

Embryo Freezing and Transfer of Frozen Embryos



**Southern Ontario
Fertility Technologies**

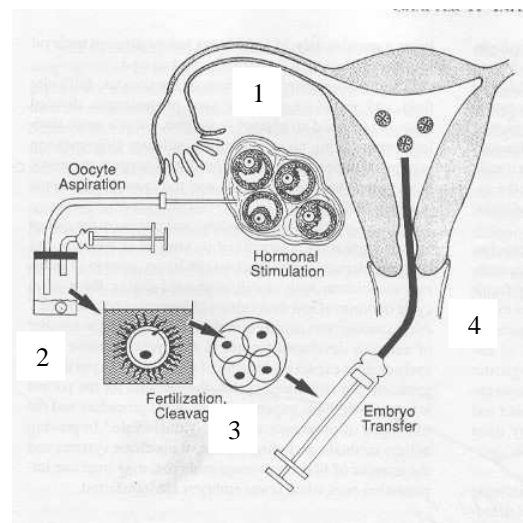
Introduction

In an in vitro fertilization (IVF) or IVF with intracytoplasmic sperm injection (ICSI) cycle, if more embryos are available than can be transferred, you will be offered **embryo freezing**. A fee \$650.00 is charged for freezing the embryos and storing them for one year. Embryos have an 80 to 90% chance of surviving the freeze-thaw and can be transferred later in medicated or spontaneous cycles with vaginal progesterone for maximizing the preparation of the endometrial lining.

The IVF cycle

In-vitro refers to something occurring outside of the body, fertilization is the penetration of the egg by a sperm and the mixing of their genetic information. With IVF, the cycle is stimulated to produce multiple eggs. This is not by definition part of IVF but is necessary to improve the success of IVF. In fact, natural-cycle IVF is offered in some countries in the world but its success rate is lower than 5% per cycle.

Once the multiple eggs have matured, they are retrieved from the body. This is depicted in the diagram to the right beside #1. The next step is fertilization of the eggs with sperm. This is the step that gives IVF its name and is depicted beside #2 in the diagram. Once the eggs are fertilized, they become embryos. These are cultured in the lab. During culturing, the number of cells in the embryo increases and this is depicted beside #3 in the diagram. Once the embryos have cell divided, a predetermined number are transferred back into the woman's uterus hopefully to implant and produce a baby. This is depicted beside #4 in the diagram. If extra embryos form and are of good quality they can be frozen for future use.



8-Cell Day 3 Embryo



Day 5 Blastocysts

Embryo Freezing

Embryos are frozen by replacing the water inside them with antifreeze and lowering their temperature to that of liquid nitrogen (-296 C). Only high quality embryos can be frozen. At ISIS extremely good quality embryos will be frozen on day 3 (the day of embryo transfer) but any viable embryos will be cultured to day 5 and frozen then if they look good. These are blastocysts and may freeze better because of the smaller individual cell size.

Once frozen, they can be kept frozen for long periods of

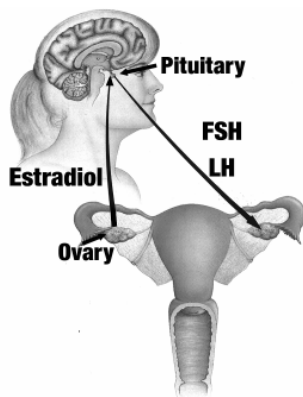
time. Some human embryos have been thawed after 10 years of storage without appreciable deterioration or a decrease in survival rate. Similar freezing has been done for up to 20 years in animals.

Embryo Thawing

Embryos can be thawed and transferred into the uterus of their owner as long as the endometrium is at the receptive stage. Two ways are available to do this. One way for this to occur is to time it with a woman's own spontaneous cycle. If spontaneous cycles are not available (ovarian failure, PCOS or hypothalamic amenorrhea) the embryos can be replaced in a medicated cycle.

Day 3 embryos are usually replaced on day 3 and day 5 embryos are replaced on day 5. Embryos are thawed the day before they are intended to be transferred into the uterus. This allows the lab staff to assess their viability (whether they are alive or not). Sometimes embryos will resume cell division after they are thawed. This, of course is a very good sign.

