EMBRYOLOGIST PROFILE:

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In this article, we will share the tips and tricks of Kathy Miller when considering single blastocyst transfers. In the following article, Kathy provides insights from her experiences with single blastocyst transfers; one of her key success factors is utilizing one source for all the media and reagents to reduce shock to the embryo induced by exposure to different base components, buffering systems or pH culture requirements. Her recommended choice of culture media is SAGE In Vitro Fertilizaton.

Kathleen Miller is the Vice President of Laboratory Services for MedTech For Solutions, a full-service healthcare consultant company focusing on the Assisted Reproduction Technology (ART) laboratory market. They offer strategic tools, innovative solutions and cutting-edge technology to meet the specific needs of the ART center.

She is currently the Laboratory Director at Vancouver's Genesis Fertility Centre. The facility is dedicated to providing single embryo transfer therapy to patients undergoing IVF treatment. Previously, she was the Director of the Embryology Laboratory at Reproductive Medicine Associates of New Jersey and prior to that, the joint program at Southeastern Fertility Center, the Medical University of South Carolina, and the University of South Carolina School of Medicine. The author of more than 100 abstracts and papers on fertility and assisted reproduction technologies, Ms. Miller is well known for research advances in the field of blastocyst culture and transfer and pre-implantation genetics.

She is board certified in the disciplines of embryology and andrology. She is currently working on her PhD in global health science at Nova Southeastern University in Fort Lauderdale, Florida. A former president of the College of Reproductive Biologists (CRB) a subdivision of American Association of Bioanalysis (AAB), she is still an active member and serves on many committees of ASRM, ESHRE and AAB.

Single Blastocyst Transfer: The results are in the details

Blastocyst transfer facilitates the selection of good quality embryos and has been reported to generate high implantation rates while lowering multiple gestation rates following IVF. In addition, the number of blastocyst-stage embryos transferred are lower than earlier stages thereby reducing the risk of higher order pregnancies. Over the last decade, numerous IVF centers[1-4] have demonstrated an increase in implantation rates for both selected and non-selected patient groups using blastocyst-stage rather than day 3 embryo transfer. IVF treatment of selecting and transferring only one blastocyst embryo (elective single embryo transfer, or e-SET) can virtually eliminate the twinning rate associated with a two-blastocyst embryo transfer while maintaining equivalent pregnancy rates. [5,6]

Photo provided by Kathy Miller

The limiting factor in every IVF laboratory is the ability to grow "viably competent" embryos. With increasing emphasis to place fewer or a single embryo in patients, the IVF laboratory needs to maintain a culture system and environment that can sustain consistent yields of viable blastocysts. There are contrasting opinions on whether a less-thanexpected outcome in the *in vitro* progression of day 3 embryos to high quality blastocysts is prognostic of intrinsic oocyte or sperm factors, stimulation issues, embryo development issues, or a sub-optimal culture system. Some clinicians prefer to perform a day 3 Embryo Transfer, which could reflect their concern that the blastocyst culture system utilized in their laboratory may in fact be sub-optimal.

How can the IVF laboratory maximize the development of viable blastocyst embryo and confidently and routinely perform single blastocyst transfer? First, the IVF laboratory design should reflect a controlled environment imitating *in vivo* conditions for the collection and culture of oocytes, sperm and embryos [7]. Second, the culture system used in the IVF laboratory should be designed to meet the various and diverse needs of an embryo to develop *in vitro* and avoid unnecessary stressors to the embryo. When an embryo is stressed in culture, the embryo may grow slowly and fail to progress to the blastocyst stage. Additionally, blastocyst embryos produced under stress may have altered metabolism, gene expression, and imprinting, reduced viability, and inability to implant.[8] Third, the laboratory's culture system which includes the environment, equipment, culture media and technical staff performance are significant factors for success. The system's utilization should be habitually scrutinized to evaluate its ability to create a cohort of viable blastocyst embryos capable of producing live births in both fresh and subsequent frozen cycles. Finally, avoid the impulse to introduce something new into your culture system to overcome the inability of your culture system to produce viable blastocysts. Instead of implementing new or different media, oils, supplements, incubators, dishes, and/or gases, focus on reducing stressors that may be influencing the ability of your culture system to produce the most viable blastocyst embryos. The results are in the details: frequently what is considered some of the most insignificant minutiae of culturing embryos can collectively contribute to the success of developing viable blastocyst embryos and performing eSET routinely.

