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History of KSOM, A Single Medium for Embryo Culture

by John D. Biggers DSc, PhD
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Since 1993 a family of media for the culture of preimplantation embryos has been designed that has acquired the generic name KSOM (Biggers, 2002). The initial work on the design of these media was done as part of the National Cooperative Program on Non-Human In Vitro Fertilization and Preimplantation Development, sponsored by the NICHD between 1986 and 1996, with the objective of improving embryo culture. Since the original formulation of a medium called SOM (Lawitts and Biggers, 1991), four modifications have been published: KSOM (Lawitts and Biggers, 1993) mKSOM (now called KSOMg) (Summers et al., 1995) KSOMAA (Ho et al., 1995, Biggers et al., 2000) KSOMgAA (Biggers and McGinnis, 2000).

In the early days of preimplantation mouse embryo culture, heralded by the pioneer paper of Whitten (1956), a major problem had been an arrest of development at the two-cell stage, called the two-cell block. Over the years several seemingly unrelated ways of overcoming this block were described (review: Biggers, 1993), which led to the notion that the media being used were unbalanced and hence not optimal. Optimizing culture media in general is a complex matter because of the need to take account of the possible interaction of effects between its components (Biggers et al., 1957). This is a general problem in many areas and was first studied in the optimization of industrial processes such as the manufacture of chemicals. One solution that was introduced was called sequential simplex optimization (see sidebar). Our research involved determining the proportion of embryos that developed beyond the two-cell stage as the concentrations of constituents in a start medium were simultaneously varied, according to the rules determined by the optimization protocol. After twenty iterations, taking about two years, a medium called SOM was developed which overcame the two-cell block (Lawitts and Biggers, 1991). Soon after, the composition of the medium was slightly modified to formulate KSOM (Lawitts and Biggers, 1993), based on measurements of the intracellular ionic composition of the blastomeres determined by electron probe microanalysis. An unexpected bonus of this research was the fact that KSOM supported a high yield of mouse blastocysts from zygotes that were capable of developing into fetuses after transfer into the uterus of surrogate mothers (Erbach et al., 1995). The medium was greatly improved when Ho et al. (1995) supplemented KSOM with 19 natural amino acids (glutamine was already in the medium). In another study very high yields of expanded blastocysts were obtained containing nearly double the numbers of inner cell mass and trophectoderm cells compared to KSOM alone (Biggers et al. (2000a). This medium is called KSOMAA.

A variant of KSOM (mKSOM) was introduced to support IVF in the mouse. The original version of KSOM contains a low concentration of glucose (0.2mmol/l), which does not support the viability of sperm and therefore cannot be used for IVF. When the concentration of glucose in KSOM was raised to that found in blood (5.56mmol/l), IVF in the mouse was successful (Summers et al., 1995). This result was particularly surprising since this concentration of glucose did not prevent the subsequent cleavage divisions, contrary to the widespread dogma asserting that glucose inhibits the development of the preimplantation embryo. Subsequent studies have confirmed that glucose in a concentration as high as 5.56mmol/l in KSOMAA does not inhibit the early cleavage divisions of the mouse embryo (Biggers et al., 2000b). This medium is now called KSOMgAA.

The inclusion of glutamine in media used for the culture of cell lines has always been of concern because its instability leads to the formulation of a medium called SOM (Lawitts and Biggers, 1991), four modifications have been published: KSOM (Lawitts and Biggers, 1993) mKSOM (now called KSOMg) (Summers et al., 1995) KSOMAA (Ho et al., 1995, Biggers et al., 2000) KSOMgAA (Biggers and McGinnis, 2000).

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The inclusion of glutamine in media used for the culture of cell lines has always been of concern because its instability leads to
accumulation of ammonium in the media. Gardner and Lane (1993) alerted the IVF community to the putative dangers of including glutamine in embryo culture media by reporting a disturbing incidence of exencephaly in mice whose preimplantation embryos had been cultured in a medium containing the compound. More recent studies, by two independent laboratories, have failed to detect any incidence of gross abnormal development in fetuses and newborn mice whose preimplantation embryos have been cultured in KSOM-type media. Possible reasons for the discrepancies have been discussed by Biggers et al. (2004a). Nevertheless, concern about the potential adverse effects of glutamine in media can be alleviated by replacing glutamine with a dipeptide containing glutamine. Alanylglutamine is commonly used for this purpose. However, glycyglyutamine may be preferable since there is evidence that it favors the development of the ICM (Biggers et al., 2004b). Glycyglyutamine is therefore included in all currently used variants of KSOM in our laboratory.

It has become almost universal to use a two-step protocol when it is desired to culture human zygotes to the blastocyst stage following the early recommendations of Gardner (1998). The protocol involves the sequential culture of the embryos in media of different chemical composition. There are several pairs of such media available commercially. The justification put forward for changing the medium in the middle of the culture period is either to remove putative toxic substances that have accumulated, or to imitate the natural environment which changes as the embryos pass from the oviduct into the uterus. Unfortunately there have been few experimental investigations to verify the need for a two-step protocol. A paper that has been submitted for publication describes results which show that there is no gain in renewing KSOMAA during the culture of the mouse preimplantation embryo. The studies of Biggers and Racowsky (2002) using KSOMAA, and Macklon et al. (2002) using the so-called “Rotterdam” medium, failed to show an advantage of the two-step procedure, for the culture of human preimplantation embryos, suggesting that a more thorough examination of the practical advantage of two-step procedures should be undertaken.

The ultimate medical objective of treatment for infertility is the production of normal healthy babies. The techniques used to evaluate treatment protocols has involved such parameters as the rates of embryo development before transfer, and the rates of biochemical pregnancies and delivery rates. There has always been a lingering concern that some constituents of media may have deleterious effects (review: Summers and Biggers, 2003), particularly epigenetic effects that may be passed on to later generations (review: Johnson, 2005). Advances in molecular genetics are beginning to open up studies on these questions. For example, Rinaudo and Schultz (2004) have clearly shown that media can effect the expression of genes in mouse blastocysts cultured from the zygote to the blastocyst stage; 114 genes were mis-expressed in Whitten’s medium and only 29 genes in KSOMAA. Whether this difference can account for the better development in KSOMAA needs detailed analysis.

References


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Imagine a medium with only two components. There are an infinite number of media possible, each defined by the paired concentrations of each of the two components. Imagine also that we know the response of an embryo when cultured in each medium. Then the totality of responses can be represented by a concentration-response surface (Figure 1). We assume that the maximum response is the optimum response. How do we locate the maximum of the concentration-response surface?

With only two components we can choose a set of media that form a grid over the surface. The grid defines a set of media to be compared in a factorial experiment. From the results of this experiment a concentration response surface can be fitted and the maximum located.

When media contain more than two components, the concentration surface will be multi-dimensional. In producing SOM we optimized 10 media so the response surface was modeled in 11-dimensional space. Locating a maximum in this space is logistically impossible using a set of factorially arranged media since a prodigious number of media combinations would be required. An alternative approach is to climb sequentially the surface. Sequential simplex optimization is one of several algorithms that allow this climb to be made.

Data already obtained is used to locate a point of the putative hill; this point defines the composition of a START medium (Figure 1). If the medium consists of two components only, a set of three media are chosen in the neighborhood of the START medium, defined by the loci of three vertices of a triangle (a triangle is a simplex in two-dimensional space). The responses to these media, (in this case passage through the two-cell block) are observed experimentally and the medium that gives the worst response is identified. A new medium is then determined from these results which is higher up the hill, generating a new simplex consisting of the two non-rejected original media and the new medium. The new set of three media are then compared and the experimental procedure repeated. By repeating this procedure the hill will be climbed and its top reached. In the case of 10 media, 11 media are compared at each step. A detailed description of the procedure can be found in the website http://www.multisimplex.com/algorithms.htm.

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FIGURE 1
Oxygen & Embryo Culture

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Since the inception of IVF, it has been typical to use a gas phase of 5 to 6% carbon dioxide in air, a common practice of tissue culture laboratories. This approach has resulted in thousands of successful pregnancies. Therefore, it is evident that ambient oxygen is compatible with embryo development. With that said, why should we consider culturing human embryos at concentrations of oxygen below that of air (which is around 20%, although this depends on where you live, of course. Those of us at altitude already have an oxygen deficit!). Well first of all, there is no tissue in the human body, save the alveoli of the lungs, that comes into contact with such high oxygen concentrations. Furthermore, oxygen is an aggressive molecule, giving rise to superoxide radicals and numerous harmful intermediates, all capable of inducing several different pathologies. To this end, aerobic organisms have had to develop an antioxidant strategy, that includes several enzymatic systems (see section below on antioxidants).