A Description of a Spontaneous Cycle



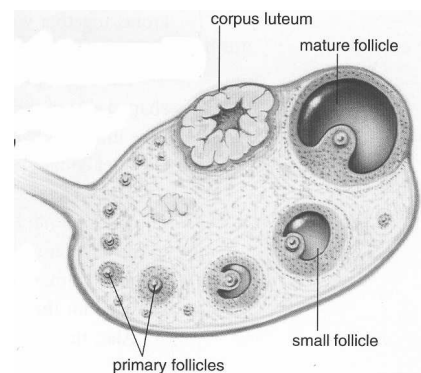
The menstrual cycle is described in terms of days. Day one being the first day of vaginal bleeding requiring protection as long as it occurs before midnight. At this point most of the hormones are at very low levels. The cycle begins with **Follicle stimulating hormone (FSH)** being released by the pituitary and stimulating both egg-maturation and production of estrogen (estradiol). In a successful (ovulatory) cycle, the combination of FSH and estrogen facilitates a rapid release of Luteinizing Hormone (LH). This rapid, high level of LH is often referred to as the **LH Surge** and is thought to induce release of the egg (ovulation) about 34 to 36 hours after its peak. The LH surge is used to time the return of the embryos.

Once the surge occurs and the egg is released, the follicle it was growing in starts to produce progesterone. The estrogen in the first half of the cycle causes the cells of the endometrium to divide. The progesterone in the second half of the cycle matures the endometrium.

Timing the Procedure – Spontaneous Cycle

To time the transfer of the embryos back into the uterus, the cycle is monitored to determine that it is a normal ovulatory cycle and to pinpoint the LH surge. An embryo that was frozen at 3 days after egg retrieval and fertilization will be thawed 2 days after the LH surge and transferred the next day (day 3). An embryo that was frozen at 5 days after egg retrieval and fertilization is thawed 4 days after the LH surge and transferred back the next day (day 5).

The success of frozen embryo transfer depends on timing it with **ovulation or release of the egg from the ovary**. Ideally, the transfer should be done at the exactly the number of days after ovulation as the embryos age. Three days after ovulation for embryos frozen on day three and 5 days for embryos frozen on day



5. Frozen embryo return cycles use **both blood tests and vaginal ultrasounds to determine when this occurs.**

The beginning of the cycle is referred to as “day 1”. “Day 1” is the first day of menstrual bleeding requiring more than a panty liner for protection. All hormone levels are very low and the ovary should contain only very small follicles. Follicles are small cystic structures which contain the eggs and produce the ovarian hormones. We can figure out how the cycle is progressing by measuring the growth of the follicle(s) and measuring the blood level of key hormones.

The first thing that happens in the cycle is that the pituitary gland produces **follicle stimulating hormone (FSH)**. This causes a follicle to grow (one in 20 spontaneous cycles has two follicles). The follicle(s) produces **estrogen** in increasing levels as it grows. In most cycles the estrogen increases each day and we measure this increase during the critical time of the cycle. Another hormone, luteinizing hormone (LH) stays low during the first part of the cycle. However, once the estrogen reaches a certain level or a follicle becomes a certain size; a **LH surge** occurs (a doubling or tripling of the level). The LH surge is then used to time the insemination. In spontaneous cycles the LH surge usually occurs on day 12 or 13. Once the LH surge has occurred the estradiol level usually drops. In fact, with our monitoring, we can determine if we are at the beginning or end of the LH surge by the estradiol level. If it is still rising, we are at the beginning of the LH surge; if it has dropped from the previous day, we are at the end. The peak of the LH surge occurs **34 to 36 hours before the release of the eggs.**

The dynamics of a spontaneous cycle is presented in this table. **This table is an example only and individual cycles may vary widely.**

Usually, blood tests (estradiol and LH) are performed daily from day 11 of the cycle. In the cycle, the estradiol should increase slowly and the LH should stay consistently low until one day it rises dramatically. The “**LH surge**” refers to a rapid release of luteinizing hormone from the pituitary gland before ovulation occurs and is reflected in the LH blood test by a doubling or tripling of the baseline level. The transfer is performed the prescribed number of days after the “LH surge”. A LH surge does not always occur in cycles being monitored for frozen embryo transfer. This can happen even if there is normal egg development. In natural cycles this has been named “luteinized, unruptured follicle syndrome” and is thought by some to contribute to some infertility. If a surge does not occur, the cycle will be cancelled. In women with extremely regular cycles we may try monitoring another cycle or we may suggest you go directly to a medicated cycle.

