IVM in humans: more risks than benefits
by Marc-André Sirard, DMV, PhD

Microfluidics in ART: Time to go With the Flow?
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The global® Family of Media: a Unified Approach to Human Embryo Culture
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Group versus Individual Culture: Effect of Embryo-Secreted Factors
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Normally the best time to recover eggs in all mammalian species is when they are ready to be fertilized or matured. In some cases, the recovery of immature eggs could offer some advantages if the maturation process can be mimicked in vitro. In-vitro oocyte maturation has been used for decades in laboratory and farm animals. The natural ability of the mammalian oocyte to resume nuclear maturation spontaneously when removed from the follicle was first shown by Pincus in 1935. This “by default” maturation comes from the removal of a still unknown inhibitor present in the follicular fluid. Consequently, the mature oocyte can then be fertilized in vitro and develop into an embryo, if properly treated.

During the period preceding ovulation, the follicle secretes hormones that are important for uterine receptivity, but also for communication with the brain to ensure that ovulation will triggered once the oocyte is ready. The follicle also signals the oocyte, by an unknown mechanism that stimulates the oocyte to get ready for the final maturation. When oocytes are matured in vitro, they lack the final instructions given by the follicle before ovulation.

The maturation process takes between 12 and 48 hours depending on the species (approximately 34 hours in the human) and cannot be arrested or reversed. In vivo, the process is triggered by the pre-ovulatory LH surge, and the maturation of the oocytes occurs within the pre-ovulatory follicle. Once the oocyte starts to mature, a time window opens for fertilization and after that period the oocyte will die if not fertilized.

Studies in animals over the last two decades have shown that the more time the oocyte spends outside the follicle, the less chance it has to become a normal embryo. Some of the problems associated with IVM in animals are implantation failure, spontaneous abortion, and post-natal problems, including the large-offspring syndrome.

In-vitro maturation of oocytes happens in human in the same way as in animals, in that most oocytes aspirated from antral follicle will spontaneously resume nuclear maturation (preparing the oocyte for fertilization). There are three main approaches to obtaining immature oocytes from human follicles:

1. Immature oocytes are recovered following ovarian stimulation and ovulation induction with hCG, normally along with mature eggs.

In this scenario, the immature oocytes are very much likely to come from follicles that are still in the growing phase and devoid of LH receptors (on the granulosa cells) at the time of hCG treatment. These follicles are still actively growing and have had insufficient time to differentiate. The oocytes are often incapable of normal meiotic maturation and are associated with poor developmental competence. This direct negative link between FSH-stimulated growth and developmental competence has been demonstrated in several species, particularly in cattle.

2. Oocytes at varying degrees of maturation are recovered following LH/hCG treatment during the follicular phase.

The effects of this approach are difficult to assess because, until follicles reach 14 mm in diameter in humans, the LH receptor is almost exclusively limited to the theca cells. This means that the hCG injection will likely stimulate androgen synthesis and could induce the onset of atresia, which, has been associated with improved ability to produce an embryo (in cattle...
The initial status of the follicles at the time of injection will determine the type of response. If the largest follicle is already dominant (positive for LH receptor in the granulosa cells), it will induce ovulation similar to that in an unstimulated cycle, but if the follicles have not reached dominance (smaller than 13-14 mm), the growth of the cohort of follicles will be influenced by a potential androgen rise. Consequently, the ability of the enclosed oocyte to be mature might be improved, but without necessarily having all the required differentiation to achieve full normalcy.

Another important factor to consider before exploring the use of in-vitro maturation in humans is the possible risk of epigenetic changes that may affect the health of the offspring at or after birth. In several species, the large-offspring syndrome has been clearly associated with in-vitro culture. At present, there is not enough data to determine whether the syndrome results from collection of the oocytes from immature follicles, in-vitro oocyte maturation, or culture after fertilization. The large-offspring syndrome may have similar causes to Angelman and Beckwith-Wiedemann syndromes in humans (for which the risk increases after ART), and therefore it would be important to do additional studies in animals to identify the cause of the problem.

Doing IVM in human, knowing the risks described in farm animals and not knowing the cause, is likely to increase the risk of imprinting problems and unhealthy babies. Worse yet, some experiments are being reported in which additives are added to the culture medium to improve the in-vitro maturation of oocytes that are not differentiated enough to do it by themselves. This triggering of maturation without knowing what signals are missing from the follicle may result in the adverse outcomes observed in animals.

In conclusion, since mature oocytes can be obtained in most cases, it is, at present, unjustified to harvest them before they have completed their natural maturation within the follicle.

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Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect

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Dr Yves Ménézo DSc began his career with INRA, becoming head of the assisted reproductive technology department at Laboratoire Marcel Mérieux in 1990. He owns several patents and has received various awards, including the 2001 Gold Medal of the Department of Obstetrics and Gynecology of the Instituti Dexeus, Barcelona. He has published over 250 international scientific publications and book chapters and is on the editorial board of Genesis, References En Gynecology Ey Ostetrique and Zygote. Dr Ménézo has served on many organizing committees and is currently a member of the France–USSR commission for the study of Fertility.

Abstract

Reactive oxygen species (ROS) have a negative impact on sperm DNA, leading to the formation of oxidative products such as 8-oxo-7,8-dihydroxyguanosine. This compound causes fragmentation and, thus, has a mutagenic effect. Patient treatment with oral antioxidant vitamins is, therefore, standard practice for male infertility, in an attempt to decrease formation of ROS and improve fertility. In this study, the DNA fragmentation index and the degree of sperm decondensation were measured using the sperm chromatin structure assay before and after 90 days treatment with antioxidant vitamins associated with zinc and selenium. Antioxidant treatment led to a decrease in sperm DNA fragmentation (−19.1%, $P < 0.0004$), suggesting that at least part of the decay was linked to ROS. However, it also led to an unexpected negative effect: an increase in sperm decondensation with the same order of magnitude (+22.8%, $P < 0.0009$). The opening of interchain disulphide bridges in protamines may explain this aspect, as antioxidant vitamins, especially vitamin C, are able to open the cystin net, thus interfering with paternal gene activity during preimplantation development. This observation might explain the discrepancy observed concerning the role of these antioxidant treatments in improving male fertility.

Introduction

Sperm quality is one of the most important areas of concern in treatments involving assisted reproductive technology. Although progress has been made via the use of intracytoplasmic sperm injection (ICSI), the question of sperm DNA quality remains of major importance. Human spermatozoa have a high content of polyunsaturated fatty acids and a limited capacity for DNA repair. This renders them extremely sensitive to attack by reactive oxygen species (ROS). Sperm DNA fragmentation as an indicator of quality has recently become an area of considerable interest, and a variety of methods have been developed in order to evaluate paternal DNA integrity. This assessment of sperm quality is independent of all basic semen parameters including sperm morphology, concentration and motility. The different technologies that have been applied have reached the same conclusion: sperm DNA fragmentation leads to reduced fertility (Kodama et al., 1997; Henkel et al., 2004), particularly for artificial insemination with partner’s spermatozoa and in routine IVF (Evenson et al., 2002). Results suggest that ICSI may sometimes, but not always, improve embryonic developmental potential for sperm samples that display this type of DNA fragmentation (Lopes et al., 1998a).

Reactive oxygen species have a seriously deleterious effect on sperm DNA. They lead to formation of 8-oxo-7,8-
dihydro-2’-deoxyguanosine (8-oxodGuo), the major oxidative product of sperm DNA. This compound causes fragmentation (Lopes et al., 1998b), and therefore has a mutagenic effect. During the process of fertilization, factors in the oocyte cytoplasm should theoretically repair any decays in the integrity of sperm DNA (Hamatani et al., 2004; Zeng et al., 2004). The DNA repair capacity is decreased with age. In the absence of repair, such DNA lesions have profound effects on cell viability and have the potential of oncogenic transformation: transmission of altered DNA is associated with an increase in childhood cancer (Boiteux and Radicella, 1999; Zenzes, 2000; Cline and Hanawalt, 2005). It has therefore been tempting to try to reduce or avoid the generation of DNA fragmentation related to free radical exposure. Numerous controversial studies have evaluated the efficacy of antioxidants in the treatment of male infertility (Agarwal et al., 2004; Greco et al., 2005a,b). The majority agree that these treatments result in only partial success. This study used sperm chromatin structure to investigate the impact of antioxidant treatment, not only in terms of sperm DNA fragmentation but also with respect to sperm head decondensation. Antioxidants may have an effect by opening the interchain disulphide linkages in protamines, especially P2, leading to sperm DNA compaction (Bedford and Calvin, 1974).

Materials and methods

The experiments were performed in two centres of assisted reproductive biology, one near Lyon, France (Centre de FIV de la clinique du val d’Ouest) and one in Paris (Clinique le Muette, laboratoire d’Eylau). Patients who had at least two previous failures of IVF or ICSI were enrolled in the study. A sperm chromatin structure assay (SCSA; Evenson et al., 2002) was performed on sperm samples. After acidic denaturation, cells were dyed with acridine orange. Then sperm samples were analysed in a flow cytometer (Beckman Coulter Epics XL-MCL). SCSA software allows the separation and analysis of spermatozoa with red fluorescence and those exhibiting green fluorescence. Lack of appropriate maturation or anomalies in compaction result in an increase in the amount of DNA staining (green fluorescence). Red fluorescence is a marker of single-stranded DNA decay. A minimum concentration of 1 × 10^6 spermatozoa/ml is required for the assay. In addition, a strict minimal abstinence of 3–5 days and a negative semen culture were required because these factors could increase DNA fragmentation. If either the DNA fragmentation index (DFI) or the degree of sperm decondensation was > 15%, the patients were prescribed a daily oral antioxidant treatment consisting of vitamins C and E (400 mg each), β-carotene (18 mg), zinc (500 μmol) and selenium (1 μmol): this is the classic treatment given by andrologists (see Agarwal et al., 2004). The value of 15% for both parameters corresponds to half of the threshold at which fertility is impaired: 30% for DFI (Evenson et al., 2002) and 28% for decondensation of spermatozoa (authors’ own observation). The duration of treatment was 90 days, in order to provide protection during the complete cycle of spermatogenesis. At the end of the treatment period, the DFI and the degree of sperm decondensation were checked and recorded. Statistical analysis was performed using Student’s t-test and the Wilcoxon test.

Results

Fifty-eight patients fulfilled the enrolment requirements of the study. When these patients were analysed, a significant decrease (–19.1%) in DFI was observed, from 32.4% (σ = 12.2) to 26.2% (σ = 10.8), (P < 0.004 for Student test, P < 0.001 for Wilcoxon test) (Table 1, Figures 1, 2). In contrast, a significant increase in sperm decondensation (+22.9%) was observed from 17.5% (σ = 12.2) to 21.5% (σ = 13.6) (P < 0.009 for Student’s t-test, P < 0.001 for Wilcoxon test) (Table 1, Figures 1, 2).