Significantly, analysis of the levels of oxygen within the female reproductive tract has shown that gametes and embryos in situ never see an oxygen concentration greater than 10%, and typically less than this. For example, the concentration of oxygen in the lumen of the rabbit oviduct is 2-6% (1,2), whereas the oxygen concentration in the oviduct of hamster, rabbit and rhesus monkey has been determined to be 8% (3). Of interest, the oxygen concentration in the uterus is lower than that in the oviduct, ranging from 5% in the hamster and rabbit, to 1.5% in the rhesus monkey.

Furthermore, several studies on the preimplantation embryos of different mammalian species have clearly demonstrated that culture at a reduced oxygen concentration (5 to 7%) results in enhanced embryo development in vitro (mouse, (4-6); sheep and cow, (7); goat, (8); human, (9)). More significantly, exposure to 5 to 7% oxygen in vitro leads to higher fetal development following embryo transfer (6,9,10). However, the embryos of the human and certain F1 mice can develop well in culture in the presence of an atmospheric oxygen concentration (20%). This has therefore produced some confusion regarding the optimal concentration for these species. Interestingly, unlike mouse embryos from an F1 strain, the embryos derived from outbred mice, such as CF1, develop significantly better in culture in a reduced oxygen environment (11). In our own experience, human embryos cultured in a low oxygen environment (5%) produce more blastocysts, with significantly more cells, than those embryos cultured in a high oxygen environment (20%) (9,12).

More recently, it has been shown that oxygen affects gene expression in the preimplantation embryo. Harvey et al. (13), have demonstrated an effect of oxygen concentration on the expression of hypoxia-inducible factors in bovine embryos. Using gene expression arrays, we have observed a significant difference in the expression of several genes when mouse zygotes were cultured to the blastocyst stage in either a 5% or 20% oxygen environment. It was determined that nineteen genes were up-regulated in the 20% O2 group, while twelve genes were down-regulated compared to the 5% O2 group (14).

Antioxidants
Several studies have examined the effect of known antioxidants on preimplantation embryo development, although the data to date remain rather controversial. Supplementation of medium with superoxide dismutase (SOD), which dismutates superoxide radicals, increased the development of mouse zygotes beyond the 2-cell block to the blastocyst stage (15,16). However, several studies have reported that SOD had no effect on mouse (17), rabbit (18) or bovine (19) embryo development in vitro. Similarly, Legge and Sellens (20) reported that addition of glutathione to the medium stimulated development of mouse zygotes in culture, whereas Nasr-Esfahani and Johnson (21) reported that the addition of glutathione to the medium did not increase embryo development in culture. Glutathione is present in fluid of the reproductive tract and therefore may have a role in embryo development (22). Moreover, the beneficial effects of the addition of cysteamine to the medium for bovine (23) oocyte development have been attributed to an increase in intracellular glutathione levels (24). Therefore, it is feasible to suggest that the maintenance of a high intracellular pool of glutathione may be important for high rates of development of the oocyte and early embryo.

The conflicting reports as to the benefits of addition of antioxidants to culture media may in part be explained by their use in isolation and not as part of a more complete antioxidant system. For example, when SOD is present to dismutate superoxide radicals to hydrogen peroxide, then catalase and/or glutathione may be required to remove the peroxide formed.
Alternatively, the generation or otherwise of superoxide radicals will depend on the medium used for culture.

Interestingly, although it has been shown that pyruvate when present in the culture medium is a powerful antioxidant (25) and readily decreases intracellular hydrogen peroxide levels within the embryo (26,27). Since pyruvate is present in all media for embryo development then, by default, embryo culture media are supplemented with an antioxidant. Similarly, the amino acid taurine present in such media can function as an antioxidant (19).

Therefore, perhaps it is serendipitous, that embryo culture media have always contained powerful antioxidants, and may help explain the ability of embryos from certain strains/species to tolerate higher oxygen in the culture environment.

Conclusions

Gone are the days when we can remain stationary when there is growing data from animal models to indicate that there can be improvements in human ART. For some of the systems currently being used in human IVF, such as atmospheric oxygen, there is increasing animal and human data to indicate lower levels of oxygen are less stressful and more effective. Arguments of costs cannot be indulged as a modern tri-gas incubator is now only a few hundred dollars more than a conventional tissue culture incubator. A practical alternative for clinics with small patient volume, is to use a modular chamber (or glass desiccator, with which Drs. Edwards and Steptoe used so effectively to grow Louise Brown), which can then be purged with a premixed gas cylinder. This also has the added advantage of providing a pure gaseous environment, and can greatly assist in trouble shooting in an IVF laboratory; as such chambers effectively isolate the embryos form the incubator and laboratory air supply. This is a useful strategy when setting up a new laboratory.

Finally, from our own animal studies, we have shown that 20% oxygen has a negative effect on embryo development at all stages. Therefore, moving to a reduced oxygen concentration is not something that is required solely for extended culture, but is something that should be adopted for all embryonic stages.

References


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Techniques for the culture of ovaries have been described for almost 70 years. At first researchers attempted to culture whole ovaries, with limited success. Then the idea of culturing ovarian sections was attempted to overcome the problems of maintaining the large mass associated with the whole ovary culture. While follicles can be successfully grown with this method to the antral stage it is difficult to track the growth of individual follicles. This is further complicated by the cross-talk of paracrine growth factors between follicles when cultured in groups. Hence, development of the isolated whole follicle culture system has evolved. The isolated follicle culture assay (IFCA) is based on the use of a homogenous narrow class of intact early pre-antral follicles of approximately 80 to 120 µm in diameter (Figure 1). Selection of such a narrow class of follicles that are isolated from the ovary and cultured either in 96-well plates or micro-drops allows for the observation of individual follicle dynamics under various culture conditions. Methods for the recovery of immature follicles are most advanced and successful in rodent models. In general, the theca cell layer and basement membrane, of small ovarian sections, is degraded by a short exposure to collagenase. Further manipulation with 28 gage needles or small pulled glass pipettes allows for the recovery of follicles of the desired size with an intact theca layer for culture. The mouse follicle culture system allows growth and development of intact early pre-antral follicles up to the ovulatory stage. At the end of 13 days of culture, mature metaphase II oocytes can be harvested after induction of ovulation by an ovulatory stimulus. From these oocytes, healthy mouse pups have been obtained by IVF and transfer of embryos into pseudo-pregnant foster mothers (Cortvrindt and Smitz, 2002; O’Brien et al., 2003).

Reproductive Biology Applications
A key feature of the IFCA is that individual follicles can be observed daily, both morphologically and biochemically, throughout the entire culture period, at the end of which oocytes can be harvested for further analysis. During culture the follicles can be exposed to test compounds either continuously, or specifically at a defined phase of development. Test compounds could be drugs potentially important to fertility, reproductive toxicants or dietary factors thought to affect fertility. The IFCA provides insight into the mechanism of beneficial versus deleterious effects of a test compound by allowing one to focus on: (i) the cell type affected; (ii) the vulnerable stage of folliculogenesis; (iii) the effect of peripheral pathways such as steroid or protein production; (iv) oogenesis (affect on meiosis); (v) and mechanism of cell survival versus death (apoptosis). The value of the IFCA as a tool in reproductive biology has been demonstrated by several groups (Cortvrindt and Smitz, 2002) including Sun et al., (2004) who recently used it to identify direct and indirect effects of environmental chemicals on the somatic compartment, the follicle and

Figure 1: Isolated rat follicle approximately 100 um in diameter. The developing oocyte is visible in the centre surrounded by granulosa cells in an envelope of theca cells.
Summary

In vitro follicle culture systems have been developed with the aim of growing immature oocytes from early follicle stages to fertilizable oocytes. They have tremendous potential to be a valuable tool to study the basic mechanisms involved in the process of folliculogenesis and oogenesis. Great potential exist to develop these technologies from the experimental phase to clinical applications. Before this application becomes routine it must first be perfected and proven to be safe in larger animal models and primates. We are still a long way from being able to use follicle culture as a strategy to obtain competent oocytes. Investigation of the impact of in vitro culture on the health of the offspring, which includes studies on epigenetics and gene expression, is necessary to advance this technology to clinical application.

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Neal MS, Zhu J, Stampfli MR, Holloway AC, and Foster WG. (2004) Mechanism of cigarette smoke toxicant (benzo-[a]-pyrene) impairment of folliculogenesis in vitro (Neal et al., 2004). Therefore, this assay is a valuable in vitro test of ovarian function and response to test chemicals since it can provide information on mechanisms that would be both difficult and inefficient to aquire using an in vivo approach.

Preservation of Fertility

Down the road another potential application of follicle culture is in the area of fertility preservation. Ovarian cryopreservation and improvement in follicle culture efficiency offers exciting and practical applications for rescuing fertility. Banking of ovarian tissue is considered a potential method for safeguarding fertility of young women undergoing sterilizing chemotherapy and radiation treatment for cancer and other diseases. Freezing ovarian tissue has the advantage that the ovarian cortex contains the majority of primordial follicles which can be frozen with relatively good success. In the past decade there have been many reports of successful cryopreservation and autografts in many species including humans. However, in view of the tremendous wastage of follicles during transplantation ischemia and the risk of returning the disease by autografting, the ability to grow and mature follicles in vitro from frozen ovarian tissue would be desirable. Not only would this capability be efficient, but also it would eliminate the risk of reintroducing the disease to the individual if in vitro matured oocytes are used in combination with IVF to produce embryos for transfer back to the patient. However, for the foreseeable future transplantation remains the only practical solution since IFCA technology needs to be refined so that precious germ cells can be used most efficiently.
The current topic for The Patient’s Corner is polycystic ovarian syndrome, or PCOS, which is the most common endocrine disorder in women of reproductive age, afflicting between 5-10% of premenopausal women.

Stephanie, a 34-year-old, and her husband Gerry were referred to a Reproductive Endocrinologist. Stephanie is suffering from primary infertility of 2 years duration. Based upon her irregular menstrual cycle, she was diagnosed by her primary gynecologist as having PCOS. She underwent 4 cycles of Clomid therapy which unfortunately did not result in pregnancy. Stephanie reports only having 3-4 menstrual cycles per year, experiencing menstrual bleeding mostly after withdrawal of progesterone supplementation (Provera). She has experienced a weight gain of approximately 40 lbs over the past 48 months.

Following a complete history and physical and laboratory evaluation, the diagnosis of PCOS was confirmed. Stephanie was a moderately obese patient with a definitive male pattern hair growth. Briefly, laboratory analyses revealed an elevated level of male hormones (androgens), an elevated fasting insulin/glucose ratio and no evidence of ovulation. Testing ruled out other ovarian disorders or disorders of the pituitary or adrenal glands. Ultrasound evaluation of the ovaries indicated multiple cystic follicles beneath the capsule of the ovary. An endometrial biopsy was abnormal indicating an overgrowth of the uterine lining (complex hyperplasia). The hysterosalpingogram revealed patent tubes with bilateral fill and spill. Gerry’s semen analysis parameters were all within normal limits. Stephanie consented to a hysteroscopy and D&C to ensure that there are no advanced stages of uterine lining abnormalities confirmed by pathology. The surgical pathology report came back as normal.

Stephanie and Gerry had many questions about PCOS, not exclusive to treatment for infertility.

Can you summarize PCOS?
Multiple features such as ovulation dysfunction, elevated androgen production, an abnormal ratio of pituitary gonadotropins (LH and FSH), insulin resistance and polycystic ovaries can characterize the disorder. Recently a PCOS consensus workshop recommended that a diagnosis of PCOS should be based upon the findings of at least 2 of the following 3 criteria: (1) oligo- and or anovulation (infrequent or absence of ovulation, respectively), (2) polycystic ovaries on ultrasound, and (3) hyperandrogenism (elevated levels of androgens or “male hormones”).

What is insulin resistance?
Insulin facilitates the utilization of glucose as a source of energy for the cells in the body. In some patients the cellular response to circulating insulin is inadequate; therefore, insulin synthesis is increased to compensate for the deficiency in glucose metabolism. Through the years this over production may exhaust the pancreas and lead to diabetes. Additionally, the inefficient use and absorption of glucose together with high levels of insulin causes an increased storage of fat, hence the increased obesity seen in many women with PCOS. However, a large proportion of thin women with PCOS can exhibit a degree of insulin resistance. The variation in insulin resistance is dependent upon age, body mass index (BMI), body fat distribution and family history of diabetes.

How does this affect my menstrual cycle and my ability to conceive?
In order to completely appreciate PCOS and its heterogeneous presentation, it is important to understand the dynamics of follicular growth within the ovary. The follicle which houses the oocyte, or egg, is composed of layers of cells which support the growth of the oocyte through the follicular phase of the menstrual cycle. One cell type, the theca-interstitial cells, responds to LH to produce androgens. Androgens are then converted to estrogens by the granulosa cells in response to FSH. The
ovarian response to the gonadotropins is modulated by both ovarian derived and peripheral factors. One such regulator, insulin, augments androgen production by the theca cells and also promotes the proliferation of these cells within the ovary (Figure 1). Although peripheral tissues exhibit resistance to insulin (thus stimulating overproduction), the ovary, however, has an increased sensitivity to insulin and when combined with elevated LH produces the high androgen levels and polycystic ovaries observed in some women. The conversion to estrogens is not as efficient in the PCOS patient therefore the estrogen concentration per follicle is lower than normal. However, the abundance of small follicles together produces an elevated estrogen level. As the development of the oocytes within the ovarian follicles is subject to even

the slightest alteration of hormonal environment, the quality of the oocyte(s) and thus resulting embryo(s) can be compromised. This may influence the predisposition of the PCOS patient to experience early pregnancy loss.

Does PCOS have any effects on my long term health?
For many years PCOS was dismissed as a cosmetic problem, one interfering with the reproductive processes only. PCOS is now known to have long term ramifications on women’s health including an increased risk of diabetes, hypertension and cardiovascular disease, due to the impact of androgens on the lipid profile. Lifestyle modifications both in diet and exercise can not only lead to an improved quality of life but reduce the risks of the long term sequelae of PCOS. Moreover, the infrequency of

![Figure 1. Illustrates the action of peripheral insulin on the ovary and the pituitary. Insulin augments androgen synthesis directly in the ovary and by purportedly over-stimulating LH production by the pituitary.](image-url)
ovulatory and consequently menstrual cycles causes an increased frequency of endometrial growth from simple changes (hyperplasia) to cancer.

What are the options for us to assist in conception?

Although not common practice today, surgical ovarian drilling may be employed to reduce the amount of ovarian derived androgens. This procedure involves destruction of some of the theca tissue. Because of the tendency for formation of significant scar tissue following this procedure, a more conventional methodology is preferred. The more common approach concentrates on the biochemical abnormalities (abnormal insulin levels) of the PCOS patient. Insulin sensitizers such as Glucophage are used to reduce the levels of circulating insulin and thus the level of insulin at the ovary. This class of agents enhances insulin action at its target site, thus causing a decrease in circulating insulin levels.

Clomid (ovulatory drug) is typically the first line of intervention with >75% success with ovulation and a 40% singleton take home baby rate, however failure to respond to Clomid necessitates the consideration of insulin resistance and circulating androgen levels. Clomid resistance can be approached by co-administering insulin sensitizers such as Glucophage and/or addition of low levels of Dexamethasone, which will reduce androgen production from the adrenal gland. For patients resistant to this treatment, gonadotropin stimulation (Gonal-F, etc.) may be prescribed. This option, however, is not without risks and challenges.

Stephanie and Gerry agreed to undergo ovulation induction with Clomid and Glucophage. She conceived three times with this treatment. Unfortunately all three pregnancies ended in first trimester losses. Chromosomal analyses of the last two pregnancies indicated severe abnormalities. During a return consultation, the couple expressed their frustration with their history and inquired about possible reasons and resolutions. Since an increase of chromosomal abnormalities has been recently reported in pregnancies in PCOS patients, in vitro fertilization (IVF) in conjunction with preimplantation genetic diagnosis (PGD) was discussed. This option will reduce the chance of transferring a chromosomally abnormal pregnancy which may result in an early pregnancy loss.

Stephanie and Gerry elected to undergo IVF with PGD. During her stimulation cycle 18 oocytes were retrieved, 12 fertilized and following PGD, 2 embryos were identified as chromosomally normal. The couple underwent a day 5 embryo transfer which resulted in a single intrauterine pregnancy. Stephanie remained on Glucophage treatment for 12 weeks. The pregnancy was complicated by gestational diabetes which required insulin therapy; however, a healthy female infant was born.

Despite this pleasant ending, PCOS represents a significant challenge to both patients and physicians. Not only do the questions of infertility, repeat pregnancy losses and pregnancy-related complications need to be addressed, but the long-term ramifications pose great challenges. We often manage the treatment of young women who do not desire fertility but inquire of the long-term effects. We hope that as information about PCOS at the molecular level evolves, concurrent improvements in therapy will reduce this challenge.

In the subsequent issue we will discuss the clinical and laboratory aspects of recurrent pregnancy loss.

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Tyho-Galileo Research Laboratories is an organization created by world-renowned specialists in reproductive science and molecular genetics. In founding the Tyho-Galileo Laboratories, these individuals have joined together to stimulate progress in medical research, particularly in the areas of reproductive medicine, gametogenesis, human pre-implantation embryology and genetics. The research that Tyho-Galileo promotes will enhance the understanding of human reproduction and the origin of human disease and facilitate development of treatment modalities that can lead to the eradication of infertility and certain genetic diseases.

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Alternative healing, and our body’s own innate ability to heal itself, can help control and reduce stress. Stress has been linked to infertility problems. We have all heard a story of a woman who fails to conceive, adopts a child, and 3 to 6 months down the road finds herself pregnant.

Alternative healing modalities have been around for centuries. Reiki, Reflexology, Hypnosis, and Aromatherapy, to name just a few are ever so prevalent as part of the Eastern culture. Not only are they used as complimentary healing modalities but many of them are considered accepted medical practices. For centuries, the Eastern culture, has known about and widely used natural herbs for treating different ailments. Many people in our Western culture are now turning to natural herbs and supplements to aid in their healing process. For example, Echinacea is best known for its immune enhancing ability; St. John’s Wort has been known to be effective in treating mild to moderate depression; FertileAgeM for Women and FertileAgeM for Men combine fertility & anti-aging ingredients that may help restore and preserve your reproductive health throughout your child-bearing years; Lotus FlowerM and Lotus TigerM are fertility supplements for women and men who want to conceive now and may help promote sexual vigor and performance.

What we term ‘alternative healing’ may not have as broad a spectrum as it does in many Eastern societies but it certainly does lend a helping hand to our modern medical practices within our Western culture, where we are increasingly accepting and appreciating their worth.

Many modern medical practitioners are embracing the benefits of alternative healing modalities such as Reiki, Reflexology, Hypnosis and Aromatherapy as complimentary aids to, and enhancements of, modern medical practices. They are accepting of the fact that more and more people are turning to natural herbs and supplements for different ailments. They understand that one enhances or compliments the effects of the other and can work well hand-in-hand. In our Western society, now, more prevalent than ever are Holistic Health Clinics and Wellness Centers that promote the healing of the mind, body and spirit. This shows we are making some progress. In a ‘perfect world’ Eastern and Western medicine can blend seamlessly.

Another very important issue in our society today is stress. Stress is believed to be the root cause of many illnesses and disease. We hear it mentioned everywhere in the media. Millions of people worldwide now suffer from some form of tension or stress. Every aspect of our lives can suffer due to stress related illnesses. Stress can be defined as a mentally or emotionally disruptive or upsetting condition occurring in response to adverse external influences and capable of affecting physical health. It is only when we are in a totally relaxed state that our bodies are able to harness the full benefits that modern and alternative medicines can provide us which in turn help trigger our body’s innate ability to heal.

Reiki, Reflexology, Hypnosis and Aromatherapy have been known to be effective in promoting both physical and mental relaxation, to greatly reduce stress, to relieve pain and to help the body maintain homeostasis. Homeostasis is the ability or tendency of an organism or cell to maintain internal equilibrium by adjusting its physiological processes. Hence these alternative healing modalities help our bodies return to a balanced and healthy state. Our natural state is one of perfect health. One of the main benefits of these alternative healing modalities is that there are no contraindications, meaning that they are totally safe, simple and effective. And, let us not forget, that natural herbs and supplements have been known to help promote and maintain both our physical and mental well-being.

Imagine the enhanced effects such modalities could offer fertility and infertility patients. Whether couples or individuals are wanting to become pregnant, are already pregnant, are undergoing or in the process of going through in vitro fertilization procedures, the added benefits of any one of these alternatives could be astounding. We are all well aware that with any procedure whether it be in vitro fertilization, surgery, delivering a baby or the recovery from any one of...
these the less stressed and more relaxed we are is the better the final outcome will be.

Reiki is an ancient, energy-based, hands-on healing art dating back to the 1800s in Tibet. Different cultures believe that all living things have a life force energy running through them. Reiki by definition means ‘Universal Life Force Energy’. It enhances medical and psychological healing methods. It is very relaxing and has been known to relieve stress, anxiety and pain. It is believed that it speeds up the healing process and enhances the immune system. It may increase your energy level and promote a sense of well-being. Reiki is very powerful and at the same time gentle and relaxing.

Since the beginning of time, reflexology has been known as a healing art. There is evidence of cultures around the world with no apparent contact with each other having used reflexology as a healing modality. Reflexology is another hands-on healing art that promotes vitality and well-being. Reflexology is a method for activating the body’s natural ability to heal itself. The use of reflexology among medical professionals that deal with pregnancy and childbirth is greatly increasing. There are also studies that vouch for the benefits of reflexology during labour and delivery. In The Effects of Reflexology on Labour Outcome (UK, 1989), Dr. Gowri Motha and Dr. Jane McGrath found that pregnant women who had a schedule of ten sessions prior to giving birth experienced shorter labour and less pain during labour and while giving birth.

Hypnosis refers to a state in which a subject becomes highly responsive to suggestion. If a subject is highly responsive to the suggestions given, he or she hears, sees, feels, smells, and tastes in accordance with the suggestions that have been given. Hypnosis has been used to induce relaxation and reduce stress and anxiety. It has been known to be highly effective in pain management, both with analgesia, touch but no pain, and anesthesia, no touch or pain. A certain depth level of hypnosis must be reached in order for analgesia or anesthesia to be produced. Hypnosis preparation prior to childbirth reduces anxiety, fear and tension, reduces birth complications, and promotes a rapid recovery process. The best results are achieved when the mom is introduced to hypnosis a few weeks before labor begins. More and more, scientific studies are showing how useful and effective hypnosis can be during childbirth. The mind, body, spirit connection is extremely strong with hypnosis and can be used to ease a woman’s birthing experience.

Aromatherapy, is yet another very old healing art which utilizes essential oils and has been known to be very effective in the promotion of relaxation and stress reduction. The essential oils are all natural and are extracted from plants and trees. Aromatherapy uses essential oils such as Cypress, Clary-sage, Thyme, Nutmeg, Coriander, Geranium, Fennel and Chamomile Roman to help female infertility issues related to stress and uses Thyme, Cumin, Sage, Clary-sage, Basil, Cedarwood, Vetiver and Angelica to help male infertility issues related to stress.

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Experience one or more of these natural healing modalities and see the judge. You will be amazed at the results. Take a natural supplement such as FertileAge™ for Women, FertileAge™ for Men, LotusFlower™ or LotusTiger™ enjoy a Reiki session, make time for a relaxing Reflexology session, take time out of your busy schedule for a relaxing Aromatherapy massage or why not be really adventurous and give Hypnotherapy a whirl. You have nothing to lose and everything to gain. It is a win/win situation.

You can contact Debbie Nagy, C.Ht. at: debbie@PersonalDynamix.com
**ART in the UK**

by Christopher Jones, BSc (Hons), MSc (Oxon), DPhil (Oxon Exp ’05)
Nuffield Department of Obstetrics & Gynaecology

*The author would like to acknowledge the gracious support of The Bertarelli Foundation, Switzerland*

**Introduction**

One of the key differentiating factors between the Human Fertilisation and Embryology Authority (HFEA) in the UK and similar authorities in other countries is that the UK alone requires all treatment cycles of IVF, or any procedure whereby gametes or embryos are manipulated, stored and/or used for treatments or research, to be reported to the authority along with details of both procedures and outcomes. In the UK, clinics are required by law to record and submit clinical data on all such treatments and they can lose their license if they fail to comply with this policy. Therefore, this legal requirement attaches a greater degree of accuracy to this database compared to others such as SART in the USA and FIVNAT in France. As such, the HFEA dataset provides a perfect opportunity to test key hypotheses related to clinical preferences and outcomes following procedures of assisted conception.

Established in August of 1991, 13 years after the birth of Louise Brown, the first IVF conception of Professor Robert Edwards and the late Mr. Patrick Steptoe, the HFEA maintains one of the world’s largest IVF datasets as it oversees activities in accordance with the 1990 Human Fertilisation and Embryology Act (Her Majesty’s Stationary Office, 1990, c. 37). As a non-departmental, government body the HFEA regulates and inspects all clinical facilities providing IVF, donor gametes or donor embryo services, or provisioning the cryostorage of sperm, eggs or embryos. Its main purpose is to safeguard the interests of patients or donors, children, embryos, gametes, doctors, scientists, the wider public, and future generations (HFEA 2003).

The principal tasks of this authority are to license and monitor all UK clinics that provide in vitro fertilisation (IVF), donor gametes or donor embryo services, or provisioning the cryostorage of sperm, eggs or embryos. Its main purpose is to safeguard the interests of patients or donors, children, embryos, gametes, doctors, scientists, the wider public, and future generations (HFEA 2003).

How the HFEA collects treatment data

Licensed clinics are required to complete several forms of consent for each patient, which are sent to the HFEA where they are held in confidence and reviewed periodically to guide policy in making changes to medical practice. These forms require at various stages the commitment of doctors, nurses, embryologists and the patients themselves. Doctors oversee the process to ensure accurate information is recorded in a timely fashion. Embryologists record data at the time of egg collection and transfer, and patients themselves report their own results of the pregnancy test(s), complications, birth event(s) and any abnormalities.

Once the information is received by the HFEA, data are entered in a master database and analysed. Under no circumstances is identifying information disseminated to the public.

In addition to the number of HFEA forms that are completed by patients and hospital carers, patients’ IVF and obstetric histories are also recorded, along with number of prior treatment attempts per specified clinic. Reasons for infertility are indicated on each of the forms as: male-factor, female-factor or idiopathic.

Storage of Gametes and Embryos

Patients must indicate from the outset the duration they wish their samples to be stored. In instances where samples are to be stored for more than 5 years, a designated HFEA form must be signed by a qualified medical practitioner. In addition, most clinics require a preconceptual agreement that lists choices for disposition of their sperm, eggs and/or embryos in the event of death.
or mental incapacitation of the putative mother or biological father. Sperm, eggs and/or embryos may be allowed to perish or else continue in storage for an indicated purpose that must be stated in advance. Patients can withdraw their consent at any time, also, provided their samples have not already been used.

Gamete and embryo details are recorded on the HFEA Treatment and Embryo Creation and Use Form. This form indicates which patients are using whose samples for their treatment, as would be the case with a donor for another patient's treatment, or in a surrogacy arrangement. Patients and/or donors must consent to and provide details of the reasons for egg collection to create embryos and indicate whether this is for contemporaneous treatments or use at a later date, and whether embryos are to be stored cryogenically. If some embryos are to be donated for research purposes, then patients must state so prominently on this form. In addition, total number of previous IVF pregnancies attempted is recorded along with the resultant number of live births. Duration of infertility is recorded along with the duration of treatment. Number of eggs retrieved and the number of those mixed, fertilised, used, stored, thawed, and/or transported are likewise indicated on this form.

The date of egg collection (day/month/year) is recorded along with, where applicable, the method of hormone stimulation (e.g. anti-oestrogens, gonadotrophins, or another type). If no eggs were collected, the reason is stated from a choice of contraindications in light of risk of Ovarian Hyperstimulation Syndrome (OHSS), inability to retrieve eggs, or another reason. When eggs are collected, the number retrieved are recorded and their fates determined by the patient. Possible embryo fates include: storing for later use, discarding, using a certain amount in fresh cycle IVF and donating some or all to research facilities.

As sperm, egg freezing and embryo freezing are becoming increasingly popular techniques in assisted conception more forms are required to ensure each treatment is documented. When gametes or embryos are to be stored, patients must complete either the Form of Consent to Storage and Use of Eggs and Embryos, or the Form for Consent to Storage and Use of Sperm and Embryos. On each of these, patients indicate their consent to donate their eggs or sperm for the purpose of treating themselves (with or without a named partner), treating any others, and/or donating gametes or embryos for research purposes.

In the case of gamete donors, a Donor Information Form is completed for the HFEA which lists the donor’s: name(s), date and place of birth, sex, height and weight, ethnic group, eye colour, hair colour, skin colour, religion, occupation, interests, the date the gametes were first used or supplied for use in treatment, any donations to other centres, any children of their own, and a brief narrative of one’s self to pass to any child born as a result of their help, as well as to their legal parents.

When it is decided to undergo a treatment cycle of IVF, the type of treatment is indicated (e.g. fresh/frozen, stimulated/unstimulated, IVF or IVF with ICSI, etc.). Then the date of embryo transfer (day/month/year) is recorded along with any consequent outcomes of no pregnancy, biochemical pregnancy only, miscarriage, ectopic pregnancy, heteartopic pregnancy, molar pregnancy and foetal heartbeat confirmed. If embryos are not transferred in a given treatment cycle, the reasons are recorded.

In addition to the requested data, patients may state particular conditions as to use of their gametes or embryos. The normal maximal allowable storage time for gametes (eggs and sperm) is 5 years and 10 years for embryos. Storage of gametes or embryos for durations of more that 5 or 10 years, respectively, requires medical consent. If no other indications are given, samples are thawed and discarded.

Outcomes
Outcomes are recorded on the HFEA Pregnancy Outcome Form and include: number of gestational sacs detected by foetal heart pulsation, the age of gestation in weeks, and whether a treatment cycle and pregnancy resulted in a live birth surviving through 27 completed days post-delivery. Miscarriages, terminations, embryo reductions, still births and/or neonatal deaths are also recorded, along with their cause(s) if known. In the event of a live birth, plurality is listed along with weight of each child in grams. The sex (male/female) of each baby is indicated along with their place of birth and county of registration, if known. The delivery day (day/month/year), method of delivery, NHS number, and any congenital abnormalities also are recorded.

How the clinics and HFEA collect outcome data
Many units operate a satellite system whereby smaller district general hospitals (DGHs) refer patients into a tertiary centre for treatments such as IVF with or without ICSI. Under such circumstances, a patient may initially present to a larger clinic but receive their treatments in a smaller clinic closer to home. However, satellite centres are the responsibility of the licensed centre, and it is the responsibility of the latter to ensure that all forms are complete accurately and in a timely fashion.

Advantages and Limitations of the HFEA Database
It goes without saying that the construction and maintenance of a complex database such as described above is not without limitations as well as advantages. The primary advantage is that, because all births from assisted conception are included, long-term follow-up studies are possible on subsets of the data. A secondary advantage is that data reliability and completeness tend to bolster public confidence in policies and reports directed toward patients as well as practitioners. A third and vitally important advantage is that members of the public who wish to avail

CONTINUED ON PAGE 20
themselves of infertility treatment are able to compare clinic success rates from a totally independent source. Lastly, the data lend themselves to a variety of research endeavours.

Amongst the most obvious limitations of the present system in the UK is that the number of forms required for each patient constitutes a bureaucracy, notwithstanding the fact that without the forms the database could not exist. Further, the need to fill out the forms in every instance is time consuming and financially costly which certainly is factored into the overall cost of each treatment cycle. Third, the creation of comparative results in tabular form allows the public to “cherry pick” to attend one clinic or another based entirely on numerical success rates (positive or negative) rather than making a choice based on the quality of medical care. Finally, the promulgation of national policies that regulate reproduction should be based on sound science, but the implementation of these policies may abrogate the patient’s right to choose her therapy in the manner that she feels most appropriate. This is particularly evident in terms of the numbers of embryos transferred per cycle.

The policies of HFEA are unique to that body and to the United Kingdom. They do not copy nor act in concert with other regulatory bodies in different countries – the principle ones being SART in the United States and FIVNAT in France. Interested readers are free to consult the HFEA website as well as the respective websites of SART and FIVNAT for further details which are in the public domain.

Quality Management in Assisted Reproduction

Reproductive medicine is among the fields in which the introduction of quality management has made great strides. Certainly, this has been true of European IVF centres and the UK in particular. In the past, a series of specific quality management systems for various industries came into existence worldwide such as the Good Production Practice (WHO directive, 1964), which was developed for the pharmaceutical and food industries and the European Community Pharmaceutical Directive which was established for the clinical and research settings (European Community Pharmaceutical Directive, 1965).

In June 2004, the Oxford Fertility Unit, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, became the first University-based fertility centre in the United Kingdom to achieve certification of ISO 9001:2000. Use of the International Standard Organisation (ISO 9001 manual series: 2000) manuals became globally widespread during the 1980s and created regulations for quality management systems with the standards series 9001 through 9004. The ISO 9001 standard is applicable for manufacturing and complicated service enterprises including hospitals and fertility units. Certification of this quality assurance standard ISO 9001 is given when

assessment of clinical, nursing and laboratory procedures has been made. Patient care, facilities and customer satisfaction are evaluated by this organisation. The implementation of ISO 9001 guarantees quality and standardises management systems to provide quality service to all patients, employees and associates working within the centre.

Conclusions

As the HFEA dataset is comprehensive it serves as an excellent model for other countries, particularly where clinics are not absolutely required by law or policy to report treatment and outcome data for IVF. Eliminating underreporting of treatments and outcomes in countries beyond the UK can be expected to lead to a more accurate indication of healthy baby rates within and between populations that differ in age and treatment histories. The ISO 9001 standard will guarantee a high standard of treatment provision and patient satisfaction in existing and emerging fertility clinics around the world.

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A View of Legislation in Canada

by Haimant Bissessar, BSc
Vice President, CAN-AM Cryoservices Corp.

Canada has one of the most comprehensive legislative and regulatory frameworks governing the practice of assisted human reproduction anywhere in the world. This became a reality on March 29th, 2004 with the passage into law of the Act Respecting Assisted Human Reproduction and Related Research (also known as the Assisted Human Reproduction Act).

The guiding principles of the Act are to protect the health and well being of children born through the application of assisted reproductive technologies; prohibit ethically unacceptable practices such as human cloning and to ensure that assisted human reproduction research takes place within a regulated environment.

Health Canada, in its commitment for an open and transparent process began consultations with the public to gather information to be used in the development of the regulatory framework of the Act. The Act will be implemented in stages with full implementation expected in 2008.

Certain activities deemed unethical will be prohibited while others will be allowed with a license and regulations.

Activities that would be prohibited include:
- Knowingly creating a human clone for any purpose.
- Creating an in-vitro embryo for any purpose other than creating a human being or providing instructions in assisted reproduction procedures.
- Creating an embryo from another embryo or foetus.
- Maintaining an embryo outside the body of a woman after 14 days of development, except where the embryo has been frozen.
- Sex selection or identifying the sex of an embryo except to prevent, diagnose or treat sex linked disorders or disease.
- Altering the genome of a cell such that the alteration can be transmitted to descendents.
- Transplanting non-human gametes, embryos or foetus into a human being.
- Use of in-vitro embryos or reproductive material that was transplanted into a non-human life form to create a human being.
- Creating or transplanting a chimera (an embryo that has cells from a non-human life form, other embryos, foetus or human being) into a human being or non-human life form.
- Creating a hybrid (embryos created using human and non-human genetic material) for reproduction.
- Commercializing reproductive capability.
- Obtaining gametes from any individual under the age of 18 except for cryopreservation or creating a human being to be raised by the donor.
- Use of reproductive material without informed written consent.

Controlled activities, which are to be undertaken with a license and regulations, include:
- Creating, altering, manipulating, treating or making use of an in-vitro embryo.
- Obtaining, storing, transferring, importing or exporting gametes or an in-vitro embryo.
- Undertaking transgenic research.
- Reimbursement of expenditures.
- Licensing to permit the use of a particular premise to undertake a controlled activity.
- Accepting human reproductive material or an in-vitro embryo without obtaining the required health reporting information.
- Accessing, disclosing, distributing or destroying health-reporting information except under specific circumstances.

This Act was the result of over a decade of debate, consultation with stakeholders, public opinion polls and included the comprehensive report of the 1993 Royal Commission on New Reproductive Technologies. This Act was not universally endorsed and was not without controversy. The Canadian Fertility and Andrology Society (CFAS) in their brief to the members of the Senate Committee on Social Affairs, Science and Technology on October 8th, 2003 was quite decisive in their opposition to the proposed legislation as indicated by their position.

“It is with regret that the CFAS cannot support the passage of Bill C-13 due to some of its restrictive provisions and deficiencies. To do so would be to abandon the needs and wishes of the patients and the larger society that we serve as well as violate CFAS guiding principles”.

On the other hand, the Society of Obstetricians and Gynaecologists of Canada (SOGC), while recognizing that this bill has its shortcomings, overwhelmingly urged its members in a media release on October 14th, 2003 to

“Vote in favour of Bill C-13”

More recently, on December 16th,
2004 the government in the province of Quebec launched a legal challenge on the constitutionality of certain sections of the Act and introduced its own legislation (Bill 89) to regulate assisted reproductive practices. It would not be surprising if other special interest groups across Canada launch similar constitutional challenges to this ACT.

The responsibility for issuing and renewing licenses, inspecting assisted human reproduction facilities, maintaining a donor/offspring registry and collecting and maintaining health-reporting information will fall under the jurisdiction of the Assisted Human Reproduction Agency of Canada (“Agency”) to be established in 2005. This Agency will report directly to the Minister of Health. The penalties for any violations of the Act are quite severe and range from a monetary fine of between $100,000.00 and $500,000.00 or imprisonment for a term between 2 and 10 years.

Stakeholders and the public will have an opportunity to present their views, concerns and opinions on how well the act is working, as a parliamentary review will be conducted within 3 years after the establishment of the Agency.

You can contact Haimant Bissessar, BSc at: info@canamcryo.com
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Introduction

a) Embryo Culture Media Based on Oviduct and Uterine Fluids

Much of the early development of embryo culture media was based on simple salt solutions derived from Krebs-Ringer Bicarbonate (see Summers and Biggers 2003). An alternative approach was the formulation of media based on the measured concentrations of the components of oviduct and uterine fluids. Differences in such concentrations have led Gardner and Lane (2002) to suggest that “in order to optimize mammalian embryo development in culture, sequential media are required, each designed to meet the changing requirements of the developing embryo.” Although this “back to nature” approach seems logical, it relies on several questionable assumptions. First, the measurements of the components of oviduct and uterine fluids are highly variable (Summers and Biggers 2003), and almost certainly subject to physiological inductance. Second, such measurements only reflect the overall composition of the tract fluids and not the micro-environment around the embryo. Third, as shown in Figure 1, the physical and chemical environment of the embryo in vivo is completely different from its environment in vitro. Clearly, the stresses on the embryo in vitro are very different from those in vivo, and culture media must be designed to optimize embryo development under in-vitro conditions.

b) Embryo Culture Media Designed by Simplex Optimization

In a radical departure from the traditional methods for designing embryo 150 13th World Congress on In Vitro Fertilization, Assisted Reproduction & Genetics culture media, Lawitts and Biggers (1991; 1992) applied the principles of simplex optimization to determine the optimal concentration of each component for the culture of mouse embryos. This resulted in the formulation of Simplex Optimization Medium (SOM) which was marked by a low NaCl concentration, and could overcome the mouse 2-cell block (Lawitts and Biggers, 1991; 1992). Blastocyst development was improved by increasing the concentration of KCl to 2.5 mM (KSOM, Erbach et al. 1994). Embryo development was further improved by the addition of amino acids to (KSOM-AA, Biggers et al. 2000). KSOM, with or without amino acids, has been shown to support the development of cattle (Liu and Foote 1995), rabbit (Liu et al. 1996), rhesus monkey (Weston and Wolf 1996), pig (Machaty et al. 1998), rat (Zhou et al. 2003), and human (Biggers and Racowsky 2002, Table 1) embryos.

Figure 1. A comparison of the interaction of an embryo with its environment, in vivo and in vitro.

EDITOR’S NOTE: See article by J.D. Biggers, Page 4
Table 1. A comparison of the morphological development of zygotes after culture in KSOM-AA for 5 days or in P-1 from Day 1 to 3 and in CCM from Day 3 to 5. (Biggers and Racowsky 2002)

<table>
<thead>
<tr>
<th></th>
<th>KSOM-AA (%)</th>
<th>P-1 → CCM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrested</td>
<td>20 (24.4)</td>
<td>41 (29.3)</td>
</tr>
<tr>
<td>Morula</td>
<td>11 (13.4)</td>
<td>18 (12.9)</td>
</tr>
<tr>
<td>Early Blastocyst</td>
<td>20 (24.4)</td>
<td>28 (20.0)</td>
</tr>
<tr>
<td>Expanding Blastocyst</td>
<td>6 (7.3)</td>
<td>19 (13.6)</td>
</tr>
<tr>
<td>Expanded Blastocyst</td>
<td>25 (30.5)</td>
<td>34 (24.3)</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>140</td>
</tr>
</tbody>
</table>

There was no significant difference between the distributions of the stages of development of the media groups.

c) The Development and Use of global® Medium in Human ART

Wiemer and his colleagues showed that a modified version of KSOM-AA could support high rates of development of Day 3 human embryos to the blastocyst stage (Wiemer et al. 2002). The medium was subsequently further modified for human use, and this is now available as global®.

Clinical Studies of the Culture of Human Embryos in global®

global® medium has been successfully used for the culture of early human embryos from Day 1 to 3 (Tables 2 and 3), as a “blastocyst medium” for culture from Day 3 onward (Tables 4 and 5), and as a single medium for culture from Day 1 to 5, with a change to fresh medium at Day 3 (Tables 6 and 7). It is important to note that these studies were independently designed, supervised and conducted by the individual clinical laboratories, according to their own requirements and protocols.

Table 2. A comparison of global® and HTF for the culture of human embryos from Day 1 to Day 3. (Neal et al. 2004)

<table>
<thead>
<tr>
<th></th>
<th>global®</th>
<th>HTF</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean composite embryo score on Day 2</td>
<td>13.1</td>
<td>11.2</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean composite embryo score on Day 3</td>
<td>29.4</td>
<td>23.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pregnancy Rate</td>
<td>55.0</td>
<td>39.0</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Table 3. A comparison of global®, P1 and Quinn’s medium for the culture of human embryos from Day 1 to 3. (Moodie et al. 2004)

<table>
<thead>
<tr>
<th></th>
<th>global®</th>
<th>P1</th>
<th>Quinn’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cleavage score</td>
<td>8.2</td>
<td>7.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>48.0</td>
<td>55.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>21.3</td>
<td>26.0</td>
<td>26.3</td>
</tr>
</tbody>
</table>

There were no significant differences in number of embryos transferred, cleavage score, pregnancy rates or implantation rates among the media groups.

Table 4. The development of human embryos cultured in HTF from Day 1 to 3 and then in global® from Day 3 to 5. (Wiemer et al. 2002)

<table>
<thead>
<tr>
<th></th>
<th>ICSI (N = 538)</th>
<th>IVF (N = 433)</th>
<th>Combined (N = 971)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early blastocyst (%)</td>
<td>10.4</td>
<td>12.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Full blastocyst (%)</td>
<td>13.4</td>
<td>13.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Expanded blastocyst (%)</td>
<td>17.1</td>
<td>18.5</td>
<td>17.7</td>
</tr>
<tr>
<td>Hatching, hatched blastocyst (%)</td>
<td>2.0</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Total blastocysts (%)</td>
<td>42.9</td>
<td>44.8</td>
<td>43.8</td>
</tr>
</tbody>
</table>
Table 5. Implantation and pregnancy rates of human embryos cultured in P-1 from Day 1 to 3, and then either in γ-glucose global® or in IBM before being frozen on Day 5 and thawed and transferred in a subsequent cycle. (Unpublished data from Dr. T.B. Pool, Fertility Center of San Antonio, San Antonio, TX, USA)

<table>
<thead>
<tr>
<th></th>
<th>P-1 → γ-glucose global®</th>
<th>P-1 → IBM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation rate (%)</td>
<td>62.9</td>
<td>25.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>77.8</td>
<td>39.8</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Table 6. A comparison of the development of human embryos cultured in γ-glucose global® from Day 1 to 3 and Day 3 to 5, or in IVC-One from Day 1 to 3 and then in G2 from Day 3 to 5. (Freeman and Rieger 2004)

<table>
<thead>
<tr>
<th></th>
<th>γ-glucose global®</th>
<th>IVC-1 → G2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;3 cells on Day 2 (%)</td>
<td>60.1</td>
<td>51.9</td>
<td>0.090</td>
</tr>
<tr>
<td>&gt;7 cells on Day 3 (%)</td>
<td>53.3</td>
<td>43.3</td>
<td>0.040</td>
</tr>
<tr>
<td>Expanded blastocyst on Day 5 (%)</td>
<td>35.9</td>
<td>26.5</td>
<td>0.036</td>
</tr>
</tbody>
</table>

Table 7. A comparison of the development and pregnancy rates of human embryos cultured either in γ-glucose global® from Day 1 to 3 and from Day 3 to 5, or in G1 from Day 1 to 3 and then in G2 from Day 3 to 5. (Unpublished data from Dr. A.J. Carrillo, The Fertility Center, Louisville, KY, USA)

<table>
<thead>
<tr>
<th></th>
<th>γ-glucose global®</th>
<th>G1 → G2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleavage rate (%)</td>
<td>99.4</td>
<td>94.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;6 Cells on Day 3 (%)</td>
<td>57.7</td>
<td>48.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Blastocysts on Day 5 (%)</td>
<td>50.3</td>
<td>55.5</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Conclusions

The results presented above clearly demonstrate that γ-glucose global® can support the development of human embryos at all stages from the zygote to the blastocyst.

Acknowledgements

Dr. Thomas B. Pool of the Fertility Center of San Antonio, San Antonio, TX, USA, and Dr. Alberto J. Carrillo of The Fertility Center, Louisville, KY, USA graciously provided their unpublished data.

References


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Delaware Valley Reproductive Biology — Spring Seminar 2005

The Delaware Valley Reproductive Biology Group held its Spring Seminar on April 16, 2005 at the Radnor Hotel at Saint Davids. The symposium included three world-renowned speakers presenting the most current research available in the disciplines of Embryology and Andrology. The distinguished guest lecturers included Don Rieger, Ph.D., Harry Fisch, MD and Mina Alikani, M.Sc. The intimate setting afforded an opportunity for both laboratory and clinical members of the DVRBG to interact directly with experts who are truly committed to understanding and improving so many aspects of infertility and its treatment. Summaries of the lectures are provided.

The Delaware Valley Reproductive Biology Group would like to express its gratitude to the following generous benefactors for support of the Spring Seminar.

- Serono, Inc.
- IVFonline
- Embryotech Laboratories
- Mid-Atlantic Diagnostics
- Rosemont Pharmacy
- Ferring Pharmaceuticals

Don Rieger, PhD, Scientific Director of LifeGlobal, LLC, presented “Metabolism and Non-Invasive Selection of Embryos”.

According to the SART statistics for 2002, overall, 17% of ART embryos transferred result in the birth of a baby, ranging from 23% for women <35 years of age to only 5% for women 41-42. To offer a reasonable chance of pregnancy, multiple embryos are transferred, but this leads to unacceptably high rates of multiple births. Being able to select and transfer only embryos with a high probability of survival would be a partial solution to this dilemma. The measurement of metabolic activity to select embryos is theoretically very promising because energy metabolism is required for all aspects of early development. The uptake of glucose by cattle and mouse embryos has been shown to be predictive of subsequent viability in vivo, but not for human embryos. The uptake of pyruvate by human embryos has been related to subsequent development in vitro, but not to development in vivo. Amino acid turnover by human embryos has been shown to be predictive of subsequent in-vitro and in-vivo development, but the HPLC technique is probably beyond the capability of most human ART labs. Oxygen uptake is related to the stage and morphological quality of cattle embryos but has not been shown to be predictive of subsequent viability. Most of these studies have relied on single point measurements which are unlikely to be sufficient to evaluate the developmental potential of the embryo. A definitive metabolic test will almost certainly require repeated measurements, and/or measurements under mild stress, and/or combinations of measurements.

Harry Fisch, MD, Director of Male Infertility, Department of Urology, Columbia Presbyterian Medical Center, presented “The Male Biological Clock”.

Say “biological clock” and most people immediately think “women”. Female fertility, after all, strikes “midnight” with the cessation of menses. This occurs because of distinct-and dramatic-declines in estrogen production. And as women age, the genetic quality of their eggs and the efficiency with which their bodies reject genetically damaged embryos both decline, leading to an increased risk of genetic problems in their offspring. This triad of declining fertility, declining hormone levels, and increasing risk for genetic problems is what most people mean when they say “biological clock”.

Until recently, that is. Although it’s an idea that has not yet filtered down to the general public, we now know that men have biological clocks too. And those clocks involve the same physiological triad experienced by women. Male fertility and male sex hormones do decline with age. And the genetic quality of sperm does decline, leading to an increased risk of genetic problems in offspring above and beyond any contributed by the female.

Data obtained in the past decade suggested a worldwide decline in male fertility. Although initially thought to be the result of external variables such as exposure to pollution, we now understand a real culprit: men are simply waiting longer to have children and aging is adversely affecting their fertility. The increase in paternal age is both a personal problem for many couples and a public health problem because of the simple (but still largely unrecognized) fact that male fertility declines with age.

As with women, the levels of sex hormones in men declines with age. The drop is not as steep or as sudden as that
associated with menopause, but it can be equally significant for fertility and overall well-being. In fact, changes in men’s hormones are just as important as changes in women’s hormones. The roughly 1 percent per year decline in testosterone levels after age 30 has been termed “andropause”, though this is a somewhat unfortunate choice because testosterone levels don’t actually “pause” in the same way that estrogen levels do. A more technically accurate (though clumsy) term is “symptomatic hypogonadism in the aging male”. Whatever you call it, declining testosterone causes problems ranging from decreased libido and erectile dysfunction, loss of muscle mass and strength, weight gain, and declining cognitive function. Hypogonadism is also associated with type II diabetes, insulin resistance, central obesity and the metabolic syndrome. Newer treatments for hypogonadism such as exogenous testosterone replacement and stimulation of endogenous testosterone production are gaining tremendous popularity. However, indiscriminate use of testosterone supplements can raise the risk for prostate problems, blood disorders, and infertility.

Although increasing maternal age has long been known to be associated with increased incidence of birth defects, the age of the male as been seen as irrelevant. New data show what we should have suspected all along: the age of the male does matter and the genetic quality of sperm does decline with age. Studies indicate that older men are at higher risk of fathering a child with schizophrenia and Down Syndrome. Additionally, the risk of pregnancy loss is elevated.

The reality is that men have biological clocks that affect their fertility, hormone levels, and the genetic quality of their sperm. This clock plays a role on a personal level (when couples must grapple with infertility or birth defects) and on a public health level (when society must decide policies governing, for instance, insurance coverage for advanced fertility treatments such as in vitro fertilization.) Women should no longer be viewed as solely responsible for age-related fertility and genetic problems. Infertility is not just a woman’s problem and with the new awareness of a male biological clock couples and their physicians can much more accurately proceed with proper testing, diagnosis and (if needed) treatment of the male. The field of male-factor infertility is still young, and much more research is needed to fully characterize risks and to find more effective treatments. We also need to better understand the cellular and biochemical mechanisms of “gonadal” aging in order to find safe, effective ways to delay this process and, in effect, “rewind” the male biological clock. Doing so will lessen the potential for adverse genetic consequences in offspring, improve the sexual and reproductive health of aging males, and increase a woman’s chance of having healthy children by correcting defects in the male reproductive machinery.

Dr. Fisch is the author of the Male Biological Clock, published by Free Press, a Division of Simon and Schuster, Inc.

Mina Alikani, MSc., Senior Research Scientist, Tyho-Galileo Research Laboratories, presented “Cytoplasmic Fragmentation: A Matter of Life or Death?”

Cytoplasmic fragmentation is commonly observed during preimplantation development in-vitro. Both the extent of fragmentation and the distribution of fragments determine the development potential of fragmented embryos. This phenomenon is not well understood, although it has been widely viewed as the manifestation of cell death—principally apoptosis or genetically programmed cell death. However, the high incidence of fragments, the occurrence of fragmentation patterns, and the different development potentials associated with fragmented embryos are suggestive of other underlying mechanisms. Experiments with mouse oocytes and embryos have now shown that contrary to what apoptosis would imply, fragmentation is a precisely timed event that is exclusive to mitotically active cells in the cytokinetic phase of the cell cycle; one trigger for fragmentation in lieu of normal division is altered cytoskeletal organization. Although one consequence of fragmentation may be cell death, it appears that cell death is not the trigger for fragmentation in activated eggs. The nature of the cytoskeletal alterations that lead to fragmentation has been further investigated but not yet reported. These findings have important implications in a number of areas where fragmentation is considered an impediment, including assisted reproduction and nuclear transplantation.

The 13th World Congress on In Vitro Fertilization, Assisted Reproduction and Genetics was held in Istanbul, Turkey on May 26-29, 2005.

The goal of the Congress was to present topics of particular interest to clinicians and scientists working in the field of reproductive medicine, and provide up-to-date information delivered by world-class scientists. The Organization Committee managed to craft a superb scientific Program and interesting social activities.

The fields of IVF, Assisted Reproductive Technologies and Reproductive Genetics are witnessing a significant expansion both clinically and in basic research. Greater numbers of scientists are devoting their professional lives to the understanding and mastering of human fertility. The field will continue to undergo innovative and revolutionary changes in the new millennium especially in molecular reproduction and genetics.

During the Congress the leading scientists, clinicians recruited from around the world covered the most relevant aspects of this field. This distinguished international faculty of clinicians and basic scientists in the related disciplines addressed and discussed the recent information, controversies and future directions on infertility, assisted reproduction, human reproductive genetics. The Congress was a very good opportunity for the participants to update the relevant research data and their clinical applications.

Istanbul in the spring was the charming venue of the Congress. A very rich social and cultural heritage with unrivaled beauty complimented by unique juxtaposition of ancient and modern, the capital of successive Roman, Byzantine and Ottoman empires, Istanbul offered visitors all the sophisticated delights of a big modern city. Excellent Congress facilities, sightseeing, dining out, nightlife or shopping ...it was all there.

In summary 13th World Congress on IVF, Assisted Reproduction & Genetics was a rewarding educational and social and travel experience!
The 13th World Congress on In Vitro Fertilization, Assisted Reproduction and Genetics was held in Istanbul, Turkey on May 26-29, 2005. The meeting was organized by Dr. Timur Gürgan, under the auspices of World IVF and the Turkish Society for Reproduction Medicine.

The opening ceremony featured a lecture on the significance of the congress by Dr. Robert Edwards, an overview of the history of Istanbul by Dr. Victor Gomel, and a dance performance by the Fire of Anatolia. The evening concluded with a very convivial get-together reception. On subsequent evenings, the speakers were treated to a dinner cruise on the Bosphorus, and the gala dinner was held at the Esma Sultan Mansion.

The scientific content of the congress began with four pre-congress courses on controlled ovarian stimulation, preimplantation genetic diagnosis, practical aspects of embryo laboratory practice, and ART for nurses.

Seven keynote lectures were given in two plenary sessions:

- **Recent Advances in Assisted Conception and Its Derivatives** (R. Edwards)
- **Careers, Babies and Biologic Clocks: What Medical Science Can and Can Not Do?** (G.D. Adamson)
- **Genetics, Epigenetics and Developmental Outcome of Art Children** (A. Van Steirteghem)
- **IVF 2005 and Beyond** (J. Van Blerkom)
- **Human Genome 2005: Changing The Practice Of Reproductive Medicine** (M. Hughes)
- **Outcome Measures of IVF: from Pregnancy Rate Per Cycle to Health Economics of Healthy Singleton Per Started Treatment** (B.C. Fauser)
- **Manipulation of Development by Nuclear Transfer** (G.D. Palermo)

The remainder of the scientific program included oral sessions on ovulation induction, the oocyte, andrology, the embryo, infertility, PGD, PCOS, reproductive surgery, implantation, endometriosis, quality management in the ART laboratory, IVM, reproductive aging, and reproductive imaging. Among these were presentations by D. Sakkas on paternal effects on embryo development, H.J. Out on gonadotrophins, Jean Cohen on infertility, S. Munné and Y. Verlinsky on PGD, Jacques Cohen on cytoplasmic transfer, R.S. Legro on PCOS, C. Racowsky on QC/QA/QI in the IVF laboratory, S.L. Tan and R.-C. Chian on In-vitro oocyte maturation, Y. Ménézo on imprinting, M. Alikani on monozygotic twinning, A. Trounson on stem cells, and P. Quinn on the impact of culture media on IVF. In addition to the oral presentations, a wide variety of topics were covered in 198 posters.

Dr. Timur Gürgan and his colleagues are to be congratulated for their out-standing scientific organization of this diverse and stimulating meeting. The opportunity to experience Istanbul, an historic cross-roads of civilization, made it an even more memorable occasion.

The proceedings of the World Congress on In Vitro Fertilization, Assisted Reproduction and Genetics are available in print or on CD from Medimond International Proceedings (http://www.medimond.com/proceedings/detail.asp?id=20050526).
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Lotus Flower™ allows women to experience the beauty and aid of the lotus flower’s ancient legends to influence sexuality, love and life.

Designed by fertility experts and nutritional collaborators, Lotus Flower™ has been formulated to help enhance the reproductive health of women and help alleviate the stress related to infertility and increase your chances of conceiving now.

Lotus Tiger™ arouses the natural prowess of the male enhances the sexual experience while improving sperm motility, morphology and cellular structure, and production of healthy sperm.

Lotus Tiger™ contains safe, proven vitamins, minerals and herbal supplements to increase energy, libido and improve erectile function and your overall performance.

…Unleash the Tiger…

Millions of couples suffer from some form of infertility. Female and male infertility may be caused by many factors such as environment, your habits, diet and age. Our fertility experts have explored the solutions and come up with the most effective fertility supplements in the market today. They utilize years of experience, scientific findings and published documentation to develop the concise balance of safe, proven, natural ingredients, and when taken daily may immediately improve your reproductive health.

Daily doses of FertileAge™ supplements will provide your body with important scientifically proven ingredients that comply to our strict quality standards to enhance your reproductive system and your chances of conceiving. FertileAge™ products are available worldwide through direct distribution and online channels and are designed to enhance the reproductive systems of women and men.

www.FertileAge.com