Cycle Day	Estradiol E ₂	LH	Follicle Size (mm)
Day 1	50	4	2
Day 3	100	3	5
Day 6	200	4	8
Day 8	250	3	11
Day 10	320	4	14
Day 11	400	3	15
Day 12	550	6	17
Day 13	600	42	19
Day 14	300	25	21

Unfortunately, the results of **the blood test required for this monitoring have to be available the same day.** This usually requires them to be done at S.O.F.T. We have been able to arrange same-day blood testing in some cities (IE Windsor, Kitchener and Sarnia). This can only be done on weekdays and on days that you don’t have to have an ultrasound. If you are interested in doing some of your blood testing in these labs, you should speak to the nurse about it on your first day of monitoring. This is usually reliable, but S.O.F.T. cannot take responsibility for the

result being sent to us the same day. This can make doing an IUI cycle very stressful and fatiguing. We try to make this less stressful by performing the monitoring blood tests at the clinic (one stop shopping!). Also, instead of rigidly having to do the tests first thing in the morning, they can be done as late as 1 pm on most weekdays.

Blood testing, ultrasounds and inseminations may need to be performed 7 days a week at the clinic. For this purpose, S.O.F.T. is open from 7:30 am to 1 pm weekdays and 8:30 am to 10 am on weekends. On weekends and holidays only the back door of the building will be open.

Vaginal ultrasound monitoring of spontaneous cycles for frozen embryo transfer is also be important. It is employed in spontaneous cycles on day 10 or 11 to determine how many potential eggs are developing and to ensure that the endometrium is of adequate thickness. (Vaginal estrogen may be prescribed if the lining is too thin.) Follicles that may go on to ovulate are usually 11 mm or more in diameter by your first monitoring day. If all follicles are smaller than this it may mean that you will not ovulate in this cycle or will have an unusually long cycle. If the follicles are very large, it may mean that



Ultrasound of an ovary with many small follicles but one central follicle, which has become larger



Ultrasound of a uterus, which demonstrates a triple layer endometrium of adequate thickness

they are left over from the last cycle or that you are going to ovulate earlier than usual in this cycle. Follicles that are 16 mm or greater in diameter probably contain a mature egg. It used to be thought that if follicles got to large, they may not be good. However, we now know that even large follicles 30 to 34 mm can produce a pregnancy.

Usually, blood tests are performed daily from day 10 until an “LH surge” is detected. The “LH surge” refers to a rapid release of luteinizing hormone from the pituitary gland before ovulation occurs. The insemination is performed the day after the “LH surge”.

The LH surge is used to time a frozen embryo return. re follicles (depending on ovarian response and medication) will grow. Once the estrogen reaches a certain level or a follicle becomes a certain size; a LH surge occurs (a doubling or tripling of the level). The LH surge is then used to time the insemination. In spontaneous cycles the LH surge usually occurs on day 12 or 13. Once the LH surge has occurred the estradiol usually drops.

Cycle Monitoring – Day By Day – Return of Frozen in a Spontaneous Cycle

Cycle Day	Instructions	Patient Notes
Day 1	Call S.O.F.T. (519-685-5559) and inform us that your cycle has started	

Day 3	Baseline blood and/or vaginal ultrasound (Only if necessary – the default is none)	
Day 11	Blood and ultrasound	
Day 12-15	Daily blood testing until LH surge	
Any Day Indicated	Blood and ultrasound	
Day 17 on	If medium-sized follicles, continue monitoring until adequate size follicle develops If no good follicles – cancel cycle	
Day of Surge On	Vaginal Progesterone	
2 or 4 Days After Surge	Thaw Embryo(s)	
3 or 5 Days After LH surge	Frozen Embryo Transfer	
14 Days After Transfer	If no menses, serum <i>BHCG</i>	
40 Days After Transfer	If pregnancy test positive, an early pregnancy vaginal ultrasound	

The day-to-day instructions will remain the same unless S.O.F.T. calls you with a change.

If an extra ultrasound is indicated, or a spontaneous LH surge has occurred or we want you to cancel your cycle, this will be discussed at your visit or we will telephone you.