Table 1. Difference in DNA fragmentation index (DFI) and degree of high DNA stainability (HDS) in 58 patients following treatment with antioxidants.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After treatment</th>
<th>P-value</th>
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<tbody>
<tr>
<td>DFI (%)</td>
<td>32.4</td>
<td>26.2</td>
<td>0.0004</td>
</tr>
<tr>
<td>HDS (%)</td>
<td>17.5</td>
<td>21.5</td>
<td>0.0009</td>
</tr>
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</table>

Figure 1. Decrease in DNA fragmentation index (DFI) and increase in sperm decondensation in 58 patients after treatment with antioxidants. Grey: before treatment; white: after treatment. DFI = DNA fragmentation index, HDS = degree of decondensation of spermatozoa.

CONTINUED ON PAGE 12
Discussion

Even under physiological conditions, gamete and zygote genomes are continuously submitted to attack. In human IVF and ICSI, in-vitro manipulations undoubtedly increase these assaults, especially in relation to ROS exposure. Patient treatment with oral antioxidant vitamins is a standard practice in male infertility, in an attempt to improve sperm quality. The rationale behind this is to decrease the formation of ROS by neutralizing superoxide anions ($\text{O}_2^-$), hydrogen peroxide ($\text{H}_2\text{O}_2$) and hydroxyl ($\text{OH}^-$) radicals, thus decreasing the potential decay of membrane lipid and DNA. *In vitro*, vitamin C and alpha-tocopherol may have ambiguous effects, intensifying the DNA decay even if the production of ROS is reduced (Donnelly et al., 1999). This confirms that DNA strand breaks are not solely induced by ROS. A careful analysis of the various dosages described in the literature shows that, *in vivo*, these treatments are either effective or useless, irrespective of a significant decrease in ROS generation observed in sperm and seminal plasma.

However, several of these antioxidant treatments improve the fertilization rates for ICSI, even in the absence of significant improvements observed for sperm parameters (Lewin and Lavon, 1997). Intake of oral vitamins does reduce DNA fragmentation, but it also increases sperm decondensation by more than 25%. This is definitely a negative effect, as the nuclear structure of sperm chromatin influences the initiation and regulation of paternal gene activity during preimplantation development (Haaf and Ward, 1995). An unwanted high degree of sperm decondensation can result in asynchronous chromosome condensation, and may lead to cytoplasmic fragments in the embryo. In the authors' clinics, no pregnancy has been observed following IVF or ICSI when sperm decondensation is > 28%. Contrary to the situation concerning DNA breaks (Greco et al., 2005a,b), ICSI does not improve the results for high decondensation. The negative effect on chromatin packaging seems to
originates from the high reducing potential linked to these reducing agents. In a small cohort of patients, the same observations (chromatin decondensation and reduction in DFI) were observed when a modified superoxide dismutase was given orally alone instead of vitamins. In a small cohort of 12 patients the same variations were observed with an even greater increase in sperm decondensation (data not shown). This secondary effect is probably due to disulphide bond reduction in protamines. Due to its high redox potential, vitamin C can reduce cysteine to two cysteine moieties, and thus open the interchain disulphide bridges.

It is difficult to compare this data with the situation in vivo (Moustafa et al., 2004; Hammadeh et al., 2006; Appasamy et al., 2007). Here again the results are controversial: it is obvious that DNA damage is induced by oxidative assaults, but the physiological antioxidant situation in the seminal plasma does not seem to be strongly associated with either a reduction in the DNA damage or an increase in the decondensation (Hammadeh et al., 2006). Moreover it has to be pointed out that the results usually originate from small cohorts of patients and that, following antioxidant treatment, the ‘total antioxidant status’ reached in vivo is the most important factor and this is likely to be different from the situation observed in vitro.

In conclusion, care must be taken in the use of antioxidant treatment for improving sperm quality, especially considering the decay in the integrity of DNA. The quality of DNA packaging seems to be equally important. Antioxidants should not be recommended in men whose semen samples show a degree of decondensation over a threshold of 20% in order to avoid reaching the critical value of 28%. Below this value, the risk:benefit ratio is in favour of antioxidant treatment. This observation might ultimately explain the wide discrepancy observed in the scientific literature concerning the role of longterm antioxidant treatments in improving fertility (Agarwal et al., 2004), especially if individual variations between patients are considered.

References
Lopes S, Sun JG, Jurisicova A et al. 1998a Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertility and Sterility 69, 528–532.
Protect embryos from harmful volatile organic compounds (VOCs) and other air contaminations in the air present in IVF incubators, CO₂ gas tanks and IVF laboratories.
Microfluidics in ART: Time to Go With the Flow?

by Jason E. Swain, PhD and Thomas B. Pool, PhD
Fertility Center of San Antonio, San Antonio, TX

Introduction
Improving the production of preimplantation embryos for use in assisted reproductive technologies (ART) has been a central goal of reproductive scientists since the inception of the field, and methods have continually been refined to aid in this endeavor. Although approached from different perspectives, the common theme is to minimize the external stresses on the gametes and embryos. Perturbations in environmental and intracellular conditions such as osmotic imbalances, shifts in temperature, energy imbalances, and pH fluctuations, can all have devastating effects on embryo quality. However, even with tremendous improvements in the field, relatively little attention has been paid to the platform on which gametes and embryos are cultured and manipulated.

Clinical embryology laboratories have historically used polystyrene test tubes, Petri dishes, organ culture wells, or 4-well plates to accommodate varying numbers of gametes or embryos and volumes of media. Recently, embryo-specific culture dishes have been produced that offer some “ease-of-use” benefits for embryo culture [1]. Although each of these approaches offers certain advantages, it is evident that the environmental conditions in all of these culture platforms are very different from the conditions in vivo. Investigations of the differential effects of physical and structural environment experienced by gametes and embryos in vitro versus in vivo may provide a means to further improve the results of clinical ART. In vivo, gametes and embryos are exposed to the constricted “moist” environment of the female reproductive tract and surrounded by various oriented glycoproteins as they are moved by ciliated epithelia toward their destination. This is in stark contrast to the static environment to which gametes and embryos are exposed in vitro, resting on inert synthetic polymers, bathed in a relative ocean of media. Microfluidic devices may make it possible to mimic the in-vivo conditions, in the hopes of creating an environment more suitable to gamete and embryo development and function.

Basis of Microfluidics
The term “microfluidic” refers to the characteristics of fluid movement in a micro- or nano-environment. At this small scale, fluid has streamlined and predictable flow patterns. These predictable flow patterns impose laminar flow upon fluids, allowing multiple streams of media to move in parallel through the same microchannel, with no mixing, except by diffusion across the fluid-fluid interface (Figure 1). In contrast, at the macro level, fluid flow results in chaotic particle movement within the fluid stream, producing turbulence. It is these inherent qualities of microfluidic channels that offer tremendous potential. Practically speaking, a microfluidic device houses the microfluidic channels through which fluid flow is accomplished. Microfluidic devices vary greatly in design and are used in a variety of scientific disciplines, including ART.

Media Flow/Cellular Manipulation in ART Microfluidic Devices
Although the approach of dynamic media flow in ART is not new, prior attempts at perfusion systems for embryo culture on the macro-scale have proven inefficient and consequently not been widely implemented [2, 3]. Fortunately, the nature of microfluidic flow allows for alternate approaches to media movement that are more amenable to wide-spread use. Studies have shown advantages of media flow in microfluidic devices for all aspects of ART, including sperm processing [4-8], in-vitro maturation [9, 10], fertilization [11-13], and embryo culture [14-22].

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achieved in microfluidic devices through manipulations of flowing media, including removal of cumulus cells [24] or the zona pellucida [25], as well as handling of a single oocyte in a manner that may be suitable for ICSI [26].

How Do Embryos Benefit from Microfluidics?

The question arises, how does microfluidic culture improve embryo development and/or quality? It has been suggested that unknown autocrine/paracrine factors secreted by gametes or embryos are extremely localized due to the confining nature of microfluidic devices, and that this localization of factors may be beneficial. Indeed, oocytes and embryos do secrete various factors, and research is ongoing to identify them as non-invasive markers of embryo viability [27, 28]. If the secretion of beneficial factors from the embryos is responsible for their improved development, then the numbers of embryos, volume of medium and surface area occupied would be extremely important factors to consider (see reference 29).

Furthermore, in dealing with group culture, another important variable to consider is quality of companion embryos [30, 31]. However, experiments exploring effects of embryo density within microfluidic devices are lacking.

Although the secretion of trophic factors from embryos offers an attractive explanation for the means by which embryo development may benefit from microfluidic culture, it does not explain the observations of improved embryo development within microfluidic devices with dynamic conditions. With media flow, any such trophic factors would presumably be diluted or removed, yet advanced development has still been reported (17-19). Alternatively, benefits within dynamic devices could be the result of removal of harmful embryo metabolic byproducts, such as ammonia [32]. However, it should be noted that studies removing spent culture media and replacing it with fresh media from microdrops at various intervals shows no benefit to embryo development [33, 34]. Thus, removal of byproducts may not be the sole reason for improved embryo development within microfluidic devices.

Due to the constrictive nature of the microfluidic devices, confining embryos to a very small area, benefits may somehow be related to cell proximity and spacing. Indeed, close spacing of embryos during group culture at the macro-scale improves development. If embryo spacing was too great, blastocyst development was not achieved [35, 36]. This spacing theory is supported by studies showing advanced embryo development in confining ultra-micro drops [34], culture within glass capillary tubes [37, 38], culture within small concave wells (GPS dish) [1], as well as culture within extremely small volumes in the “well of a well” or WOW technique [39]. If spacing is important and can improve embryo development when
cultured in groups, then another variable to consider in designing microfluidic devices for ART is shape of the culture chamber [29], which will determine if embryos are in direct contact or not. It remains to be seen if microfluidic embryo culture improves development of individually cultured embryos.

It has been suggested by some that the benefits of culturing embryos within extremely small volumes, including microfluidic culture, may result from a reduction in localized oxygen tension due to culture in these confined spaces. In traditional culture approaches, reducing the oxygen concentration from atmospheric levels of approximately 20% to 5-7% is beneficial for embryo development and quality [40-43]. Interestingly, the majority of reported microfluidic culture experiments were performed in 5% CO2 in air, and thus, reduction in localized oxygen would appear to be a plausible theory. However, bovine embryos cultured in dynamic microfluidic devices in 5% O2 still showed improved development over static culture in the same device [18]. Also, it should also be noted that hypoxic conditions are detrimental to embryo development [42, 44]. Thus, if localized oxygen depletion is occurring in microdevices, culturing in reduced oxygen tension could in fact result in hypoxia, decreasing embryo quality. Fortunately, as indicated by the results of Bormann and colleagues using bovine embryos (27), this does not appear to be the case. In support of this, mathematical modeling suggests oxygen depletion does not appear to be a factor in microdrops, regardless of the number of mouse embryos cultured, as diffusion and convection currents mix the environment to prevent anoxic regions [45]. Thus, localized reduction in oxygen levels is questionable, especially when one considers the microfluidic devices used in ART studies are composed of polydimethylsiloxane, which is extremely gas permeable. However, at least one mathematical modeling study by Byatt-Smith et al. suggested that, due to their slightly larger size, of human embryos cultured in static microdrops may become marginally hypoxic, especially when cultured in 5% O2 [46]. As a preventative measure to hypoxia, these same authors hypothesized that “embryos may develop more successfully in stirred, as opposed to still medium”. Therefore, in studies using dynamic microfluidic devices, media renewal due to flow should prevent any localized build-up or depletion of factors, including oxygen, thereby arguing against the possibility that advanced development is due to oxygen depletion.

