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Decisions for the IVF laboratory: comparative analysis of embryo culture incubators

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Abstract Incubators in the IVF laboratory play a pivotal role in providing a stable and appropriate culture environment required for optimizing embryo development and clinical outcomes. With technological advances, several types of incubators are now available and careful consideration is required for selection. Examination of variables, such as recovery/stabilization of temperature, gas atmosphere and humidity, as well as understanding various approaches utilized by each device to regulate these variables, is critical. Additionally, a comprehensive examination of clinical studies that compare various incubators may provide insight into their efficacy. Other factors, both technical and practical, must also be considered when selecting an incubator. Importantly, proper management, including patient volume and workflow, is paramount in optimizing function of any incubator, regardless of the technology incorporated. This review highlights incubator function and reviews key environmental variables controlled and the technology utilized in various units. Additionally, existing comparative studies focused on incubator recovery and clinical outcomes are critically analysed. Finally, strategies employed for incubator management, as well as future potential incubator improvements are discussed. While existing reports indicate that smaller benchtop/topload incubators provide faster recovery of environmental variables, there is no clear advantage of any particular incubator based on clinical outcomes.

KEYWORDS: benchtop, blastocyst, box, embryo, incubator, topload

Introduction

Minimizing environmentally induced stress within the IVF laboratory is crucial in creating a culture system optimized for embryo development and to achieve maximal assisted reproductive outcomes. Key environmental variables to consider within the culture system include pH of the culture medium, temperature, media osmolality and air quality.

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Importantly, all of these potential stressors can be impacted by the laboratory incubator. As a result, the incubator is arguably the most important piece of equipment in the laboratory, controlling multiple environmental variables and housing the embryos for the vast majority of their time *in vitro*. Thus, incubator selection and management is critical to ensure success of an IVF programme.

With advances in technology, multiple incubator types exist with varying capabilities and differing methods of regulating their internal environment (**Table 1**). As a result, selection of an appropriate culture incubator for the IVF laboratory has become a complex process. The objective of this review is to summarize key aspects of incubator function, critically examine comparative studies on incubator performance in the laboratory, provide insight into proper incubator management and discuss possible future incubator advancements.

Incubator function

The primary function of an incubator within the IVF laboratory is to provide a stable environment to optimize gamete function and embryo development *in vitro*. To achieve this goal, an incubator must regulate several environmental variables, including gas concentrations, temperature and humidity. Importantly, a variety of approaches and technologies are utilized by different incubators to regulate these environmental variables and each has benefits and disadvantages that should be considered when selecting a unit. Additionally, other functional and practical considerations exist that require consideration before implementation of the equipment into the IVF laboratory.

Gas monitoring and recovery

A critically important function of the laboratory incubator is to reliably provide an appropriate gas atmosphere. Specifically, regulation of CO_2 concentration is of paramount importance, as this gas helps regulate pH of the culture medium. pH of the culture medium is an important variable that can significantly impact gamete function and embryo development (Swain, 2012). Additionally, reduced O_2 concentration in the culture environment has, for many years, repeatedly been found beneficial for both animal and human embryo development and outcomes (Bavister, 2004; Bontekoe et al., 2012; Mantikou et al., 2013), most notably when used throughout the entire culture period to the blastocyst stage (Kovacic and Vlaisavljevic, 2008; Meintjes et al., 2009; Waldenstrom et al., 2009). Thus, modern IVF incubators should monitor and regulate both CO_2 and O_2 concentrations.

Accurate and rapid monitoring of gas concentrations by the incubator is critical to achieve target values in a timely fashion and ensure appropriate growth conditions. Central to this function is the type of gas sensor used. The primary method utilized by IVF incubators to monitor CO_2 concentration includes one of two sensor types; thermal conductivity (TC) or infrared (IR). Thermal conductivity sensors function through measurement of resistance between two thermistors, with one enclosed and the other exposed to the incubator chamber (Chou, 1999a). The presence of CO_2 in the incubator chamber changes the resistance between the two thermistors and permits elucidation of gas concentrations. Importantly, the resistance, and therefore CO_2 readings, of incubators using TC sensors are

Table 1 Incubator technology variables that should be considered when evaluating and selecting a unit for the laboratory.

Gas type	*CO_2 sensor*	*O_2 sensor*	*Temperature control*[a]	*Design*[b]	*Humidity control*	*Contamination control*[a,c]	*Other*
CO_2-only	Infrared	Zirconium	Air jacket	Benchtop	Yes[d]	Heat	Data logging
Low O_2 — mixer	Thermal conductivity	Galvanic (fuel-cell)	Water jacket	Two-chamber	No	UV	Cost
Low O_2 — premixed cylinder			Direct heat	Multichamber		H_2O_2	Patient capacity
				Other (i.e. time-lapse imaging)		Copper alloy	Service
				Small box		External HEPA	Technology integration
				Large box			

HEPA = high-efficiency particulate absorption.
[a]May be influenced by presence/absence of an internal fan.
[b]Other novel designs exist, but these are general terms to refer to the most commonly used incubators in the IVF laboratory; actual volumes will vary from unit to unit.
[c]Ease of removing inner parts and/or wiping interior is also important to consider.
[d]Some units bubble gas through a water pan to expedite rehumidification.

impacted by temperature and humidity. Conversely, IR sensors are largely humidity and temperature independent. Infrared sensors emit a light and utilize optics to detect IR absorbance, which is relative to the concentrations of CO_2 in the chamber atmosphere (Chou, 1999b). As a result, incubators utilizing TC CO_2 sensors tend to take longer to stabilize gas atmosphere following door opening compared with incubators outfitted with IR sensors, as CO_2 concentrations cannot be fully determined and subsequently adjusted until both temperature and humidity stabilize. Thus, considering improvements to IR sensor lifespan and reduction in cost, incubators outfitted with IR sensors have become the preferred option in many laboratories to help to improve environmental stability and recovery.

Similarly to incubator CO_2 sensors, two primary types of sensor are used to assess incubator O_2 concentration; galvanic/fuel cell or zirconium sensors (Hitech Instruments Technical Note). A galvanic sensor is a diffusion-limited metal/air battery (Chou, 1999a). Oxygen diffuses through the outer barrier of the sensor to reach the inner cathode where it is reduced to hydroxyl ions which, in turn, oxidize the metal anode. A current, proportional to the rate of consumption of O_2, is generated when the cathode—anode circuit is completed. The rate of O_2 diffusion to reach the cathode and the cell current is a direct function of this diffusion rate, this in turn being a direct function of the concentration of O_2 in the sample. A zirconium sensor is an impervious tube with a zirconia element with a closed end and is coated externally and internally with porous metal electrodes. At elevated temperatures, the element becomes an O_2-ion conductor, which results in a voltage being generated between the electrodes. The value of the voltage is dependent upon the differences between the partial pressures of the O_2 in the sample and the O_2 in a reference gas (generally air). Although more recent galvanic sensors have improved the rapidness of their response reading, they tend to yield slower response times compared with zirconium sensors and more frequent replacement is often required to ensure adequate functioning.