"Choose a reliable sequential media system capable of producing high quality blastocyst embryos."

What details are important?

Use of a culture media system designed for blastocyst culture

The composition of culture media used in the IVF laboratory has evolved over recent years with the increasingly prevalent usage of "sequential culture media systems." These systems comprise a range of media and buffers designed to provide optimized support to each stage of the IVF process, from oocyte isolation through fertilization and embryo cleavage out to the blastocyst stage of development.[9] Although many publications report the ability of embryos to grow to the blastocyst stage in a wide variety of culture media systems, the ability of those blastocyst embryos to implant and produce a live birth is significantly dissimilar among different culture conditions.[10-12] Great care should be taken to select and validate a culture media system that produces highly competent and implantable blastocyst embryos. Once a culture system is employed, the laboratory should utilize all the media and reagents consistent with one vendor in an attempt to reduce shock to the embryo induced by the introduction of different base components, buffering systems or pH culture requirements.

Maintaining thermal stability

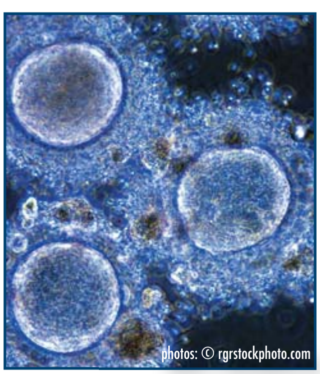
Thermal control is one of the most important culture system variables in the IVF laboratory requiring close attention and management. Oocytes and embryos are particularly sensitive to alterations in temperature. Temperature fluctuations have been known to disrupt spindle and chromosomal organization of human oocytes and cause chromosomal abnormalities of embryos developing from those oocytes.[13-16] Although 37ºC is the widely accepted tolerance limit for culturing human embryos, the optimal temperature is still debated and many investigators are still researching the best temperature range for embryo culture performance and outcome.[17,18] Several IVF laboratories are reporting increased pregnancy rates when embryos are exposed to, manipulated and cultured in thermal environments between 36.5 and 36.9 ºC.[16,19]

"Thermal control is one of the most important culture system variables."

The IVF laboratory should use equipment with a steady temperature profile because incubators, warming surfaces and devices are prone to drift in and out of calibration during normal operation. Equipment with a thermal component should be monitored with an external measuring device with a measurement sensitivity of at least + 0.2 ºC accuracy.[19-21] Additionally, the actual microenvironment the oocytes and embryos are manipulated and cultured in should be monitored for thermal stability given that the actual temperature of the heated surface is not reflective of these microenvironments. Most designs of the disposable culture dishes do not allow the base of the dishes to come into direct contact with the heated surface due to an air gap. Since air is a poor conductor of heat and reduces efficiency of heated surfaces, the actual microenvironment to which the oocytes or embryos are exposed to is cooler than the observed temperature of the heated surface. Because temperature loss occurs rapidly and is related to the amount of time a culture dish stays out of the incubator, manipulating and observing oocytes and embryos in a temperature- and gaseous-controlled closed environment, such as an isolette chamber, may contribute to more competent embryos.[22-25]

Likewise, having an inadequate number of incubators will result in excessive door openings and fluctuations in the thermal and gaseous phases of the incubators.[21,25,26] If at all possible, separate incubators for media equilibration and embryo culture should be used in the laboratory to minimize the embryos exposure to unnecessary door opening. Several studies have shown that an incubator's inability to maintain sufficient thermal and gaseous state control or recovery times of either state can affect the ability of embryos to develop and maintain their competency.[27,28] A new generation of micro-incubators (0.3 cubic feet) is now available to use for embryo culture and have been reported to have temperature, humidity and CO_2 recovery times up to fifteen times faster than traditional large volume 6.5 cubic feet incubators.[21,29] On the horizon, incubators are being developed with time-lapse image capture capabilities that allow for the dynamic and flexible scoring of the developing embryo and allow the embryologist to review analyze embryos throughout their development without removing them from their incubator environment.[30, 31]