Timing the Procedure – Medicated Return

In women where a spontaneous cycle does not exist or may be unreliable return of frozen embryos will be done in a medicated cycle. This method is preferred if the cycles are irregular. It is the method of choice for women with PCOS, ovarian failure or hypothalamic amenorrhea.

The spontaneous cycle, as described earlier in this information sheet, is dominated by estrogen in its first half. Estrogen causes the cells of the endometrium to divide. After the LH surge, the dominant hormone is Progesterone.

The medicated cycle simulates this. Estrace ® 2 mg is given three times per day by mouth. The dose of estrace can be individualized and sometimes some estrogen is given vaginally. This usually lasts for 11 to 13 days (the same as a spontaneous cycle).

Once the endometrium is thick enough, the estrace is decreased and vaginal progesterone is started. The usual dose of progesterone is 200 mg given vaginally twice a day. The estrace is usually decreased to two per day when the progesterone is started and then either stopped or decreased to one per day when the embryos are transferred.

Monitoring usually involves a baseline blood and ultrasound. The next monitoring is day 8 and is only an ultrasound. The purpose of this is to make sure the endometrium is responding to the estrace. Endometrial thickness should be at least 6 mm. If it is the cycle will be continued and transfer will occur at the pre-planned time. If it is not, the dose of estrace will be adjusted and the cycle will probably continue a little longer than expected.

Cycle Monitoring – Day By Day – Return of Frozen in a Medicated Cycle

Cycle Day	Instructions	Patient Notes
Prior to Day 1	Call S.O.F.T. (519-685-5559) and inform us that you would like to start	
Day 1	In women with absolutely no cycle Day one can be picked arbitrarily. In women with some cycling it will be the first day of bleeding or bleeding will be produced by medication	
Day 1	Baseline blood and ultrasound (if necessary)	
Day 1	Start Estrace ®	
Day 8	Ultrasound endometrium	
Day 8 to 11	If endometrium 6 mm or greater, continue estrace and plan for transfer If endometrium < 6 mm, adjust dose of estrace ®	
Day 10 or 11	Recheck ultrasound for endometrium If OK - Vaginal Progesterone If not OK - cancel	
2 or 4 Days Vaginal Progesterone	Thaw Embryo(s)	
3 or 5 Days After LH surge	Frozen Embryo Transfer	
14 Days After Transfer	If no menses, serum B HCG	
40 Days After Transfer	If pregnancy test positive, an early pregnancy vaginal ultrasound	

The day-to-day instructions will remain the same unless S.O.F.T. calls you with a change. If an extra ultrasound is indicated, or a spontaneous LH surge has occurred or we want you to cancel your cycle, this will be discussed at your visit or we will telephone you.

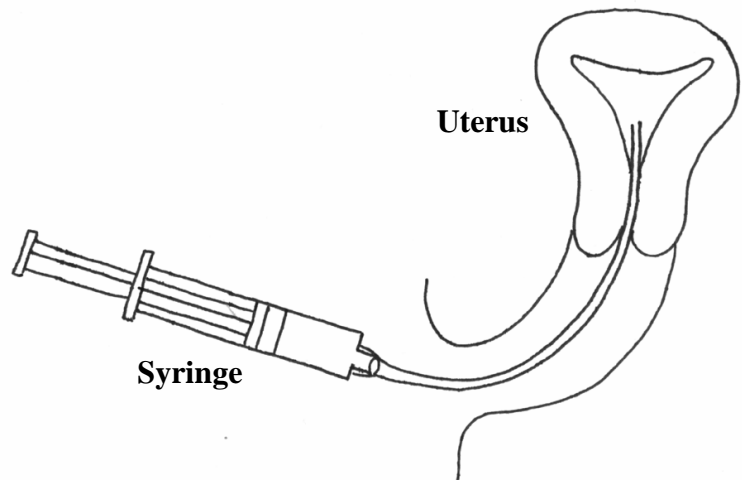
Other Medications

Low dose aspirin (81 mg) is indicated for recurrent pregnancy loss. It has not been tested or proven to improve the chance of a pregnancy in return of frozen embryos. However, despite this many clinics suggest it for this. Because it is non-prescription, the patient may choose for themselves.

Vaginal Viagra ® has been demonstrated to improve implantation rates in IVF cycles in patients with recurrent implantation failure. This is a circumstance where people had multiple embryo transfers without an implantation occurring. It has not been tested for return of frozen embryos. Despite this, we have used it in many cycles and have the impression it may help. It is usually not used on the first attempt but may be employed after a number of failures.