Importantly, for both incubator O_2 and CO_2 readings, external incubator display values should not be relied upon to determine atmospheric recovery during re-equilibration, as some incubator displays return to their programmed set points prior to actual re-equilibration of internal gas concentrations. A more accurate assessment or comparison of gas recovery rate entails independent measurement of O_2 and CO_2 concentrations using real-time measuring devices placed inside the incubator chamber.

In addition to the two primary sensor types used to measure CO_2 and O_2 concentrations, accurate gas concentrations can be achieved in the absence of gas sensors through the use of supply cylinders of premixed gas. Cylinders of premixed gas can be supplied directly to an incubator or to a sealed modular chamber placed inside the incubator, rather than requiring the incubator to mix the gases into the proper ratios. Using this approach, appropriate CO_2/O_2 concentrations are quickly achieved as soon as the incubator volume has been filled with the premixed gas. However, proper quality control is required to ensure that the premixed gas concentrations/ratios within the supply cylinder yield the desired medium pH and growth conditions. Different culture media may contain different bicarbonate and/or protein concentrations, which might require differing CO_2 concentrations to achieve the desired pH. Similarly, different laboratories may require different CO_2 concentrations, as variables such as laboratory elevation can impact pCO_2 and resulting pH. Once an appropriate gas mixture is determined based on the requirements of a particular culture medium and/or laboratory, accurate mixing may be verified through routine pH testing, independent gas measurement or through a formal certificate of analyses supplied by the vendor.

Independent of gas sensor or gas supply type, incubator volume also influences gas equilibration and recovery timing. Following door opening, traditional 'large-box' incubators (~150—200 l) may require an extended time to refill

Table 2 Approximate culture chamber volumes of various incubators/devices utilized for clinical IVF.

Model	*Approximate chamber volume (l)*
K-systems G185	0.299 × 10 chambers
Astec EZ Culture	0.310 × six chambers
Cook K-MINC	0.43 × two chambers
Planar BT-37/INC-A20	0.43 × two chambers
Labotect Labo C-Top	0.5 × two chambers
Astec IVF Cube	0.775 × four chambers
ESCO Miri Multiroom	0.886 × six chambers
Fertilitech Embryoscope	2.4
Billups-Rotheburg MIC-101 Modular Chamber	5.8
Galaxy 14S	14
Labotect C-16	16
Astec Penguin DH	30
Astec CCM IVF	30
Astec APC30/APM30	32
IKS IVS-9000	33
IKS INB-203	42
Galaxy 48R	48
Panasonic MCO 5M	48
Binder CB 53	53
Astec APC50/APM50	57
Labotect C-60	60
Memmert Inc108med	108
Binder CB 150	150
Forma series II 3130	150
Heracell 150i	150
Memmert Inc153med	153
Astec CDI/SMA	163
Galaxy 170R	170
Panasonic MCO 18/19	170
IKS INB-203XL	179
Nuaire Autoflow NU4950	188
Ruskinn Ac-tive IVF	394

A complete list of all incubators is not feasible. This list focuses on currently produced incubators with low-O_2 capability commonly sold for use in IVF laboratories. Volumes were obtained from manufacturer/sales websites, product literature or via communication with company technical personnel. In addition to chamber volume and number, ability to minimize incubator opening/closing is important for atmospheric stability.

with CO_2 and/or nitrogen gases. These large units were initially designed for use with multiple flasks of somatic cells placed onto each shelf. However, dishware used for IVF is considerably smaller, few dishes are used, and often only one patient is placed onto a single shelf at any given time. Thus, 'small-box' incubators (~14–48 l) have come into use and, depending on workflow, may help improve gas recovery, reduce environmental stress and improve embryo development compared with larger incubators (Avery and Greve, 1992). More recently, benchtop/topload units of varying sizes/configurations designed specifically for clinical IVF have extremely small chambers (~0.31–0.5 l), further improving gas atmosphere recovery time (**Table 2**). Of note, while smaller incubator volumes are beneficial in terms of rate of atmospheric recovery following repeated opening, large volume isolator-based incubator systems can also yield acceptably high outcomes, likely due, in part, through limiting or eliminating excursions from the enclosed system (Hyslop et al., 2012). Thus, as will be discussed, incubator management is also a key component for optimized incubator function.

Air quality

An additional variable related to gas atmosphere that impacts incubator function is air quality. Air quality, specifically presence and amounts of volatile organic compounds (VOC), may compromise embryo development (Cohen et al., 1997; Hall et al., 1998; Khoudja et al., 2013; Merton et al., 2007), although the relevant concentrations of VOC are still unknown. As a result, most laboratories have dedicated air handling systems to filter out particulates as well as VOCs and various studies indicate a benefit to embryo development and/or outcomes once air quality is improved (Boone et al., 1999; Khoudja et al., 2013). However, while outside air quality may be important, it is the quality of the atmosphere inside the incubator that is likely of more concern.

Outside air quality has an obvious impact on atmospheric quality within the incubator, especially in CO_2-only incubators, which carry a balance of ~94% room air. However, the quality of gas from the supply tanks must also be considered, especially in low-O_2 incubators, which flood their interiors with nitrogen from these tanks to reduce O_2 concentration to ~5%. VOCs have been detected in gas supply tanks used for IVF incubators (Hall et al., 1998). In these cases, filtering the supply gases through inline filters prior to incubator entry may be an effective approach to improve incubator atmosphere. These inline filters not only contain high-efficiency particulate absorption (HEPA) filtration to reduce particle counts but also methods to reduce VOC as well, including activated charcoal or potassium permanganate. At least one preliminary study has shown improvements in embryo development and outcomes following implementation of inline gas-filtration systems (Esteves et al., 2006). Placement of specialized VOC filtration units inside incubators can also improve air quality and outcomes (Mayer et al., 1999; Merton et al., 2007; Schimmel et al., 1997), although this is not always the case (Battaglia et al., 2001; Higdon et al., 2008; McLellan et al., 2001) and, depending on their size, fitting into smaller incubators may be problematic. An emerging approach to improve air quality to some incubators includes recirculating atmosphere past a UV light source for photocatalytic breakdown of VOCs (Chapuis et al., 2002; Sharmin and Ray, 2012). Whether one of the aforementioned approaches to remove VOCs is superior to another or provides superior results in the IVF laboratory incubator is unknown.