Yet another possibility for benefits of microfluidic culture is the gentle agitation of embryos or media surrounding embryos resulting from fluid flow. This mechanical stimulation could theoretically aid in clearing of receptors or stimulate signaling pathways, subsequently promoting embryo development. Though interesting, this hypothesis still awaits further exploration.

Unfortunately, each microfluidic device utilized to date is different in its construction (culturing in channels, culturing in funnels, culturing with/without flow, using co-culture, utilizing different number of embryos/volume), thereby making it difficult to ascertain where the benefit to embryo development actually lies. Regardless, it is obvious that a combination of factors contributes to embryo development and quality within any culture device, including microfluidic platforms. Further research to examine divergence of gene expression patterns, molecular signaling pathways, or other biochemical endpoint are required to begin to elucidate possible explanations for observed effects in microfluidic culture and to optimize this promising technology for use in ART. Furthermore, microfluidic platforms may benefit from specialized culture media, addressing potential concerns inherent with fluid at such a small scale, such as pH or osmolarity shifts (Table 1).

Table 1. Suggested variables that should receive future study to assist in wide-spread implementation of microfluidic platforms in ART.

<table>
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<th>Variable</th>
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<td>Effects of embryo/gamete density</td>
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<td>Effects of embryo/gamete density</td>
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<tr>
<td>Importance of embryo/gamete spacing</td>
<td></td>
</tr>
<tr>
<td>Effects on cell transcriptome, proteonome, metabolome, and secretome</td>
<td></td>
</tr>
<tr>
<td>Influence on embryo developmental competence, implantation, and pregnancy</td>
<td></td>
</tr>
<tr>
<td>Optimization of media flow rates/patterns</td>
<td></td>
</tr>
<tr>
<td>Development of specialized culture media</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion/Future Directions

In a now increasingly used quote, Bavister stated “Ideally, the culture medium should change progressively during development to keep pace with the embryos needs, but continuous flow or perfusion culture systems are difficult to maintain on a micro scale and may be impractical” [47]. It is apparent that advances in microfluidic technology may offer a solution to this impasse, as designs are quickly approaching a stage where continuous flow of media will no longer be problematic. Characteristic fluid flow patterns in microfluidic devices are amenable to applications requiring precise fluid sampling or manipulations; including examination of cellular behavior and interactions. Thus, microfluidics has an immense potential for improving clinical ART by
offering the ability to seamlessly adjust the composition of media flowing to developing gametes and embryos. In this way the medium can be adjusted to meet the evolving nutritional requirements of the embryo, without the exposing them to the external stresses inherent in the manual manipulation required with current sequential culture systems. In addition, microfluidic technology uses minimal amounts of media in a constricted environment, similar to that experienced by gametes and embryos in vivo. Thus, the scale of microfluidic devices offers the ability to usher in a new era of embryo culture. Emerging technological advancements offer the ability to regulate not only the embryo culture fluid dynamics, but also the substrate composition and dynamics, essentially marking the end of “fixed” chemistry in media. By combining a microfluidic platform with 3-dimensional matrices of oriented macromolecules to create a “moist” rather than a fluid environment, more like that of the female reproductive tract, embryos may benefit in ways previously unavailable in standard culture systems. Furthermore, and perhaps more importantly, the scale of microfluidic platforms allows multiple procedural steps of IVF on the same device. This “lab-on-a-chip” would not only save space, but also time, as it allows for automation of processes as well as inclusion of diagnostic assays aimed at non-invasively identifying the healthiest cells for subsequent use. Indeed, diagnosis of preimplantation embryo metabolism and respiration as an indicator of quality have been performed in microfluidic devices [48, 49].

Although theoretical advantages for microfluidics platforms in ART should be now readily apparent, it is important to recognize that several potential issues must be addressed in order to achieve wide-spread acceptance.

These considerations can be grouped into 4 major categories: 1) Material/Design Biocompatibility, 2) Device Operation/Failure, 3) Manipulation/Handling of Embryos, and 4) IVF Laboratory Compatibility (Table 2). Diligence in these pursuits is called for. Although the field has received tremendous initial interest, the lack of peer-reviewed publications demonstrates the infancy of the technology. Continued research and exacting experimentation are required to realize the full potential of microfluidic technology for ART. Current approaches and future directions for use of microfluidic platforms in ART are discussed in greater detail in a thorough review by Swain et al. [50].

References

Table 2. Practical considerations that must be addressed in order for microfluidic devices to gain wide-spread acceptance into IVF laboratories.

<table>
<thead>
<tr>
<th>Material Design/Biocompatibility</th>
<th>Device Operation/Failure</th>
<th>Manipulation/Handling of Embryos</th>
<th>Laboratory Compatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-toxic fabrication materials</td>
<td>perfusion system (recirculating or not)</td>
<td>easy visualization</td>
<td>fit in conventional incubators</td>
</tr>
<tr>
<td>sterilization of device</td>
<td>ability to be used over 5-6 days</td>
<td>rapid loading/unloading</td>
<td>user friendly external apparatus</td>
</tr>
<tr>
<td>packaging to pass “in-house” QC</td>
<td>detection of failure (air bubbles)</td>
<td>ability to isolate/monitor individual gametes or embryos</td>
<td>short set-up time</td>
</tr>
<tr>
<td>fit in conventional incubators</td>
<td>easy rectification of failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>user-friendly external apparatus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
for selecting motile with with higher DNA integrity Fertil Steril; 88: S76.


38. Lane M, Gardner DK. 1992. Effect of incubation volume and

CONTINUED ON PAGE 20


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Throughout the last several decades there have been multiple breakthroughs that have advanced assisted reproductive technologies, including gamete and embryo freezing. Which of these has had the greatest impact on animal offspring production? This short article will identify some of the key events in the development of assisted reproductive technologies in cryobiology in domesticated animals. It should not be overlooked that some preservation methods are more advanced in humans than in animals.

**Sperm Freezing**

The first production of a few viable human sperm after freezing to –196°C was reported in 1938 (Jahnel, 1938). Correspondingly, the first attempt at freezing frog sperm using sucrose dehydration was also reported in 1938 (Luyet and Hodapp, 1938). The first use of glycerol as a cryoprotectant for avian sperm was reported in 1949 (Polge et al., 1949).

Using the freezing method of Polge and co-workers (1949), Stewart (1951) reported the first birth of a calf following insemination with frozen-thawed sperm using glycerol as the cryoprotectant. Five cows were artificially inseminated, resulting in the first mammal (a calf named ‘Frosty’) born from frozen-thawed semen in the world in England. Dr. Chris Polge of Cambridge has often told the story of this event, saying that this first frozen-thawed sperm pregnancy was ‘pure luck’. The first calf from frozen-thawed bull semen in the USA occurred in Wisconsin in 1953. This Holstein calf was also named ‘Frosty’.

The first frozen-thawed human sperm used for AI was reported in the USA occurred in 1953 (Bunge and Sherman, 1953). However, after more than 20 years of effort, the first litter of four pigs was produced from frozen-thawed sperm in Minnesota in 1970. The sperm pellets of boar sperm, prepared by Dr. Ed Graham, were thawed on a kitchen hot plate and then surgically transplanted into recipient sows.

Today, in the United States, more than 85% of all dairy cattle are artificially inseminated with frozen-thawed semen, in contrast with less than 5% of beef cattle. It is estimated that over 25 million cattle are artificially inseminated annually throughout the world. The largest percentage increase in AI in recent years in the USA has been in performance horses and pedigreed dogs.

**Advances in Sperm Preservation**

Viable sperm have been harvested from testes of dogs postmortem and stored for 24 hours at 4ºC (Stilley et al., 2000). More recently, the first frozen-thawed sperm offspring in mammals (mice) have been born after whole body freezing after death (Ogonuki et al., 2007). In this case, the males were frozen for 15 years prior to the harvesting of sperm.

The ultimate dream of the andrologist is to store dried sperm on the shelf at room temperature. Freeze dried sperm research is presently underway in several laboratories. There has been recent progress with dried sperm producing live mouse offspring (Wakayama and Yanagimachi , 1998; Ward et al., 2003). Also, there has been some success with dried sperm producing blastocysts in the cow (Keskintepe et al., 2002) and in the domestic cat (Moisan et al., 2006).

**Oocyte Freezing**

Frozen-thawed human oocytes were first used to produce IVF embryo pregnancies in the mid to late1980's (Chen, 1986; Van Uem et al., 1987). This method remained
dormant for a number of years, but its use has increased in recent years. During the last five years, vitrified cattle oocytes have produced viable IVF and nuclear transfer embryos, and offspring have been born. This area is under intense investigation in farm animals.

The first foals from oocyte vitrification were produced at Colorado State University (CSU) in 2002 (Macelllan et al., 2002). This procedure along with oocyte transfer is becoming the procedure of choice for ‘problem breeder’ mares.

Embryo Freezing

The first frozen-thawed embryo offspring were produced in mice in England, 1972 (Whittingham et al., 1972). Shortly thereafter, the first frozen-thawed embryo calf was born in England in 1973 and was named ‘Frosty II’ (Wilmut & Rowson, 1973a,b). Although initially there was concern about the viability of offspring resulting from a frozen-thawed cattle embryo, it should be noted that at 10 years of age (old for the life span in cattle) Frosty II remained healthy and fertile.

A one-step method for direct nonsurgical transfer of frozen-thawed cattle embryos was initially developed in Texas in the early 1980s (Leibo, 1984). At present, most of the frozen-thawed cattle embryos are transferred to recipient females using this procedure. Today, 45 to 55% of all the embryos collected commercially from cattle are frozen for subsequent transfer. A large portion of these frozen embryos are marketed to other countries (see Thibier, 2007).

The first frozen-thawed embryo lambs were produced in England in 1976 (Willadsen et al., 1976) and the first frozen-thawed lambs in the USA were produced at LSU (Sims et al., 1982). Correspondingly, the first frozen-thawed goat offspring were reported in Australia in 1976 (Bilton & Moore, 1976) and the first frozen-thawed goat offspring in the USA were also produced at LSU in 1987 (Pendleton et al., 1987). The first frozen-thawed embryo foal was produced in Japan in 1982 (Yamamoto et al., 1982), which was then followed by a subsequent frozen-thawed embryo foal reported from CSU in 1984 (Takeda et al., 1984). For the last and most difficult of the farm animals, the first frozen-thawed embryo piglets were produced in Japan in 1989 (Hayashi et al., 1989).

The first frozen-thawed baboon embryo to produce a live offspring was reported in 1984 (Pope et al., 1984). Shortly thereafter, the first frozen-thawed embryo offspring were produced in humans in Europe (Zeilmaker et al., 1985), and in Melbourne, Australia on March 28, 1984 (Downy et al., 1985). The Australian new-born resulted from an 8-cell embryo that had been frozen for two months before autologous transfer to the donor. Baby Zoe Elizabeth Leyland weighed 5 lbs. and 13 oz. at birth.