Maintaining pH stability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Over the past decade, the optimization of culture media systems has focused on emulating the *in vivo* environment as closely as possible to the oocyte and embryo's specific stage of development.[9] It is now evident that throughout the developmental processes, human oocytes and embryos not only have different chemical and nutrient requirements, but physical ones as well. Increasing attention is being made to determine and validate the optimum pH values needed to culture oocytes and embryos through the different steps of the IVF process. Several studies have demonstrated that oocytes and embryos require culture environments equilibrated at dissimilar pH values.[32-33] Typically, oocytes undergoing conventional insemination need a more basic milieu around 7.3-7.4, and several studies have shown in both animal and human models that fertilization rates are compromised if the pH of the fertilization medium falls below 7.2.[9, 34] Data has also shown that cleavage stage embryos develop better when the pH values of cleavage embryo-specific culture media is at 7.2 and equally cleavage stage embryos develop into higher quality blastocysts when the extended culture medium is at 7.3.[9, 19, 35-36]

The embryologist is not only faced with the challenges of deciding the target pH that provides the highest fertilization and embryo development rates, but maintaining the environment to stabilize pH and reduce the fluctuations of pH that may induce stress to the developing embryos. Minor changes in pH can disrupt many intracellular metabolic processes in the embryo.[37-38] Although the dogma that overexposure of oocytes and embryos to HEPES-buffered media is toxic is still unrefuted, most embryologists shy away from using bicarbonate-buffered media for all oocyte and embryo manipulations and culture due to the difficulty of keeping these media pH-stabilized outside of the incubator.[39] As previously discussed, the usage of a temperature and gaseous controlled closed environment, such as an isolette chamber, can allow the oocytes and embryos to be manipulated and observed in bicarbonate-based media, reducing or alleviating the toxicity concerns related to using media containing non-bicarbonate buffering systems.

> "Directed daily management of pH values is essential…"

The correct application of a culture media system requires the use of a special environment that is at the very least enriched with carbon dioxide, humidity and temperature and maintained in appropriate culture vessels. Medium is a dynamic entity that reacts to its climate and atmosphere. Although commercial vendors of culture media have recently been formulating their media products to specific pH values, embryologists are still reluctant to fine-tune the CO_2 concentrations in their incubators to obtain the most precise pH levels.[40-41] pH should never be adjusted by any other means than titrating CO_2 levels in the laboratory's incubators. Directed daily management of pH values is essential to obtaining the highest outcome levels in the embryology laboratory since every laboratory's environment is different due to many parameters: for example, incubators and other equipment, culture media, plasticware, temperature, humidity, and/or altitude.

Appropriate application and equilibration of culture media and dishes

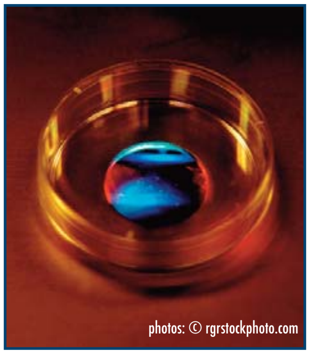
Today the culture of embryos *in vitro* has evolved into the placement of oocytes and embryos into microdroplets of culture media overlayed with culture oil to protect against changes in temperature, humidity and pH. Although an oil overlay is an essential protective and supportive element to an embryo culture system, culture oil in tandem with plasticware are the least defined components used in embryo culture systems and usually are the source of toxins in the culture systems which can disrupt embryo development.[42]

> Culture medium is not inert and reacts rapidly to climate. . .

Even though commercially available materials usually arrive with detailed quality control specifications outlining endotoxin levels, pH, osmolality, sterility, and results of a bioassay, IVF laboratories should still conduct their own independent appraisal of materials using set quality control guidelines.[43-50] Confirmation of osmolality and pH of culture media will verify the accuracy of the media preparation process and should be evaluated before media usage. The implementation of several different validation procedures of laboratory materials will decrease variation in gamete and embryo development and should successfully avert a total collapse of the culture system.

Culture medium is not inert and reacts rapidly to the climate and atmosphere of its surroundings. Great care should be taken when storing and handling all media solutions used for culturing embryos in the IVF laboratory. Media should be utilized within the manufacturer's reported expiration date and stored refrigerated until usage. Culture media should be opened using aseptic technique in a laminar flow hood and used within 5 days of its first opening. Each patient should have their dishes prepared individually and no more than two microdroplet culture dishes should be prepared at one time to avoid evaporation of media during dish preparation and excessive outgassing of CO_2 from the medium and oil overlay if these two componets have already been partially equilibrated with a CO_2 atmosphere.