It should be mentioned that incubators that utilize cylinders of premixed gas have the ability to filter the entirety of the gas supply prior to it entering the incubator chamber. Incubators that mix gases themselves, either CO_2-only or low-O_2 incubators, have at least some portion of room air present, although if room air is of high quality this likely poses little problem. Also important to note, plasticware or internal incubator components may de-gas inside the elevated temperatures of the incubator chamber (Cohen et al., 1997). Thus, despite having acceptable outside air quality or a prefiltered gas supply, VOCs may still be present inside any incubator. In these cases, proper initial cleaning of incubators and de-gassing of devices and supplies may help address concerns. Additionally, placement of modular VOC filter units in the incubator chamber or recirculation of chamber atmosphere through external filters may also be effective.

Temperature

Another primary function of the laboratory IVF incubator is to maintain an appropriate temperature for gamete function and embryo development. It is also well known that temperature can impact various aspects of gamete and embryo function, most notably meiotic spindle stability (Sun et al., 2004; Wang et al., 2001, 2002) and possibly embryo metabolism (Leese et al., 2008). However, data indicate that temperature gradients may exist in the female reproductive tract (Hunter, 2012; Hunter and Einer-Jensen, 2005; Hunter et al., 2006). Thus, while the optimal target temperature for IVF incubators that contain varying cell types and embryos at different developmental stages is still unknown (Higdon et al., 2008; Hong et al., 2012) and beyond the scope of this review, maintaining an accurate temperature while inside the incubator is mandatory for reducing environmental stress.

Three main types of heating approaches are employed by IVF incubators. Two common warming methods used primarily in box-type incubators include a water jacket or air jacket, both of which warm the air in the incubator chamber and may or may not include an internal fan to circulate. The third heating approach used by benchtop/topload units entails contact of the warmed incubator surface, upper and lower, and direct heat transfer to the culture dish and enclosed medium. Importantly, each incubator warming approach has benefits and limitations. Water-jacketed incubators retain heat longer in case of incubator opening or power failure. However, units are heavy, tend to have higher power consumption, which may burden emergency power supplies, and are accompanied by concerns that contamination may originate from inside the water jacket. Air-jacketed incubators warm up quickly, but do not retain heat for long periods. Air-jacketed, but not water-jacketed, units are also compatible with heat-sterilization decontam-

ination cycles and may help with contamination concerns. Finally, direct heat/contact results in very rapid heat recovery following opening of the unit or dish removal, but maintenance of this temperature for any period of time can be problematic if power interruption occurs. Of note, while not necessarily a component of the incubator *per se*, any incubator can and should be connected to commercially available battery backup units and/or generator-protected electrical outlets to avoid concerns associated with power loss.

Temperature gradients can exist in any incubator, regardless of the warming system used. Such occurrences are most commonly noted in box-type incubators utilizing air- or water-jacketed warming. A preliminary report indicated slight temperature variations when culture dishes were placed in various locations within a large-box water-jacketed incubator, with measurements of 36.97°C, 37.17°C and 37.23°C (Stoddart et al., 2003). Similar findings were also recently reported between two large-box incubator, where minor (~0.07—0.17°C), but significant temperature differences were identified between shelves and between incubators (Walker et al., 2013). Whether such minor fluctuations have an impact is unknown, but independent temperature measurement between shelves on box-type units, between individual culture chambers or across warmed surfaces of various benchtop/topload unit configurations can provide insight into temperature accuracy, as well as possible variation that could impact gamete and embryo development and function. As will be discussed, examination of possible temperature gradients is useful in optimizing incubator management.

Humidity

Many incubators regulate humidity to avoid media evaporation during culture to avoid harmful rises in medium osmolality that can compromise embryo development (Lane et al., 2008; Swain et al., 2012). This humidification is usually supplied in a passive fashion, via evaporation of a water reservoir commonly placed in the bottom of incubator chambers. However, the presence of a water pan for humidity control is also a potential source of contamination and should be monitored and replaced regularly. A variation on this approach includes bubbling inlet gases through the water pan or a supplied water bottle, which may help humidify the air more rapidly, but also acts as an additional filter of inlet gases. Of note, humidity inside the incubator is not necessarily required to culture embryos if adequate amounts of oil overlay are used and may be dependent upon the number of days of continuous culture, although this should be validated within each laboratory. Furthermore, lack of humidity may benefit incubators using TC CO_2 sensors, as humidity recovery is no longer a limiting factor.

Other considerations

Other considerations for incubator selection include approaches available for cleaning and sterilization to reduce chances of contamination. Various incubators are constructed with copper-containing alloys, as copper can act as antimicrobial and antifungal agent (Borkow and Gabbay, 2004; Grass et al., 2011). However, at least one study suggested that oxidized copper particles from incubator walls may have detrimental effects on bovine embryo development (Avery and Greve, 1992), although experimental design precluded any conclusive correlation and several copper-containing incubators are used successfully for human embryo culture. Alternatively, some air-jacketed incubators feature heat decontamination cycling capability. Other incubators can be outfitted with hydrogen peroxide sterilization capability by the manufacturer. Ultraviolet light treatment of water pans is also available to reduce incidence of contamination, although this feature is often turned off to avoid damage to cells within the incubator. Additionally, any incubator can be sterilized and/or cleaned by removing inner pieces for autoclaving and wiping down the interior of the unit with embryo-safe products, such as hydrogen peroxide or other commercial IVF cleaning solutions, preferably with low VOC content. Units with fewer removable shelves or lacking internal fans are easier to clean and may help reduce the risk of contamination.

Quality control may be another variable to consider when selecting a laboratory incubator. Several optional features may be available on units than can assist with routine monitoring, including data logging capabilities to monitor real-time temperature fluctuations or number of door openings. Some incubator designs may make other aspects of quality control more difficult, although perceived limitations can often be overcome. For example, in benchtop/topload incubators, it may be difficult to measure pH of the culture medium. However, specialized pH meters or blood gas analysers can be purchased in some cases, or pH can be tested directly from the gas supply if using a cylinder of premixed gas. Alternatively, small test tubes can often be laid inside units to permit medium equilibration for subsequent measurement.

Finally, additional consideration must be given to cost and capacity of each incubator, as well as space occupied. Several incubators are required in any IVF laboratory to help avoid overcrowding and promote proper incubator management, as well as provide backup capabilities in case of unit malfunction or scheduled downtime for routine maintenance.