Embryo Refreezing and Assisted Hatching

What could one do, if the wrong embryos were thawed? Refrozen intact embryos (mice) resulted in viable post-thaw embryos at LSU in 1992 (Vitale et al., 1992). Successful refreezing of mouse embryos after blastomere removal was reported in 1993 (Snabes et al., 1993). The first report of refrozen cattle embryos producing viable post-thaw embryos was reported at LSU in 1994 (Vitale et al., 1994).

Assisted hatching was first developed for use in human embryos in 1989 (Malter et al., 1989; Cohen et al., 1992). In farm animals, laser-assisted hatching has recently been developed for use in frozen-thawed in-vitro-produced cattle embryos at LSU in 2004 (Gao et al., 2004). Today, this laser-assisted hatching technology has been refined and now commercially available to farm animal embryo transplant units.

Summary

Since these advances in gamete and embryo cryopreservation there have been more recent developments in assisted reproductive technologies in animal models. The advancements in these areas hopefully will allow the medical community to visualize ways to develop new assisted reproductive technologies in humans.
References


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Group versus Individual Culture: Effect of Embryo-Secreted Factors

by Michael Neal, MSc
Laboratory Manager, Hamilton Health Sciences Centre for Reproductive Care
Hamilton, ON, Canada

The benefit of culturing embryos in groups compared to individual culture has provided for an interesting debated among embryologists. The notion of a group culture effect has come from several animal studies demonstrating that group culture promotes better embryo development when compared to individually-cultured embryos. The favourable effects of culturing embryos in groups on blastocyst formation and hatching rates have been demonstrated for cattle (O’Doherty et al. 1997), sheep (Gardner et al. 1994), and mouse (Kato and Tsunoda 1994; Lane and Gardner 1992; Paria and Dey 1990; Salahuddin et al. 1995) embryos. Culturing human embryos in groups is also thought to enhance development (Jones et al. 1998). More specifically, it has been suggested that specific factor(s) are secreted from embryos that influence the growth and development of companion embryos. Furthermore, it has been suggested that this positive interaction among human embryos in vivo explains the disproportionate increase in implantation rate when more than one embryo is transferred (Walters et al. 1985).

The question of group culture has been of interest to our IVF Lab Team. The recent introduction of the Corral IVF culture dish (IVFonline, Guelph, ON, Canada) has provided a unique opportunity to study embryos that share media within a group or are cultured individually. The design of the dish allows monitoring of individual embryo performance, which is still our primary criterion for embryo selection. We found no difference in embryo growth rate or quality of sibling zygotes randomly allocated to group (n = 208) or individual culture (n = 210) from 44 patients (mean age ± S.D. = 32.9 ± 3.4 years). Embryos were selected for transfer based on cell number and morphological appearance. There was no difference in embryo quality as calculated by a cumulative embryo score on day 3 of culture. For transfer, 15 patients received only embryos from individual culture, 12 received only embryos from group culture, and the remaining 12 patients received at least one embryo from each of individual and group culture (Table 1). Pregnancy rates were similar between the individual (46.6%) and mixed embryo transfer groups (41.2%). However, the highest pregnancy success (66.7%) was observed among patients that had embryos exclusively from embryos cultured as a group (Table 1).

Because there was no observed difference in the morphological appearance of the embryos between the groups, there may be some factor(s) in the media of grouped embryos that results in better pregnancy success. One of these factors may be embryo-derived platelet-activating factor which has been shown to be secreted by mouse embryos and stimulates embryo development to blastocyst (Stoddart et al. 1996). The other aspect to consider is the timing of secretion of these paracrine or autocrine effects. Fujita et al. (2006) demonstrated that embryo-secreted factors released or accumulated in the culture media are important for embryo development up until day 4 after IVF. This suggests that the developmental fate has been established at the time of blastocyst formation. Similarly, a beneficial effect has been shown on the development of individually cultured mouse embryos with the addition of epidermal growth factor or transforming growth factor (Paria and Dey 1990).

It has been established that the expression of specific genes can be effected by the presence of other embryos during culture in vitro, in several species (Fujita et al. 2006; Gardner et al. 1994; Paria and Dey 1990). It is not known whether or not human pre-implantation embryos secrete factors that may have a long-lasting effect on the establishment and maintenance of pregnancy. If products of genes are involved with determining the subsequent fate of the embryo, then a careful assessment of the culture conditions (group vs individual) and their influence on gene expression will lead to better selection of high implantation embryos and better pregnancy success with IVF.

Table 1. Comparison of pregnancy rates between women who had embryos transferred from individual or group culture, or mixtures of embryos from both individual and group culture.

<table>
<thead>
<tr>
<th>Source of transferred embryos</th>
<th>Number of transfers</th>
<th>Number pregnant</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual culture</td>
<td>15</td>
<td>7</td>
<td>46.6</td>
</tr>
<tr>
<td>Group culture</td>
<td>12</td>
<td>8</td>
<td>66.7</td>
</tr>
<tr>
<td>Mixed</td>
<td>17</td>
<td>7</td>
<td>41.2</td>
</tr>
</tbody>
</table>
References


You can contact Michael S. Neal, MSc at: nealm@hhsc.ca
Comparison of Two Media for the Culture of Human Embryos up to Day 3: A Prospective Randomized Study

Sidney Verza Jr., Danielle T. Schnneider and Sandro C. Esteves
Androfert, Centro de Referência em Reprodução Masculina, Campinas, São Paulo, Brasil.

Introduction

The successful development of human embryos in vitro and their subsequent development after transfer to the uterus can be affected by any or all of the entire series of events in ART. These include the stimulation protocol, the quality of the oocytes, the quality of the sperm, fertilization, embryo culture, transfer, and preparation and maintenance of the uterus. Among these factors, embryo culture is one of the most essential components.

The choice of the best culture medium is an important challenge for the embryologist, and should be based on both (i) published studies and (ii) in-house studies in each IVF laboratory in order to determine whether the published findings are applicable to the specific work conditions.

Objective

The aim of this study was to compare in-vitro embryo development to day 3 following culture in two different commercially-available culture media intended of human embryos.

Materials and Methods

A total of 763 metaphase II (MII) oocytes retrieved for intracytoplasmic sperm injection (ICSI) were blindly randomized for culture. After ICSI, the injected oocytes were cultured for three days using two different media, as follows, (i) Global (LifeGlobal, IVFonline, Canada, n=376) and (ii) IVF (Vitrolife, Sweden, n=387). The MII oocytes from an individual patient were split equally, microinjected and cultured in the tested media.

Ovulation Induction and Oocyte Recovery

Ovarian hyperstimulation was achieved using GnRH analogue (nafarelin acetate) along with recombinant FSH (rFSH: Gonal-F®, Serono) in a long down-regulation protocol. Starting gonadotrophin dose varied from 150-300 IU and was based on female age, body mass index, ovarian volume, basal FSH levels and the number of preantral follicles. Human chorionic gonadotrophin (hCG) was administered when at least one follicle reached 18 mm in diameter on ultrasound scan. Oocytes were collected 34-36 hours after hCG administration under transvaginal ultrasound guidance.

Preparation of Oocytes and Spermatozoa

Cumulus-oocyte complexes were treated with 80 IU/ml hyaluronidase for 30 seconds (Hyase, Vitrolife, Sweden) and then stripped of the remaining cumulus cells by mechanical aspiration through a 130-μm pipette (Flexipet, Cook, USA). Ejaculated sperm samples were processed for ICSI using a discontinuous two-layer density gradient or simple washing. Percutaneous epididymal (PESA) or testicular (TESA) sperm aspirations were performed to retrieve sperm from men with obstructive azoospermia. Testicular sperm aspirations (TESA) or testicular sperm extraction with microdissection (micro-TESE) were used in non-obstructive azoospermia cases.

Intracytoplasmic Sperm Injection (ICSI)

Oocytes showing first polar body extrusion were injected with a single spermatozoon. Sperm selection and microinjection were performed using 400X magnification. The ICSI procedure was carried out in a Class 100 clean-room equipped with volatile organic compounds (VOC) filtration units (VECO, Brazil). The microscope and manipulators were set up on anti-vibratory table. All injections were performed at 37°C under an inverted microscope (Eclipse, Nikon, Japan) equipped with Hoffman modulation contrast optics, and electro-hydraulic manipulators and microinjectors (Narishige, Japan). Injected oocytes were washed and transferred to 50 μL droplets of each medium.

Embryo Culture

Fertilization was checked 18-20 hours after ICSI. Normal, fertilized zygotes were cultured in microdroplets of culture media covered with mineral oil (Ovoil, Vitrolife, Sweden) at 37°C in a humidified atmosphere of 6.0% CO₂ in air.
**Main Outcomes Analyzed**

Fertilization rate, cleavage rate, and the proportion of top quality embryos (TQE) on days 2 and 3 were compared. TQE were defined as those presenting with 3-4 and 7-9 equally size blastomeres on days 2 and 3, respectively, and with grades I or II of cytoplasmic fragmentation.

**Statistical Analyses**

Student’s t-tests and Mann-Whitney U-tests were used to compare the groups, as appropriate, with P<0.05 considered significant.

**Results**

Normal fertilization after ICSI was not statistically different between groups (Table 1). However, we found that embryo culture using Global media yielded a higher proportion of top quality embryos on day 2 (P = 0.01) and on day 3 (P < 0.01), in comparison with IVF. In addition, cleavage rate was greater for embryos cultured in Global medium compared with those cultured in IVF (P < 0.01). There was no difference in the proportions of embryos without fragmentation on Day 3 between the media culture groups.

**Discussion and Conclusion**

Our results suggest that the proportion of TQE is increased by culturing human zygotes obtained after ICSI with Global media as compared to IVF. As shown in Table 2, Global medium is considered a complex medium that contains amino acids, different from IVF medium, and this observation may be related to the results found. Global medium is a medium that contains all the nutrients needed to support human embryos development until day 5, while IVF medium is a sequential medium, intended for use only until day 3 of culture.

The results observed may be useful to optimize the number of TQE for transfer and cryopreservation, and it may be especially relevant for the cases in which a low number of mature oocytes is expected.

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AV. Dr. Heitor Penteado, 1464 – Taquaral – Campinas, São Paulo, Brasil

**Table 1.** Comparison of fertilization, cleavage and quality for human embryos cultured in either Global or IVF medium.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group 1 (Global) (n=376)</th>
<th>Group 2 (IVF) (n=387)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate (%)</td>
<td>72.5 ± 22.0</td>
<td>75.6 ± 19.9</td>
<td>NS</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
<td>98.8 ± 5.0</td>
<td>93.5 ± 14.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Top quality embryos on Day 2* (%;n)</td>
<td>77.8 ± 33.6 (198)</td>
<td>63.4 ± 30.3 (187)</td>
<td>0.01</td>
</tr>
<tr>
<td>Top quality embryos on Day 3* (%;n)</td>
<td>70.1 ± 32.0 (180)</td>
<td>45.8 ± 33.5 (143)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Embryos without fragmentation on Day 3** (%;n)</td>
<td>25.0 ± 30.2 (68)</td>
<td>19.6 ± 27.5 (59)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant

*Embryos with 3-4 and 7-9 blastomeres on day 2 and 3, respectively, with grade I or II of fragmentation.

**Table 2.** The components of Global medium and IVF medium

<table>
<thead>
<tr>
<th>Global</th>
<th>IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chloride</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>EDTA</td>
<td>EDTA</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>Magnesium sulfate</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>Sodium chloride</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Sodium lactate</td>
<td>Sodium lactate</td>
</tr>
<tr>
<td>Sodium piruvate</td>
<td>Sodium piruvate</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Penicilin</td>
</tr>
<tr>
<td>Amino acids</td>
<td>HSA</td>
</tr>
<tr>
<td>Phenol red (+10%HSA added)</td>
<td></td>
</tr>
</tbody>
</table>
• Proven Results
• Over 20 independent studies with published results
• Stringent Quality Control

Figure 1. Development of human embryos cultured in γ-glutamyl® from Day 1 to Day 3 and Day 3 to Day 5, or in G1 from Day 1 to Day 3 and then in G2 from Day 3 to Day 5. The proportion of embryos reaching the blastocyst stage by Day 5 was significantly greater in γ-glutamyl®. The pregnancy and implantation rates were significantly greater for embryos cultured in γ-glutamyl® than for those cultured in G1/G2. (Zech et al., Human Reprod. 21, Suppl. 1, i162, 2006)

Figure 2. Development of human embryos cultured in γ-glutamyl® from Day 1 to Day 3 and Day 3 to Day 5, or in BAS1 from Day 1 to Day 3 and then in BAS2 from Day 3 to Day 5. The proportion of embryos having 6 or more cells on Day 3 was significantly greater in γ-glutamyl®. The blastocyst rate on Day 5 and the pregnancy rate were not different between media treatments (Matsubara et al., Proc. 24th Ann. Meet. Japan Soc. Fert. Implant. 206, 2006)

Figure 3. Development of human embryos cultured in γ-glutamyl® from Day 1 to Day 3 and Day 3 to Day 5, or in G1 from Day 1 to Day 3 and then in G2 from Day 3 to Day 5. The proportion of embryos reaching the blastocyst stage by Day 5 was significantly greater in γ-glutamyl®. The pregnancy rates were not different between the culture media treatments. (Angus et al., Fert. Steril. 86 Suppl 2, S229, 2006)

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6. Removes the high amounts of Chemical Air Contaminants.

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The global® Family of Media: a Unified Approach to Human Embryo Culture

by Don Rieger, PhD
Vice President, Research and Development
LifeGlobal, LLC

Introduction

Clinical studies have shown that the use of a single medium, global®, for culture of the human embryo from the zygote to the blastocyst stage, results in better in-vitro development (Angus et al. 2006; Freeman and Rieger 2004; Greenblatt et al. 2005; Matsubara et al. 2006; Vansteenbrugge et al. 2007), and/or pregnancy and implantation rates (Sepulveda et al. 2008; Zech et al. 2006) compared with culture in sequential media. These observations strongly support the suggestion of Biggers and Racowsky (2002) that “The switching of embryos from one medium to another may cause additional osmotic or other shock to the embryo …”

In view of the clinical results using global® medium for embryo culture, a family of media based on global® have been developed for human ART procedures. The overarching philosophy is to maintain, as closely as possible, the same chemical environment throughout all stages of gamete and embryo handling and culture, in order to minimize the stress of changing from one medium to another.

In addition to global®, for embryo culture from the zygote to the blastocyst stage, the family includes global® for Fertilization, for conventional in-vitro fertilization; global® w/HEPES, for ICSI and embryo handling; and LifeGlobal PGD Biopsy Medium, for the removal of cells for preimplantation genetic diagnosis. The components of the four media are shown in Table 1.

global® for Fertilization

In conventional in-vitro fertilization, the medium must support the metabolic requirements of three cell types: the oocyte, its surrounding cumulus cells, and the spermatozoa. The oocyte itself metabolizes little or no glucose (Biggers et al. 1967; Rieger and Loskutoff 1994). Rather, the cumulus cells metabolize glucose to pyruvate, lactate, and other intermediates which are passed to the oocyte for oxidative

Table 1: The components of the global® family of ART media

<table>
<thead>
<tr>
<th>Component Type</th>
<th>global® for Fertilization</th>
<th>global® w/HEPES</th>
<th>LG PGD Biopsy Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts</td>
<td>NaCl</td>
<td>NaCl</td>
<td>NaCl</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>KCl</td>
<td>KCl</td>
</tr>
<tr>
<td></td>
<td>CaCl₂</td>
<td>CaCl₂</td>
<td>CaCl₂</td>
</tr>
<tr>
<td></td>
<td>KH₂PO₄</td>
<td>KH₂PO₄</td>
<td>KH₂PO₄</td>
</tr>
<tr>
<td></td>
<td>MgSO₄</td>
<td>MgSO₄</td>
<td>MgSO₄</td>
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metabolism (Biggers et al. 1967; Downs et al. 1996; Leese and Barton 1984). Human sperm also metabolize glucose (Rees et al. 1990), and capacitation, the quality of motility, and the ability to penetrate the oocyte depend on glucose (Rogers and Ferreault 1990).

Conventional human IVF is typically carried out in HTF, which contains pyruvate, lactate and a relatively high concentration of glucose (Quinn et al. 1985). However, the ionic composition of HTF is quite different from global® and it contains no amino acids. In some circumstances, global® works well as a fertilization medium. However, global® contains a relatively low concentration of glucose, which may be insufficient to satisfy the metabolic requirements of fertilization if there are large numbers of cumulus cells and/or spermatozoa present. Consequently, global® for Fertilization was formulated with a glucose concentration equal to that of HTF, but with all the other components present in the same concentrations as in global®.

global® w/HEPES

All human embryo culture media include approximately 25 mM sodium bicarbonate (NaHCO₃), which, when placed in an incubator containing 5-6% CO₂ results in a physiological pH of approximately 7.30. However, when removed from the incubator, the pH of bicarbonate-buffered media rises very rapidly. ART procedures such as ICSI and assisted hatching are performed outside of the incubator, and consequently the medium for such procedures must use an acid-base buffer that does not require CO₂, usually HEPES (Good et al. 1966).

Quinn et al. (1984) have suggested that the use of a HEPES-buffered medium for manipulating embryos in the absence of an atmosphere containing 5% CO₂ is an important factor in the success of human IVF. However, the use of relatively simple media like HEPES-buffered HTF may be stressful to the embryo, because of differences in ionic composition and the lack of amino acids. For that reason, global® w/HEPES contains all of the salts, energy substrates, amino acids, and other components, in the same concentrations, as in global®. It is important to note that global® w/HEPES does contain a low concentration of bicarbonate, because bicarbonate is important for embryo development in some way unrelated to acid-base buffering (Kane 1975).

LifeGlobal PGD Biopsy Medium

Preimplantation diagnosis of early human embryos for chromosomal or gene defects requires that a cell be removed, usually at the 6-8 cell stage, on Day 3 of development. However, at about that time, as a prelude to compaction, adhesion between cells increases; partly mediated by epithelial cadherins (calcium-dependent adhesion molecules) (see Alikani 2005). Because of the adhesion between the cells, separation of a cell from its neighboring cells may cause stress or damage to the embryo. Consequently, it is advisable to temporarily disrupt the E-cadherin connections and reduce cell-cell contact. This is typically done by placing the embryo into a calcium- and magnesium-free medium (Grifo et al. 1990).

Although the embryo is exposed to the biopsy medium for a very short time, usually not more than 5 minutes, the composition of the biopsy medium can affect the subsequent development and viability of the embryo following return to culture. Hill and Li (2004) found that the clinical pregnancy rate for biopsied embryos was significantly better if the biopsy medium was supplemented with alanyl-glutamine, EDTA, pyruvate and sodium lactate, compared with unsupplemented biopsy medium. They concluded that “We believe this step -- that of using embryo biopsy media that contain essential amino acids and nutrients found in the primary culturing milieu -- to be critical in maximizing clinical outcomes.” Based on their results, and in collaboration with Dr. Santiago Munné, (Reprogenetics, Livingston, NJ, USA), LifeGlobal PGD Biopsy Medium was formulated without calcium chloride and magnesium sulfate, but with all the energy substrates, amino acids and other components, at the same concentrations, as in global®. Based on the experience of Munné and his colleagues, sucrose was added to cause the cells to shrink slightly and thus make it easier to remove the biopsy. LifeGlobal PGD Biopsy Medium is HEPES-buffered, and contains human serum albumin to make it ready-to-use. As for global® w/HEPES, and for the same reason, LifeGlobal PGD Biopsy Medium contains a low concentration of bicarbonate.

The Unified Approach to Human Embryo Culture

As noted above, when used together, the global® family of media have been designed to minimize the stress of changing from one medium to another throughout ART procedures from fertilization to transfer.

Figure 1 shows the time course of the use of global® for Fertilization, global® w/HEPES, global® and LifeGlobal PGD Biopsy Medium from Day 0 to Day 6.

Conclusion

Use of the global® family of ART media allows the embryologist to maintain, as closely as possible, the same chemical environment throughout all stages of gamete and embryo handling and culture, and thereby minimize the stress on the embryo. By minimizing stress, the inherent developmental potential of the embryo can be better maintained.

CONTINUED ON PAGE 32
Figure 1: The time-course of culture of human embryos in the globa® family of ART media from fertilization on Day 0 through to blastocyst transfer on Day 5 or 6.

Acknowledgement

My thanks to Dr. Mina Alikani, (Tyho-Galileo Research Laboratories, Livingston, NJ, USA) for a very helpful discussion of E-cadherin binding in the early embryo, and for her critique of the manuscript.

References

Kane MT (1975) Bicarbonate requirements for culture of one-cell rabbit ova to blastocysts. Biol Reprod 12, 552-5.
Zech N, Stecher A, Zech H, Uher P, Vanderzwalmen P (2006) Prospective analysis of embryo development to day 5 and transfer outcomes in sequential medium (G1.3-G2.3) vs a one step protocol (Global medium). Human Reprod. 21, Suppl. 1, i162 (Abstract).
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- Same chemical environment throughout all stages of oocyte and embryo handling and culture
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- Easy to use

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Our Bodies, Our Stem Cells

by John Hambor, PhD (CEO of CellDesign, Inc.)

The carousel of change is a fundamental tenet of life. Just as seasons turn on a yearly cycle and organisms evolve on a millennial timeline, our bodies have the amazing capacity to transform through regeneration. Over the ebb and flow of our entire lifetime, worn out cells die and are replenished by new cells so that we can continue to breathe, move, eat, smell, feel, read and think. We only need to consider our growing children, attend our 25th high school reunions, or look at earlier pictures of ourselves to realize that all of us inevitably, imperceptibly and imperfectly morph throughout our lives. But how and why does this happen? The answers may be found in our stem cells.

For most of us, the process of transformation is so gradual that we don’t seem to notice the progressive physical changes that occur on a daily basis. As you read this article, you will produce billions of new blood cells and millions of new skin cells. In fact, we continuously produce new cells for every organ and tissue; even the neurons of our brains are slowly replaced over time. Sunburned skin peels and is restored; broken bones mend; we can even sculpt our muscles much like the Play-Doh™ figurines we molded as children. With this plasticity comes the opportunity to direct cellular fates and dictate the biological outcome as we age. The basics of a healthy diet, sanitary habits and exercise can increase fitness, prolong life expectancy, and resist aging. With growing knowledge of the intricate mechanisms of developmental and regenerative processes, it may even be possible to create new medical treatments that either replace lost or damaged tissues or control fundamental aging processes. Imagine one day being able to take a pill that will enhance our body’s ability to regenerate tissue or inject cells that will rejuvenate or replace failing organs. With the help of modern science, will we find Ponce de Leon’s proverbial “Fountain of Youth” and prolong life expectancy?

In essence, regeneration is healing. We realize the healing power of nature, vis medicatrix naturae, as our inborn regenerative processes kick into high gear in response to injury. We scrape a knee and are appreciative of how quickly the body can heal itself. In a few short days, evidence of damaged tissue has vanished and the scraped knee is virtually forgotten. Underlying this outcome is a well-orchestrated sequence of events that begins with inflammation resulting in the removal of damaged tissue, followed by the generation of replacement tissue. Replacement cells, each with a specialized function for that tissue or organ, arise from a restricted reservoir of regenerative stem cells that exist in a specified niche in each tissue. A complex set of cues signal these stem cells to expand and then differentiate into the replacement cells needed to restore the damaged tissue. Thus, it is our stem cells that drive the regeneration process that is evident in healing. And it is our stem cells that reveal a diminishing ability to regenerate that ultimately results in aging.

Since stem cells are the facilitators of regeneration, understanding their origins, development and fates holds the key to discovering new mechanisms and pathways that could result in the development of new regenerative medicines. Every cell of the trillions of cells that constitute our human bodies, including stem cells, can be traced back to the fertilized egg that came into existence with the initial union of sperm and egg. All of the over 200 different types of cells of our bodies are derived from a pool of stem cells in the early embryo. During embryonic development, as well as later in life, lineage-restricted stem cells give rise to the specialized or differentiated cells that perform specific functions of the body, such as skin, blood, muscle, and nerve cells. Not surprisingly, the same molecular instructions that guided our early development in utero also provide the biologic indicators that prompt differentiation decisions as we mature from childhood through...
adolescence into adulthood, as well as mediate the processes of homeostatic tissue regeneration and wound healing. Since the same biologic blueprint that specifies cell fate during early development is used again and again throughout our lives, a greater appreciation of developmental biology will provide important insights into the biology of stem cells.

Stem cells are found in all of us, from the early stages of human development to the end of life. All stem cells may prove useful for medical research, but each of the different types has both promise and limitations. Embryonic stem cells (ES cells), which can be derived from a very early stage in human development, are pluripotent, having the potential to produce all of the body's cell types. Human ES cells have been the topic of much ethical debate in that current methods used for their isolation result in the destruction of pre-implantation human embryos. On the other hand, adult stem cells, which have been found in almost all tissues in fully developed humans, from babies to adults, may be lineage-restricted, limited to producing only certain types of specialized cells. Multipotent stem cells found in umbilical cord blood and matrix (Wharton's Jelly), as well as the placenta and amniotic fluid, appear to be less restricted, giving rise to a wide variety of cell types and tissues. Recently, artificially-derived ES cell-like cells, termed induced pluripotent stem cells (iPS cells), have been generated by introducing key stem cell-associated genes into non-pluripotent, somatic cells such as skin cells or fibroblasts. These induction genes are thought to epigenetically re-program or de-differentiate somatic cells resulting in a pluripotent stem cell that is remarkably similar to an ES cell. While new delivery techniques and induction controls are still needed to increase the efficiency of the reprogramming method, iPS cells, created to have authentic ES cell-like properties without the controversial use of embryos, represent an important advancement allowing the derivation of patient-specific cells for therapeutic uses and diseasedisposed cells for research applications.

The daily work that occurs in laboratories around the world studying stem cells begins with developing ways to identify stem cells, isolate cell lines in culture, and stimulate stem cells to differentiate. Self-renewing human ES cells are isolated from the inner cell mass of a pre-implantation blastocyst by placing these cells in a culture dish with nutrient-rich media that induces their replication in an undifferentiated state for very long periods of time. The largest source of blastocysts is from in vitro fertilization (IVF) clinics, but blastocysts can also be generated from eggs after nuclear transfer – the insertion of a nucleus from an adult somatic cell into a donated egg. Similar means are used to culture iPS cells after the re-programming step. Just a few human ES cells or iPS cells can build a large bank of stem cells to be used in experiments aimed to define the conditions needed for stimulating them to create specialized cells such as neurons, hepatocytes or cardiomyocytes. ES cells and iPS cells seem to be far more flexible than stem cells found in adults, because they have the potential to produce every cell type in the human body. ES cells are generally easier to collect, purify and maintain in the laboratory than adult stem cells. Improved derivation techniques may also make iPS cells easier to manipulate in the lab.

Adult stem cells are hidden deep in defined niches within organs, in times of need replenishing the millions of ordinary cells that surround them in that tissue. Some adult stem cells are currently being used in therapies such as bone marrow transplants or skin grafts. Though every organ has a characteristic turnover rate ranging from rapid (such as intestine, skin and blood) to slow (such as brain and pancreas), stem cells have been identified and isolated from every organ and tissue of the human body. Unlike ES cells, adult stem cells are already somewhat specialized. Though adult stem cells may be lineage-committed, scientists are working on finding ways to stimulate adult stem cells to be more versatile. In addition, extra-embryonic material such as cord blood, matrix, placenta and amniotic fluid are being explored to provide alternative sources of multipotent, unspecialized cells.

As early as 1961, adult bone marrow was found to contain cells that could make all of the blood cell types. But it wasn't until 1988 that these stem cells were isolated as pure populations. Why did it take so long? The techniques for identifying stem cells have only recently been developed. Partly, this is because adult stem cells are, by their very nature, inconspicuous in shape, size and function. They also tend to hide deep in tissues, don't replicate unless needed and are present only in low numbers, making their identification and isolation like finding a needle in a haystack.

How do scientists know when they have found a stem cell? Every cell displays an array of proteins on its surface; different cell types have different proteins. Scientists can use the surface proteins as “markers” that characterize individual cell types – a type of “molecular fingerprint”. For example, using antibodies that recognize and attach to specific
surface proteins that are chemically modified to fluoresce under specific wavelengths of light, scientists can visually tell the difference between a blood stem cell and a mature white blood cell. Unfortunately, not all stem cells can be currently identified in this manner because markers for all stem cell types have yet to be identified. Stem cells can also be identified by their behavior in the laboratory: stem cells must be able to remain unspecialized and self-renew for long periods of times. With new isolation techniques and improved methods to grow stem cells, the search for additional adult stem cell types continues.

Cell culture refers to the growth and maintenance of cells in a controlled environment outside an organism. Successful stem cell culture, keeping the cells healthy, dividing and unspecialized, is the first step in establishing a stem cell line — a propagating collection of genetically identical cells. Cell lines are important because they provide a long-term supply of multiplying cells that can be distributed for research and therapy development. However, over time, all cell lines change, typically accumulating harmful genetic mutations. Though there is no reason to expect that stem cell lines will behave differently, it is possible that the intrinsic nature of stem cells may enable them to maintain their genomic integrity via physiologic mechanisms that either resist or correct genetic mutations. While there is much that can be learned using existing stem cell lines, such concerns necessitate continued monitoring of these cells as well as the development of new stem cell lines in the future.

Once a stable stem cell line has been established, the process of causing the stem cells to differentiate into specialized cell types can begin. The cellular environment in which stem cells naturally reside provides clues as to how to make them differentiate in a culture dish. For example, in the bone marrow, where blood stem cells reside, bone cells send physical and chemical signals that tell the blood stem cells when to differentiate. The nature of these signals is just beginning to be understood so that ways to mimic the natural process in cell culture can be developed. Usually, the technology involves adding certain proteins to the cell culture and, in some cases, introducing specific genes into the stem cells. Significant advancements in this area have made it possible to generate a wide variety of stem cell-derived cell types such as neurons, hepatocytes and cardiomyocytes. These physiologically-relevant human cells have characteristics of their counterparts found in the human body and have demonstrated important functional properties making them a novel renewable source of human cells and tissues for transplantation, research and drug discovery.

Currently, only a few diseases are treatable with stem cell therapies because only a limited number of cell types can be efficaciously and safely regenerated. However, the success of the most established stem cell-based therapies — blood and skin transplants — gives hope that someday stem cells will allow physicians to provide therapies for a variety of diseases previously thought incurable. Many major diseases are caused by the loss of a single cell type or tissue. For example, type I diabetes (Juvenile-onset) is caused by the loss of the insulin-producing cells of the pancreas, and its treatment is limited to merely alleviating symptoms by giving the patient insulin replacement therapy. Finding a cure for such a disease would be much easier if it were possible to simply regrow the missing or damaged cells and implant them into patients.

The list of medical achievements stem cells could offer seems to be expanding at an incredible pace. The role of stem cells in medicine is already very real, but there is a danger of exaggerating the promise of new medical developments. As scientists and physicians get caught up in the excitement of breakthrough discoveries, not only the potential outcomes of both embryonic and adult stem cell research are “over-promised”, but also the time scales that are involved. The basic research needed to develop viable therapeutic options is a lengthy process that may extend over many years and decades. Even after science has moved from basic research to developing medical applications, it still takes many years to thoroughly test those applications and demonstrate that they are safe to prescribe for patients. This is true for all medical treatments, including the development of new drugs, procedures, and medical equipment, and is not specific to the living cell.
therapies made possible by stem cell research.

Stem cells offer opportunities for scientific advances that go far beyond cell-based regenerative medicine. They offer a window for addressing many of biology’s most fundamental questions. Watching embryonic stem cells give rise to specialized cells is like peeking into the earliest development of the many tissues and organs of the human body. Stem cell research may help clarify the role genes play in human development and how genetic mutations affect normal processes. They can be used to study how infectious agents invade and attack human cells, to investigate the genetic and environmental factors that are involved in cancer and other diseases, and to decipher what happens during aging.

Stem cells may also revolutionize traditional chemical medicine. Because stem cells can continue to divide for long periods of time and produce a variety of cell types, they could provide a valuable source of human cells for testing drugs or measuring the effects of toxins on normal tissues without risking the health of a single human volunteer. In the future, millions of compounds could be quickly tested on a wide assortment of cell types derived from stem cells, making drug discovery more efficient and cost effective. Access to a renewable source of human, physiologically-relevant, functional cells could revolutionize cell-based screening in all aspects of discovery and pre-clinical development of potential drugs.

Generating iPS cells from genetically-predisposed individuals could be particularly useful for testing drugs for disorders that are of genetic origin. For example, it is difficult to study the progression of Alzheimer’s and Parkinson’s diseases in the brains of live patients – but by using the cells from such patients to create iPS cell lines, it may be possible to trace the development of the disease in a culture dish and test drugs that regenerate lost nerve cells with no danger to the patient.

Since stem cells can serve as a model to study developmental or regenerative processes, they may also help scientists to calculate the effect of toxic substances in drugs, foods, and the environment. For example, a simple test measuring the differentiation of ES cells into beating heart cells is being used to identify potential teratogens that may cause reproductive toxicities. In addition, a slew of novel compounds and target proteins have recently been identified using stem cell models that modulate the regeneration of brain, bone, blood, fat, muscle, and heart tissues, providing the fodder for developing prospective new regenerative drugs.

Currently, all forms of stem cell research in the U.S. are legal at the federal level. That is, it is not illegal to make or work with new embryonic stem cell lines. However, the use of federal funds for human embryonic stem cell research is restricted to the “Presidential” cell lines that were available before August 9, 2001. Therefore, the derivation of new embryonic stem cell lines can only occur when scientists are working with non-federal funding. Many prominent scientists and physicians have publicly taken the stand that stem cell technology is too important to be limited by restrictions on any lines of investigation. To take up the slack, some states and private foundations have been supporting the work that is currently encumbered by federal funding limitations. Appropriately, some requirements of federal law, such as protection of human subjects, apply to state- and privately-funded stem cell research. With changing political winds beginning to blow, perhaps it is time for us to realize that the true power to unleash the enormous potential of the stem cell lies within us, both morally and scientifically.

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Human embryos cloned from adult cells

by David Cyranoski

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A California company has brought human cloning research to a new level with efficient production of cloned human blastocysts – an early stage of embryos.

The company, Stemagen in La Jolla in California, hopes that its achievement will be the first step towards using cloning techniques for biomedical research and, potentially, therapy. But first they will need to go to the next step – using such blastocysts to establish self-propagating lines of embryonic stem cells that, as clones, would be genetically identical to a patient.

Cloned human blastocysts have been reported before, but not at this level of achievement. The five cloned blastocysts produced by Stemagen are the first ones to be made with adult human cells – in this case male fibroblasts.

Korean researcher Woo Suk Hwang claimed in 2004 and 2005 to not only have created cloned human blastocysts, but also to have produced stem-cell lines from them. His results turned out to be fraudulent. In May 2005, Miodrag Stojkovic and his group at Newcastle University, UK, reported that three cloned embryos had made it to the blastocyst stage, but they could not produce a cell line. Stojkovic’s group used embryonic stem cells from discarded embryos from in vitro fertilization procedures, a less impressive achievement because these cells were already in a flexible embryonic state, and because they were not matched to any patient’s genetics.

Real, healthy clones?

Stojkovic, now at the Cellular Reprogramming Laboratory at Prince Felipe Research Centre in Valencia, Spain, and an associate editor at Stem Cells, says that this is a “huge difference” from what his group had achieved. Stojkovic also congratulated the group for doing extensive genetic tests. One of the blastocysts successfully went through rigorous tests to prove its identity. “After Hwang, the field is very sensitive,” says Stojkovic. “With these analyses, there is no doubt that at least one is a real clone.”

Robert Lanza from Advanced Cell Technology in Los Angeles, California, a competitor in the field, says that the article lacks data to show that the cells were fully reprogrammed and that the resultant blastocysts were in good condition. He says the photos of the blastocysts “look very unhealthy”.

Though most researchers have agreed not to use cloning techniques to produce human babies, there are fears that this sort of work might open the door to reproductive cloning says Marcy Darnovsky of the Center for Genetics and Society, a science watchdog group based in Oakland, California.

Good eggs

Lead author Andrew French credits the group’s success to the quality of the eggs they used. The company set up a laboratory next to a fertility centre and were able to get to work on eggs donated by women within two hours of extraction. Their 5 successful blastocysts came from 20-30 eggs.

Technologically there was nothing much new here, French admits. The team didn’t use the cutting-edge visualization technique that a group in Oregon claimed to be crucial for their success in creating an embryonic stem cell line from cloned monkey blastocysts, reported in November 2007 (see Cloned monkey stem cells produced [http://www.nature.com/uidfinder/10.1038/news.2007.24]). French says that there are no patents attached to their achievement. The company hopes to make money through agreements with drug companies that want access to specific stem-cell lines for different diseases.

Surprisingly, the group sent all five blastocysts out for independent DNA tests, foregoing a chance for the ultimate goal - the establishment of a cloned embryonic stem-cell line. French says they wanted to “make sure the right DNA was in the blastocyst” and rule out contamination. Since only 10-20% of such blastocysts are expected to produce cell lines, French says that he does not think they stood much of a chance anyway. “We would have loved to go for the holy grail and get the stem-cell line, but we wanted to get this first step sorted out first,” he says.

Harvard stem-cell expert George Daley describes the article as an “important first step” but he says the true test will be the derivation of cloned embryonic stem-cell lines.

Researchers in the field continue to take two parallel approaches to making patient-matched stem-cell lines. Some, like Stemagen, are using cloning; others are attempting to bypass eggs and embryos completely by instead reprogramming adult cells directly into embryonic-like stem cells.

References
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A Prospective Randomized Comparison of global® Medium with Sequential Media for Culture of Human Embryos to the Blastocyst Stage

In a recent report, Sepulveda et al. (2008) compared Global medium with ECM/Multiblast sequential media for the culture of human embryos from the zygote to the blastocyst stage. Oocytes were collected from 47 oocyte donors and fertilized with sperm of the partners of 80 patients who had been randomly assigned to have their embryos cultured in either Global (N = 287 zygotes) or ECM/Multiblast (N = 322 zygotes). The zygotes were cultured from Day 1 to Day 3 in droplets of Global and then in fresh droplets of Global from Day 3 to Day 5 or 6, or in droplets of ECM from Day 1 to 3 and then in droplets of Multiblast from Day 3 to Day 5 or 6.

On Day 2, cell number was significantly greater (P = 0.022) and multinucleation was significantly less (P = 0.020) for embryos cultured in Global than for those cultured in ECM/Multiblast. As shown in Figure 1, the proportion of zygotes that developed to ≥ 6 cells on Day 3, compacted on Day 3, morula on Day 4, blastocyst on Day 5 and full to hatching blastocyst on Day 5 were significantly greater in Global than in ECM/Multiblast. There were no other significant differences in developmental parameters between the culture medium treatments.

On Day 5 or 6, the best (usually two) embryos in each cohort were transferred to 40 patients in the Global group and to 38 patients in the ECM/Multiblast group (mean of 2.0 embryos/transfer in both groups). As shown in Figure 2, there were no significant differences in pregnancy rates, as measured by being positive for hCG or for fetal heart beats (FHB), between the culture treatments. However, the implantation rate, as measured by FHB, was significantly greater for embryos cultured in Global than for those cultured in ECM/Multiblast.

The authors conclude that “A single medium was as good as or better than a sequential media system for human embryo culture from the zygote to blastocyst stage,” and that “The idea that a sequential media system is required for optimal development of the human embryo to the blastocyst stage is highly questionable.”


(Text and figures by D. Rieger, LifeGlobal LLC)
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**Level 3:** Gives a ‘HIGH’ level, or maximum vacuum for aspiration, for clearing the tubing, by pressing the button on the front panel.

The ranges are those recommended for aspiration by current practices and have shown to be safer for aspiration if the flow rates are between 50 and 100 mmHg.

The TriVac gives you a Large vacuum gauge which gives you an excellent view of the vacuum level during operation, and reads up to 300 mmHg. A smaller gauge allow you to view low as well as higher vacuum readings, making it adaptable for additional procedures. Tests have shown that correct usage of our aspiration units have a significant improvement in oocyte quality, with less tearing, less damage, with far more usable oocyte. This aspiration pump with proven dependability, long life is a must for your laboratory.

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Accessory Kit . . . . .PTAK-010

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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.

HEPA filters alone cannot remove all of these impurities. The Coda® Air and Gas Filtration System can maximize the air quality of your incubators and laboratories by removing up to 99.97% of these contaminants.

The Coda® System consists of the Coda Incubator Filtration Unit for use within your incubator and environmental chamber; the Coda® CO₂ and Tri-Gas Inline Filter for use with incoming gas lines; and the Coda Tower® which filters the air in your laboratory, procedure rooms and working environments.

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

<table>
<thead>
<tr>
<th>Removes up to 99.97% of</th>
<th>Particulates</th>
<th>Mold Spores</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Toluene</th>
<th>Benzene</th>
<th>Chloroform</th>
<th>Xylenes</th>
<th>Formaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coda® System</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>HVAC Filter System</td>
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<tr>
<td>Equipment Filter System</td>
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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.

If improving results is your goal, then Coda® is the solution. For more information call IVFonline or visit our website at www.IVFonline.com.

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 1. Deep-pleated high efficiency filter with separators. (Klocke and Whyte 2002)

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

Molecules of embryotoxic compounds are 100 to 1000 times smaller than 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

Reprinted from Fertility Magazine, 7, 15-16
1995. Some environmental effects from other events involving construction in neighbouring spaces and the use 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.

Figure 3. The effect of Coda® incubator units and towers in a human IVF laboratory on pregnancy and abortion rates. (Racowsky et al. 1999)

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
<th>Ongoing</th>
<th>Abortions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Coda</td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Coda Tower</td>
<td>a</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>Coda Tower + Coda Unit</td>
<td>a</td>
<td></td>
<td></td>
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</tbody>
</table>

Figure 4. The effect of Coda® incubator units in a human IVF laboratory on pregnancy rates (Mayer et al. 1999).

Figure 5. The effect of Coda® incubator units in a bovine IVF laboratory on embryo development and pregnancy rates (Merton et al. 2007)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Blasts</th>
<th>Fresh</th>
<th>Frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant (Day 90+)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P ≤ 0.05
N.S.

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


You can contact Antonia V. Gilligan at: antoniagilligan@sprynet.com
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The Basics of Breastfeeding

by Michele Brown, MD
Beauté de Maman, Westport, CT, USA

Breastfeeding has become a national priority. The American College of Obstetrics and Gynecology, the American Academy of Pediatrics, and the United States Public Health Service have issued strong support for the practice and have outlined the advantages to both infant and mother.

For the mother, breastfeeding provides the following benefits:

1. Increases uterine contractions – an increase in the hormone oxytocin helps decrease post partum bleeding and returns the uterus to normal size.

2. Enhances maternal relaxation by increasing the levels of the hormone prolactin.

3. Long term benefits include a reduction in breast cancer and a reduced incidence of osteoporosis with its associated risk of hip fractures.

For the infant, breast-feeding provides the following advantages:

1. Improved immunity to infection:
   - Colostrum, the fluid immediately secreted after birth, provides the infant with maternal antibodies and thus protection from infections.
   - Anti-bacterial effects of human milk also protect the infant from bacterial infections. The American Academy of Pediatrics mentions a decreased risk of meningitis, bacterial infections in the blood stream, diarrhea, respiratory tract infections, colitis, ear infections, and urinary tract infections in breastfed infants.

2. Protection from other diseases including sudden infant death syndrome, diabetes, obesity, hypercholesterolemia, and asthma have been reported in breastfed children.

Many women attempt breastfeeding but stop due to breast and nipple pain. Once interrupted, few women resume nursing thus losing the advantages of this age-old practice.

Breast pain: The causes of post partum breast pain are varied.

- Breast engorgement occurs in up to 25% of breastfeeding women. This results from excessive milk accumulating in the breast. The breast becomes hard and tender. If left unattended milk can seep into surrounding tissue and cause inflammation and mastitis. Inflammatory mastitis occurs within 12 to 24 hours after milk blockage. This incidence of inflammatory mastitis can be greatly reduced by proper lactation training encouraging infant latch-on, and increasing the frequency of feedings.

- Sore nipples are commonly experienced by 80% of mothers within the first few days of feeding. This can lead to maternal anxiety, and interruption of breastfeeding followed by engorgement and infection. Proper technique can help avoid this problem.

- Clogged milk ducts – treatment includes warm compresses and continuing to feed or use a breast pump to relieve the congestion.

- Broken skin and the use of irritating products can also cause breast discomfort. Management of this problem includes restoring the moisture barrier and avoiding excessive drying, which allows for healing of the ulcerations of the nipple. When a cracked nipple occurs, skin bacteria can enter the deeper layers of the skin causing an infection called mastitis.

- Bacterial and fungal infections can occur when a milk duct with poor drainage becomes stagnant and skin bacteria from the nasopharynx of the infant enter the skin. This condition, infectious mastitis, presents with a unilateral local skin
redness and inflammation of the surrounding connective tissue, and is accompanied by fever, chills, and muscle aches. It occurs in 3 to 5% of breastfeeding women in the United States.

Aggressive antibiotic therapy for 10-14 days can prevent the development of an abscess. Antibiotics used to treat mastitis include dicloxacillin, erythromycin, cephalosporins, clindamycin, and vancomycin. Bacteria are emerging that are resistant to the traditional penicillin-like antibiotics, making prevention even more important. Continuation of breastfeeding or manually emptying the breast between feeds is considered safe, and promotes drainage of milk from the involved area, helping to prevent an abscess. Abscesses require surgical drainage or aspiration.

Treatment of mastitis includes pain medication in the form of Tylenol or ibuprofen along with warm compresses to help breast drainage. Cool packs may be used initially to decrease swelling. Breast shells and tight fitting bras should be avoided. If symptoms persist, despite the use of antibiotics, a physician must be notified to rule out the rare possibility of an inflammatory carcinoma. Chronic mastitis can occur when infections relapse due to poor drainage of the ducts, strictures, or residual milk remains infected. Rarely does mastitis enter the bloodstream and cause a severe blood-borne infection.

The role of an obstetrician/gynecologist is to not only be aware of the problems that their patients encounter, but also to be supportive and knowledgeable in helping women understand the importance and benefits of breastfeeding. Beaute de Maman’s goal is to provide high quality products to enable more women to breastfeed successfully thus improving the health of their infants as well as their own wellbeing.

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www.FertileAge.com
A lternative 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; FertileAge™ for Women and FertileAge™ for Men combine fertility & anti-aging ingredients that may help restore and preserve your reproductive health throughout your child-bearing years; Lotus Flower™ and Lotus Tiger™ 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.

FertileAgeTM for Women is a fertility & anti-aging supplement for women that may help restore and preserve your reproductive health throughout your child-bearing years. FertileAgeTM for Men is a fertility & anti-aging supplement for men that may help produce more quality sperm with better morphology and motility and may help maintain the reproductive system. Lotus FlowerTM is a fertility supplement for women who want to conceive now and may help to influence sexuality, love and life and may help the reproductive system. Lotus TigerTM is a fertility supplement for men that may help sexual vigour and may help the reproduction system.

Experience one or more of these natural healing modalities and you be the judge. You will be amazed at the results. Take a natural supplement such as FertileAgeTM for Women, FertileAgeTM for Men, LotusFlowerTM, or LotusTigerTM, 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.

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About the Author

Debrah Frank 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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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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The Delaware Valley Reproductive Biology Group recently held its spring meeting at Restaurant Pearl in Philadelphia, PA. Dr. Jacques Cohen, Tyho-Galileo Research Laboratories, presented “An overview of clinical tools and methods developed by an independent gamete and embryo research laboratory” to the society members. The lecture focused on the ground-breaking reproductive research being performed by the Galileo team and its associate scientists and laboratories.

It was a refreshing evening of discussion surrounding the efforts of the Tyho-Galileo team. Topics addressed included their recent success with a new vitrification method (S3) that has been developed for human blastocysts. This approach combines the safest aspects of traditional cryopreservation such as having a DMSO-free environment and the use of a large straw, with aspects of super-fast cooling and high success rates. Blastocyst survival rates with this method have exceeded 90% and pregnancy rates are as high as the best rates reported for regular vitrification. Over 100 babies have been born from this approach. Survival and pregnancy results with the conventional straw system were impressive, and the benefits for use of the cryo-straw rather than cryo-loop or micro-straw are obvious. The Tyho-Galileo team is also developing S3-vitrification systems for earlier embryos, oocytes and stem-cells.

Dr. Cohen also discussed Tyho-Galileo’s research and collaboration with Reprogenetics on the development of small peptides, ligands, to improve recognition of cell types such as stem-cells, spermatozoa, eggs and embryos for the purpose of identifying stages of development, parts of cells or sperm-oocyte interaction. A stem-cell identification kit for instance may alleviate the need for expensive and complicated antibody-based assays. The research center has adopted a novel approach by examining ligand binding between sperm and the zona pellucida. By
targeting specific protein sequences, they have been able to synthesize peptides which can enhance or specifically block sperm binding to the zona pellucida. Their preliminary findings may significantly impact the future direction of research in both fertility and contraception. Tyho-Galileo and Reprogenetics have also developed a ligand that specifically binds to the human sperm tail, and another ligand that identifies nuclei with DNA fragmentation. The group is also investigating the use of ligands developed against different cancer types for diagnostic and therapeutic purposes.

Dr. Cohen spent the third portion of his lecture explaining the design of the state-of-the-art culture ware manufactured by IVFonline, the GPS and Coral dishes. In addition to being the only culture dishes designed and quality-tested exclusively for IVF and embryo growth, the unique design of both dishes permits the safe culture of embryos and rapid embryo identification for scoring. This and the ease of dish preparation are time saving qualities that every embryology laboratory would appreciate. The ingenious construction of the dishes and their lids ensure maximal heat transfer, optimal gas exchange and safe handling of the dishes from incubator to microscope surface, and back. The strategic placement of the inner and outer wells promotes ease of oocyte/embryo identification, while the well side wall prohibits droplet movement or merging. The concavity of the wells ensures an exact consistency between the focal points of all the specimens. Consequently, daily assessment can be performed efficiently and the dishes returned to their incubation environment in a much more timely fashion than with traditional droplet cultures. Data were presented showing that there was significant reduction in time spent in setting up cultures and locating and checking eggs and embryos in the GPS wells, compared to a standard flat dish culture with microdroplets. The Coral dish offers the added benefit of group culture, or medium sharing, while maintaining the embryos separately for individual scoring. As the benefits from embryo “cross talk” or paracrine communication have been well documented, the use of the Coral dish in IVF is well justified.

There is ample opportunity for participation in scientific research and in clinical trials as a member center of the Tyho-Galileo Research Laboratories. Membership benefits include collaboration with leading scientists at the forefront of reproductive research; research that may alter our methods of practice and thus enhance our success. For additional information on Tyho-Galileo membership, please contact Kelly Ketterson at Kelly.ketterson@embryos.net or at 973-436-5012.

Thank you to IVFonline, Sepal, and Serono for their sponsorship of the evening. A special thank you to Bob King of Serono for his generous support of the dinner venue for the past 4 years, during my presidency.

Charlene A. Alouf, PhD
Laboratory Director, Crozer-Chester Medical Center
President, DVRBG 2003-2008
Dear Colleagues,

The 20th Anniversary of IVF in Turkey was celebrated by the 2nd Congress of Assisted Reproduction and Reproductive Medicine together with the 1st Congress of Reproductive Medicine Society, held in Izmir, Çeşme, Turkey, April 17-20, 2008.

The first studies on IVF in Turkey started in 1985 with my return from Kiel, Germany, where we had the opportunity to practice IVF and endoscopic surgery in Professor Kurt Semm's Clinic, at Christian Albrechts University. At this stage Professors Mettler and Michelmann supported us by sharing their knowledge and experience. Soon thereafter, we began mouse IVF studies at the Ege University, Izmir. After 3 years of experimental laboratory studies, we built the first IVF laboratory at the Ege University, and the first IVF baby was born in April 18, 1989.

At that time, the supplies and technical equipment for IVF laboratories were not as easy to obtain as they are now. I remember how hard it was to find Petri dishes, tubes, aspiration needles, embryo culture media and embryo transfer catheters. When I remember making culture media, checking the osmotic pressure, and doing the mouse toxicity tests ourselves, it is clear that the development and availability of disposable materials has made our work easier and safer. The role of pharmaceuticals, equipment, culture media, and other supplies are as important as the IVF teams for better success rates. Notably, culture media that require cold chain shipping has a very important place among all other laboratory equipment. Tarhan Bora, whom I met in 1999 at our IVF meeting, with his hard working and sincere attitude, has played an important role in the achievements of PM group in Turkey.

The congress began with seven full-day pre-congress workshops on Endocrinology,
Embryology, Andrology, Laparoscopy, Hysteroscopy, Data Collection and Problems of national IVF Centers, and IVF Nursing. The laparoscopy, hysteroscopy and andrology courses included hands-on practice. Over 300 participants attended these workshops.

The main congress had 1200 attendees, including speakers from the USA, England, Canada, Spain, Italy, Germany, Egypt, Lebanon, Jordan and France. International and national experts presented papers in 30 scientific sessions covering ART, Embryology, Stem Cells, Reproduction and Cancer, Preimplantation Biology, Endoscopy, ART Babies, Contraception, Andrology, PGD, Multiple Pregnancies, Menopause, and Adolescence. Two keynote lectures were presented. The first was by Professor Victor Gomel, entitled “Women: the Changing Horizon,” and was a very interesting view of the subject. Professor John Biggers’ lecture was entitled “Historical Development of Embryo Culture Medium.” Hearing about the development of culture media throughout the years from the “father” of culture media was very impressive. Two satellite symposia were also organized.

The congress was supported by the Reproductive Medicine Society of Turkey, the Turkish – German Association, and the Turkish Andrology Society. The International Society for In-vitro Fertilization (ISIVF), the Middle East Fertility Society (MEFS), and the Mediterranean Society for Reproductive Medicine (MSRM) also supported the congress. The ISIVF assigned this Congress as their first regional meeting, and the MSRM and MEFS had their own sessions.

The participants had the opportunity to exchange and learn scientific data throughout the three days, as well as sharing friendship and the social atmosphere. An award was presented to the pioneers of ART and IVF in Turkey, as well as to Professors Biggers and Gomel, and the MEFS, MSRM, ISIVF, Turkish-German Andrology Societies.

As the president of this congress I want to thank all the contributors and especially Ms. Monica Mezezi (IVFonline, Guelph, Canada) and Mr. Tarhan Bora (PM Group, Ankara, Turkey) for their great support for the scientific and social aspects of the Congress.

Hoping to welcome you to Izmir in the near future...

Professor Dr. Erol Tavmergen
Congress President
Saturday, October 25, 2008

The New England Infertility and Family Building Conference

RESOLVE of the Bay State presents, The New England Infertility and Family Building Conference, Saturday, October 25, 2008 at the Best Western Royal Plaza Hotel, Marlborough, MA.

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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.

Conjoint Annual Meeting
MEFS & STGO 2008

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