Embryo culture strategies are diverse and there is no consensus or standardization of culture dish type, open or oil system, media or oil volume and embryo density within the media volume. A culture strategy should be chosen that provides and maintains the most stable environment of temperature, humidity and pH, is simplistic in function, and develops and supports the most competent embryos. Prior to placing any oocyte or embryo into a culture, culture dishes containing any type of media should be pre-equilibrated for temperature and if bicarbonate-buffered media are utilized for pH. Equilibration times should be at least 4 hours, although a recent study demonstrated that an 8-10 hour equilibration window was needed to stabilize target pH in culture dishes containing 50 uL microdroplets or 500 uL of culture medium overlayed by culture oil[51]. Culture dishes containing culture media without oil overlay equilibrated in one hour. Equilibration times should not exceed 18 hours, as the degradation of amino acids, protein sources, vitamins and the antibiotics will begin. Embryos should be changed into fresh medium every 48 -72 hours to counteract this breakdown of media components.



When using a bicarbonate-buffered media system it is essential to minimize the amount of time a culture dish is out of the heated gaseous humidified incubator environment. Temperature loss occurs rapidly and is related to the amount of time a culture dish stays out of the incubator. Several studies have shown rapid de-equilibration of both temperature and pH after removal of culture dishes from the incubator and slow re-equilibration.[21, 51-52] These differences are due to the relative magnitudes of the differential CO_2 contents between the equilibrated medium and air and between the incubator's atmosphere and the partially out-gassed medium.[52]

Appropriate manipulation of zygotes and gametes

Oocytes and embryos are extremely sensitive. Rough handling of oocytes and embryos during their processing can cause irreparable damage.[53] Shear stress over 1.2 dyn/cm_2 has been reported to cause lethality within 12 hours to blastocyst embryos.[54] Using a pipette that is too narrow or with a jagged edge can also cause damage to the oocyte or embryo.[55] The diameter of the pipette should be slightly larger than that of the oocyte (approximately 130–175 μm), the cleavage embryo (175 μm) or the blastocyst embryo (275-300 μm).

Minimizing embryo observations

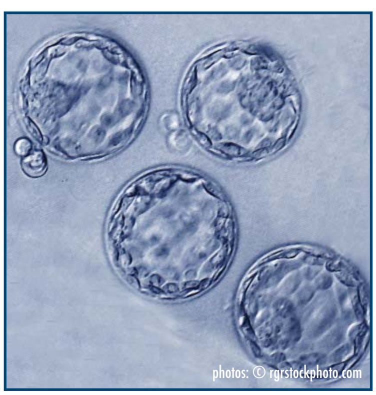
Many studies have illustrated a positive correlation with implantation

> "Determine the target pH... Fine tune the CO_2... Only adjust pH valves by titrating CO_2 levels."

rates when embryo morphological appearances and rate of embryo development are used in tandem to select the highest quality embryos for transfer.[56-59] Despite the development of these more sophisticated embryo grading systems, the majority of IVF laboratories worldwide are unable to define and or trace with certainty the embryos that create live births. Thus, the embryos in their laboratory may be subjected to unnecessary observations that generate data never used conclusively to select the embryos with the greatest potential to cause a live birth. Studies have shown that improvements in IVF outcome with respect to embryo quality, cryopreservation rate, and possibly implantation rate, may result from reduced embryo observations, which contribute to the increased stabilization of the culture environment.[60-61]

Are single blastocyst embryo transfers a reality?