Comparative studies and clinical outcomes

While literature from various commercial companies can be found demonstrating performance characteristics of a particular incubator against a competing incubator or technology, comparative studies within a laboratory and resulting clinical outcomes reported in the peer-reviewed literature may offer better insight into incubator performance within the context of the IVF laboratory. Unfortunately, very few studies comparing environmental stability and recovery of particular incubator units exist, and even fewer studies comparing outcomes of embryo development or assisted reproductive outcomes are available (**Table 3**). Furthermore, careful examination of the existing literature is required to understand why differences may exist, and scru-

Table 3 Incubator comparison studies.

Study	Incubator comparison	No. of patients	No. of embryos	Method	Outcome: results, statistical significance	Conclusions and notes
Cooke et al. (2002)	MINC versus Forma large-box (water jacketed, TC CO_2 sensor)	NA	NA	Compared time to temperature recovery from 35°C to 37°C of 1.0 ml media with 0.1 ml oil overlay versus 50 μl media with 1 ml oil overlay	MINC recovered temperature within 5.5–6.5 min depending on media volume	Direct heat transfer of the benchtop unit resulted in faster temperature recovery than the indirect-warming system of the larger incubator
					Forma did not recover temperature by 20 min for either volume (reached 36.2 and 36.7°C)	An aluminium block can help with temperature recovery in the large indirect-warming incubator
Fujiwara et al. (2007)	K-MINC benchtop versus ASTEC small-box (TC CO_2 sensor, galvanic O_2 sensor, water jacketed)	30	334	RCT: sibling embryos randomized after 2PN check	Temperature recovery time: 5 min versus 30 min, $P < 0.01$	Benchtop incubators offer improved culture environment recovery times
				5-s door opening, repeated 10 times and averaged	O_2 recovery time: 3 min versus 8 min, $P < 0.01$	Recovery time may influence the formation of good-quality embryos
					Early 'good' embryo rate: 40% versus 38%, $P < 0.05$ 'Good' blastocyst formation rate: 15% versus 8%, $P < 0.05$	
Lee et al. (2010)	K-MINC benchtop versus Forma large-box (high O_2, TC CO_2 sensor, water jacketed, inner doors)	97	1,189	RCT: sibling embryos randomized	Temperature recovery time: 1 min versus 180 min, $P < 0.01$	Benchtop incubators offer improved culture environment recovery times
				10-s door opening and outcomes compared	CO_2 recovery time: 8 min versus 120 min, $P < 0.01$	No differences were found between incubators in respect to embryo quality or outcomes
					Humidity recovery: 12 min versus 180 min, $P < 0.01$ Fertilization rate: 72% versus 67%, $P < 0.05$ Day-3 grade, NS Implantation rate: 39% versus 32%, NS Clinical pregnancy rate: 64% versus 54%, NS	

Table 3 Incubator comparison studies (continued).

Study	*Incubator comparison*	*No. of patients*	*No. of embryos*	*Method*	*Outcome: results, statistical significance*	*Conclusions and notes*
Cruz et al. (2011)	Embryoscope versus Heracell 150 large-box (air jacketed, high O_2)	60	478	RCT: sibling embryos were randomized after 2PN check	Blastocyst formation rate: 55% versus 51%, NS	No difference between the Embryoscope and a standard incubator in blastocyst formation blastocyst viability rate or ongoing pregnancy rate
				Morphological assessment was made at days 2 and 3	Blastocyst viability: 29% versus 35%, NS	Study also compared Embryoslide individual culture versus microdrop individual culture
				Embryos were *not* removed from Embryoscope for assessment	Ongoing pregnancy rate: 43% (6/14) versus 42% (8/19), NS	
Kirkegaard et al. (2012)	Embryoscope versus Galaxy R 170 large-box (direct heat, high O_2, IR CO_2 sensor)	59	676	RCT: sibling embryos were randomized after insemination	No. of 4-cell embryos on d2: NS	No difference was found between the Embryoscope and standard incubator for embryo development, implantation rate or clinical pregnancy rate
				Morphological assessment at days 2, 3 and 5	No. of 7—8-cell embryos on d3, NS	Study also compared Embryoslide individual culture in small microvolume versus group culture in Nunc wells of larger volume
				Embryos were removed at equal time points for *both* incubators and outcomes compared	No. of blastocysts on d5, NS	
					Implantation rate: 37% (7/19) versus 33% (6/18), NS	
					Clinical pregnancy rate: 38% (8/21) versus 30% (7/23), NS	

2PN = 2 pronuclei; IR, infrared; NA = not applicable; NS = not significant; RCT = randomized controlled trial; TC = thermal conductivity.

tiny often points out limitations in study design that should be considered when interpreting results.

For example, when comparing a small benchtop incubator unit with two topload chambers (~0.43 l) and a small-box incubator (~32 l), it was found that, after a 5-s opening, the benchtop/topload unit had improved temperature recovery (5 min versus 30 min), O_2 recovery (3 min versus 8 min), improved 'good' early embryo development (40% versus 38%) and improved 'good' blastocyst formation (15% versus 8%; Fujiwara et al., 2007). Interestingly, authors measured O_2 recovery rather than CO_2 recovery. While O_2 and CO_2 will recover at the same rate in the benchtop unit due to using a premixed gas supply, O_2 will recover much more slowly than CO_2 in the frontload unit, which uses separate gas supplies due to the larger amount of nitrogen needed in the larger volume. It is unknown if such large differences would exist when measuring CO_2, which is likely to be more important. Furthermore, in this case, the small-box unit was outfitted with outdated technology, utilized a TC CO_2 sensor and was water jacketed. Whether the

same differences would be apparent if using the faster IR CO_2 sensor and air-jacketed unit is unknown. Finally, no oil overlay was used and overall blastocyst conversion rates in both incubators were low. It is possible that use of oil overlay would help stabilize pH and temperature and perhaps improve the suboptimal growth conditions in the study. Thus, while the benchtop/topload unit likely recovers atmosphere and temperature more rapidly, closer examination of the study reveals that the discrepancies between the two incubators may not be as pronounced if compared with an updated box-type incubator optimized technology.