Embryo Transfer:

Embryo transfer occurs three to five days after the egg retrieval providing fertilization of the eggs with the partner's sperm has been successful. No preparation is required before the procedure is performed. Breakfast can be eaten normally and medication is not necessary before or during the procedure. You can drive yourself. We usually ask you to keep your bladder full for the procedure and to rest for



at least 10 minutes after the procedure. This can be a little uncomfortable but helps to straighten the cervical canal, improves visualization on ultrasound and makes the embryo transfer easier.

A speculum is placed in the vagina (similar to a Pap smear) and the catheter is fed up through the cervix (the opening to the uterus) and into the endometrial cavity. Once the tip of the catheter is in the endometrial cavity, the embryos are gently injected into the uterine cavity. **The number of embryos transferred is individualized; the usual number is two on day 3 or day 5.** This maximizes the chance of pregnancy while minimizing the risk of multiple pregnancies. Complications of pregnancy occur nine times as frequently in a twin pregnancy and thirty times more often in a triplet pregnancy.

Ultrasound-guided embryo transfer is always used. Historically; the embryo transfer catheter is placed into the uterine cavity “by feel” and with knowledge of a previously performed uterine length measurement. Using ultrasound guidance when placing the actual embryo-containing catheter gives additional reassurance that the embryos are being placed in the correct place in the uterine cavity. The exact placement of both the inner and outer catheter can be seen using the ultrasound and, although the embryos themselves cannot be seen, a small bubble of air on either side of the embryos is visible.

The Success Rate

Frozen embryo return is very successful! The lab director at ISIS is very selective about freezing only embryos which will provide a good chance of pregnancy. With return of a single embryo, the chance of a pregnancy is 16 to 17%. With return of two frozen embryos, the chance is about 25%. The chance of twins with return of two frozen embryos is 10 to 15%. Figures for transfer of more than two frozen embryos are not available as this is seldom done.

Time and Expense

Human embryos have been frozen for almost 10 years, unfrozen, transferred and produced successful pregnancies. No increase in congenital abnormalities or long-term consequences to the resulting child has ever been observed. Embryo freezing is discussed in more detail in its own information sheet.

Embryo freezing and the first year of storage is **\$850.00** and each year of storage after the first year is \$200.00. The return of frozen embryos costs \$350.00 for setup and monitoring of cycle and \$650.00 for the thaw, transfer and lab fees for a total of **\$1,000.00**.

For your convenience, S.O.F.T. carries a supply of most of the drugs that we use for infertility treatment. These can be sold to you at our cost plus a very small handling charge with no dispensing fee. In most cases this will make them less expensive than from pharmacies. A receipt will be given for tax purposes or for reimbursement from your insurance company. Procedures or drugs can be paid for with VISA, MasterCard or Debit. The small “profit” we make on these drugs is used to keep other fees at a minimum. You can use your prepaid drug coverage for medications at any pharmacy. We often recommend Commissioners Pharmacy as it is close, works closely with us and is knowledgeable about our drugs and procedures.

Side Effects

Embryo transfer **is extremely safe!** When the embryos are injected into the uterine cavity, many women will experience **mild, short-duration cramping**. Performing a “uterine measurement” before embryo transfer are done can avoid some of this. The uterine measurement involves a “dry-run” of the embryo transfer procedure (without sperm injection) and measuring the distance from the end of the uterine cavity to the cervix. Then, when the actual inseminations are performed the catheter is introduced a shorter distance avoiding it hitting the top of the uterine cavity. A uterine measurement is often performed during a couple’s initial instructional visit for IUI.

Rarely (less than 1 in 3,000-10,000 transfers), an **infection** can be introduced into the uterine cavity during the insemination. This will present as increasing pelvic pain in the day or two after the transfer. It is sometimes accompanied by a vaginal discharge, fever or chills. If any of these symptoms occur after an embryo transfer, you should contact our program or go to the nearest hospital emergency department immediately. Prompt treatment will usually lead to an uneventful recovery.

Sometimes **spotting** will occur after the embryo transfer. This happens because of an injury to a small blood vessel on the cervix at the time of the transfer. It will resolve by itself and does not decrease the chance of pregnancy.

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