Faced with the possibility of transferring only one embryo in the future due to elective or regulatory pressures attributed to the concerns regarding multiple pregnancy rates, many IVF programs are integrating blastocyst embryo transfer into their clinical therapy due to the higher implantation and pregnancy rates.[57,62-63] The potential disadvantage of committing to an aggressive blastocyst culture and transfer philosophy is the possibility that the patient will have no blastocyst embryos for transfer.[63] The laboratory should have in place functional quality assurance and improvement programs that enable the monitoring of key laboratory performance indicators. To successfully culture blastocyst embryos that will have high competency, utilization and implantation rates, and give the clinician and the patient the confidence to transfer only one blastocyst, IVF laboratories must be able to detect laboratory variations beyond usual and accepted before the results manifest into a less than desirable clinical outcome.[64] As more IVF laboratories move forward in their pursuit of improving success rates and lowering multiple births by transferring fewer, more competent embryos, attention to fine detail and monitoring relevant laboratory indicators will assist the laboratory in integrating crucialtreatment options for patients.



A SAGE interview with Kathleen Miller

When was the first time you used SAGE Media and why do you prefer SAGE over other IVF media?

I have been using media formulated by Dr. Quinn in my laboratories for over twenty years. Dr. Quinn has been a mentor and sounding board for me as I have developed my embryo culture systems throughout my career in clinical embryology. SAGE IVF Media consistently produces high quality embryos cost effectively. In addition, SAGE is always open to suggestions on how to improve their media or develop complimentary products to enhance your laboratory's culture system.

One of the positioning points for using SAGE is its quality and consistency of media. Do you find this to be true and do you have any examples you can share?

One of the key features I look for when considering media for Single Blastocyst Transfers is the consistency of the media to produce the highest quality of blastocyst. As part of my laboratory quality assurance program, we look at lot-to-lot consistency by assessing developmental rates, pregnancy rates and implantation rates. From my experience, SAGE Media has no variability from lot-to-lot and consistently produces high quality embryos.

Out of the array of SAGE products, what is your favorite product to use and why?

The Quinn's Advantage Protein Plus Media line due to its ready-to-use application and its ability to produce high quality embryos.

Is there a specific tip or trick you use when using the SAGE Media?

Measuring the pH levels of the media in the setting of its usage and adjusting incubators to achieve the optimal pH level for the production of the highest quality embryos.

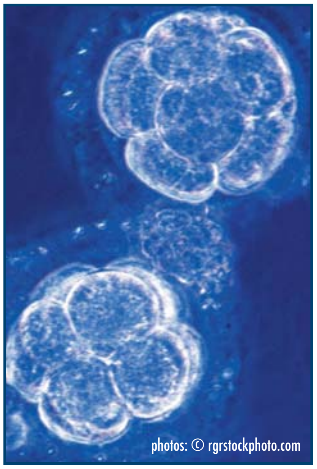
If you had one thing to say about SAGE, if you were presenting the media to another clinic, what would resonate with the other clinic the most?

An easy-to-use media product line that consistently produces high quality embryos for transfer and cryopreservation.

The details…

- Choose a reliable sequential media system capable of producing high quality blastocyst embryos.
- Choose a culture strategy that provides and maintains the most stable environment of temperature, humidity and pH, is simplistic in function, and develops and supports the most competent embryos.
- Use all the components of the manufacturer's sequential media system. Do not interchange media components between vendors.
- Understand your laboratory's environment with regards to equipment, temperature, humidity and/or altitude.
- Remember that thermal control is one of the most important culture system variables in the IVF laboratory and requires close attention and management.
- Determine the thermal tolerance limits for culturing human embryos in your laboratory.
- Use equipment with steady temperature profiles because incubators and warming surfaces and devices are prone to drift in and out of calibration during normal operation.

- Use temperature and gaseous-controlled closed environment equipment, such as an isolette chamber, as much as possible.
- Have a sufficient number of incubators to limit excessive door openings and fluctuations in the thermal and gaseous phases inside incubators.
- Use separate incubators for media and dish equilibration.
- Determine the target pH, which provides the highest fertilization and embryo development rates.
- Fine-tune the CO_2 concentrations in your incubators to obtain the most precise pH levels.
- Only adjust pH values by titrating CO_2 levels in your incubators.
- Directed daily management of pH values is essential to obtaining the highest outcome levels in the embryology laboratory.