A subsequent study again compared a box-type incubator and a small 2-chambered benchtop/topload unit, examining CO_2, temperature and humidity recovery as well as fertilization rate, embryo quality, clinical pregnancy and implantation rates (Lee et al., 2010). Following a 10-s incubator opening, it was found that there was a significant difference in temperature recovery (1 min versus 180 min), CO_2 recovery (8 min versus 120 min) and humidity recovery (12 min versus 180 min), with faster recovery occurring in the benchtop/topload unit. Interestingly, the large-box incubator was outfitted with nonairtight inner doors which may not provide as stable a gas environment as new units which employ air-tight inner doors. Again, the large-box incubator used the slower TC sensor and was water jacketed. Finally, the benchtop/topload unit utilized low-O_2 culture via premixed gas, while the large-box incubator utilized CO_2 only. As mentioned previously, low O_2 appears to yield better embryo development and outcomes (Bavister, 2004; Bontekoe et al., 2012; Mantikou et al., 2013). Furthermore, the use of the cylinder of premixed gas in the benchtop/topload unit may provide improved air quality over use of ~94% room air in the large-box incubator. Indeed, one preliminary study comparing the same types of large-box and small benchtop/topload incubators reported that air quality/gas composition may be partially responsible for improved mouse blastocyst development observed in two out of five different culture media in the benchtop unit compared with the large-box incubator, although why benefit was not observed in the other three media is unclear and other variables that could impact outcomes between incubators also existed (Morbeck et al., 2011). These same confounding variables exist in another preliminary study that compared the same large-box and small benchtop unit (Mortimer et al., 2003) and make it impossible to truly determine the impact of the incubator alone. Importantly, despite the differences in the culture parameters and suboptimal culture conditions provided in the large-box incubators in these studies, there were no reports of significant difference in human embryo development, clinical pregnancy or implantation rates (Lee et al., 2010).

In a comparative examination of incubators using oocytes from donors, outcomes between a benchtop/time-lapse imaging incubator and a standard large-box incubator were assessed (large-box incubator size confirmed via M Cruz, personnel communication). Despite significant differences in embryo handling, including not removing embryos from the benchtop incubator while removing embryos at least twice from the large-box incubator, as well as use of low O_2 in the benchtop unit but not the large-box incubator, authors reported no significant difference in blastocyst formation, quality or ongoing pregnancy (Cruz et al., 2011). Additionally, embryos were cultured individually in microdrops in the large-box incubator, while being placed into individual microwells for the benchtop (M Cruz, personnel communication). This is important to note because the type of culture dish/conditions can influence embryo development through modifications of the microenvironment (Swain and Smith, 2011). While no significant difference between the number of day-3 or day-5 transfers based on a particular incubator was reported (benchtop/time-lapse versus standard incubator; Cruz et al., 2011), upon reanalysis of the reported data using a different statistical program, it appears that more day-5 transfers were performed from the larger boxincubator (34/58) compared with the smaller time-lapse incubator (19/50; chi-squared 0.052, chi-squared with Yates correction 0.038, Fishers test $P = 0.038$, Fisher's exact two-tailed $P = 0.036$). Often day-5 transfer is dictated by superior quality or number of embryos; although no differences in outcomes were noted. Thus, use of smaller benchtop incubators do not necessarily equate to better embryo quality, as several other variables are involved in efficacy of the culture environment.

A similar study comparing a benchtop/time-lapse incubator versus a standard large-box unit (large-box incubator size confirmed via JJ Hindkjaer, personal communication) used embryos from infertile patients and compared embryo development, pregnancy and implantation rates. Despite confounding variables, such as use of different culture dishes (Embryoslide versus Nunc 4-well) and embryo density (single versus group), no significant differences in any endpoint were found between incubators (Kirkegaard et al., 2012). While high O_2 was used in each incubator (K Kirkegaard, personal communication), other conditions in each specific incubator, such as pH or humidity, were not reported. Failure to properly control all variables between incubators, which is a difficult task, makes it hard to determine 'superiority' of a particular incubator. Thus, while these studies help demonstrate safety of time-lapse imaging of embryos and use of a novel benchtop/time-lapse incubator, it could also be used to demonstrate that a large-box incubator, with proper management, can yield similar outcomes to a benchtop unit using time-lapse imaging. Indeed, if limiting patient number and the associated door openings of large-box incubators, with proper management, traditional box-type incubators can perform similarly compared with benchtop/time-lapse units (M Tucker and M VerMilyea, personnel communication). However, in this case, several large-box incubators are needed to facilitate the required management and match the capacity of the benchtop unit.

A more recent retrospective observational multicentre cohort study comparing clinical pregnancy rates from embryos grown in a benchtop/time-lapse incubator compared with a large-box CO_2 incubator with a TC sensor, demonstrated a 20.1% increase in clinical pregnancy rate per oocyte retrieval or 15.7% per transfer (Meseguer et al., 2012). However, as pointed out by the authors in their discussion, this could be due to a variety of factors including, but not limited to, improved embryo selection from use of time-lapse imaging and not removing embryos for daily observation from the benchtop/time-lapse unit. Interestingly, in this study, air quality in the smaller incubator, but not the large-box incubator, was extensively filtered through HEPA and activated carbon and was UV-treated

every 10 min. An improved approach to assess the impact of the incubators themselves may include comparison of outcomes using time-lapse imaging inside a large-box incubator, with similar filtering and air quality to those from a benchtop incubator with time-lapse imaging.

A comparison of a small two-chamber benchtop/topload unit (~0.43 l) using direct heat versus a large-box (~170 l) incubator using a water jacket and no inner doors demonstrated a significantly faster recovery of temperature in the benchtop/topload direct heat unit (Cooke et al., 2002). Temperature in the benchtop/topload unit recovered to 37°C within 5.5–6.5 min, dependent upon the volume of medium tested, while the large-box incubator failed to reach the set point following 20-min recovery (36.2°C and 36.7°C). Whether the same would hold true with an air-jacketed box-type incubator, small or large sized, or with units using sealed inner doors is unknown. Interestingly, use of milled aluminium blocks to hold culture dishes within the box-type incubator were able to maintain temperature and help lessen/avoid temperature decrease in the culture dish (Cooke et al., 2002). This demonstrates the importance of incubator management in optimizing performance of various units.

Finally, a more recent preliminary study retrospectively analysed laboratory data following equipment change and suggests that mini bench-top incubators can significantly improve laboratory outcomes compared with single-chambered large-box incubators (Hill et al., 2013). When changing from 12 large-box incubators using low O_2 to 16 bench-top incubators using cylinders of premixed gas ($CO_2/O_2/N_2$ 6:5:89), a significant improvement in 2 pronuclei formation was observed, with no significant improvement in blastocysts frozen, cycles with embryos frozen or donor pregnancy rate. However, again, differences in air quality with differing gas supplies could be a possible explanation for results. Perhaps more likely, with the addition of four additional benchtop units compared with the large-box-type incubators, undoubtedly fewer patients were placed into each bench-top incubator. Thus, it is not possible to determine if one incubator type/technology was actually superior to the other, as differences in patient distribution between incubators impacts opening/closing and environmental stability.

