 Apr 19-22 †	38	34	24	--	4
1947 Sep 12-17 †	40	25	27	--	8
1941 Mar 21-26 ‡	38	24	23	--	15

(vol.) = Volunteered response
^ If you were to have a child right now, would you rather have a boy or a girl?
† If you had another child would you rather have a boy or a girl?
‡ If you could have only one (one more) child, which would you prefer to have -- a boy, or a girl?

GALLUP

Gender Selection, Boy or a Girl

3. Preference: some prefer a child of certain sex due to social factors. Recent poll in The UK indicates that when 2,129 recently married couples were surveyed, found that 47% admitted that they would prefer to have a son first, with the majority citing practical reasons like boys being “less hard work”. Only 21% of respondents said they would like to have a daughter as their firstborn, and 32% reported having no preference either way. Couples who wanted to have a daughter first see older girls as ‘better role models’ to their younger siblings. In the US a Gallup poll yielded similar answers by American parents, especially men, since 1940s. American women do not have a proportionate preference for girls. American women show essentially no preference either way: 31% say they would prefer a boy and 33% would prefer a girl. More recent

trends indicates that American couples prefer girls.

In contrast couples on a waiting list for adoption prefer girls both in the US and India. There is also some evidence that sexual orientation may influence that preference. Gay men are more likely to have a gender preference for their adopted child whereas heterosexual men are the least likely. Couples in heterosexual relationships are more likely to prefer girls than people in same-gender relationships.

The preference is also influenced by geography and politics. The official family planning policy in China, applied to large portions of Chinese, allow only for one child and does not allow sex selection. In the US many couples desire to limit the number of children to 2. If the first child is of one sex they desire the second child to be of the opposite sex

How is the sex of the embryo determined?

Older methods of selecting sex through change in the position or timing of intercourse or sperm sorting are not accurate and are not suitable for sex selection in modern couples seeking a specific sex (the other sex maybe conceived in 30% or more of couples). Modern sex selection depends on genetics. After stimulation of the ovaries, egg retrieval and fertilization, one or few cells from the embryo is obtained. The cells are analysed for each embryo for the X and Y chromosome. Results are obtained and are accurate >99% of the time.

After identification of the X and Y chromosomes, the desired embryo is transferred into the uterus. The embryo that carries the correct chromosome, should survive and be of good quality. Sex selection is more likely to succeed in women with good ovarian reserve, producing a good number of eggs. The larger the number of embryos available for testing, the more likely a healthy embryo of the desired sex is available for transfer.

Learn more about **gender selection**.

Should You Test Embryos Created after IVF for Chromosomal Abnormalities?

Should You Test Embryos Created after IVF for Chromosomal Abnormalities?

Many of the embryos created after IVF carry abnormal chromosomes. Normal embryo cells carry 46 chromosomes. The most common abnormalities are extra chromosome e. +21 (47 chromosomes) or missing a chromosome e.g -X (45 chromosomes). By far, abnormalities in the egg is the source of abnormal chromosome number.

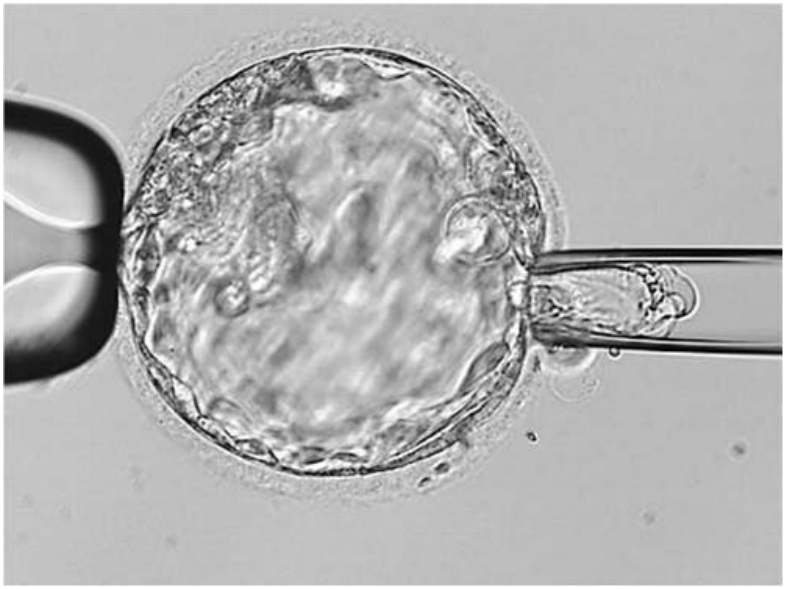
PGD: Testing of embryo chromosomes

Finding a ‘normal’ embryo is clearly advantages as it will theoretically lead to 1. The transfer of a single embryo instead of many embryos and 2. can produce higher pregnancy rate than an embryo selected based on morphology (looks) alone. The process of embryo testing for the purpose of improving pregnancy rate is, however, not simple in relation to the accuracy of testing and many other issues

Preimplantation genetic screening for chromosomal abnormalites (PGS)

PGS require two steps: 1. Biopsy: obtaining a cell or a group of cells from the embryo and 2. genetic testing of the cells for chromosomes ideally in 1-2 days to obtain results and allow fresh transfer

Biopsy



Biopsy of trohoectoderm cells of blastocyst

Obtained by removing a. a single cell of a day 3 embryo or b. group of cells from the trophoectoderm (the outer part of the embryo that makes the placenta) of a day 5 embryo (blastocyst). Removal of cells nowadays uses a laser beam. Cells are fixed on a glass slide and sent for analysis.

Genetic Analysis of Embryos

In the past old technologies (FISH) was limited in its ability to test all chromosomes. Multiple studies in the past few years proved that PGD using FISH actually reduce the chance for pregnancy in many IVF populations and should not be used. Two newer technologies can test all the chromosomes in an embryo: cGH (comparative genomic hybridization) array and SNP (single nucleotide polymorphism) array. Some of these methods can report the results in 3 days allowing for delayed fresh transfer (day 6) and others require about a month for accurate testing, necessitating embryo freezing and transfer in frozen-thaw cycle. Labs offering these methods claim accuracy of 95 to 97%. There are more advanced methods e.g genome screening, that can test embryo chromosomes in as short as 6 hours. The

ultimate method for testing is still evolving.

Should women test their embryos before transfer to the uterus?

My short answer is no, not routinely. The pros of testing embryos could be transferring less embryos , improving IVF outcomes (pregnancy rates) and avoiding pregnancy with a baby carrying chromosomal abnormalities. The cons are these aims are still not proven facts due to

1. The biopsy may hurt the embryo, reducing its ability to implant

2. The assumption that one cell represent the whole embryo may not be true (mosiacism); the cell may be abnormal while the rest of embryo is normal or vice versa

3. The methods of testing was not validated by independent large studies from multiple centers and maybe less accurate than claimed

4. Delay in transferring the embryo in the fresh cycle may reduce its implantation potential

5. Cost associated with biopsy and testing the embryo is approximately $5500 to $8000

6. Testing of an embryo will not improve the ‘pregnancy’ potential of that embryo. It will just tell you if the embryo is ‘normal’ or not. The potential from all the embryos obtained from IVF after an egg retrieval is not changed by testing. Assuming a very accurate test and an excellent freezing program, tested embryo transfer should yield similar outcome as transferring untested embryo(s) in multiple cycles. That is the most important point to consider. If you are willing to be patient and transfer one or few embryos resulting from one ovarian stimulation successively in the fresh cycle then frozen cycles, the cumulative pregnancy and

delivery rate should be the same at the end. For example in young women transferring one embryo, approximately 30- 40% of them will just achieve pregnancy in the fresh cycle. In the first frozen-thaw transfer another 30% or so will get pregnant. Frozen cycles are not as demanding as fresh IVF. Many women can have the embryo transferred in a natural cycle with no medications and minimal monitoring.

Embryo testing may help younger women, producing a large number of embryos and want to transfer only one. An alternative approach is to transfer one embryo at a time as their pregnancy rate is high even with a single untested embryo.

Testing of embryos from older women (40 or older) producing few embryos (<6) is of little value as the alternative is to transfer 5 or so untested embryos in that age group because of the very high rate of chromosomal abnormalities in the embryos.

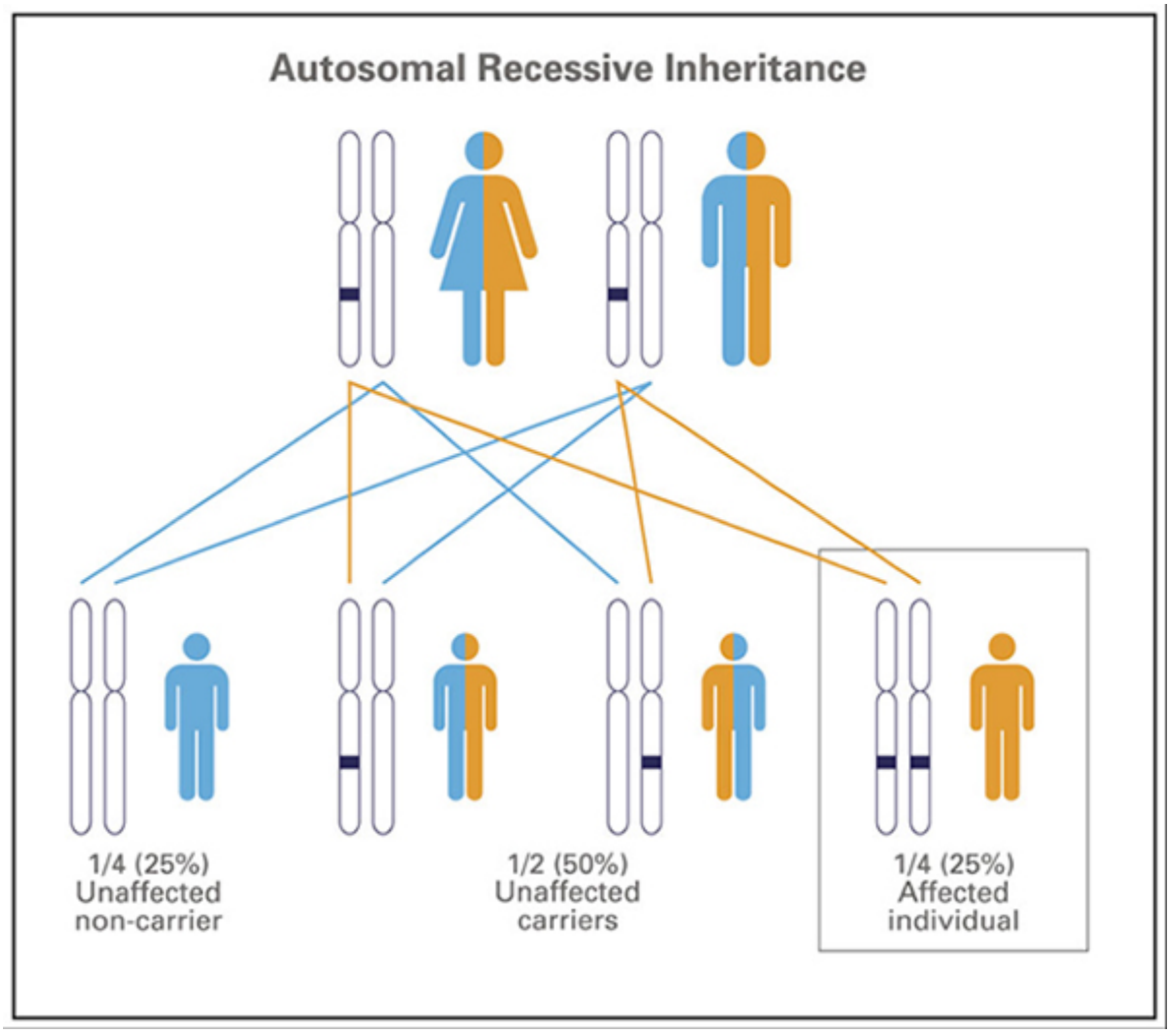
Testing may be helpful for older women (40 or older) producing a very large number of embryos (e.g >10 embryos) to eliminate the need for multiple transfers to get to the healthy embryo. This category (older women and very large number of eggs / embryos) is rare in IVF population.

Women contemplating testing of their embryos after IVF should consider many issues including age, number of embryos, history of unsuccessful fertility treatment if any, cost and sometimes tolerance for multiple pregnancy and fetal reduction. Moreover women should consider all these factors and be ready to modify their decision during the cycle depending on the number of available embryos.

All this does not apply to women testing the embryos for chromosome translocation, a specific genetic disease or sex.

How to Avoid Conceiving a Baby with Cystic Fibrosis

Cystic Fibrosis is a recessive genetic disease that affects a child mostly because he or she inherited two abnormal copies of the gene, one from the father and one from the mother. If both the mother and father are carriers, there are 1 in 4 chances for the baby to be affected. The odds of carrying a mutation are variable and are approximately 1 in 29 in Caucasian populations.



Autosomal Recessive Inheritance

Prior to conceiving a baby, one of the partners can be tested for common cystic fibrosis mutation, using a simple blood test. If one partner is a carrier the other partner is tested. One partner does not carry CF gene mutation: no need to test the other partner and the risk of CF transmission to the baby is very low.

Both partners carry CF gene mutation: the risk of CF transmission to the baby is 25%. In this case the couple can consider IVF with preimplantation genetic diagnosis (PGD) for the specific mutation. Embryos that do not carry the mutation are transferred to the uterus, avoiding the disease.

Consulting with a reproductive endocrinologist can identify the risk and prevent the transmission of cystic fibrosis to your baby.It is a recessive genetic disease that affects a child mostly because he or she inherited two abnormal copies of the gene, one from the father and one from the mother. If both the mother and father are carriers, there are 1 in 4 chances for the baby to be affected. The odds of carrying a mutation are variable and are approximately 1 in 29 in Caucasian populations.