- Implement several different validation levels of laboratory media, reagents and contactware to decrease variation in gamete and embryo development and prevent poor performance outcomes.
- Use culture medium within manufacturer's specifications. Culture media should be stored properly and used aseptically.
- Culture dishes should be made no more than two at a time.
- Pre-warm and pre-equilbrate culture media and dishes prior to placing any oocyte or embryo into a culture. Equilibration times should be at least 8-10 hours, not to exceed 18 hours prior to use.
- Limit the amount of time a culture dish is out of the heated, gaseous, humidified incubator environment. Temperature loss occurs rapidly and is related to the amount of time a culture dish stays out of the incubator.
- Oocytes and embryos are extremely sensitive. Rough handling of oocytes and embryos during their processing can cause irreparable damage.
- Reduce embryo observations.
- Implement a system to interpret the true meaning of

quality assurance data and apply the information methodically towards laboratory improvement and optimization.

REFERENCES

[1] Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Hum Reprod 1998; 13:3434-3440.

[2] Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil Steril 1998; 69:84-88.

[3] Marek D, Langley M, Gardner DK, Confer N, Doody KM, and Doody KJ. Introduction of blastocyst culture and transfer for all patients in an in vitro fertilization program. Fertil Steril 1999; 72: 1035-1040.

[4] Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, and Lyles R. Integration of blastocyst transfer for all patients. Fertil Steril 2002; 77:693-696.

[5] Elective single blastocyst transfer reduces twin rates without compromising pregnancy rates. Amy Criniti, Angela Thyer, Gregory Chow, Paul Lin, Nancy Klein, Michael Soules. Fertil. Steril. December 2005 (Vol. 84, Issue 6, Pages 1613-1619).

[6] Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA (April 2010). "Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age." Fertil. Steril. 93 (6): 1837–43.

[7] American Society for Reproductive Medicine. Guidelines for human embryology and andrology laboratories, Fertil Steril, 58(Suppl I) 1S, 1992.

[8] Gardner D. Road to Single Embryo Transfer. The Clinical Embryologist 2004; 7: 1.

[9] Quinn P. Media systems used the assisted reproductive technologies laboratories. A Color Atlas for Human Assisted Reproduction: Laboratory and Clinical Insights. Eds Patrizio P et al. 2003. Lippincott Williams & Wilkins. Philadelphia.

[10] Gardner DK and Lane M. Towards a single embryo transfer. RBM Online 2003; 6: 470-81.

[11] Gardner DK and Lane M. Development of viable mammalian embryos in vitro: Evolution of sequential media. eds. Cibelli J, Lanza RP, Campbell KHS, and West MD. Principles of Cloning. 187-213. 2002. New York, Academic Press.

[12] Gardner DK and Lane M. Culture media for the human embryo. eds. Revelli A, Tur-Kaspa I, Holte JG, and Massobrio M. Biotechnology of human reproduction. 181-199. 2002. Parthenon Press.

[13] Paula A. Almeida and Virginia N. Bolton (1995). The effect of temperature fluctuations on the cytoskeletal organisation and chromosomal constitution of the human oocyte. Zygote, 3, pp 357-365.

[14] Wang WH, Meng L, Hackett RJ, Odenbourg R, and Keefe DL. Limited recovery of meiotic spindles in living human oocytes after cooling-rewarming observed using polarized light microscopy. Hum Reprod 2001: 16, 2374.

[15] Wang WH, Meng L, Hackett RJ, Odenbourg R, and Keefe DL. The spindle observation and its relationship with fertilization after intracytoplasmic sperm injection in living human oocytes. Fertil Steril 2001: 75, 348.

[16] Wang WH, Meng L, Hackett RJ, Odenbourg R, and Keefe DL. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertil Steril 2002; 77: 1274

[17] Brinster RL. In vitro cultivation of mammalian ova. Adv Biosci 1969; 4: 199–233.

[18] Hunter RHF. Temperature gradients in female reproductive tissues and their potential significance Anim. Reprod. 2009; 6: 7-15.

[19] Higdon III, HL. Blackhurst DW, and Boone WR. Incubator management in an assisted reproductive technology laboratory. Fertil Steril 2008; 89: 703–10.

[20] Yeung QS, Briton-Jones CM, Tjer GC, Chiu TT, Haines C. The efficacy of test tube warming devices used during oocyte retrieval for IVF. J Assist Reprod Genet 2004; 21:355–60

[21] Cooke S, Tyler JP, Driscoll G. Objective assessments of temperature maintenance using in vitro culture techniques. J Assist Reprod Genet 2002; 19:368–75

[22] Testart J, Lassalle B, Frydman R. Apparatus for the in vitro fertilization and culture of human oocytes. Fertil Steril 1982; 38:373-375.

[23] Khabani A, Tufts K, Craig L, Soules M, Scott L. Cooling and warming rates in microdrops for embryo culture. Fertil. Steril. 2003; 80: 117.

[24] Kalan MJ, Francis MM, Lewis DE, Allen RB, Landay MF, Paulson RJ. Temperature Fluctuation in Culture Media in the IVF Lab: Is the Isolette a Better Environment Than the Bench Top? Fertil Steril 2008; 89: S21.

[25] Kelly PB, Deignan KC, Emerson G, Mocanu E. Assessment of temperature variation during in vitro culturing: a comparison of different IVF micro-environments. Fertil Steril 2009; 92: S234.

[26] Sharma L, Tarchala S, Nakagawa J, Perry J, Rawlins R. Next Generation Quality Control in the IVF Laboratory: Using data loggers to monitor real time incubator temperature. Technical Bulletin, Marathon Products, San Leandro CA. (date?)

[27] Morrison L, Carney S, Portmann M, Tucker M, McGuirk B, Feinberg R. Do Busier IVF Days From Grouped Cycles Impact Pregnancy Rates and Embryo Development? Fertil Steril 2005; 84: S18.

[28] Takahashi MFK et. al. Mika Izuno · Ya Ru Duan, Maya Kazono Fuminori Kimura Yoichi Noda . Effect of micro-environment maintenance on embryo culture after in-vitro fertilization: comparison of top-load mini incubator and conventional front-load incubator J Assist Reprod Genet (2007) 24:5–9

[29] Lee MA etc, R Grazi, DB Seifer Incorporation of the cook K-Minc – 1000 triple gas incubator and media system into the clinical IVF lab. Fertil Steril 2008; 90: S413-S414.

[30] Ramsing NB, Callesen H. Detecting timing and duration of cell divisions by automatic image analysis may improve selection of viable embryos. Fertil Steril 2006; 88:S88.

[31] Pribenszky C, Losonczi E, Molnar M, Lang Z, Matyas S, Molnar K, Lovacs P, Nagy P, Conceicao J, Vajta G. Prediction of the in vitro developmental competence of early cleavage stage embryos with compact timelapse equipment. Reproductive Biomedicine Online 2009/4551.

[32] Dale B, Menezo Y, Cohen J, DiMatteo L, Wilding M. Intracellular pH regulation in the human oocyte. Hum Reprod 1998;13:964 –70.

[33] Phillips KP, Leveille MC, Claman P, Baltz JM. Intracellular pH regulation in human preimplantation embryos. Hum Reprod. 2000;15:896–904.

[34] Miyamoto H, Toyoda Y, Chang MC. Effect of hydrogen-ion concentration on in vitro fertilization of mouse, golden hamster and rat eggs. Biol Reprod 1974; 10:487-493.

[35] Quinn P, Stone BA, Marrs RP. Suboptimal laboratory conditions can affect pregnancy outcome after embryo transfer on day 1 or 2 after insemination in vitro. Fertil Steril 1990; 53:168-170.

[36] Ferring Pharmaceuticals. Council for the Advancement of Ovulation Induction and Assisted Reproductive Technology. Marrs RP, Steinkampf MP, chairpersons. The assisted reproductive global monitor. May Council meeting, special meeting reporter, Session 1. 1999.

[37] Edwards LJ, Williams DA, Gardner DK 1998 Intracellular pH of the preimplantation mouse embryo: effects of extracellular pH and weak acids. Molecular Reproduction and Development 50, 434–442.

[38] Nematollahi-mahani SN, Nematollahi-mahani A, Moshkdanian G, Shahidzade-hyazdi Z, Labibi F. The role of co-culture systems on developmental competence of preimplantation mouse embryos against pH fluctuations. J Assist Reprod Genet (2009) 26:597–604

[39] Swain J. New pH-buffering system for media utilized during gamete and embryo manipulations for assisted reproduction. Reproductive BioMedicine Online; 2009: 18; 799-810.

[40] Pool TB. Optimizing pH in clinical embryology. Journal of Clinical Embryology 2004; 7:1–17.

[41] Quinn P and Cooke S. Equivalency of culture media for human in vitro fertilization formulated to have the same pH under an atmosphere containing 5% or 6% carbon dioxide. Fertil Steril 2004; 81:1502– 6.

[42] Morbeck DE, Khan Z, Barnidge DR, Walker DL. Washing mineral oil reduces contaminants and embryotoxicity. Fertil Steril 2010 DOI: 10.10 16/j. fertnstet. 2010.03.067

[43] Bavister BD and Andrews JC (1988) A rapid sperm motility bioassay procedure for quality control testing of water and culture media. JIVET, 5, 67-75.

[44] Bertheussen K, Holt N, Forsdahl F and Hoie KE. A new cell culture assay for quality control in IVF. Hum Reprod 4:531, 1989.

[45] Chatot CL, Ziomek CA, Bavister BD et al. An improved culture medium supports development of random-bred 1-cell mouse embryos in vitro. J. Reprod. Fertil. 86: 679,1989.

[46] Critchlow JD, Matson PL, Newman MC, et al. Quality control in an in vitro fertilization laboratory: use of human sperm survival studies. Hum Reprod 4: 545, 1989.

[47] Davidson A, Vermesh M, Lobo RA et al. Mouse embryo culture as a quality control for human in vitro fertilization: the one-cell versus the two-cell model, Fertil Steril, 49, 516, 1988.

[48] Gerrity M. Mouse embryo culture bioassay. In Wolf DP, Bavister BD, Gerrity M, and Kopf GS (eds) In Vitro Fertilization and Embryo Transfer: A Manual of Basic Techniques. Pg 57-76. Plenum Press, New York, 1988.

[49] Naz RK, Janousek JT, Moody T, et al. Factors influencing murine embryo bioassay: effects of proteins, ageing of medium, and surgical glove coatings. Fertil Steril 46: 914, 1986.

[50] Weiss TJ, Warnes GM, Gardner DK. Mouse embryos and quality control in human IVF. Reprod Fert Devel 4: 105, 1992.

[51] Conaghan J, Steel T. Real-time pH profiling of IVF culture medium using an incubator device with continuous montioring. Journal of Clinical Embryology 2008;11: 15-16.

[52] Blake DA, Forsberg AS, Hillensjo T, Wikland M. The practicalities of sequential blastocyst culture. Presented at ART, Science and Fiction, the Second International Alpha Congress, Copenhagen, September 1999.

[53] Xie Y, Wang Y, Zhong W, et al., 2006. Shear stress induces preimplantation embryo death that is delayed by the zona pellucida and associated with stress-activated protein kinase mediated apoptosis. Biol. Reprod. 75, 45–55.

[54] Xie Y, Wang Y, Puscheck EE, et al., 2007. Pipetting causes shear stress and elevation of phosphorylated stress-activated protein kinase/jun kinase in preimplantation embryos. Mol. Reprod. Dev. 74, 1287–1294.

[55] Payne D, Flaherty SP, Barry MF and Matthews CD. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. Hum Reprod vol.12 no.3 pp.532–541, 1997

[56] Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M and Ryckaert G. Characterization of a top quality embryo, a step towards single embryo transfer. Hum Reprod 1999; 14: 2345-2349.

[57] Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000; 73:1155-1158.

[58] Neubourg DD, Gerris J, Mangelschotscc K, Van Royen E, Vercruyssen M, Elseviers M. Single top quality embryo transfer as a model for prediction of early pregnancy outcome. Hum. Reprod 2004; 19:1476-1479.

[59] Racowsky C, Stern JE, Gibbons WE, Barry B, Pomeroy KO, Biggers JD. National collection of embryo morphology data in SARTCORS: associations among cell number, fragmentation and blastomere asymmetry on day 3 (d3) with live birth rate. Fertil Steril 2009: 92: S82.

[60] Khabani A, Shen S, Fujimoto VY, Houmard BS, Rainer C, and Battaglia DE. Improved Embryo Quality and Rates of Cryopreservation With Modifications in an In Vitro Fertilization (IVF) Culture System. Fertil Steril 2000; 74: S104.

[61] Skiadas CC, Biggers JD, Racowsky C. Correctly classifying viability of day 3 embryos is compromised by evaluations performed on multiple days before transfer. Fertil Steril 2009; 92: S159.

[62] Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil

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