Thus, in examining existing comparative studies on incubators, differences likely exist between environmental recovery between various units, including gas atmosphere and temperature. This depends largely on the size of the incubator, but also on the technology incorporated into the unit, such as gas sensor type or temperature regulation approach. However, careful attention should be paid to the use of optimal available technology/approaches for each type of incubator to better analyse the comparisons being made. Many existing studies compare newer smaller benchtop units to older outdated large-box units. While this reflects many real-world system changes, comparison of new smaller units to an 'optimized' large-box or small-box unit might be more insightful. Additionally, it becomes apparent that, while smaller units recover gas atmosphere and temperature more rapidly, and this undoubtedly results in less environmental stress imposed upon the system, this may not necessarily equate to better outcomes. Furthermore, the available comparative incubator studies fail to properly control other confounding variables, such as patient number per incubator, making it very difficult to determine the true impact of a particular incubator.

Incubator management

Following critical review of existing comparative studies, it becomes apparent that it is impossible to determine the 'best' incubator. This will vary from laboratory to laboratory based on specific use and needs. Certainly results can vary between incubators for a variety of reasons (Avery and Greve, 1992; Higdon et al., 2008). This reinforces the need for strict quality control as well as proper management of laboratory IVF incubators to optimize their functions and maximize outcomes (Higdon et al., 2008). Insight into specific incubator units, both benchtop/topload and standard box type, their functioning and potential drawbacks can be found elsewhere (Meintjes, 2012). Regardless of the specific model of incubator utilized within the laboratory, without proper incubator management, environmental stability and embryo development can be compromised in even the most cutting-edge unit employing the newest technology.

Proper incubator management includes various approaches aimed at maintaining environmental stability inside the unit. This includes distribution of patient samples and proper workflow to avoid overuse of any particular incubator. Overuse of an incubator results in the inability to maintain a stable culture environment due to repeated opening/closing. Thus, management requires an adequate number of incubators based not only on total cycle volume, but also on the time frame of when these cycles are performed. For example, an IVF laboratory that performs 200 cycles spread over the entire year will have a different incubator requirement than an IVF laboratory that performs the same 200 cycles in four batches a year because the workload for a single incubator will be greater for the laboratory that batches. The number of incubators required can be determined through analysing a particular laboratory's workflow, taking into account how many patients can fit into an individual unit (i.e. one patient per shelf or two patients per unit), how many patients will be seen within a period of time (i.e. ~6 d) and other relevant variables.

In addition to an appropriate number of incubators, workflow between incubators must also be considered. Preferred use of a single incubator over others due to a convenient location can compromise the environmental stability of the individual incubator due to increased opening/closing. It was demonstrated that reducing door opening from six to four times over a 6-d period on a small-box incubator utilizing a water jacket with TC CO_2 and galvanic O_2 sensors resulted in significantly improved human blastocyst formation (53% versus 43%) and good-quality blastocyst rates (60% versus 51%), although no differences in day-3 embryo quality, implantation or clinical pregnancy rates were observed (Zhang et al., 2010). Also supporting the benefit of reduced incubator opening and improved embryo development, use of a gassed/sealed modular chamber placed inside the incubator to stabilize gas atmosphere resulted in significantly improved mouse blastocyst development and increased cell number compared with embryos cultured in a standard box incubator opened approximately

11 times per day (Gardner and Lane, 1996). Similar improvements in mouse embryo development and clinical outcomes were also observed with use of a large enclosed isolator-based culture system, likely due, in part, to improved environmental stability due to reduced incubator opening (Hyslop et al., 2012). Finally, limiting patient number in small two-chambered benchtop/topload units to a maximum of four patients, compared with the full unit capacity of eight patients, has been used to improve blastocyst formation (M Tucker, M Vermilyea, W Caswell, personal communication). Thus, IVF cases should be distributed between all available incubators to avoid overuse or excessive opening, regardless of the size or format of the incubator.

Other approaches to avoid excessive incubator opening include the use of 'holding' incubators that can be used for transient procedures, such as dish equilibration, sperm swim-up/capacitation and even brief culture of thawed embryos prior to same-day/immediate transfer. Using older 'outdated' incubators for these purposes may help avoid overuse of incubators that are used primarily for extended embryo culture.

Finally, use of various commercially available adjuncts can help with incubator management and maximize environmental stability. Various gas or air filters have been mentioned and can be used to improve air quality. Use of inner doors on box-type incubators can help prevent excessive gas exchange. Desiccator jars or modular chambers can maintain gas atmosphere within box-type incubators during repeated opening/closing. Additionally, use of specialized milled aluminium blocks designed to hold culture dishes can help maintain a stable temperature during door opening or during routine observations performed outside the incubator.

Incorporating new technology

Another important consideration for incubator selection entails the ability to implement new technology. Recent advances in dynamic embryo culture include motorized tilting devices, vibrating platforms and even piezo-actuated pin systems (Smith et al., 2012; Swain and Smith, 2011; Swain et al., 2013), all which require standard box-type (large or small) incubators for placement. Similarly, some emerging real-time embryo imaging devices that use portable modular cameras also require large- or small-box incubators for implementation (Chavez et al., 2012; Conaghan et al., 2013; Wong et al., 2010). With proper management, box-type incubators used to accommodate these devices aimed at improving embryo development and/or selection can likely perform in a similar fashion as benchtop units. Similarly, with continued development, perhaps novel dynamic culture devices can be scaled down to permit incorporation into small benchtop/topload incubators.

Looking towards the future, incubator size and design will likely continue to change. An emerging trend for benchtop/topload units is apparent and further modifications or advancements may continue to help revolutionize and drive incubator design. For example, emerging time-lapse imaging devices could potentially be modified to incorporate dynamic vibrational culture. One could envision a small vibrating motor, similar to those used to vibrate cellular phones, attached to the area housing the embryo dish to provide gentle mechanical stimulation for brief periods between image capture. Prior studies indicate that 5 s of vibration at 44 Hz improved embryo development and outcomes (Hur et al., 2013; Isachenko et al., 2010, 2011). Accordingly, incubators could be modified to incorporate these devices, utilizing the strengths of both large-box and benchtop units, including housing each in a small, independently sealed chamber. Indeed, at present there are already incubators that utilize individual or removable chambers, which remain sealed/gassed and separate from the rest of the larger unit, thereby permitting isolation of individual patient material while not compromising the gas environment of other patient samples when accessed. Some of these even include real-time imaging. Furthermore, reliance on tanks of medical gases for incubators could potentially be alleviated through use of a safe, clean, chemically supplied atmosphere. As demonstrated by prior studies supplying CO_2 from a simple citric acid and sodium bicarbonate reaction in a closed test-tube system, proper pH can be obtained and maintained over extended periods of time, permitting production of mouse blastocysts at similar rates and with similar cell numbers to embryos grown in standard large-box incubators (Swain, 2010, 2011). This approach would be conducive to simplifying the incubator system and offers a means of promoting low-cost IVF (Swain, 2011). Indeed, this method was later adopted for clinical use as part of a low-cost IVF initiative (Klerkx et al., 2013; Van Blerkom et al., 2013). Authors demonstrated that fertilization and embryo development could proceed in test tubes filled with a chemically generated atmosphere and the approach has resulted in seven live births. Similar approaches may result in more cost-effective modular culture devices, perhaps further lending itself to individualized culture platforms to help stabilize the growth environment.

Conclusions

Incubator selection is an important decision for the IVF laboratory, as these devices regulate several environmental variables that can impact embryo development. While novel culture approaches may reduce the need for traditional incubators (Blockeel et al., 2009; Hyslop et al., 2012; Itoi et al., 2012; Ranoux and Seibel, 1990; Ranoux et al., 1988; Suzuki et al., 1999; Swain, 2010, 2011; Taymor et al., 1992; Vajta et al., 1997, 2004; Van Blerkom et al., 2013; Varisanga et al., 2000), for the time being they remain a central part of a modern IVF laboratory. Functional aspects of the incubator, such as gas capability and sensor type, as well as temperature control and size/patient capacity need to be considered. Smaller incubator units, especially benchtop/topload devices, result in faster gas atmosphere and temperature recovery. However, no study has clearly demonstrated a distinct advantage of any specific incubator type in terms of human embryo development or clinical outcomes. Regardless of the unit, low-O_2 capability should be available and utilized and a IR CO_2 probe is preferable for those units that mix the gases to permit the fastest CO_2 recovery. Practical issues, such as cost and space, must also be weighed. The proper number and type of incubators to

adequately support a laboratory's caseload must be determined on a laboratory-by-laboratory basis. A mix of incubator types, including both large-box, small-box and benchtop/topload within a laboratory helps cover multiple scenarios and offers several options for utilization, including implementation of emerging technologies.

Paramount in appropriate functioning and optimizing incubator performance is proper incubator management. Regardless of incubator size or the technology incorporated/utilized, improper management of case workflow or failure to perform proper quality control can compromise the culture conditions provided by any incubator. Management should consider the daily, rather than annual, patient caseload to help avoid overcrowding and maintain a stable environment. As technology continues to advance and new culture platforms and embryo selection technologies become available, incubators will undoubtedly need to be adapted to meet the changing needs of the field.

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References

Avery, B., Greve, T., 1992. Impact of incubator type on the yield of in vitro produced bovine blastocysts. Acta Vet. Scand. 33, 341—348.

Battaglia, D., Khabani, A., Rainer, C., Moore, D., 2001. Prospective randomized trial of incubator CODA filtration unites revealed no effect on outcome. Fertil. Steril. 75, s6.

Bavister, B., 2004. Oxygen concentration and preimplantation development. Reprod. Biomed. Online 9, 484—486.

Blockeel, C., Mock, P., Verheyen, G., Bouche, N., Le Goff, P., Heyman, Y., Wrenzycki, C., Hoffmann, K., Niemann, H., Haentjens, P., de Los Santos, M.J., Fernandez-Sanchez, M., Velasco, M., Aebischer, P., Devroey, P., Simon, C., 2009. An in vivo culture system for human embryos using an encapsulation technology: a pilot study. Hum. Reprod. 24, 790—796.

Bontekoe, S., Mantikou, E., van Wely, M., Seshadri, S., Repping, S., Mastenbroek, S., 2012. Low oxygen concentrations for embryo culture in assisted reproductive technologies. Cochrane Database Syst. Rev. 7, CD008950.

Boone, W.R., Johnson, J.E., Locke, A.J., Crane 4th, M.M., Price, T.M., 1999. Control of air quality in an assisted reproductive technology laboratory. Fertil. Steril. 71, 150—154.

Borkow, G., Gabbay, J., 2004. Putting copper into action: copper-impregnated products with potent biocidal activities. FASEB J. 18, 1728—1730.

Chapuis, Y., Klvana, D., Guy, C., Kirchnerova, J., 2002. Photocatalytic oxidation of volatile organic compounds using fluorescent visible light. J. Air Waste Manag. Assoc. 52, 845—854.

Chavez, S.L., Loewke, K.E., Han, J., Moussavi, F., Colls, P., Munne, S., Behr, B., Reijo Pera, R.A., 2012. Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. Nat. Commun. 3, 1251.

Chou, J., 1999a. Electrochemical sensors. In: Hazardous Gas Monitors: A Practical Guide to Selection, Operation and Applications. McGraw Hill, New York, pp. 27—35.

Chou, J., 1999b. Infrared sensors. In: Hazardous Gas Monitors: A Practical Guide to Selection, Operation and Applications. McGraw Hill, New York, pp. 55—72.

Cohen, J., Gilligan, A., Esposito, W., Schimmel, T., Dale, B., 1997. Ambient air and its potential effects on conception in vitro. Hum. Reprod. 12, 1742—1749.

Conaghan, J., Chen, A.A., Willman, S.P., Ivani, K., Chenette, P.E., Boostanfar, R., Baker, V.L., Adamson, G.D., Abusief, M.E., Gvakharia, M., Loewke, K.E., Shen, S., 2013. Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. Fertil. Steril. 100, 412—419.

Cooke, S., Tyler, J.P., Driscoll, G., 2002. Objective assessments of temperature maintenance using in vitro culture techniques. J. Assist. Reprod. Genet. 19, 368—375.

Cruz, M., Gadea, B., Garrido, N., Pedersen, K.S., Martinez, M., Perez-Cano, I., Munoz, M., Meseguer, M., 2011. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J. Assist. Reprod. Genet. 28, 569—573.

Esteves, S.C., Verza Jr., S., Gomes, A.P., 2006. Comparison between international standard organization (ISO) type 5 and type 6 cleanrooms combined with volatile organic compounds filtration system for micromanipulation and embryo culture in severe male factor infertility. Fertil. Steril. 86, S353—S354.

Fujiwara, M., Takahashi, K., Izuno, M., Duan, Y.R., Kazono, M., Kimura, F., Noda, Y., 2007. 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. 24, 5—9.

Gardner, D.K., Lane, M., 1996. Alleviation of the '2-cell block' and development to the blastocyst of CF1 mouse embryos: role of amino acids, EDTA and physical parameters. Hum. Reprod. 11, 2703—2712.

Grass, G., Rensing, C., Solioz, M., 2011. Metallic copper as an antimicrobial surface. Appl. Environ. Microbiol. 77, 1541—1547.

Hall, J., Gilligan, A., Schimmel, T., Cecchi, M., Cohen, J., 1998. The origin, effects and control of air pollution in laboratories used for human embryo culture. Hum. Reprod. 13, 146—155.

Higdon 3rd, H.L., Blackhurst, D.W., Boone, W.R., 2008. Incubator management in an assisted reproductive technology laboratory. Fertil. Steril. 89, 703—710.

Hill, D., Darway, J., Surrey, M., Danzer, H., Ghadir, S., Barritt, J., 2013. Significant improvement in laboratory outcome data following following replacement of 'traditional' large, single chamber incubators with 'mini' incbators. Fertil. Steril. 100, s533—s534.

Hong, K., Forman, E., Lee, H., Ferry, K., Treff, N., Scott, R., 2012. Optimizing the temperature for embyo culture in IVF: a randomized controlled trial (RCT) comparing standard culture temperature of 37C to the reduced more physiologic temperature of 36C. Fertil. Steril. 98, s167.

Hunter, R.H., 2012. Temperature gradients in female reproductive tissues. Reprod. Biomed. Online 24, 377—380.

Hunter, R.H., Einer-Jensen, N., 2005. Pre-ovulatory temperature gradients within mammalian ovaries: a review. J. Anim. Physiol. Anim. Nutr. (Berl.) 89, 240—243.

Hunter, R.H., Einer-Jensen, N., Greve, T., 2006. Presence and significance of temperature gradients among different ovarian tissues. Microsc. Res. Tech. 69, 501—507.

Hur, Y.S., Park, J.H., Ryu, E.K., Park, S.J., Lee, J.H., Lee, S.H., Yoon, J., Yoon, S.H., Hur, C.Y., Lee, W.D., Lim, J.H., 2013. Effect of micro-vibration culture system on embryo development. J. Assist. Reprod. Genet. 30, 835—841.

Hyslop, L., Prathalingam, N., Nowak, L., Fenwick, J., Harbottle, S., Byerley, S., Rhodes, J., Watson, B., Henderson, R., Murdoch, A., Herbert, M., 2012. A novel isolator-based system promotes viability of human embryos during laboratory processing. PLoS One 7, e31010.

Hightech Instruments Technical Note TN01. Oxygen Sensors: Theory and Application. <http://wwwhitech-instcouk/ pdfs/technical/oxygen_sensorspdf>.
Isachenko, E., Maettner, R., Isachenko, V., Roth, S., Kreienberg, R., Sterzik, K., 2010. Mechanical agitation during the in vitro culture of human pre-implantation embryos drastically increases the pregnancy rate. Clin. Lab. 56, 569—576.
Isachenko, V., Maettner, R., Sterzik, K., Strehler, E., Kreinberg, R., Hancke, K., Roth, S., Isachenko, E., 2011. In-vitro culture of human embryos with mechanical micro-vibration increases implantation rates. Reprod. Biomed. Online 22, 536—544.
Itoi, F., Tokoro, M., Terashita, Y., Yamagata, K., Fukunaga, N., Asada, Y., Wakayama, T., 2012. Offspring from mouse embryos developed using a simple incubator-free culture system with a deoxidizing agent. PLoS One 7, e47512.
Khoudja, R.Y., Xu, Y., Li, T., Zhou, C., 2013. Better IVF outcomes following improvements in laboratory air quality. J. Assist. Reprod. Genet. 30, 69—76.
Kirkegaard, K., Hindkjaer, J.J., Grondahl, M.L., Kesmodel, U.S., Ingerslev, H.J., 2012. A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. J. Assist. Reprod. Genet. 29, 565—572.
Klerkx, E.P.F., Janssen, M., van Blerkom, M., Campo, R., Ombelet, W., 2013. First pregnancies with a simplified IVF procedure: a crucial step to universal and accessible infertility care ESHRE. Hum. Reprod. 28, i6 (O—O14).
Kovacic, B., Vlaisavljevic, V., 2008. Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. Reprod. Biomed. Online 17, 229—236.
Lane, M., Mitchell, M., Cashman, K.S., Feil, D., Wakefield, S., Zander-Fox, D.L., 2008. To QC or not to QC: the key to a consistent laboratory? Reprod. Fertil. Dev. 20, 23—32.
Lee, M., Grazi, R., Seifer, D., 2010. Incorporation of the Cook K-Minc incubator and media system into the IVF lab: the future of IVF. J. Clin. Embryol. 13, 21—32.
Leese, H.J., Baumann, C.G., Brison, D.R., McEvoy, T.G., Sturmey, R.G., 2008. Metabolism of the viable mammalian embryo: quietness revisited. Mol. Hum. Reprod. 14, 667—672.
Mantikou, E., Bontekoe, S., van Wely, M., Seshadri, S., Repping, S., Mastenbroek, S., 2013. Low oxygen concentrations for embryo culture in assisted reproductive technologies. Hum. Reprod. Update 19, 209.
Mayer, J., Nechchiri, F., Weedon, V., Jones, E., Kalin, H., Ochniger, S., Toner, J., Gibbons, W., Muasher, S., 1999. Prospective randomized crossover analysis of the impact of an IVF incbuator air filtration system (coda, genx) on clinical pregnancy rates. Fertil. Steril. 72, s42.
McLellan, S., Panagoulopoulos, C., Dickinson, K., Wright, D., Toght, T., Lanzendorf, S., 2001. Effect of incubator air filtration system on IVF outcomes. Fertil. Steril. 76, s103.
Meintjes, M., 2012. CO_2 and low-O_2 incubators. In: Nagy, Z., Varghese, A., Agarwal, A. (Eds.), Practical Manual of In Vitro Fertilization. Springer, New York, pp. 61—70.
Meintjes, M., Chantilis, S.J., Douglas, J.D., Rodriguez, A.J., Guerami, A.R., Bookout, D.M., Barnett, B.D., Madden, J.D., 2009. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. Hum. Reprod. 24, 300—307.
Merton, J.S., Vermeulen, Z.L., Otter, T., Mullaart, E., de Ruigh, L., Hasler, J.F., 2007. Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos. Theriogenology 67, 1233—1238.
Meseguer, M., Rubio, I., Cruz, M., Basile, N., Marcos, J., Requena, A., 2012. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil. Steril. 98, 1481—1489, e1410.
Morbeck, D., Fredrickson, J., Walker, D., Daftary, G., 2011. Culture in a bencthop incubator reduces in vitro stress in a senstive mouse embryo QC assay: potential role of air quality. Fertil. Steril. 96, s249.
Mortimer, S., Fluker, M., Yuzpe, A., 2003. Effect of culture system upon fertilization rate and embryo development for patients 40 years and older. Hum. Reprod. 80, s168.
Ranoux, C., Seibel, M.M., 1990. New techniques in fertilization: intravaginal culture and microvolume straw. J. In Vitro Fert. Embryo Transf. 7, 6—8.
Ranoux, C., