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New Hope for Infertility Therapy: Fabricating Gametes from Stem Cells
by Huai L. Feng, PhD

HEPA Is Not Enough
by Antonia V. Gilligan, BS

A Comparison of GPS and Standard Dishes for Embryo Culture: Effects on Set-Up and Observation Times and Embryo Development
by D. Rieger, T. Schimmel, J. Cohen and M. Cecchi

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New Hope for Infertility Therapy: Fabricating Gametes from Stem Cells

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Introduction
In recent years, the field of reproductive medicine has been revolutionized by recent studies demonstrating that mouse and human embryonic stem (ES) cells are capable of forming primordial germ cells (PGCs) in vitro (Kehler et al., 2005). The mouse germ cells appear to be capable of undergoing meiosis and forming both male and female gametes in vitro. Although the full function of these ES-derived germ cells and gametes remains to be demonstrated, these findings give new hope for infertility therapy and novel approaches to regenerative medicine.

Male Gamete Formation In Vivo
Approximately 50% of human infertility is attributable to male defects including oligo-, astheno-, teratospermia and azoospermia (Sigman et al., 1997). Treatments for male infertility are limited and a range of in-vitro fertilization (IVF) techniques are used to circumvent, rather than treat, male infertility problems. Intracytoplasmic sperm injection (ICSI) is most frequently used in cases of male infertility. With ICSI, it is possible to use individual sperm or germ cells recovered from various maturation stages for fertilization. To develop true therapies, for example to restore spermatogenesis by using stem cell transplantation, will require a deeper understanding of spermatogenesis, which might be provided by studies in the mouse (Cooke et al., 2002). The transplantation of donor mouse male germ stem cells into recipient seminiferous tubules resulted in the formation of spermatocytes morphologically characteristic of the donor species, and production of offspring from donor germline stem cells (Kanatsu-Shinohara, et al., 2003). In the testis, Sertoli cells interact with the germ cells and defects in the Sertoli cells compromise spermatogenesis, leading to male infertility. However, it has not been possible to restore spermatogenesis from endogenous stem cells in infertile testis with environmental defects. This defect in the male germine microenvironment can be corrected by Sertoli cell transplantation and result in normal offspring (Kanatsu-Shinohara et al. 2005), which indicates a promising opportunity to develop a new strategy for the treatment of human male infertility.

A series of investigations failed to establish a complete human spermatogenic line in the testes of mutant aspermatogenic (W/Wv) mice or severe combined immunode®-cient (SCID) mice. The SCID mice received human spermatogenic germ cells obtained from testicular biopsies of non-obstructed or obstructed azoospermic men undergoing infertility treatment. However, sections from the recipient testes examined up to 150 days after transplantation showed recipient seminiferous tubules lined mainly with recipient Sertoli cells, but human germ cells were not found. The authors speculated that the donor germ cells were unable to survive and colonize the mouse testes due to non-compatible cellular interactions and immunological rejection resulting from interspecies differences (Reis et al., 2000).

Therefore, new therapeutic approaches can be envisaged for human spermatogenic failure. A germ stem cell or Sertoli cell transplantation procedure combined with developments in freezing, long-term culturing or enriching germ cell populations will be particularly useful for restoring fertility to those who become infertile after malignancy therapy by chemicals or radiation. Although no method for fertility protection is currently available for prepubertal boys who do not have sperm, stem cell transplantation will provide a method to recover their fertility, since spermatogenesis occurs by transplantation of spermatogonial stem cells even from immature donors (Shinohara et al., 2001). Assay and cell sorting techniques must be developed to minimize the risk of transferring malignant cells back into the patient (Sofikitis et al., 2003).

Male Gamete Formation In Vitro
Recent development of spermatogonial stem cell culture techniques enables in-vitro expansion of stem cells from a small biopsy sample for autogous transplantation (Kanatsu-Shinohara et al., 2005). As the method to culture Sertoli cells develops, it will be possible to correct the defect in Sertoli cells to be used for autologous transplantation in vitro as well. A more recent study indicated that mouse embryonic stem cells can form germ cells in vitro (Toyooka et al., 2003). Furthermore, mouse embryonic stem cells are capable of forming primordial germ cells and haploid male gametes (spermatocytes),
which, when injected into oocytes, restore the somatic diploid chromosome complement and develop into blastocysts (Geljsen et al., 2004; Surani, 2004) (Figure 1). The possibility to derive germ cells and male gamete from embryonic stem (ES) cells could have a very significant impact on assisted reproduction, enabling male infertile individuals to have their own genetic children.


Follicular Development and Female Gamete Formation In Vivo and In Vitro

A basic doctrine of reproductive biology is that most mammalian females lose the capacity for germ-cell renewal during fetal life, such that a fixed reserve of germ cells (oocytes) enclosed within follicles is endowed at birth (Franchi et al., 1962). A recent study indicated that juvenile and adult mouse ovaries possess mitotically active germ cells that, based on rates of oocyte degeneration (atresia) and clearance, are needed to continuously replenish the follicle pool. Consistent with this, treatment of prepubertal female mice with the mitotic germ-cell toxicant busulphan eliminates the primordial follicle reserve by early adulthood, without inducing atresia. Furthermore, wild-type ovaries grafted into transgenic female mice with the mitotic germ-cell toxicant busulphan eliminates the primordial follicle reserve by early adulthood, without inducing atresia. Therefore, the existence of proliferative germ cells that sustain oocyte and follicle production in the postnatal mammalian ovary.

Earlier and recent in-vivo and in-vitro studies have shown that mouse embryonic germ cells differentiate from a somatic lineage, and that adult ovaries of prosimian primates and mice possess mitotically active germ cells of uncertain origin (Bulkovsky et al., 2004). Bulkovsky et al., (2004) provided direct evidence that in the human, the components for new primary follicles, primitive granulosa, and germ cells, differentiate sequentially and de novo from the cytokeratin(CK)+ mesenchymal progenitor cells residing in the ovarian tunica albuginea, and that new primary follicles are formed by assembly of oocytes with nests of primitive granulosa cells in the ovarian cortex. The number of newly-formed primary follicles in adult human ovaries appears to be determined by the number of developing nests of primitive granulosa cells supplied by a higher number of available oocytes. Formation of new primary follicles throughout the reproductive period may compensate for the well documented atresia of a significant proportion of the follicular pool (Bulkovsky et al., 2004). These data may contribute to the selection of the best possible oocytes and ensure preservation of the relatively constant number of primary follicles found in human females between 18 and 38 years of life.

Furthermore, research has shown for the first time that human eggs may develop directly from cultured ovarian surface epithelium (OSE) (sources of ovarian germ stem cells) derived from adult human ovaries that were obtained from five women aged 39 to 52. Oocytes derived from the culture of OSE cells developed in vitro into mature eggs suitable for fertilization and development into an embryo (Bukovsky et al., 2005). The ability to produce mature human eggs from adult ovaries in vitro has several potential applications in human reproduction. The technique of harvesting cells from the ovarian surface is relatively easy, can be accomplished by a laparoscopy technique, and yields more cells for use for in-vitro fertilization. The ability to develop human eggs from OSE cells may help women with reduced fertility and premature menopause, who lack follicles in their ovaries, to have a better chance of conceiving through in-vitro fertilization. Eventually, frozen OSE cells from younger females may be preserved for later production of fresh eggs. This may prevent the occurrence of fetal genetic alterations, which are often associated with an advanced maternal age. In addition, a colonization of premenopausal ovaries with younger oocyte and granulosa stem cells may establish a new cohort of primary follicles. This may result in a 10- to 12-year delay of the onset of natural menopause. Also, these ovarian stem cells could be used to generate several cell types used in stem cell research, and fertilized eggs produced in this way could produce cells capable of giving rise to embryonic stem cells for use in research and therapeutic applications (Bukovsky et al., 2005).

The results of a study by Johnson et al., (2005) suggest that adult mouse ovaries rapidly generate hundreds of CONTINUED ON PAGE 10
oocytes, despite a small premeiotic germ cell pool. In considering the possibility of an extragonadal source of germ cells, it also showed expression of germline markers in bone marrow (BM). Further, BM transplantation restores oocyte production in wild-type mice sterilized by chemotherapy, as well as in ataxia telangiectasia-mutated gene-deficient mice, which are otherwise incapable of making oocytes. Donor-derived oocytes are also observed in female mice following peripheral blood transplantation. Although the fertilizability and developmental competency of the BM and peripheral blood-derived oocytes remain to be established, their morphology, enclosure within follicles, and expression of germ-cell- and oocyte-specific markers collectively support that these cells are bona fide oocytes. These results identify BM as a potential source of germ cells that could sustain oocyte production in adulthood (Johnson et al., 2005).

Hubner et al. (2003) have shown that mouse ES cells are capable of differentiating into oocytes and forming structures very similar to normal follicle. Release of these oocytes from the surrounding cells led to spontaneous activation and development to the blastocyst stage, thereby demonstrating that these cells are totipotent even in vitro. Future experiments will reveal whether the oocytes that have generated in culture from ES cells can be fertilized, whether they have undergone a gender-specific resetting of the epigenetic marks (imprinting), and whether they can be used as starting material to derive ES cell lines after nuclear transfer (Hubner et al., 2003) (Figure 1). All data suggest that human eggs derived in culture could have an even more exciting use. By following the same procedure, it might be possible to use these eggs to generate ES cells that produce diseased tissues - the adult nuclei for the process being taken from patients with complex diseases such as diabetes. Such ES cells may be repaired or corrected in defect gene, the repaired or health ES cell would provide an unlimited resource, allowing approaches to the treatment of disease that are currently impossible. This might, in turn, lead to new treatments in male and female infertility.

In summary, the possibility of fabricating gametes from stem cells, and generating viable embryos provides hope for the development of new infertility therapies, and may have a significant impact on assisted reproductive medicine in the next decade. Although such therapies may seem like science fiction, they merit international discussion to develop a unified set of medical, ethical, and safety (such as genetic, epigenetic and infection related risks) guidelines that accommodate the moral convictions of patients, physicians, and politicians from diverse social and religious backgrounds (Kehler et al., 2005).

References


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Growing Embryos in a World That is Flat: A Short History of Embryo Culture in Petri Dishes

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We are now two million babies, children and adults into this adventure we call ART (assisted reproductive technology) and there is still not a single scientific publication dedicated to the successful marriage between IVF and the Petri dish. The Petri dish, the temporary home to embryos growing in the laboratory, is used in more than 99.9% of ART procedures. By inference, embryologists may have used over 100 million of them to date! Despite its broad use, even in specialized areas such as cell tissue culture, microbiology and pre-clinical embryonic research, few alterations have been made to the dish since its inception one hundred and twenty years ago. However, this flat and plain area of embryo culture finally seems to be changing. One area of interest is to move away from the Petri dish altogether and replace it with such systems as micro-fluidics (Suh et al., 2003). Another option, explored by Tyho-Galileo Research Laboratories in collaboration with Sun-IVF, is to improve on the original Petri dish by implementing desired features of embryo culture. These include providing the ability to locate embryos in predetermined positions, improving temperature stability, enhancing safety, and growing the embryos in groups while allowing individual tracking.

To best understand how these changes have evolved and the potential benefits they offer, it is important to start at the birth of the Petri dish. The Petri dish was developed in the latter part of the 19th century because there was a need in vaccine research to grow micro-organisms on a solid substrate rather than in a broth. The famous German scientist, physician and Noble Prize recipient (in 1905) Robert Koch, known as the master of bacteriology, was the first to suggest replacing the commonly used liquid phase. The problem was that Dr. Koch’s assistant had difficulty using glass flasks for this purpose. Koch’s assistant, Julius Richard Petri (1852-1921), decided one day in 1887, to cut off the flask and use only the bottom to contain the solid media. A larger glass cover was loosely placed over the flask bottom and the first glass Petri dish was “born”. Chronologically Petri was not the first inventor though. At least three individuals developed the dish independently from one another, but Petri had the advantage of working in the most famous experimental microbiology laboratory of the time. The reputation of his employer elevated his name, and put it into every biology laboratory in the world.

Early material changes to the glass dish
The dishes were first manufactured in glass. In the mid 1960’s, injection molding technology sufficiently evolved so that Petri dishes could be manufactured from clear sterilized polystyrene plastic. However, the plastic dish was merely a clone of the first glass Petri dish. It was large, thick, and bulky. It weighed almost twice as much as the conventional plastic dish in use today. Objectives on older microscopes were sometimes unable to accurately focus through these thick layers of plastic. The dish’s main advantages included its relatively low cost, and its durability compared to the...
easily breakable glass dish.

In the mid-1970’s, this cost advantage was lost because the price of petroleum-based products, including plastic Petri dishes, skyrocketed. Customer-pressure forced Petri dish manufacturers to cut back on the excess size and weight to reduce cost. By the mid-80’s, further developments in the processing of raw materials, and a better understanding of the injection molding process allowed most manufacturers to reduce the weight of their Petri dishes to between 15 and 17 grams. These new, thin plastic Petri dishes have remained largely unchanged ever since.

Microdrop Culture under Oil for IVF

One of the most important steps towards contemporary embryo culture was developed by the maverick scientist Ralph Brinster (of sperm stem-cell fame). In 1963, he successfully cultured mouse eggs to blastocysts. He decided to do away with ‘open’ culture and protect small amounts of culture medium using a transparent viscous fluid overlaying the media. He used paraffin oil for this purpose. This system moved away from Koch’s solid substrate approach for which the Petri dish was designed. The advantage of the oil-medium bi-layer was enormous. Oil prevented most microbial infections, and allowed fertilization and embryo growth to take place in less stringent conditions. The gametes and embryos could be observed for long periods without concerns about evaporation or contamination. The method also allowed the study of minute quantities of metabolites released or absorbed by the cells. It later facilitated the introduction of micromanipulation methods such as ICSI. The high heat capacity of oil also helped to maintain temperature when the dishes were removed from the incubator for observation or manipulation.

It is estimated that more than 90% of clinical IVF laboratories now use some variation of the method originally proposed by Brinster. The current changes to the method address some of its key issues: toxicity and batch-to-batch variation of the oil. Paraffin oil has now been largely replaced by other oils such as mineral oil. Mineral oil is a variation of light hydrocarbon oils – a distillate of petroleum – and significantly reduces toxicity. Batch-to-batch variation persists, but can be addressed by pre-washing the oil with water or media to partition volatile organic compounds. Also, pre-gassing with 5% or 6% CO2 is advantageous because equilibration with CO2 is slower with the use of mineral oil; otherwise dishes must be set up well ahead of time.

However, these variations in the Brinster method do not address the fact that the embryos cultured under oil are still located in a world that is flat and vulnerable. Their fluid environment can deteriorate if droplets are flattened or move together, making separation and identification difficult. The droplets are still fairly large on a microscopic scale, much larger than embryos. Embryologists have to continually change to different magnifications to locate and handle embryos when moving from drop to drop. Sometimes flattening can be so severe that pipetting becomes arduous. Also, the current system does not allow embryologists the advantage of pooling embryos, a method that clearly assists the growth of mouse embryos in vitro, but makes it impossible to trace embryos from day to day. This is a problem particularly in PGD testing where individual identification is mandatory (Alouf, 2006).

In 1999, Tim Schimmel of Tyho-Galileo Research Laboratories, Michael Cecchi of Gen-X/IVF-online and I decided to see whether we could address these issues of concern with the oil overlay/underlay system by making changes to the Petri dish. The droplet issue was the first problem to be tackled, and a dish was designed around shallow wells placed in a circle. The well walls were made shallow enough to allow pipetting at various angles, and the use of relatively small volumes of medium. One problem that remained was that some embryos could be located against the walls, making them difficult to locate or pipette. This resembled the wall problem seen in the much larger wells of four-well dishes. To address this, we decided to slope the bottom of the well so that eggs and embryos would roll towards the lowest point by gravity. The angle was kept small enough to prevent any distortion of the view. We named the lowest point in the well the GPS location, and placed it in the middle of each well. The dish that finally developed from this in 2006 was aptly named “GPS”. Not only was the bottom not flat anymore, but the GPS point was at the thinnest location, possibly enhancing heat transfer to the area surrounding the egg or embryo.

Numerous changes were made to the dish lid and bottom as well. Changes to the lid were designed to reduce the risk to embryos during transport. In the standard plastic

---

**Figure 3.** A diagram of the side view of a well in the GPS dish. The size of the embryo is exaggerated.
Petri dish, oil may seal the space between the lid and the dish, altering gas exchange and possibly jeopardizing diffusion of gaseous compounds such as CO₂. This can increase the pH to levels clinically detrimental to the embryos. As a result, many embryologists limit the amount of oil in a dish or remove the lid altogether while carefully moving dishes. The new GPS lid has been elevated and stabilized, allowing for the use of larger amounts of oil. The combination of enclosed droplets and the re-designed lid permits embryologists to process dishes faster. The GPS dish can be loaded and transported over great distances without fear of spillage. For the bottom of the dish, we reduced the height of the elevated area that was added to the standard Petri dish some years ago to avoid scratching of the bottoms during packaging. This feature may also improve heat transfer from warmed surfaces when the dish is outside the incubator.

Allowing for group culture without losing the ability to track individual embryos was a much harder improvement to design. The concept was initially simple: place the embryos in small porous wells allowing fluid exchange while keeping the embryos separated. But this was easier said than done! We developed a dozen or so dishes, one model more exotic than the other until we arrived at the dish we now call the “Corral”. In the Corral dish, individual embryos are placed in the four quadrants of the Corral well, separated by shallow solid walls that are several times taller than the embryos. On the top of the walls are posts with apertures narrower than the diameter of the human embryo. The medium extends above the posts, allowing for the passage of medium and among the four quadrants. The wells are slanted and have GPS points that are deeper than that of the GPS dish. Eight standard GPS wells surround the Corral wells for washing and performing other embryological activities.

The GPS and Corral dishes offer significant improvements over the traditional flat world of embryos growing in a Petri dish. Pre-clinical trials have shown that the GPS dish reduces set-up and search times for even experienced embryologists. Pre-clinical trials of the Corral dish are currently in progress. The dishes are now available from Sun-IVF, and feedback from embryologists is much appreciated. Please contact Sun-IVF or email me at jc@embryos.net.

References

You can contact Jacques Cohen, PhD at: jc@embryos.net.

Figure 4. A diagram of the top view of a Corral well. The yellow circles represent individual embryos. The size of the embryos is exaggerated.
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HEPA Is Not Enough

by Antonia V. Gilligan, BS, President, Alpha Environmental Jersey City, NJ, USA

Most embryologists are familiar with the HEPA (High Efficiency Particulate Air) filters used in laminar flow hoods and, in some cases, in central air supply systems (Figure 1). Interestingly, HEPA filters do not resemble a sieve, but rather are a mat of bound fiberglass fibers, with approximately 10-micron spaces between the fibers (Figure 2). Particles in the air stream adhere to, or become imbedded in, the fibers, and HEPA filters will trap almost all particles greater than 0.3 microns in diameter. This is sufficient to remove airborne particles, fungi, mold spores, and bacteria, and therefore HEPA filters can significantly reduce microbial contamination. However, HEPA filtration is not enough, and will never be enough, for the stringent air quality requirements of human ART.

Figure 2. Photomicrograph of high efficiency filter medium. (Klocke and Whyte 2002)

Molecules of embryotoxic compounds are 100 to 1000 times smaller that the effective pore size of a HEPA filter. Volatile organic compounds (VOCs) such as benzene, formaldehyde, acetaldehyde, acetonitrile have been found in laboratory air (Hall et al. 1998), and they are not trapped by a HEPA filter. To trap or destroy these materials requires a smaller trap, such as is provided by activated charcoal. The spaces between the carbon particles contain a cloud of delocalized electrons that acts as an electronic glue (van der Walls forces), to bind chemical contaminants onto the carbon. Compounds such as alcohols and ketones are not easily removed by carbon, but they can be oxidized, and thereby detoxified, by potassium permanganate. Again, this is a chemical reaction happening at a scale of a thousand times smaller than the particles trapped by a HEPA filter. The essential point is having an effective removal device that fits the scale of the particle or chemical molecule.

The possible significance of air quality on IVF was raised by Cohen et al. (1997) who observed decreases in in-vitro embryo development and pregnancy rates associated with the move of an IVF lab from suburban Naples, Italy to the downtown area in 1992, and associated with construction around another IVF lab in New Jersey in 1995. Some environmental effects from other events involving construction in neighbouring spaces and the use

CONTINUED ON PAGE 16
of toxic materials in lab spaces preceeded these periods. They consequently measured significant concentrations of VOCs in the laboratory air, and in the compressed CO2 used for the incubators. Based on these observations, they designed the Coda® incubator units for use inside the incubators, in-line Coda® filters for the CO2 supply, and larger Coda® towers to filter the laboratory air, all of which contain HEPA filters, activated charcoal and potassium permanganate.

A number of studies have shown improved pregnancy rates with the use of Coda® air filtration in human IVF labs (Racowsky et al. 1999, Figure 3; Mayer et al. 1999, Figure 4) and cattle IVF (Merton et al. 2007, Figure 5). It is important to note, however, that it is not realistic to expect the use of Coda units to improve in-vitro development and/or pregnancy rates in every case. A difference in clinical outcome is only likely if there is an ongoing problem with air quality, or if a there is a dramatic decrease in air quality, from nearby construction, for example, during the study period. Such environmental crises can and do occur, but they are often intermittent and rarely predictable.

In conclusion, HEPA air filtration is highly effective for the reduction of particulates and bacteria, but cannot reduce the concentrations of embryotoxic VOCs in the ART laboratory. Coda® filters contain activated charcoal and potassium permanganate and can significantly reduce VOCs. Coda® units should be considered as common-sense safety devices. That is, they should always be in place, in order to deal with unforeseeable changes in air quality.

References


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Air contaminants present in research and clinical laboratories interact with specimens, samples, tissues, media and oils. Studies show that Chemical Air Contaminants (CACs) and Volatile Organic Compounds (VOCs) introduced from many sources may seriously distort your results.

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The following table shows the filtration power of the Coda® System compared to existing HVAC systems and HEPA filters used with your equipment.

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<th>Mold Spores</th>
<th>Benzene</th>
<th>Ethanol</th>
<th>Toluene</th>
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</table>

HEPA filters are designed to stop materials down to 0.3 microns. Unfortunately many of the gases found in IVF labs; benzene, acetone, ethanol, formaldehyde, etc; are 100 to 1000 times smaller than the pore size of the HEPA filter.

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Selection of the best spermatozoa at high magnification: a way to improve the rate of development to the blastocyst stage before elective single embryo transfer (eSET)

Pierre Vanderzwalmen, PhD and Herbert Zech, MD
Institute für Reproduktionsmedizin und Endokrinologie, Bregenz, Austria

Elective single embryo transfer on day 5

There is an increasing awareness in the field of assisted reproductive technology (ART) of the need to reduce the incidence of multiple pregnancies by transferring a single embryo (SET). Consequently, embryologists are faced with the challenging task of selecting one embryo from the cohort with a high chance to continue to develop and implant. The questions is whether embryo selection based on morphological assessment on day 3 is reliable enough for detecting the one embryo with great potential for further development, or whether selection at the blastocyst stage is the better strategy.

Even though this subject is still a matter of debate, studies have demonstrated the limited predictive value of morphological day 2 or day 3 embryo grading on blastocyst formation. This highlights the notion that selecting embryos based on morphological criteria on day 3 may fall short when choosing to transfer only one embryo from a cohort, all with good morphologies. In a prospective observational study including 224 cycles, we observed that approximately 50% of the pre-selected embryos arrested in their development at the 8 cell- or morula stage. The introduction of legislative rules in some countries (e.g. Belgium) limiting the number of embryo to be transferred to one was an opportunity to analyse the best timing for transfer in a prospective randomized way. Two studies from Belgium ART units, have shown that, in selected group of patients (infertile women under 36 year of age), SET of a blastocyst results in significantly higher ongoing pregnancy rates as compared to SET of a cleavage stage embryo on day 3. (Papanikolaou et al., 2006; Zech et al., 2006), especially when good quality embryos were available. Prolonging embryo culture to Day 5 may be a better strategy to correctly identify and select from a cohort those embryos with an overall higher potential to implant. Therefore, in order not to impair the chance of a positive treatment outcome, SET on Day 5 has to be offered according to the number of good quality embryos available on Day 3.

We instituted elective SET (eSET) into our ART unit in Austria almost one year ago, even though it is not required by law. After discussion with the couples on the day of transfer (Day 5), elective transfer of one blastocyst was proposed essentially to patients less than 40 years of age and also for medical or social reasons (Table1). The key to the success for eSET is being able to obtain viable blastocysts on Day 5, and we must be aware that selection of inadequate gametes may compromise embryonic development, resulting in failed implantation.
To increase the success rate of ART by selecting the most viable embryos within a cohort, other non-invasive selection criteria techniques reflecting embryo function, such as their metabolism have been developed, including glucose uptake, measurement of AA turnover, individual embryo respirometry, and secretion of factors such as sHLA-G into the embryo culture medium. However, such methods require special techniques and equipment such as microfluorometry, HPLC, an oxygen microsensor system, and ELISA, some of which are not yet accurate, sufficiently sensitive, or accessible enough to allow for routine application in an ART unit.

**Selection of the best oocyte**

With the introduction of ICSI, removal of the oocyte cumulus complex, gives us the opportunity to select oocytes based on non-invasive evaluations of the cytoplasm (granularity, pitting, vacuoles), the perivitelline space (presence of particles), and the polar body (abnormal size, fragmented, appearance). With the aid of a differential polarized light microscope (Polscope), the metaphase spindle as well as the thickness of the three layers of the zona pellucida of oocytes from ICSI cycles may aid in identifying and selecting high better oocytes with good developmental potential for transfer.

**Selection of the best spermatozoon**

It is now well recognized that the ability to improve the selection of the best spermatozoa prior to ICSI may affect positively embryo development and pregnancy outcomes. Several promising sperm selection techniques have been reported. Ainsworth et al. (2005) developed an electrophoretic sperm isolation technique for selecting functional spermatozoa free from significant DNA damage. On the basis of functional criteria, Huszar et al. (2007) reported that spermatozoa that bind to hyaluronic acid are mature, have reduced frequency of chromosomal disomy and diploidy and are similar to those of the zona pellucida in conventional fertilization.

**Intracytoplasmic morphologically selected sperm injection (IMSI)**

Inadequate knowledge of the importance of various types of sperm defects (that are not detected during conventional ICSI at a magnification 200 to 400X), may have a negative impact on the outcome of IVF. With the introduction of motile-sperm organelle-morphology examination (MSOME), it is now possible to examine the fine nuclear morphology of motile spermatozoa, in real time, at a magnification of up to 12,500X.

Recent publications have demonstrated that selection of spermatozoa at high magnification is positively associated with pregnancy rates after day 3 embryo transfers for couples with previous failures of implantation. (Bartoov et al., 2003) and for patients with elevated degree of DNA fragmented spermatozoa (Hazout et al., 2003) However, in some semen samples, it is almost impossible to find a normal spermatozoon. Berkovitz et al. (2006) reported on low fertilization rates and a low percentage of top quality embryos on day 3 after IMSI with spermatozoa exhibiting a large panel of nucleus malformations in terms of shape, size and presence of vacuole. In a recent paper, Berkovitz et al. analysed more specifically the impact of the presence of nuclear vacuoles in the head of spermatozoa on embryo development and concluded that a positive effect on the rate of good quality embryos on day 3 is not fully apparent after a strict selection for only normal spermatozoa. Berkovitz et al. (2006) reported on low fertilization rates and a low percentage of top quality embryos on day 3 after IMSI with spermatozoa exhibiting a large panel of nucleus malformations in terms of shape, size and presence of vacuole. In a recent paper, Berkovitz et al. analysed more specifically the impact of the presence of nuclear vacuoles in the head of spermatozoa on embryo development and concluded that a positive effect on the rate of good quality embryos on day 3 is not fully apparent after a strict selection for only normal spermatozoa at high magnification. They could show that the presence of a defect at the level of the nucleus, such as nuclear vacuoles, reduces the IR and PR and furthermore was associated with higher rates of early abortion, even though there was no apparent decrease in the embryo quality on day 3.

We have introduced IMSI into the IVF activities in our laboratory and two studies were undertaken.

### Table 1. The results of elective single embryo transfer (eSET) of blastocysts at the Institute fur Reproduktionmedizin und Endokrinologie, Bregenz, Austria.

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Total number of eSET cycles</td>
<td>118</td>
</tr>
<tr>
<td>Mean patient age (range)</td>
<td>33.9 (23-41)</td>
</tr>
<tr>
<td>Clinical pregnancies (%)</td>
<td>66 (55.1)</td>
</tr>
<tr>
<td>Miscarriages (%)</td>
<td>8 (10.1)</td>
</tr>
<tr>
<td>Total ongoing pregnancies (%)</td>
<td>58 (49.2)</td>
</tr>
<tr>
<td>Ongoing pregnancies by patient age</td>
<td></td>
</tr>
<tr>
<td>&lt; 30 years (%)</td>
<td>14/24 (58.3)</td>
</tr>
<tr>
<td>30-35 years (%)</td>
<td>26/51 (51.0)</td>
</tr>
<tr>
<td>36-39 years (%)</td>
<td>17/36 (47.2)</td>
</tr>
<tr>
<td>&gt; 39 years (%)</td>
<td>1/7 (14.3)</td>
</tr>
<tr>
<td>Ongoing pregnancies by blastocyst expansion on Day 5</td>
<td></td>
</tr>
<tr>
<td>Early (%)</td>
<td>3/9 (33.3)</td>
</tr>
<tr>
<td>Full (%)</td>
<td>7/17 (41.2)</td>
</tr>
<tr>
<td>Expanded (%)</td>
<td>34/66 (51.5)</td>
</tr>
<tr>
<td>Hatching or hatched (%)</td>
<td>14/26 (53.8)</td>
</tr>
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</table>

Total ongoing pregnancies (%) 58 (49.2)

Ongoing pregnancies by blastocyst expansion on Day 5

Early (%) 3/9 (33.3)

Full (%) 7/17 (41.2)

Expanded (%) 34/66 (51.5)

Hatching or hatched (%) 14/26 (53.8)
Study 1: The first study was designed to determine whether the existence of vacuoles in the nuclei of spermatozoa (which can not be detected with conventional ICSI at 200 or 400X magnification), was associated with the ability of the embryos to develop to the blastocyst stage. Spermatozoa were selected under a Nomarski interferential Leica AM 6000 inverted microscope, equipped with a variable zoom lens. The most normal looking spermatozoon was selected at magnification up to 12,500X and a photo was taken for further classification before injection. The spermatozoa were graded in 4 groups (Figure 1) according to the presence or size of the vacuoles: Grade I, no vacuole; Grade II, maximum of two small vacuoles (< 4% head surface); Grade III, at least one large vacuole; Grade IV, large vacuole and abnormal head shapes or other abnormalities.

![Figure 1. Sperm classification grades used for IMSI. The spermatozoa were graded in 4 groups according to the presence or size of the vacuoles:](image)

- **Gr I**: no vacuole
- **Gr II**: maximum of two small vacuoles (< 4% head surface)
- **Gr III**: at least one large vacuole
- **Gr IV**: large vacuole and abnormal head shapes or other abnormalities

The injected oocytes were cultured until day 5 in Global medium (Lifeglobal) and the development of the embryos was correlated according to the different sperm categories.

Of a total of 307 selected spermatozoa, only 5.0% were completely free of any abnormalities. The majority of the spermatozoa presented small (Grade II, 61.5%) or large (Grade III, 28.0%) nuclear vacuoles alone. On Day 5, the blastocyst formation was related to the classification grade of injected spermatozoa: in Grade I and Grade II (73.3% and 45.0%) embryos reached the blastocyst stage out of which 46.7% and 25.4% respectively were classified as good quality blastocysts. Conversely, after IMSI with compromised sperm, only 9.3% (Grade III) and 0.4% (Grade IV) of embryos developed to the blastocyst stage, and 3.5% and 0.1% of those were of good quality.

Study 2: Currently, ICSI is performed after morphological selection of spermatozoa at 200X to 400X magnification using Hoffman modulation contrast. With such magnification, spermatozoa carrying defects of the head (i.e.: enlarged head, elongated head, tapered head, macrocephale, microcephale, amorphous), the neck (broken neck, cytoplasmic droplets) or the tail (broken tail, multiple tails) can be detected, and thus are not selected for injection if there is a morphologically normal spermatozoon in the preparation. Because vacuoles can not be detected during ICSI at 400X magnification, the question is whether IMSI could be a better strategy to select spermatozoa at a magnification of 6,000X to 12,500X. In order to evaluate whether IMSI could result in improved blastocyst formation and pregnancy rates compared to traditional ICSI, a prospective study, using sibling oocytes was undertaken to compare the outcome after selection of spermatozoa using the conventional ICSI procedure or after IMSI.

This study included 26 couples with at least 2 previous failures of implantation after ICSI. A total of 384 sibling oocytes (26 oocyte retrievals) were divided and allocated randomly between ICSI (203) or IMSI (181). In the IMSI group, only 6.0% of the selected spermatozoa were completely free of abnormalities, whereas the majority of the spermatozoa presented small (Grade II 56%) or large (Grade III 43%) nuclear vacuoles alone, or were associated with other abnormalities (Grade IV 9%). On Day 5, IMSI resulted in a significantly higher proportion of blastocysts (IMSI 38% vs ICSI 20% P< 0.001) and good quality blastocysts, (IMSI 16% vs ICSI 6.% P < 0.01) The majority of the transfers (n =19) that resulted in 9 ongoing pregnancies (47%) were performed with embryos that originated only from the IMSI group. Four pregnancies were obtained after transfer of a combination of embryos from the IMSI and ICSI groups.

Our results show that the proportion of embryos developing to the blastocyst stage is directly correlated with specific morphological head malformations, such as vacuoles. Because vacuoles exert a negative effect on
embryo development, it is now time to investigate their origin and under what circumstances the frequency of such vacuoles increases. In this way, a treatment may be offered, or a strategy could be established, to reduce their appearance. These observations agree with the results of previous studies that point to possible early and late paternal effects, both of which could affect early embryonic development. We suggest that a correlation exist between the presence of irregular nuclei with vacuoles and damage to the integrity of spermatozoa such as defects in sperm chromatin packaging.

The usefulness of the IMSI technique is demonstrated in the sibling oocytes injection study comparing conventional ICSI with IMSI, in that selection of a good spermatozoon can be missed by the normal selection prior to ICSI. Therefore is it reasonable to consider IMSI as the next generation technique that will replace ICSI. Our results demonstrate that IMSI is beneficial to patients having repeated implantation failure. It remains to be determined whether IMSI would be beneficial to all patients.

The importance of establishment of a new spermocytogramme, using the IMSI criteria, will allow us to define a threshold for evaluation of the developmental potential of a single spermatozoon. Moreover, analysis of the sperm according this new classification could provide information on the technique to be used, conventional ICSI or IMSI, and to allow us to decide which patients may benefit from IMSI at the first attempt. In conclusion, research in the field of gamete selection is mandatory in order to increase the rate of good embryos that will develop to the blastocyst for eSET.

References

You can contact Pierre Vanderwalmen, PhD at: pierrevdz@hotmail.com.
The Benefits of Group Culturing and Designer Dishware

by Charlene Alouf, PhD
Laboratory Director of Crozer Chester Medical Center

The group culturing of embryos has shown beneficial effects in the in vitro development of embryos, through the blastocyst stage. Several reports have shown that increased embryo density, or number of embryos cultured per medium droplet, are beneficial to preimplantation growth and development in vitro (1-8). These studies support the theory that mammalian preimplantation embryos produce autocrine or paracrine factors which may influence, respectively, their own development and the development of sibling embryos in group culture.

Group embryo culture offers a simple approach to the benefits of intercellular communication without the use of complex tissue preparations such as autologous endometrial or granulosa cell cultures. Until now, however, communal culture meant sacrificing the ability to assess the embryos individually on a daily basis; a routine procedure employed in many programs worldwide (18).

Group culture appears most beneficial when embryos are cultured at a higher density in a lower volume of fluid; however, negative effects on growth and development can be observed when the embryo density is too high (9). These observations have lead to the theory of the conditioning effects of communal culture. Positive conditioning during culture is suspected when embryodevived factors stimulate their own or neighboring embryo development, along with evidence for embryo ‘cross talk’ in vitro (19). The grouping of embryos showed improvements in implantation and fetal development were seen in mice following transfer of embryos derived from group culture in comparison with single culture embryos (4).

Reports involving pronuclear scoring, early cleavage, and other observations such as multinucleation, individual blastomere characteristics and fragmentation suggest that particular embryo morphologies may correlate with a higher implantation potential and pregnancy rate (10-16).

The introduction of the Embryo Corral® culture dish may provide a unique opportunity to incorporate these benefits of communal culture in the IVF laboratory without abandoning the daily practice of individual embryo assessment. Its unique design allows maximal communication between the embryos in reduced medium volume while precisely maintaining each embryo in individual quadrants. It is time to revisit group culture in IVF; to promote an environment more similar to in-vivo conditions employing a new generation of dedicated IVF dishware (18).

References
7 O’Neill, C. 1998. Autocrine Mediators are required to act on the embryo by the 2-cell stage to promote normal development and survival of mouse preimplantation embryo in vitro. 58:1303-1309.

You can contact Charlene Alouf, PhD at: charlene.alouf@crozer.org
The Embryo Corral®

The Embryo Corral® possesses 4 quadrants within each of its 2 central wells. Our unique quadrant design allows the exchange of medium between the embryos. Fluid movement permits “crosstalk” between embryos while the individual quadrants allow singular monitoring and development tracking of each specimen.

Overall
Design allows ease in use, simple introduction of medium and oil overlay. Save time, save effort, while improving results.

Outer Wells
The outer wells may be used for additional culturing, washing, and maintaining samples. The declining floor, promotes ease in sample location by ensuring a common focal setting.

Center Wells
Allows the ‘grouping’ of embryos yet individual tracking of each specimen. Design encourages free flow of culture medium and embryo-derived, autocrine factors which assist in the development of embryos. Design allows ease in applying oil overlay and medium placement.

Materials
Clear, medical grade non-pyrogenic, polystyrene, non-toxic, and thoroughly tested.

Lid
The distinctive lid design promotes effective CO₂ equilibration and ease of use.

Vehicle for Autologous Co-cultures
The revolutionary design of the Embryo Corral® central well provides a surface to plate autologous co-cultures. Embryos located in the remaining 3 quadrants benefit from culture.

Bottom
A slight contour of the wells bottom allows the embryos to settle and allows even heat distribution; the advanced material allows greater heat retention during periods of handling.

Culture media and the dishes
The design supports the use of ALL culture media in the IVF marketplace. The Embryo Corral® and Embryo GPS™ dish may be used for egg retrieval, fertilization, culturing of ICSI eggs, oocyte and embryo culture at all stages.

The Embryo Corral® and Embryo GPS™ dishware can be used for all types of media such as single step sequential medium, multiple step sequential medium and optimized media such as global®. The well design can be used to have defined media volumes when applying an oil overlay.

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A Comparison of GPS and Standard Dishes for Embryo Culture: Effects on Set-Up and Observation Times and Embryo Development

D. Rieger¹, T. Schimmel², J. Cohen² and M. Cecchi³
¹LifeGlobal LLC, ²Tyho-Galileo Research Laboratories, ³genX international

Introduction
In standard microdrop culture, embryos are cultivated in microdrops of medium on a flat surface. Consequently, the microdrops can flatten, move, or coalesce and the embryos can be difficult to find and examine. In contrast, in GPS culture, embryos are cultivated in equally small volumes but, as shown in Figures 1 and 2, the medium is constrained within microwells. The embryos migrate to the center of the microwells and the microwell bottoms are parafocal, so that the embryos are easy to locate and examine. The objective of this experiment was to determine whether the design of the GPS dish would reduce the time required for the set-up of culture and daily examination of mouse embryos.

Figure 1. The GPS dish.

Figure 2. A side-view schematic comparison of microdrop and GPS culture. The yellow circles represent embryos (not to scale) which settle into the center of the GPS microwells.

Materials and Methods
The medium used for all cultures was KSOM + 10 mg/ml BSA. On Day 0, 13 ml of oil was placed into each of 10 GPS dishes and 10 Nunc 60 mm dishes. Fifty microlitres of medium was underlaid into each of the eleven wells of each GPS dish, and eleven 50 μl microdrops of medium were underlaid into each of the Nunc dishes. The total preparation time was recorded for each set. All the dishes were cultured overnight at 37.3°C under 5% CO₂ in air.

On Day 1, 100 BALB/cJ X C57BL/6J zygotes were collected. The zygotes were randomly assigned to individual culture in either small GPS wells (5/dish, N = 50) or in microdrops (5/dish, N = 50). The time for the preparation of each dish was recorded.

On each of Days 2, 3, 4, and 5, the embryos were evaluated for development, and the time recorded for the examination of each dish.

The times required for set-up and embryo examination on each day were compared by Kruskal-Wallis tests. The proportions of zygotes that reached a given developmental stage on each day were compared by Chi-square analysis.
Results
The time to set-up the cultures on Day 1 and the time required for embryo evaluation on Days 2, 3, 4, and 5 were all significantly less for GPS culture than for microdrop culture (Figure 3).

Figure 3. The times required for set-up and embryo examination in microdrops and GPS microwells.

As shown in Figure 4, significantly larger proportions of embryos reached the morula stage and compacted on Day 3 in GPS culture than in microdrop culture. There were no differences in development on Days 2, 4, or 5.

Figure 4. Mouse embryo development in microdrops and GPS microwells.

Conclusions
The results show that mouse embryo development in GPS culture is as good as, or better than, development in microdrop culture, and that the times required for set-up of the cultures and embryo evaluation are significantly reduced in GPS culture compared with microdrop culture. The use of GPS dishes for human ART could significantly reduce labour time and costs while increasing security, especially for PGD embryo culture.
Human Sperm Cryopreservation: Comparison of Three Cryoprotectant Solutions

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Introduction

The cryopreservation of human spermatozoa has been employed for more than 40 years (1). Since then, the technical aspects of freezing and thawing sperm and preparing cryopreservation media have been refined (2-4). Various cryoprotectants are available to protect sperm from the negative effects of cryopreservation. Among the cryoprotectants commercially available, TEST Yolk Buffer (TYB; Irvine Scientific, USA) is commonly used. Other commercially-available cryoprotectant solutions include LifeGlobal Sperm Freezing Medium and Medicult Sperm Freezing Medium. To our knowledge, these three sperm cryoprotectant solutions have never been directly compared.

The aim of this study was to compare these three commercially-available cryoprotectants solutions for the cryopreservation of human spermatozoa.

Materials and Methods

Sperm preparation and cryopreservation

In this prospective study, semen specimens from 23 normozoospermic men were obtained by masturbation after 2-3 days of sexual abstinence. After liquefaction, a small aliquot was removed for determination of total and progressive motility.

Each sample was then divided into three equal aliquots, and each aliquot diluted with either TEST Yolk Buffer with glycerol (TYB, Irvine Scientific, USA), Medicult Sperm Freezing Medium (SFM-MC, Medicult, Denmark), or LifeGlobal Sperm Freezing Medium (SFM-LG, IVFonline, Canada). An aliquot of the cryopreservation solution equal to 25% of the original specimen volume was added to the semen aliquot. The specimen was gently mixed for 5 minutes using an aliquot mixer. This process was repeated until the ratio of freezing medium to ejaculate was 1:1.

Each aliquot was placed into a 1-ml cryovial (Corning, USA). The cryovials were held at -20°C for 8 minutes and then in liquid nitrogen vapor at -79°C for 2 hours, before being plunged into liquid nitrogen for storage at -196°C (5).

Thawing procedure and assessment of sperm parameters

After at least 48 hours in liquid nitrogen, the samples were thawed at room temperature for 5 minutes and then transferred to a 37°C incubator for 20 minutes. After homogenization, a small aliquot was removed from each specimen for the assessment of motility (%MOT), progressive motility, (%PROG) and cryosurvival rates, according to the WHO criteria (6).

Statistical Analysis

Student’s paired t-test was used to compare %MOT and %PROG between before freezing and after thawing, and to compare among the three cryoprotectant solutions. A P-value of <0.05 was considered statistically significant.

Results

There was a significant decrease in the total (%MOT) and progressive (%PROG) sperm motility from before freezing to after thawing for all the cryoprotectant solutions tested (Table 1). Post-thaw sperm survival and motility were better for SFM-MC and for SFM-LG compared to TYB (p<0.05; Table 1). There were no statistically significant differences between SFM-MC and SFM-LG for post-thaw sperm motility or cryosurvival rates.

Discussion

The fertility potential of cryopreserved semen depends, mainly on the initial quality of the sample, on the cryoprotectant used, and on the freezing technique. The use of a cryoprotective agent is necessary to prevent injury to human spermatozoa during the cryopreservation process. Lucena and Obando (7) showed that cell survival is <15% when human spermatozoa are subjected to the
freeze-thaw process without the addition of a cryoprotectant.

In this study, three cryoprotectants which contain glycerol as the cryoprotective agent were compared, with the cryosurvival rates being better for SFM-MC and SFM-LG, in comparison to TYB. TYB contains egg yolk, a compound of lipoproteins, phospholipids, cholesterol, and various other, less abundant components in combination with TES and TRIS (hydroxyethylaminomethane) buffers. Egg yolk may help reduce the deleterious effects on membrane structures that occur during rapid cooling (8,9), possibly because of an exchange of lipids between spermatozoa cell membranes and a phospholipid portion of the low-density protein of egg yolk that alters cell membrane molecular composition and maintains its fluidity (10). SFM-MC and for SFM-LG do not contain egg yolk, but both contain 15.0% glycerol, while TYB contains 12.0%, resulting in final concentrations of 7.5% and 6.0% of glycerol, respectively. A concentration of glycerol of about 7.5% (v/v) seems to be optimum for cryopreservation human sperm (11). Furthermore, the SFM-MC and SFM-LG are HEPES-buffered whereas TYB contains TES and TRIS, and SFM-MC and SFM-LG, but not TYB, contain human albumin as protein supplement. In addition, SFM-MC and SFM-LG, but not TYB, contain sucrose as an extracellular cryoprotectant. This non-permeating cryoprotectant agent, a component of the loading and freezing solution, remains outside the cell and increases extracellular osmotic pressure in relation to intracellular pressure. This osmotic imbalance induces even more intracellular water to leave the cell. These modifications may be correlated with the results found.

In a similar study, Nallella et al. (12) showed that TYB is more efficient that SFM-MC and Enhance Sperm Freeze (Conception Technologies) for the cryopreservation of human sperm, with 47.9±15.6, 36.5±13.9 and 32.2±11.6 of mean cryosurvival rates, respectively (p<0.03). However, the cryopreservation technique used in their study was different, i.e., fast-freezing with one time cryoprotectant addition instead of slow-staged cooling, with fractioned addition of cryoprotectant, as used in our study.

Finally, all the cryoprotectants tested in this study showed to be able to preserve the viability of human sperm, with better cryosurvival rates for SFM-MC and SFM-LG used compared to TYB, for normozoospermic samples. It is necessary to evaluate these freezing solutions in oligoasthenozoospermic samples, as well as spermatozoa obtained from epididymis and testis, in order to validate and extend these findings for all sperm samples.

Table 1. Comparison of pre-freeze and post-thaw sperm parameters among three cryoprotectant solutions used for cryopreservation of 23 normozoospermic human sperm samples. Values are expressed as mean ± S.D.

<table>
<thead>
<tr>
<th>Sperm Parameters</th>
<th>TYB</th>
<th>SFM-MC</th>
<th>SFM-LG</th>
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<tr>
<td>Motility (%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pre-freeze</td>
<td>62.2 ± 16.2</td>
<td>62.7 ± 14.4</td>
<td>63.3 ± 15.6</td>
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<tr>
<td>Post-thaw</td>
<td>30.7 ± 16.5</td>
<td>35.4 ± 14.6b</td>
<td>35.6 ± 17.2b</td>
</tr>
<tr>
<td>Percentage Change</td>
<td>51.7 ± 21.8</td>
<td>44.1 ± 17.8b</td>
<td>43.9 ± 22.4b</td>
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<tr>
<td>P-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.01</td>
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<td>Progressive Motility (%)</td>
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<tr>
<td>Pre-freeze</td>
<td>55.2 ± 9.9</td>
<td>56.8 ± 8.1</td>
<td>55.1 ± 11.4</td>
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<tr>
<td>Post-thaw</td>
<td>27.6 ± 16.2</td>
<td>33.8 ± 8.3b</td>
<td>34.6 ± 15.7b</td>
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<td>Percentage Change</td>
<td>51.2 ± 25.7</td>
<td>39.8 ± 15.7b</td>
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<tr>
<td>Cryosurvival Rate (%)</td>
<td>43.3 ± 21.8</td>
<td>55.9 ± 17.7b</td>
<td>56.1 ± 22.3b</td>
</tr>
</tbody>
</table>

<sup>a</sup>Within columns, P-value for comparison between before freezing and after thawing

<sup>b</sup>Within rows, P < 0.05 compared with TYB

Cryosurvival rate = %MOT after thawing / %MOT before freezing
Conclusion

All the cryoprotectant solutions tested proved to be efficient for cryopreservation of normozoospermic human sperm by the liquid nitrogen vapor method. However, we observed better cryosurvival rates when using Medicult Sperm Freezing Medium and LifeGlobal Sperm Freezing Medium, as compared to Irvine Scientific TEST Yolk Buffer. These findings may be useful for the selection of cryoprotectants in order to optimize the cryosurvival of human sperm.

References


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Successful Use of Oocyte Cryopreservation in an IVF Cycle - A Case Report

Anh-Tuan Huy La, ELD., Maria Cristina Rodriguez-Karl, B.A., David G. Diaz, M.D., and Joan E. Moody, R.N.C., West Coast Fertility Centers, Fountain Valley, CA

Introduction

Over the last four years, oocyte cryopreservation has become more prominent, “(it) has applications for both infertile and fertile women and (this technique) is gaining momentum.” (1) Oocyte cryopreservation has created more options for patients who are seeking infertility treatments and who must make decisions about their gametes and embryos.

The case described here demonstrates the potential to become pregnant using frozen oocytes. Not only did the frozen oocytes survive the freeze-thaw process, they fertilized, cleaved, and implanted for a viable pregnancy, as did the fresh oocytes in the initial cycle. The patient delivered healthy children from both fresh and frozen cycles, using oocytes from a single ovarian stimulation cycle. The use of frozen oocytes has grown more acceptable and has allowed the industry to begin promoting oocyte cryopreservation as a viable option to couples seeking infertility treatments (2).

Case Report

A 30-year-old woman with tubal factor infertility, tubal occlusion, and male factor infertility underwent controlled ovarian hyperstimulation (COH) for in vitro fertilization. Synchronization of the late luteal phase antral follicles was achieved by the use of a long GnRH-agonist protocol, and COH was initiated by the use of recombinant FSH and hMG for 9 days. An hCG injection was administered 36 hours before trans-vaginal oocyte retrieval.

Four hours after oocyte retrieval, four metaphase-II oocytes, and one metaphase-I oocyte were selected for cryopreservation. These oocytes were exposed to a modified 3-step dehydration protocol of 1, 2 – propanediol and sucrose, and then loaded into 0.25 cc straws (IMV Technology). The straws were cooled from 22°C to –7°C at a rate of –2 °C/minute before seeding with chilled forceps, to induce the formation of ice crystals. Following seeding, the straws were cooled at a rate of –0.3 °C/minute to –30 °C, and then plunged into liquid nitrogen at –196°C for long-term storage.

The three remaining (non-frozen) oocytes were fertilized by ICSI, resulting in three viable embryos that were transferred on Day 2 (Figure 1). Fourteen days following embryo transfer, the patient's initial serum beta-hCG was 133 mIU/ml, and 29 days following the transfer, ultrasound showed the presence of a single amniotic sac and cardiac activity. In November of 2003, the patient delivered a healthy baby girl by C-Section.

Three years later, the frozen oocytes were thawed at room temperature and then warmed in a water bath. The cryoprotectant was removed by passage of the oocytes through three serial dilutions of 1, 2-propanediol and sucrose. All of the 5 frozen oocytes survived thawing, as judged by the following criteria: 1) the zona pellucida and cell membrane was observed to be intact, 2) the perivitelline space was of normal size, 3) there was no evidence of cytoplasmic leakage or oocyte shrinkage (Figure 2). After thawing, the oocytes were cultured in cleavage medium (Sage, Cooper Surgical) supplemented with 10% synthetic serum substitute (Irvine Scientific) at 37 C, in an atmosphere of 5% CO2 in air. Three of the oocytes were fertilized by ICSI, and fertilization was checked 16 hours later.

All three injected oocytes were judged to have been fertilized normally, based on the number of pronuclei present in the embryo on Day 1. On Day 2, all three embryos had cleaved normally (Figure 3) and were transferred following assisted hatching. The patient's endometrium had been prepared with a combination of vaginally administered estradiol (E2) and progesterone (P4), along with intramuscular progesterone supplementation.

Fourteen days after embryo transfer, the patient's initial serum beta-hCG level was 236 mIU/ml. An ultrasound at 28 days after transfer revealed a single gestational sac and cardiac...
activity. The pregnancy was uneventful and culminated in a cesarean delivery at 40 weeks of a healthy, 7 lbs 2 oz, baby girl.

**Discussion**

This case demonstrates that the use of cryopreserved oocytes can result in a normal pregnancy and delivery of a healthy, normal baby. Also, that there is good survival of the oocytes during the freeze-thaw process. Couples that have few oocytes obtained from a fresh cycle can still utilize a smaller number of oocytes to go through a frozen cycle and produce a pregnancy. The quality and survivability of the oocytes during the freeze-thaw process has increased the percentages of pregnancies obtained by the use of frozen oocytes and decreased the previously high number of frozen oocyte needed to successfully produce viable embryos and a pregnancy.

The high survival rates of the oocytes and quality of embryos produced for transfer increase the options couples have in their fertility treatments (4). The storage of excess oocytes allows couples to inseminate the number of oocytes that they wish to transfer, instead of all oocytes obtained from the retrieval and cryopreserving excess embryos. This storage also enables couples to see oocyte disposal as a more feasible option than long-term excess embryo storage or disposal. The ethical dilemma and standpoint at which couples see embryo disposal versus oocyte disposal, ideally decreases with the increasing availability of oocyte cryopreservation. The growing technology has given patient’s more options in their infertility treatments and has allowed for a decrease in cost of a second cycle with frozen oocytes versus the pressure and stress that many patients endure going through another fresh cycle.

Storage of excess oocytes from fresh cycles has also given patients the option of donation to other couples. Instead of donating excess embryos, which many couples have moral issues in doing so, they are given the option to donate gametes and not embryos. Donation of these excess oocytes has given more options to couples seeking the use of donor oocytes and has enabled the genetic linkage from the male partner of the recipients to their embryos (3).

Frozen oocyte use and donation is a growing aspect in infertility treatment options. Currently, we are conducting an IRB-approved randomized study to observe any differences between freshly inseminated oocytes and oocytes inseminated after a freeze-thaw protocol. An interim analysis was conducted in the first 42 cycles using frozen-thawed oocytes. From a total of 271 frozen-thawed oocytes, the survival rate was 92.6% (251/271). ICSI was performed on 247 oocytes resulting in a 90.3% (223/247) fertilization rate and an 86.1% (192/223) cleavage rate. Of 42 cycles, 38 resulted in embryo transfer using embryos derived from frozen oocytes. Preliminary outcomes are 22 clinical pregnancies (57.8%) with 7 healthy live births to date. As more research and studies are performed, oocyte cryopreservation will become a more feasible and ethically appeasing option to couples. Thus, allowing patients to have a more diverse array of possible options in a second or third IVF cycle.

**References**


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Putting All Your Eggs in One Basket: The Question of the Optimal Number of Embryos per Dish

Jacques Cohen, PhD
Tyho-Galileo Research Laboratories, NJ, USA

The well-known proverb “don’t put all your eggs in one basket” is often used in financial discussions to warn against entrusting too many assets to any single investment. This warning is equally applicable to the question of how many eggs or embryos should be kept in a single Petri dish. The handling and transport of Petri dishes can lead to changes of the pH, temperature and osmolarity, the latter in cases of oil-free culture. It is also associated with physical risks that could potentially damage or result in the loss of embryos. If all of a patient’s embryos are placed in one dish, an unintended error, spillage or wastage can jeopardize the quality of patient care and significantly increase liability. Therefore, we recommend that the use of at least two dishes per patient be considered. We detail below how this minimal recommendation applies specifically to users of the new GPS dish. (See also the article on page 16)

In our view, some reproductive specialists do not seem concerned enough with the issue of overcrowding in Petri dishes. Little consideration is given in the literature or in professional fora to the changes that can occur when dishes are moved from the incubator to a heating surface. However, changes in both the temperature and pH of the culture medium and oil inside a Petri dish can be staggering when transported or handled, even when an accurately controlled hot plate is used on a microscopic stage. They can cause the oil to de-equilibrate with lasting effects on the cultured cells. The problem may be exacerbated when the number of embryos increases, since over-crowded dishes may be handled for longer periods outside the incubator. Too many embryos in a dish can increase observation and manipulation times and slow recovery once the dish is returned to the incubator or heating chamber.

In addition, sudden movements of a dish during seemingly short trips from incubator to microscope and back can lead to embryo spillage and possibly wastage. A milder variation of spillage can occur in the form of droplet mixing, in which droplets run together. In the most extreme event, the dish may be dropped or decanted. Embryos from such disarrays can be rescued by carefully pipetting all spilled liquids immediately and washing the embryos, but individual ones may become lost. Patient care can be seriously jeopardized and potential liability can significantly increase in these instances, especially if all of a patient’s embryos are contained in one affected dish.

The newly introduced GPS dish is designed to reduce some of the risks of spillage and mixing events by providing better control of the embryo. In the GPS dish, the embryo is located in a recessed area and the droplets are contained within the microwells. However, complete wastage can still occur when this dish is dropped or briskly moved. Most users say the design, while it differs from their previous set up, is appropriate for the system in their laboratory. Some users are generally satisfied with the design but would like identification numbers for each well. Other users reject the dish altogether based on the notion that it contains too few wells.

The last two responses were anticipated and require some further explanation, particularly as it relates to the overcrowding of dishes. First of all, there is not a single numeration and identification policy system on which embryologists agree. Consequently, we left the space for numbering wells blank. More importantly though, we restricted the number of culture wells in the GPS dish to eight, as both a compromise between various policies and systems of culture, and as a guideline for the maximum embryos a dish should hold. This guideline is particularly applicable in situations of large egg yields. While the best number of eggs or embryos per dish is a personal issue, we strongly recommend putting no more than eight embryos in a GPS dish and, as a best practice, using a minimum of two dishes per patient. Exceptions can occur when only one egg or embryo is present, or the embryos are cultured in isolettes and never leave the gas phase or optimal temperature setting. However, even there, spillage and damage to embryos can occur. So please reconsider putting all your eggs in one basket and spread them out instead. In an ideal situation the number of embryos per dish should probably not exceed 3-5 embryos.

Further recommended reading:

You can contact Jacques Cohen, PhD at: jc@embryos.net.
Sperm Function Testing In Evaluation of Male Fertility

Sergey I. Moskovtsev, MD, PhD
Andrology Laboratory, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada

Introduction:
Male infertility accounts for almost one half of all cases of infertility worldwide. In nearly one third of these cases, there is no identifiable cause and the patient is labelled as having idiopathic infertility. The traditional criteria established by the World Health Organization (WHO) using the standard semen parameters of concentration, motility and morphology allow separation of semen samples into normal and abnormal (1). When the patient’s sample falls into the normal (reference) range (2), we assume the patient is fertile; when the basic semen analysis reveals severe abnormalities the appropriate clinical option would likely be ICSI. However, the basic semen analysis, even if its result is within the reference range, is insufficient to judge the sperm’s fertilizing capacity, and the appropriate clinical options would be unknown.

For a sperm to fertilize an oocyte in vivo, it must be capable of successfully completing a precise sequence of events including: swimming out of seminal fluid; being transported through the uterus and fallopian tubes; undergoing maturation (capacitation); recognizing and binding to the zona pellucida; undergoing the acrosome reaction; penetration of the zona; activation of the egg (preventing penetration of the egg by other spermatozoa); decondensation (dissolution) of the sperm’s nucleus and formation of male pronucleus, and fusing with the female pronucleus (resulting in embryo formation). If any one of these steps fails, an individual sperm cannot fertilize the egg; and if defects occur in most of the sperm in an ejaculate, fertilization will likely not occur.

Sperm function tests attempt to determine the ability of sperm to mature and ultimately fertilize an egg. These tests evaluate cellular processes between the time the sperm leaves the seminal fluid and the final step of fertilization. No single in vitro test is capable of evaluating the potential to complete more than a few of these steps and not all of these steps can yet be assessed in the laboratory. In fact, even human IVF, used diagnostically, cannot replicate all of the steps in the natural fertilization process (3). Therefore the diagnostic algorithm for the investigation of the infertile male requires the addition of sperm function tests to exclude the presence of specific defects that are not evaluated in the basic semen analysis.

Hypo-Osmotic Swelling Test (HOST):
HOST assesses the durability of the sperm membrane. The test is based on the ability of live spermatozoa to withstand moderate hypo-osmotic stress. Numerous publications have shown correlations between HOST, fertilization and implantation rates. However, other studies have found no correlation with fertilization. In a study conducted in our laboratory, we found that 38% of men evaluated for male infertility had abnormal HOST results (4). In addition, we compared the relationship of sperm plasma membrane functional integrity (HOST) and sperm DNA integrity assessed by DNA fragmentation index (DFI) (Table 1).

Our results suggest that a common problem may manifest itself as a major abnormality in both the nucleus and the plasma membrane; such an abnormality would negatively affect implantation or subsequent embryo development.

Acrosome Reaction after Ionophore Challenge (ARIC):
The ARIC test measures the rate of spontaneous acrosome reaction (AR) in sperm samples where certain chemicals are used to induce AR. Only fully matured (capacitated) spermatozoa are capable of an AR which occurs at the zona pellucida after sperm binding. Similarly, only spermatozoa that have their AR synchronized with the phase of egg penetration are able to fertilize the egg. If AR occurs too early, the zona pellucida will not be able to recognize its potential, and sperm-zona pellucida binding will be compromised (premature AR). An abnormally low rate of spontaneous AR is called insufficient AR (5). Data from our laboratory revealed that 31% of our patients had abnormally low ARIC (Table 2) (6).

Zona-Free Hamster Egg Penetration Test (HEPT):
HEPT measures the ability of human spermatozoa to undergo capacitation, the acrosome reaction, penetration of the egg plasma membrane, and decondensation. HEPT does not assess the sperm’s ability to bind and penetrate the zona pellucida. The
HEPT score is calculated as the percentage of zona-free hamster eggs penetrated by one or more spermatozoa. This test has been reported to have a 70-80% predictive value for fertilization rates with 20-30% false positive and 5-30% false negative results (7).

We examined the frequency of abnormal HEPT results and DNA damage in patients expressing normal or borderline standard semen parameters for which intra-uterine insemination (IUI) would most likely be considered based on their semen analysis results (8). Almost one third of the patients had one or more abnormal tests of sperm binding (Table 3) or sperm penetration (Table 4).

**Hemizona Assay (HZA):**
HZA is one of two most common tests of verifying the ability of spermatozoa to bind to the zona pellucida (9). HZA assesses the ability of spermatozoa to undergo capacitation, acrosome reaction and the endpoint of tight sperm binding to the zona. HZA does not assess the sperm's ability to penetrate the zona pellucida. Results are calculated as the percentile difference between the zona bound patient's spermatozoa and the donor's spermatozoa to the matching halves of a human zona pellucida. HZA has been reported to have a 70-80% value for predicting fertilization results with about 25% false positive and 2-25% false negative results (10).

**Conclusions:**
It is now clear that standard semen analysis does not assess fertility; moreover, it provides insufficient information to determine appropriate infertility treatment. Almost one third of the patients in our studies had one or more abnormal tests of sperm membrane integrity (4), acrosome reaction (6), sperm binding, and penetration ability (8) with poor correlation to the semen analysis. The literature, supported by our data, suggests that sperm function testing is an important clinical strategy for the investigation of the male infertility in the presence of normal or borderline semen analysis results. These tests may help in choosing the appropriate treatment option and avoiding other unnecessary or costly and time-consuming procedures.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal HOST</th>
<th>Abnormal HOST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>63</td>
<td>39</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.6 ± 0.7</td>
<td>38.6 ± 1.1</td>
</tr>
<tr>
<td>Concentration (10⁶/mL)</td>
<td>69.5 ± 7.1*</td>
<td>41.3 ± 5.3</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>36 ± 1.8*</td>
<td>21.5 ± 1.9</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>27.9 ± 1.4*</td>
<td>16.9 ± 1.8</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>77.2 ± 0.8*</td>
<td>61.7 ± 2.2</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>16.5 ± 1.1*</td>
<td>33 ± 2.6</td>
</tr>
</tbody>
</table>

*p < 0.01

**Table 1. HOST: frequency and relationship to semen parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal ARIC</th>
<th>Abnormal ARIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>44</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.5 ± 0.8</td>
<td>36 ± 1.3</td>
</tr>
<tr>
<td>Concentration (10⁶/mL)</td>
<td>95.1 ± 17.8</td>
<td>97.8 ± 11.6</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>45.1 ± 10</td>
<td>42.5 ± 11.3</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>31.8 ± 7.7</td>
<td>27.3 ± 8.1</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>82.2 ± 4.9</td>
<td>81.6 ± 4.4</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>14 ± 1.4</td>
<td>18.1 ± 1.9</td>
</tr>
</tbody>
</table>

Note: In 15% of the patients, premature AR was combined with insufficient AR.

**Table 2. ARIC: frequency rates and relationship to semen parameters**
ARTICLES

 CONTINUED FROM PAGE 37

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal HEPT</th>
<th>Abnormal HEPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.9 ± 4.9</td>
<td>37.9 ± 5.6</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>3.8 ± 1.6</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>Concentration (10^6/mL)</td>
<td>93.0 ± 51.3</td>
<td>71.6 ± 35.2</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>48.3 ± 11.8</td>
<td>46.0 ± 11.5</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>32.8 ± 8.7</td>
<td>28.3 ± 8.3</td>
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<tr>
<td>Vitality (%)</td>
<td>82.2 ± 4.7</td>
<td>81.5 ± 4.8</td>
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<tr>
<td>HZA (%)</td>
<td>59.3 ± 30.1*</td>
<td>19.4 ± 8.4</td>
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<tr>
<td>DFI (%)</td>
<td>15.0 ± 6.6</td>
<td>17.0 ± 9.3</td>
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*p < 0.01

Table 3. HEPT: frequency and relationship to semen parameters

<table>
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<tr>
<th>Parameter</th>
<th>Normal HZA</th>
<th>Abnormal/ Subnormal HZA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.4 ± 3.8</td>
<td>38.3 ± 6.5</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>4.1 ± 1.5</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>Concentration (10^6/mL)</td>
<td>92.3 ± 51.9</td>
<td>76.1 ± 38.4</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>48.2 ± 11.8</td>
<td>46.5 ± 11.5</td>
</tr>
<tr>
<td>Normal morphology (%)</td>
<td>32.9 ± 9.6</td>
<td>28.9 ± 7.1</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>82.2 ± 4.9</td>
<td>81.6 ± 4.4</td>
</tr>
<tr>
<td>HEPT (%)</td>
<td>29.7 ± 12.1*</td>
<td>17.2 ± 18.7</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>14.9 ± 6.6</td>
<td>16.8 ± 9.0</td>
</tr>
</tbody>
</table>

*p < 0.01

Table 4. HZA: frequency and relationship to semen parameters

References:


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GILX-006......Pkg of 6
GILX-012......Pkg of 12

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GILF-006......Pkg of 6
GILF-012......Pkg of 12

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Japanese Studies

Sequential medium と Single step method の比較
岡山二人クリニック 山下陽子、平田健、鶴木美那子、川上秀子、吉岡奈々子、羽原俊宏、林伸明

【目的】Sequential mediumを使用することが主流となっているが、採卵から胚盤胞まで同一組成の培養液を使用するSingle step methodが開発されたので、Sequential mediumと比較して後方的に検討した。【方法】H18年3月～4月の期間に採卵を行った症例のうち、同一症例から得られた卵子を無作為に2群に分け、Single step methodおよびSequential mediumの2系の培養液により培養のうつ37症例398対の成熟胚を対象とした。A群: Day0→Day5までGlobal Medium (LifeGlobal)を用いて、B群: Day0→Day3 Cleavage Medium (COOK IVF)、Day3→Blastocyst Medium (COOK IVF)を用いて培養した。分割胎児ではVeeck分類により、胎盤胞ではGardner分類により胎盤の評価を行い、3日目6分割以上、fragmentation<20%、5日目3BB以上を良好胎ととした。【成績】A群とB群における受精率は67.0%、67.8%、胚発生率82.5%、83.4%、分割率99.4%、100%と有意差は認めなかったが、早期分割率は50.6%、38.5%、良好分割率は56.8%、44.6%とA群が効率であった（P<0.05）。Day3での新鮮移植胎児及び凍結胎児を除いた追加培養胎児において胎盤胞培養率は38.7%、47.3%、良好胎盤胞培養率は32%、21%、有効率（新鮮移植胎児及び凍結胎児/2PN数）44.3%、36.4%でありDay3以降においてもSequential mediumと比較して胚発育の防害は認められなかった。【結論】Single step mediumはSequential mediumと比較して胚発育を防害することなく、ラポワールの簡素化、培養液の管理面のメリットを考慮合わせた場合、2系統目の培養液として選択可能と考えられた。

Sequential Medium と Single Medium を用いた培養成績の比較
京野アートクリニック 熊谷 志麻、伊東 みゆき、柳原 裕香里、西中 千佳子、中條 友紀子、宇都 博文、京野 廣一

【目的】胚盤胞培養においてはsequential mediumが多くの施設で使用されているが、近年single mediumを用いた胚盤胞培養の報告もある。そこで我々は今回、sequential mediumとsingle mediumを用いた培養成績を比較検討した。【方法】2006年1月～2006年5月、40歳未満で当院にて実体外受精を施行した症例の全ての胚を5日目まで培養した25症例25週期を対象とした。Quinn's Advantage Cleavage Medium (Sage) (day1→day3)→MultiBlast Medium (Irvine) (day3→day5)のsequential mediumで培養した群（QM群）とGlobal Medium (LifeGlobal) (day1→day5)のsingle mediumで培養した群（GL群）の2群に分け、2PNが6個以上得られた症例に対し半数ずつQM群とGL群で培養を行った（QM群：147個、GL群：149個）。当院の胚の評価に基づきそれぞれのday3、day5の良好胚到達率とday5の胚盤胞到達率を比較した。【結果】QM群とGL群のday3良好胚到達率は43.5% vs 41.8%、day5良好胚到達率26.0% vs 28.6%、胚盤胞到達率48.6% vs 50.3%であった。【考察】今回の結果よりsequential mediumとsingle mediumの良好胚到達率、胚盤胞到達率に有意差は認められなかった。しかし、今回の結果より胚盤胞まで培養するのにはsequential mediumが必須ではないということが示唆された。今回は胚盤胞までの培養成績での比較であり、今後症例数を増やし妊娠・出産とその発育との関係についても検討も重ねる必要がある。
of Global Medium


![Graph showing the percentage of embryos for Global and BAS1/BAS2 comparisons.](https://via.placeholder.com/150)


![Graph showing the percentage of zygotes for Global and Cook Cleavage/Blastocyst.](https://via.placeholder.com/150)


![Graph showing the percentage of embryos for Global and Quinn's/Multiblast.](https://via.placeholder.com/150)

(All graphs by D. Rieger, LifeGlobal LLC, based on translations by T. Cho, ASTEC Bio.)
ARTICLES

COMPARISON OF EMBRYONIC DEVELOPMENT AFTER CRYOPRESERVATION OF MOUSE MORULA USING VITRIFICATION OR SLOW FREEZING

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Embryo cryopreservation is a widely used technique and there are several protocols available. The objective of this study was to compare the blastocyst rate of morulae exposed to vitrification or slow freezing. Vitrification and freezing solution were prepared using HTF-hepes (Life Global®, USA). The vitrification protocol consisted of exposing the morulae for 2 minutes to a solution with 10% EG, 10% DMSO, 0,25 M sucrose, 10% FCS, and then exposed for 30 seconds to a solution with 20% EG, 20% DMSO, 0,5M sucrose, 10% FCS. The slow freezing protocol consisted of exposing morulae for 10 minutes to a solution with 1,5 M ethylene glycol at room temperature, followed by 5 minutes at –7°C when seeding was performed. The straws were maintained for an additional 10 minutes at –7°C then a slope of –1,2°C/min was applied until –30°C when embryos were kept for 10 minutes before plunged into nitrogen liquid. Following thawing and warming, embryos were place in 50μl drops of Global media (Life Global®, USA) and were assessed at 24 and 48 hours. There was no difference in the blastocyst rate for slow freezing, vitrification and control at either 24 or 48 hours evaluation (Tab.1). Despite the slow freezing being the most widely cryopreservation technique used in all species, vitrification has several advantages that can turn it the standard protocol.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Embryos in culture</th>
<th>Blastocyst rate 24h</th>
<th>Blastocyst rate 48h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>195</td>
<td>90 (46,2%)</td>
<td>180 (92,3%)</td>
</tr>
<tr>
<td>Slow freezing</td>
<td>135</td>
<td>61 (45,2%)</td>
<td>114 (84,4%)</td>
</tr>
<tr>
<td>Vitrification</td>
<td>261</td>
<td>113 (43,3%)</td>
<td>225 (86,2%)</td>
</tr>
</tbody>
</table>

Table 1. Blastocyst rates following cryopreservation of mouse morulae through slow freezing and vitrification.
PGD Biopsy Medium

- A calcium- and magnesium-free medium designed to facilitate the removal of blastomeres for preimplantation genetic diagnosis
- Specially designed formulation based on global®
- Contains sucrose and HSA and is ready-to-use

LiteOil®

- Designed for IVF
- Pharmaceutical grade quality mineral oil
- Extensively washed with ultra-pure water
- Sterile hand-filtered
- MEA and endotoxin tested
- Optimal viscosity for embryo culture
- 24 Month minimum shelf life

PVP

- Used to restrict sperm motility
- High quality clinical grade
- 10% dialysed PVP in HEPES buffered solution
- Available in 2 sizes

Hyaluronidase

- Excellent for facilitating the removal of the cumulus complex and corona cells from the oocyte prior to ICSI
- Ready-to-use

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Over the years the Coda® air purification system has worked hard to keep your IVF laboratory safe from harmful environmental contaminants. Through the years we have been called on to resolve several crises and have been able to help each time. Several clinics have been endangered by brushfires, construction damage, and toxic spills. Having the Coda® products in place has helped to protect these IVF labs and can help to protect you.

**BRUSHFIRES ENGULF THE FLORIDA EAST COAST THREATEN HOSPITAL**

North Miami, Fla. (FW) -- A portion of Interstate 95 remained closed Monday morning as brushfires whipped through thousands of acres and forced about 1,000 people to evacuate their homes, authorities said. The wind blown fire and smoke engulfed neighborhoods and threatened the local hospital.

The IVF Team of the hospital was put on ‘red’ alert. The staff raced to the hospital, preparing to evacuate the embryos and the contents of the incubators. The hospital was engulfed in faint smog of smoke and a smell of the brushfire. They prepared and braced for the worst.

As the IVF Team entered the IVF laboratory, they were surprised and relieved to find that the air in the IVF laboratory was clean and clear, and that their specimens were safe and protected. They did not have to move the embryos.

**Fact:** Inside this lab were two Coda Towers® and the incubators were equipped with Coda® Units. The Team was able to relax, knowing that their embryos were safe and out of harms way. This showed the Coda Towers® to be a powerful ‘support’ system.

**Note:** Centralized systems have trouble filtering large amounts of environmental fumes such as smoke. Most systems do not contain sufficient HEPA filtration or carbon. The smoke may eventually overcome the system and enter the clinic, introducing contaminants into the lab and other rooms.

The Coda system has the ‘air power’ to fully circulate the air in your lab in minutes, and the ‘filter power’ and enough HEPA filter to work for hours, clearing the air.

Easy change-out of all filters gives you the added protection you need, and speeds your recovery time.
LARGE NEW YORK IVF CLINIC UNDER CONSTRUCTION

In anticipation of construction, a large New York IVF clinic installed several partition doors between the new laboratory construction and their current lab.

They then installed several Coda Towers® between their lab and the construction, in the halls, and in every lab. They maintained a strict regimen of replacing Coda® filters, preserving the air quality in the laboratory and operational rooms. The air of the lab was preserved and actually improved.

The Coda Towers® protected the labs from the millions of small particles which are not caught with the central HVAC systems.

The older lab was preserved during all of the construction and the specimen were protected. When the lab was opened, the same Towers and incubator units were arranged to protect them for the past 8 plus years.

The Coda® HEPA, rated at 99.997% will capture and remove the majority of contaminants and particulates from the air in, and around, your laboratory.

Coda® Incubator Units will protect the interior of the incubators from any particulates and contaminants.

Note: Whenever there is any type of construction within the building or on the property, micro particles of debris permeate the entire building as well as your IVF lab. The construction creates excessive amounts of contaminants and most system just can not keep up. The continual filtration of the Coda System, helps stop this. Unseen particulates, contaminants and particulates will be captured in the Coda 4-stage filters system.

If you are planning construction, you should plan on using Coda Towers or Low-boys to help you get through these hard times. They can then be used for continual filtering your air, maintaining your air.

YOU MAY SUFFER A TOXIN SPILL...

Over the past several years, several clinics have reported toxic spills occurring in their laboratory or adjacent rooms that share a common air system. Several clinics have experienced a reduction in performance as a result of this.

Most IVF laboratories would not be able to withstand a toxic spill. Most systems become quickly compromised when they are faced with very high levels of toxins, and quickly become saturated. Because they are only able to filter the air once, they are unable to absorb all of the toxins. Even the filters in dedicated air systems are not adequate to deal with toxic spills.

Several clinics on the other hand have been able to maintain air quality during these hard times and been able to use the Coda Tower® and air system to speed the recovery of the environment.

Note: The average filter system is not able to absorb all the contaminants from a toxic spill.

Most will not even work long enough to allow you to time to react to the spill. When a spill occurs, the level of toxins overcomes the filters as well as the duct work system of the building. The smaller filters will begin to release the contaminants that they had absorbed earlier. This means that the filter will begin to shed the chemicals it had caught earlier into your environment. The central system may also become compromised and is ‘passing through’ the gases and not catching any. Your filters will basically stop working. Coda® Canister, the only ‘canister style’ filter for IVF, is packed with activated carbon and potassium permanganate and can absorb 50-fold more VOCs and CACs than most smaller units.

The Coda® Canister filter will absorb 5 to 10 times longer than smaller units that typically last only 2 hours. If this does occur, by installing new filters, it will allow the entire room to quickly recover, while a central system may take days. A central system is not actively capturing the toxins in the room, but only pushing through air.
In the early days of mammalian embryo culture, it was believed that embryos in culture either died or else recovered to develop normally. Austin (1973) expressed this view in saying:

“The effect (of teratogenic agents) on the cleavage embryo depends on the number of cells killed or inhibited: above a certain proportion, the embryo dies; below that figure the remaining cells multiply to replace those lost and subsequent development is essentially normal.”

We now know that this view was wrong. Exposure to a variety of agents and environmental conditions during in-vitro culture can have significant effects on development after transfer to the uterus, and, most importantly, with few or no readily observable immediate effects. As for my previous articles for Fertility Magazine, this is not intended to be a comprehensive review of the topic, but rather a brief overview, using selected illustrative references. Moreover, much of the investigation of the long-term effects of in-vitro embryo culture has focused on attempting to identify the molecular mechanisms, including disturbances of genetic imprinting. The discussion here will be limited to observations of the developmental and physiological effects of a few of the many possible effectors. For comprehensive reviews of the topic, see Johnson (2005), Pool (2005) and Thompson (2007).

Perhaps the earliest report of effects of in-vitro exposure of embryos to an agent on subsequent development was the report of the effects of methylnitrosylurea (MNU) on mouse embryos by Iannaccone (1984). Mouse blastocysts were exposed to various doses of MNU for one hour, and the surviving embryos transferred to recipients. A dose of 4200 μg/kg MNU was required to kill 50% of the embryos immediately after exposure, but a dose of only 4.7μg/kg, 1000-fold less, reduced the live-birth rate by 50%. This clearly showed that a dose of MNU too low to have an immediate effect could have a significant later effect. Even more striking was the effect on post-natal survival. As shown in Figure 1, there was a significant perinatal loss of pups derived from MNU-exposed embryos compared to controls, and further losses until, at 52 weeks, the crude mortality rate was 58% for the MNU group compared with 22% for the controls. Interestingly, there were no chromosomal, histological, or anatomical abnormalities associated with the MNU treatment.

Willadsen et al. (1991) reported a high incidence of congenital deformities and very high birth weights among calves produced by cloning. This was later also shown to occur in calves and lambs produced by in-vitro maturation, fertilization and culture (IVP), and is known as the Large Offspring Syndrome. Figure 2 shows the results of a meta-analysis by Kruip and den Daas (1997) in which gestation length, birth weight, dystocia score and perinatal death rate were significantly greater in calves resulting from embryos produced by IVP compared with calves resulting from transfer of in-vivo-produced embryos. Clearly, although apparently benign, in-vitro procedures can have significant effects on subsequent development.
Figure 2. Comparison of gestation length, birth weight, dystocia score and perinatal death rate in calves produced by artificial insemination (AI), transfer of in-vivo produced embryos (ET) or in-vitro maturation, insemination and culture (IVP). (Kruip and den Daas, 1997)

It is well recognized that atmospheric concentrations of oxygen (20%) can have detrimental effects on the in-vitro development of embryos from many species, including reduced blastocyst cell numbers (Gardner et al., 1999), abnormal gene expression (Rinaudo et al., 2006), and increased DNA fragmentation (Kitagawa et al., 2004). Even more significant, high oxygen concentrations have been shown to be detrimental to development of human embryos after transfer, without having any apparent effect on development to the blastocyst stage in vitro (Catt and Henman, 2000; Figure 3).

Figure 3. The effect of oxygen concentration on the viability of human embryos (Catt and Henman, 2000)

The concentration of glucose in human serum is approximately 1 mg/ml (5.55 mM) and cell culture media such as Ham’s F-10 and MEM commonly contain this concentration of glucose. However, many studies have shown that serum concentrations of glucose are detrimental to the development of mammalian embryos in vitro. Notably, Thompson et al. (1992) showed that glucose concentrations greater than 3.0 mM inhibited the development of sheep embryos. Media for human embryo culture usually contain only low to moderate concentrations of glucose. Interestingly, however, it has been shown that glucose may be more detrimental to female embryos than to male embryos, such that exposure to even moderate concentrations of glucose in vitro may result in increased proportions of male embryos. For example, as shown in Figure 4, Kimura et al. (2005) found that although 2.5 mM glucose had no effect on the overall proportion of 8-cell cattle embryos that developed to the blastocyst stage, that concentration of glucose significantly increased the proportion of male embryos.

Figure 4. The effect of glucose concentration on the development to blastocyst and the sex ratio of cattle embryos (Kimura et al., 2005.)

Cohen et al. (1997) found significant concentrations of a wide variety of volatile organic compounds (VOC) in the atmosphere in their IVF unit in New Jersey. Surprisingly, the concentrations of many of these VOC were higher in the laboratory than in outside air, indicating that they arose within the laboratory or from adjacent areas. Possible sources included anesthetics, fumigants, adhesives, laboratory plastics and incubator gases. They were able to relate changes in air quality to implantation and clinical pregnancy rates, even though there were no significant effects on embryo development or morphology. Cohen and Dale subsequently designed small air filtration (Coda) units to be placed within the incubator. Mayer et al. (1999) found that the use of Coda units resulted in increased pregnancy rates, but without any apparent improvement in embryo quality. Using cattle embryos, Merton et al. (2007) similarly found that the use of Coda units significantly improved pregnancy

CONTINUED ON PAGE 46
rates, but had no effect on the rate of development to blastocyst or on embryo quality (Figure 5).

Figure 5. The effect of Coda air filtration on the development of bovine embryos to the blastocyst stage and on pregnancies rates (Merton et al., 2007).

In conclusion, it is becoming increasingly clear that the in-vitro environment can have deleterious effects on short and long-term embryo development and even post-natal life. However, it is important to note that the effects of specific environmental conditions differ between studies, and not all embryos will be affected by any given effector. This suggests that the ultimate fate of the embryo depends on its inherent resistance to environmental stress and the sum of the environmental stresses to which it is exposed. Therefore all factors must be evaluated before introducing them into common ART practice. The use of maternal serum for in-vitro oocyte maturation (IVM) is a case in point. Thompson et al. (1995) found that the use of human serum in sheep embryo culture medium significantly increased the incidence of large lambs, and Calder et al. (2004) showed that serum significantly inhibited the expression of a number of genes in cattle cumulus-oocyte complexes. Because it is an undefined component, the use of maternal serum has largely been abandoned for human embryo culture, and the same concern should apply to its use for IVM.

References


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Comparative Study of Two Culture Media in an IVF Programme

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Southend Rotunda, Center for Human Reproduction, Holy Angels Hospital, Vasant Lok, Vasant Vihar, New Delhi, INDIA

Introduction

Repeated IVF failures pose a special problem to the patient and to her service provider. Embryo quality, endometrial receptivity and the technique of embryo transfer have been implicated as the main causes for failure in ART. In order to overcome these problems there has been much advancement in IVF. In particular, embryo quality and pregnancy rates are said to be better if embryos are transferred at the stage blastocyst stage. Assisted hatching and PGD are other approaches that may help in cases of implantation failures.

Over the years, blastocyst culture has been promoted as a major method to better IVF results. However, lately it has been shown that closer analysis of the morphology of cleavage-stage embryos may also allow us to select better embryos and achieve the desired pregnancy results following transfer on Day 2 or Day 3. Nevertheless, in countries like India where temperature and weather conditions vary from region to region, it may be wise to make blastocysts in order to a) achieve better pregnancy rates, and b) to be sure of the quality and condition of the laboratory.

Many media have been designed for blastocyst culture based on the composition of oviduct and uterine fluids and on the patterns of embryo metabolism. All of these are sequential, labour intensive, and need a definite protocol in order to get good results. LifeGlobal global® medium, on the other hand, is a single medium that has been designed to support human embryo development from Day 1 to the blastocyst stage on Day 5 or 6. We designed a comparative study to compare the efficacy of global® medium with the existing medium (Vitrolife IVFTM) being used in our laboratory. The main outcome measure was embryo development after culture from Day 1 to Day 3. In addition, we wanted to know if we could culture embryos to the blastocyst stage in our laboratory.

Material and Methods

Fifteen age matched patients with the same diagnosis were enrolled in the study. All went through the standard long protocol for induction ovulation. Oocyte retrieval was done at 34 hrs after the hCG trigger. On Day 1, the oocytes from each patient were divided approximately equally into two and cultured until Day 3 in either Vitrolife IVFTM (N = 107), or in global® (N = 98). The components of the two media are shown in Table 1.

Embryo development was assessed on Day 2 and Day 3. Cell numbers were compared by Kruskal-Wallis tests and proportional data were compared by Chi-square analysis.

On Day 3, a total of 84 embryos having 4 or more cells (from both groups) were transferred into global® medium for further culture until Day 5.

Table 1. The components of the media used for culture from Day 1 to Day 3

<table>
<thead>
<tr>
<th>IVFTM</th>
<th>global®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>Calcium Chloride</td>
</tr>
<tr>
<td>Magnesium Sulphate</td>
<td>Magnesium Sulphate</td>
</tr>
<tr>
<td>Potassium Chloride</td>
<td>Potassium Chloride</td>
</tr>
<tr>
<td>Potassium Phosphate</td>
<td>Potassium Phosphate</td>
</tr>
<tr>
<td>Sodium Bicarbonate</td>
<td>Sodium Bicarbonate</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Sodium Lactate</td>
<td>Sodium Lactate</td>
</tr>
<tr>
<td>Sodium Pyruvate</td>
<td>Sodium Pyruvate</td>
</tr>
<tr>
<td>EDTA</td>
<td>EDTA</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Penicillin G</td>
</tr>
<tr>
<td>Human Serum Albumin</td>
<td>Human Serum Albumin</td>
</tr>
</tbody>
</table>

Results

As shown in Figure 1, the embryos cultured in global® had significantly more cells on Day 2 than the embryos cultured in IVFTM, while there was no difference in cell numbers on Day 3. The proportion of embryos that had cleaved by Day 2 was not different between the two media, but a significantly larger proportion of embryos cultured in global® had 4 or more cells compared to those cultured in IVFTM (Figure 2). On Day 3, there was a tendency for a greater proportion of embryos cultured in global® to have 6 or more cells compared with those...
cultured in IVF™ (Figure 2).

A total of 37 of the 84 (44.0%) embryos cultured in global® from Day 3 to Day 5 developed to the blastocyst stage.

**Figure 1.** Cell numbers on Days 2 and 3

<table>
<thead>
<tr>
<th>Day</th>
<th>IVF™</th>
<th>global®</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.2</td>
<td>5.1</td>
<td>0.169</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>7.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Figure 2.** Development on Days 2 and

<table>
<thead>
<tr>
<th>Day</th>
<th>Cleaved &gt; 4 cells</th>
<th>≥ 6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>3</td>
<td>80%</td>
<td>70%</td>
</tr>
</tbody>
</table>

P = 0.452

P < 0.001

P = 0.065

**Discussion and Conclusions**

Development was significantly better on Day 2, and tended to be better on Day 3 for embryos cultured in global® compared to those cultured in IVF™. This suggests that a complex medium, containing all 20 amino acids, is beneficial for the development of cleavage-stage human embryos.

The large proportion (44%) of embryos cultured in global® from Day 3 to Day 5 developed to the blastocyst stage. This demonstrates that we are able to culture embryos to the blastocyst stage in our laboratory, in global® medium. The use of a single medium is simpler and less labour-intensive for the laboratory.

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An Overview of the Effects of Age on Fertility in Women
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I. INTRODUCTION

For a variety of social and economic reasons, women (and couples) are putting off having babies until they are in their mid-thirties, or later. As shown in Figure 1, this trend is common to most industrialized countries. In the United States, the average maternal age at first birth increased from 21.4 to 24.9 years of age from 1970 to 2000. Overall, birth rates in the United States decreased from 1970 to 1980, but since then, the birth rates for women over 30 years of age have increased significantly (Figure 2).

Figure 1. The average maternal age at first birth in 1970 and 2000 for ten industrialized countries. (Mathews and Hamilton 2002)

Although some women can, and do, have babies into their forties and even fifties, in general, fertility decreases markedly with a woman's age, particularly from 35 years onward. As shown in Figure 3, the chance of being infertile is approximately twice as great for women 40-44, compared with women 30-34. It is important to note that the onset of age-related infertility occurs approximately 10 years before menopause (te Velde and Pearson, 2002). The decrease of fertility with age, coupled with the tendency toward later child-bearing has led to the suggestion that “female ageing … is now the main limiting factor in the treatment of infertility” (Ford et al. 2000).

Figure 3. The effect of a woman's age on infertility and the chance of remaining childless. Adapted from Menken et al. (1986).

* Historical data based upon the age at which a woman marries.

This paper is intended as an overview of the information available on the relationships between age and fertility in women. It is by no means an extensive review of the literature, but rather, the data have been selected to illustrate general principles. Moreover, it is becoming increasingly evident that the fertility of a couple decreases with age of the male, as has been recently reviewed by Fisch (2005). The effect of male age is beyond the scope of this paper, but where relevant and whenever possible, studies have been chosen that have been controlled or adjusted for male age as well as frequency of intercourse and lifestyle factors.

* Historical data based upon the age at which a woman marries.
II. THE EFFECT OF AGE ON NATURAL CONCEPTION

The birth of a normal healthy baby requires that a woman be able to ovulate a mature, normal oocyte (egg) at the appropriate time, that a fertile sperm be present within the oviduct (Fallopian tube) for fertilization, that the oviduct and uterus be capable of supporting the development of the embryo, and that the embryo (later the fetus) reside in uterus until fully developed and delivered without major complications. Many of these processes have been shown to be affected by the woman's age.

The development of the oocytes and the follicles in which they reside begins when the woman is herself a fetus. Primordial germ cells develop into oogonia which divide and differentiate into primary oocytes. The primary oocytes are enclosed in a single layer of granulosa cells to form primordial follicles, numbering approximately 7,000,000 in both ovaries at 4-5 months of gestation. From there onward, the oogonia stop dividing, and the primary oocytes are arrested in development until puberty. Most of the primordial follicles are lost to atresia such that only approximately 1,000,000 are left at birth, 40,000 at the time of puberty and 1,000 at menopause. Throughout reproductive life, groups of the primordial follicles spontaneously grow and develop into early antral follicles. Most of these are also lost, except for (usually) one follicle per menstrual cycle that continues to develop into a large antral (dominant) follicle in response to gonadotrophic hormones (FSH and LH) secreted by the anterior pituitary gland. A surge of LH at mid-cycle induces the final maturation of the oocyte in the dominant follicle and its release (ovulation) into the oviduct where it can be fertilized. (See Piñón, 2002)

The ability to produce and ovulate that one oocyte is directly related to the number of antral follicles on the ovaries at that time. As shown in Figure 4, the number of antral follicles present on the ovaries declines with age.

In addition to the significant decline in the number of antral follicles, increasing age is also associated with chromosomal and functional aberrations in the oocytes. Most notably, as shown in Figure 5, the frequency of aneuploidy (abnormal numbers of chromosomes) in human oocytes increases exponentially after 35 years of age. Although the sperm can also contribute to chromosomal defects in the embryo, defects in the aged oocyte are thought to be the major cause of Downs Syndrome and other chromosomal abnormalities in newborns (Figure 6). Increased maternal age is also associated defects in oocyte mitochondria, a structure responsible for energy production and many other important functions. These include an increased rate of point mutations in oocyte mitochondrial DNA (Barritt et al. 2000), and with a decreased ability of the mitochondria to produce energy (Wilding et al. 2002).

As discussed in the following section on assisted reproduction, there is evidence that maternal age can have effects on fertilization and on development of the embryo. In natural conception, it is difficult to be certain whether problems of fertility arise from deficiencies in the embryo or deficiencies in the reproductive tract. However, it is very

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clear that the ability to get pregnant, to maintain the pregnancy, and to deliver a healthy baby decreases with maternal age.

Figure 7 shows that the probability of establishing a clinical pregnancy following intercourse on the most fertile day of the cycle decreases steadily with increasing maternal age. Even for women who do eventually become pregnant, the likelihood of achieving conception within six months is dramatically reduced for women 35 years of age and older (Figure 8).

Figure 7. The effect of a woman’s age on the probability of establishing a clinical pregnancy by intercourse on the most fertile day of the cycle. The data have been adjusted for paternal age and controlled for frequency of intercourse and lifestyle factors (Dunson et al. 2002).

When pregnancy is established, increasing maternal age has severely detrimental effects on the outcome. Many of these effects probably result from the effects of maternal age on the oocyte/embryo/fetus or the embryonic contribution to the placenta. Both the miscarriage rate (Figure 9) and the frequency of preterm delivery (Figure 10) increase with increased maternal age. The risks of a wide variety of perinatal complications in the mother and the baby are significantly greater in women older than 35 years of age compared with women from 18-34 years of age (Figure 11). For example, compared with women 18-34 years old, the risk of an emergency Caesarian section was 1.5 times as great for women 35-40 years old and more than twice as great for women older than 40. Based on historic data and on animal studies, Tarin et al. (2005) have suggested that delayed motherhood may also have long-term effects on the health of the children, including impaired fertility and reduced lifespan.

Figure 9. The effect of maternal age on the incidence of miscarriage (Gindoff and Jewelewicz 1986).

Figure 10. The effect of maternal age on the frequency of preterm delivery (Astolfi and Zonta 1999).

Figure 11. The risk of perinatal complications for women 35-40 or >40 years of age compared with women 18-34 years of age (Jolly et al. 2000).
III. THE EFFECT OF AGE ON THE SUCCESS OF ASSISTED REPRODUCTION

Infertility can result from lack of ovulation, poor quality oocytes, blocked oviducts (Fallopian tubes), impotence in the man, inadequate sperm numbers, poor quality sperm, or a poor interaction between the sperm and the cervical mucus. Many of these problems (and infertility due to unknown causes) can be treated, or at least circumvented, by assisted reproductive technologies (ART). However, although ART procedures can improve the chances of a having a baby, the success rate decreases markedly with increasing age of the woman.

1. Intrauterine Insemination and Donor Insemination

Intrauterine insemination (IUI) is the simplest form of ART. Semen is collected from the male partner by masturbation and then the sperm are usually washed to remove dead cells and other possible deleterious components of the seminal plasma. The washed sperm are then placed directly into the uterus via a catheter which has been passed through the cervix. This serves to avoid any problems of passage of sperm through the cervix or cervical mucus, and provides a greater number of sperm within the uterus to increase the chances of fertilization. Intrauterine insemination is also commonly used in conjunction with ovulation induction, in order to ensure optimal timing of insemination.

Figure 12 shows that the age of the man can have a significant effect on the clinical pregnancy rate following IUI, but for any given age of the man, the clinical pregnancy rate decreases markedly with increasing age of the woman. Similarly, the pregnancy rate resulting from IUI with sperm from fertile donors is also significantly reduced with increasing age of the woman (Figure 13).

Figure 12. The effect of the woman's age on clinical pregnancy rate following intrauterine insemination with the partner's sperm (Brzechffa et al. 1998).

2. In-Vitro Fertilization

In-vitro fertilization (IVF) was originally developed to overcome the problem of blocked oviducts but is now also used to treat male-factor infertility (low numbers or quality of sperm) and infertility for which there is no apparent cause. In general, the woman is treated with gonadotrophins to increase the number of antral follicles that fully develop. It is important to note that gonadotrophin treatment has no effect on the numbers of primordial follicles that develop to the antral stage — it only acts to rescue the follicles that have already developed to the antral stage and would normally be lost to atresia. When the follicles have reached the appropriate size, the woman is given human chorionic gonadotrophin to mimic the normal ovulatory LH surge, and induce final oocyte maturation. A needle is used to recover the oocytes from the mature antral follicles. For standard IVF, the oocytes are placed together with sperm from the partner or a donor and the sperm penetrate the oocyte naturally. In cases where only small numbers or immotile sperm are available, fertilization can be achieved by injection of a single sperm into each oocyte (ICSI). After fertilization, the resultant embryos are cultured for 2 to 6 days and then transferred back into the uterus of the woman.

As shown in Figure 4, the number of antral follicles present on a woman's ovaries decreases with age and this results in a decreased number of oocytes that can be retrieved for following gonadotrophin treatment for IVF (Figure 14a). Moreover, the quality of the oocytes also decreases with increasing age (Figure 14b), resulting in a decreasing proportion of the oocytes that can be successfully fertilized in vitro (Figure 14c).

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Embryo development in culture is also affected by the age of the woman. In the example shown in Figure 15, the proportion of fertilized oocytes that developed to the blastocyst stage by Day 5 was significantly reduced with increasing age of the woman.

The effects of age of the woman on the outcome of ART using her own oocytes are strikingly demonstrated by the statistics for ART procedures for 2002 reported to the U.S. Centers for Disease Control (U.S. Department of Health and Human Services – Centers for Disease Control and Prevention 2004) that are shown in Figure 16. The data include a total of 81,888 treatment cycles which resulted in approximately 24,100 live births.

First, as shown in Figure 16a, the cancellation rate (treatment cycles in which no embryos were created or none were suitable for transfer) more than doubled for women 41-42 years old compared with women younger than 35. This reflects the decrease in the numbers and quality of oocytes retrieved with increasing age. For cycles in which embryos were produced, the proportion of those embryos that developed to the fetal stage (the implantation rate) decreased from 28% in women less than 35 years old to only 8% in women 41-42 (Figure 16b). Of the embryos that did implant, 38% were subsequently lost (miscarried) in women 41-42 years old, compared with only 14% in women younger than 35 (Figure 16b).

Overall the reduced implantation rate and increased fetal loss rate in older women resulted in only 5% of embryos transferred developing to a live baby in women 41-42 years old compared with 23% in women younger than 35 (Figure 16d). For women 41-42 years old, only 11% of cycles started yielded a live birth compared with 37% for women younger than 35 (Figure 16e). This would mean that on average, a woman 41-42 years old would need 12 IVF treatment cycles to have a 75% chance of one live birth, compared with only 3 treatment cycles for a woman younger than 35.

In addition to the decrease in live-birth rate with increasing age, pregnancies and babies resulting from ART in older women using their own oocytes are subject to the same problems of pre-term delivery, perinatal complications and chromosomal abnormalities seen with natural conception.

3. The Use of Donor Oocytes

In cases where a woman has no ovaries or is otherwise unable to produce her own viable oocytes, oocytes may be obtained from other women. The donors are most often anonymous fertile, young women but may be a relative or friend of the patient. The donor is treated with gonadotrophins and oocytes collected as described in the preceding section. Sperm from the patient's male partner is usually used for fertilization and the resulting embryos cultured and then transferred into the patient. Interestingly, it appears that the patient's age has no appreciable effect on the ability to support a pregnancy. The live-birth rate for women receiving embryos created from donor oocytes is approximately 50% at all ages from 25 to 45 (U.S. Department of Health and Human Services – Centers for Disease Control and Prevention 2004). Of course, the babies born from donated oocytes have no direct genetic relationship to the patient.

Figure 16. The effect of a woman’s age on the a) cancellation rate, b) implantation rate, c) fetal loss, d) approximate babies born per embryo transferred, and e) live birth rate with in-vitro fertilization of non-donor oocytes in the United States in 2002. (Taken or derived from: U.S. Department of Health and Human Services – Centers for Disease Control and Prevention 2004).
Clearly, common ART procedures can improve the chances of pregnancy but cannot overcome the deleterious effects of aging on numbers and quality of the oocytes. Based on a computer model, Leridon (2004) has calculated that ART can make up for only half of the births lost by postponing an attempt to become pregnant from 30 to 35 years, and less than 30% of the births lost by postponing from 35 to 40 years. Based on a literature review and their own data, Broekmans and Klinkert (2004) conclude that the prognosis for a successful pregnancy with IUI or IVF for women 44 or older “is flat zero.”

There are, however, two specialized ART procedures, pre-implantation genetic diagnosis and oocyte cryopreservation, that can, or have the potential to, circumvent the effects of aging on fertility.

IV. APPROACHES TO CIRCUMVENTING THE EFFECT OF AGE ON FERTILITY

1. Pre-Implantation Genetic Diagnosis

As noted above, the frequency of chromosomal abnormalities in oocytes increases with age in women, and this results in increased frequencies of chromosomal abnormalities in the embryos, fetuses, and babies born. An early approach to this was to obtain cells from the fetus by amniocentesis or chorionic villus sampling for evaluation of the chromosomes. Fetuses with abnormal numbers of chromosomes were then aborted, in order to prevent the birth of chromosomally abnormal babies. More recently, it has become possible to determine the chromosome status of early embryos produced by ART, before they are transferred back into the patient (preimplantation genetic diagnosis, PGD). In this case, only embryos with normal chromosome numbers are transferred.

A positive side effect of embryo selection following PGD is that the implantation and birth rates are increased because chromosomally abnormal embryos are often also developmentally compromised. An example is shown in Figure 17, where the implantation rate for embryos that had been tested and judged to chromosomally normal was 17.6% compared with 10.6% for embryos that had not been tested (and presumed to be a mixture of normal and abnormal embryos). Pre-implantation genetic diagnosis is usually used for couples with some history of chromosomal or other genetic defects, recurrent miscarriage, or in older women. Based on the improved rates of development following PGD, it has been suggested that all embryos should be tested.

**Figure 17.** Implantation rates for unselected embryos and for embryos judged as chromosomally normal by pre-implantation genetic diagnosis (Munné et al. 2003).

2. Oocyte Cryopreservation

When living tissues are deep-frozen (cryopreserved) under the appropriate conditions, all biological processes are arrested and aging of the tissue stops until it is thawed. This approach has been long used for the storage of sperm and embryos, and has recently been extended to oocytes. A major interest in oocyte cryopreservation is to preserve the possibility of fertility for young women that are due to undergo radiotherapy and chemotherapy for the treatment of cancer. Such treatments can have severely deleterious effects on the oocytes. By removing and freezing the oocytes, they are not exposed to the cancer treatments. After the patient has recovered from the cancer treatments and wants to start a family, the oocytes can be thawed and fertilized, and the embryos transferred back into her uterus.

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In the same way that cryopreservation can protect oocytes from cancer treatments, it could also be used to protect oocytes from natural loss and degeneration due to aging. Stacheccki and Cohen (2004) have suggested that this may offer an approach to preserving fertility for women wishing to delay reproduction. Oocytes would be collected from young women and then cryopreserved until they are ready to begin their families. Although as yet largely experimental, the pregnancy rates from cryopreserved oocytes are improving.

V. CONCLUSIONS

There is a tendency for women in industrialized countries to delay having babies until their mid-thirties or later. There are important social and economic reasons for doing so, but it is imperative that women be aware that fertility decreases significantly with age, particularly after 35 years of age. From a purely biological perspective, the best approach to ensuring fertility is for women to have their babies before they have reached their mid-thirties, but for many women, this is not a desirable or even practical option. At any given age, assisted reproduction techniques may improve the chances of becoming pregnant, but cannot make up for the loss of fertility due to the effects of aging on the numbers and quality of oocytes.

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On March 29, 2004, the Canadian parliament passed the Assisted Human Reproduction (AHR) Act into law to govern clinical and research activities related to assisted conception. This legislation has a comprehensive, regulatory and licensing framework for most AHR clinical activities. In addition, it explicitly prohibits certain specific activities, including the purchase of sperm and eggs for use in fertility treatment. For both providers and recipients of Donor Insemination (DI) services, this law has raised anxiety about the future availability of DI. Most of the concerns have so far focused on whether the prohibition on payment for gametes will make this option less accessible. However, other legislative and regulatory requirements of the Act also have major implications for how DI will be provided in the future.

**Governing Principles**
The Act is founded on a set of principles, requiring that consideration of the health and well-being of children be given priority. In addition, there must be ‘protection and promotion of human health, safety, dignity and rights’. While it might be difficult to argue against such principles, their interpretation and application may present considerable challenges. Some of these relate to the issue of defining and assessing the potential health and well-being of children not yet born, and the question of whose responsibility it will ultimately be to make such assessments. Donor offspring in the future might also contend that allowing gamete donors to choose on the matter of anonymity could compromise their health and safety, in terms of being denied access to a full genetic history. Those born from donors who are not anonymous will, in theory, have easier access to this information.

**Implications for Donor Counselling: Section 14:2 (b)**
While the definition of ‘donor’ under the Act is confusing in that it refers to both autologous and third party donation, there is a clear requirement for semen donors (and recipients) to receive counselling. Although technically much simpler than oocyte donation, the social and emotional consequences of sperm donation are just as complex as those for oocyte donors. However, more attention has often been paid to the counselling of oocyte donors, than that of semen donors. While space precludes a more in-depth examination of this anomaly, the fact that semen donors and recipients must in future receive counselling represents a significant change for some clinics.

It should be noted that counselling under the Act refers to activities only before the donation (or receipt) of semen. More detailed regulations about counselling are currently under development, but may address a clearer definition of counselling for the purposes of legislation, how it is to be provided, and by which groups of professionals. Although relatively few Canadian counsellors are experienced in this field, the regulations will not address the training needs of newer counsellors, or the issue of accessibility to counselling services by those needing them.

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Implications for the Privacy of Health Information (Section 17)

There has been considerable concern expressed about the security and privacy needs of those whose names will be contained in the future information registries. However, the Federal Privacy Commissioner reviewed the legislation and was satisfied with provisions for security and privacy. One implication of the birth registry for donor conceptions will be the need for more precise tracking of DI birth outcomes than has occurred previously. Follow-up of patients in regard to pregnancy and birth outcomes will be mandatory, and there will be a corresponding need for education to help donors and recipients understand how their personal information will be used.

Once the register for donor births is established, it is likely that limits will be placed on the number of offspring per donor. Regardless of whether this will be expressed in terms of the numbers of family units, or of total offspring, the number of DI offspring permitted from each semen donor is likely to be less than occurs currently. While this adds to concerns about future supply, it is essential to have a process for limiting the number of births per donor. Exactly how this can be adhered to, however, is likely to prove a further challenge if several clinics are simultaneously utilizing semen samples from the same donor.

Furthermore, if sperm from US cryobanks is to continue to be permitted, then there should be compliance with Canadian law.

To enforce these regulations, Health Canada undertakes different inspection regimes and frequencies for sperm processors and importers (yearly), distributors (2-year cycle), and health care facilities that use the donor semen for direct patient care (3-year cycle). The 2000 Semen Regulations currently remain in effect, but their future relationship to the AHR Act is unclear. Furthermore, the logistics for enforcing the non-payment of sperm donors by USA sperm banks are yet to be defined, but will presumably be regulated under the new Act. Given that Canadian clinics are heavily dependent on these sources for the supply of donor semen, it would be important to plan the enforcement of this aspect of the Act carefully in order to avoid abrupt disruptions in the supply of donor sperm for patients. Whatever one’s perspective about commercialization, the lengthy history of payment for semen has contributed to a culture of acceptance about payment, and skepticism about the feasibility of an altruistic model. Furthermore, increased reliance on USA sources by Canadian clinics means that few clinics have had recent experience of donor recruitment.

If disruptions occur and clinics have to develop their own sperm banks, this will impose several consequences including: 1) a change in the clinic's inspection status...
under the current Semen Regulations; 2) availability of reliable altruistic semen donors, especially in small centres; and c) the ability of clinics to afford the high initial investment cost in developing the sperm bank. Canadian fertility clinics, their patients, as well as their donor sperm suppliers are likely operating on the assumption that Health Canada will provide advance notification about their implementation schedule and strategy in order to avoid disruptions in donor sperm supply.

Implications for Recruitment of Sperm Donors
Health Canada has indicated that the legislation is not aimed at hindering Canadians in their ability to access fertility treatment. In response to concerns from various groups, the Assisted Human Reproduction Implementation Office in 2004 sponsored a review of sperm donation practices around the world, with a view to assessing the feasibility of altruistic sperm donation in Canada. This review identified 22 studies of sperm donation practices in 8 countries, concluding that sperm donation without payment (altruistic) is possible, and indeed is practised in some European countries, Australia and New Zealand. In particular, the altruistic system of semen donation has been the only system in place in France for the past 31 years.

Issues identified in these studies that are relevant to sperm donor recruitment include: 1) the motivation for donors who are married men with children was primarily altruistic, while the motivation for younger men was payment; 2) older donors were more likely to know persons who have experienced infertility; 3) some donors who were motivated primarily by payment, also indicated a willingness to donate without payment; 4) altruistic semen donors tended to place a limit on the number of children born from their donation; 5) a successful altruistic donor system requires an advertising campaign to enhance the public perception of semen donation.

These studies suggest that, while successful recruitment for the altruistic donor system is possible, it will not be easy. The recruitment strategy and the target population for the altruistic system may need to be considerably different from the current payment system. Given existing screening standards, the recruitment of acceptable semen donors is likely to be a time-consuming and labour-intensive activity, far removed from the practice of 20 years ago. In addition to a commitment to this new approach, donor recruitment in the future is likely to require significant increases in both personnel and overall costs.

A recent pilot study on altruistic semen donation in Canada concluded that the majority of prospective semen donors are unwilling to participate purely altruistically, independent of age or occupation. However, this study was targeted to the general population with no identified recruitment strategy suitable for the potential altruistic donor population, as suggested by the previous studies. Health Canada is planning to sponsor a study of altruistic sperm donation strategies, but has indicated that government will not participate directly in donor recruitment campaigns. However, if this legislated change in the sperm donation system is to be successful, there is still a critical role for Health Canada through funding for national initiatives, such as a public education and awareness campaign.

In summary, the wider implications of the Assisted Human Reproduction Act for Donor Insemination have received much less attention than that given to the issue of donor payment. While there is no clear indication of what will replace the current commercial semen bank model in Canada, a more comprehensive, adequately-funded, strategy than a single ‘pilot project’ is clearly needed. If there is a sincere desire on the part of government to avoid the growth of ‘reproductive tourism’, and ensure access to fertility treatment services, then a ‘made in Canada’ solution to these issues is essential. A greater focus on educating and informing the public and the various stakeholders concerned would go a long way towards alleviating current anxiety.

References

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Stress is defined as a mentally or emotionally disruptive or upsetting condition occurring in response to adverse external influences that can affect physical health. The stress response to external stimuli is usually characterized by increases in respiration and heart rate, blood pressure, muscular tension, irritability, and depression. However, stress should be balanced by that of a relaxation response; a reduction in the activity of the autonomous nervous system producing a decrease in heart rate, rate of breathing, muscle tension, etc. This opposing response occurs when the body no longer perceives stress.

The effects of chronic stress on the body are similar to that of the flight or fight response. Women, stress can alter the delicate milieu of reproductive hormones, resulting in ovulation dysfunction and irregular menstruation. In men, a reduction in testosterone in response to stress may affect the quality of sperm; however, stress can also interfere with intimacy as sexual function may be impaired.

Many of us encounter modern day stresses including employment, commuting, finances, family, and friends. These stressors can lead to improper diet, excess caffeine consumption and interruptions in rest or exercise. Stress can have a profound affect on our general health and our lives.

Many couples experience difficulty achieving a pregnancy; their lives are full of promise with the thoughts of becoming parents or extending their current family. Family building seems so natural, but, it may become stressful when dreams are unfulfilled. When difficulties arise with fertility, always remember to seek medical advice from a physician. Women under 35 should seek treatment for infertility after 1 year of trying to conceive, women 35 to 40 years of age after 6 months, and women over 40 after 3 months.

Because infertility affects approximately 10% of the population of reproductive age (CDC 1995), it is not uncommon to know someone who is experiencing difficulty in achieving a pregnancy. Stress may cause, or contribute to, the underlying diagnosis of infertility. Lifestyle stressors such as employment, personal relationships, diet, exercise and even environmental quality are numerous and affect not only our general health but that of our reproductive health.

Although infertility can be a highly stressful experience for a couple, there is little scientific or medical evidence to substantiate
claims that infertility is caused by stress. While the relationship between stress and infertility may not conclusively be defined, one cannot dispute that the stress in our daily lives is real.

Learning to cope effectively with stress is an important strategy for the quality of anyone’s life. Managing stress levels should be individualized; determining what is most effective for each person or couple. In 1978, the Mind-Body Institute of Harvard Medical School created a ten week program for women diagnosed with unexplained infertility. The program emphasized stress relief and employed guided imagery, yoga, balanced nutrition and education on topics of fertility. Although the program was intended to instruct the participants in stress management, 57% of the women became pregnant within six months of the completion of the program.

Stress management programs in general may be beneficial by simply reducing the stress in your life, whether or not it is associated with infertility. These programs may be structured, purchased regimens, or minimal lifestyle changes that can markedly improve your quality of life. Guided imagery audio CDs are very relaxing and cost effective. Hypnotherapy offers you progressive muscle relaxation and guided imagery. Reflexology for your feet, hands and head is very soothing while massage therapy or aromatherapy may be preferable. Exercise, yoga, tai-chi or leisurely walks can assist to release physical and emotional tension. Deep breathing exercises along with meditation may quickly lower acute stress levels.

It is important to point out that stress management programs are not a cure for infertility, but they may reduce the stress in your life which may, in turn, help normalize menstrual cycles and improve oocyte and sperm health. Reducing the stress in your life may also mean an improvement in the quality of your relationships and your family life in general.

Whatever the cause of the stress in your life, reducing it will be very beneficial. Experts say that a little bit of stress in our lives may be beneficial, but we need to learn how to keep it in check.

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FERTILITY MAGAZINE • VOLUME 7 • WWW.FERTMAG.COM
Will you be Fertile in 5 Years?

If you’re trying to plan a family, you need to know what your long-term – and short-term – fertility outlook is. Take our quiz to find out what your fertility future holds.

1. How old are you?
You probably already know that by your early 30s your fertility has started to decline. If you’re planning a family, it’s important to keep this in mind. While it’s a gradual process, and your fertility potential won’t vanish overnight, the longer you wait to conceive, the more difficult you may find it to get pregnant. Risk of miscarriage increases as you get older, too. (By age 42, the risk of miscarriage can be as high as 50 percent, according to one Danish study.)

2. Do you smoke?
- Yes
- Sometimes
- No
Smoking – even occasionally – can make it harder for you to get pregnant whatever your age might be. Study after study has shown that smokers find it more difficult to conceive, are more likely so suffer miscarriage and can have more problematic labors, including premature birth and fetal problems. The good news? Stop smoking now, and your fertility can return to normal - it’s not instantaneous, but within a few months, you’ll likely be back in the normal range.

3. What’s your BMI?
- 19 to 29
- Less than 19
- Great than 29
Maintaining a healthy, normal weight – with a BMI between 19 and 29 – is one of the best things you can do to protect your fertility. Being overweight or underweight can affect your ovulation patterns, making it much harder for you to conceive.

4. Have you ever had an STD?
- Yes
- No
Most of the time, an STD won’t affect your fertility in the long-term. The exception? If you have an STD that’s gone untreated for a long time, you may find it harder to get pregnant. That’s why it’s so important to pay regular visits to your OB/GYN, even when you’re not thinking about making a family.

5. Is your period regular?
- Yes
- No
- I don’t know
A major clue to long-term fertility outlook lies in your menstrual cycle: if your cycle is a regular one, lasting somewhere between 26 and 34 days, your reproductive system is likely in good working order. Longer or shorter periods could be a sign of ovulation problems, which could make it difficult to conceive.

This article first appeared in Women’s Health & Fitness. To subscribe call 1-888-881-5861.
has
infertility touched
your life?
or the life of someone you know?

RESOLVE: The National Infertility Association is here to help. For more than 30 years we have been providing resources, support and advocacy to thousands of men and women. Join today to get the support and the information you need—and deserve—through RESOLVE membership benefits.

www.resolve.org
Any time, day or night, you can access up-to-date information like local chapter events, state insurance coverage availability, publications and brochures, and participate on our bulletin boards by asking questions or sharing experiences with other members.

Subscription to Family Building
Get the latest information on all aspects of infertility treatment, adoption and childfree living. Family Building deals with the important issues surrounding infertility, your health and wellness, insurance and legal issues and much more. The leading publication for infertility information arrives at your home four times a year, wrapped to retain your privacy.

Local RESOLVE Support
Membership in RESOLVE entitles you to also have membership within your local chapter. For a list of the chapter nearest you, please visit www.resolve.org.

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Your RESOLVE membership adds strength to our collective voice for insurance coverage for infertility treatment and passage of favorable laws.

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When you have questions about your treatment, adoption, or childfree living, or just need to talk to someone who has been there, informed answers are just a phone call away. Monday 9AM – NOON, 1PM – 4PM, 7PM – 10PM, Tuesday 1PM – 4PM, 7PM – 10PM, Wednesday through Friday 1PM – 4PM, and Sunday 8PM – 10PM. (All times ET.)

Spanish HelpLine Hours: 1st, 2nd and 3rd Thursday of each month, 7PM – 10PM ET.

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- Member-to-Member Contact System
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Debrah Nagy, CH, CI

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**Your Personalized copy of “Inner Metamorphosis” can be purchased at: www.PersonalDynamix.com**

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**About the Author**

Debragh Nagy has been writing short stories, poetry and articles for most of her life. She is a Reiki Master, Certified Reflexologist and a Certified Hypnotherapist as well as instructor and has been working in the field of alternative healing for quite some time.

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**The Male Biological Clock** can be purchased at: www.malebiologicalclock.com

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**About the Author**

Harry Fisch, MD, Columbia University Medical Center, NY, author of “The Male Biological Clock”, is one of the nation’s leaders in the diagnosis and treatment of male infertility.

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Saturday, October 27, 2007

The New England Infertility and Family Building Conference

RESOLVE of the Bay State presents, The New England Infertility and Family Building Conference, Saturday, October 27, 2007 at the Best Western Royal Plaza Hotel, Marlborough, MA.

Please join us for this day-long educational event, with over 40 workshops covering all aspects of medical treatment, emotional and legal issues, and donor egg and adoption options.

If you are new to infertility or in the midst of the process and are facing difficult decisions, the volume of information and the range of feelings you must sort through can be overwhelming. The Annual Conference provides the information you need in a compassionate context with people who know what it is like to face this crisis. The conference will help you become an informed consumer of infertility treatment and services and will help you meet the challenge of your infertility and make the best possible choices.

For more information about conference workshops, fees, and registration, please visit: www.resolveofthebaystate.org or email us at admin@resolveofthebaystate.org

Pre-registration is strongly recommended.
OVERALL OBJECTIVES

During or at the conclusion of the 2008 PCRS Annual Meeting, program participants will be able to:

- Discuss the historic context of current medical practices in the field of Reproductive Medicine and Infertility.
- Evaluate new technologies in Reproductive Medicine, especially DNA arrays, PGD and Robotics.
- Assess economic factors influencing reproductive practice management.
- Evaluate the scientific basis for integrating Acupuncture into IVF practice.
- Participate in Hands-On workshops:
  - The Economics of Practice
  - Acupuncture
  - PGD
  - Robotics in Reproductive Surgery

Singapore warmly welcomes Asia Pacific’s outstanding medical professionals in the rapidly evolving field of reproduction, infertility and O&G convening for the 2nd Congress of the Asia-Pacific Initiative on Reproduction (ASPIRE) and the 6th Biennial Meeting of the Pacific Rim Society of Fertility and Sterility (PRSFS) held from 11 - 13 April 2008. Opening on 11 April, the two events, held conjointly, will offer opportunities for networking, exchange of ideas and in-depth participation in discussion forums and courses.
CONFERENCES

New England Fertility Society

SAVE THE DATE - CALL FOR ABSTRACTS

Assessing ART Outcomes:
Thirty Years After the Birth of Louise Brown

May 2-3, 2008
Woodstock Inn and Resort, Woodstock, VT

6th Annual Assembly of the New England Fertility Society

European Society of Human Reproduction & Embryology

Welcome to ESHRE 2008 - Barcelona

24th Annual Meeting

July 6–9, 2008

CFAS 2008
54th Annual Meeting
November 26–29, 2008
Calgary, Alberta

ACE 2008
January 3–4, 2008
Manchester, UK
"20th Years" in Assisted Reproductive Technologies

2nd CURRENT OPINIONS IN INFERTILITY AND ASSISTED REPRODUCTIVE TECHNOLOGIES CONGRESS

17 - 20 April 2008
Sheraton Hotel Çeşme
Izmir - Turkey

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PROF. EROL TAVMERGEN, MD

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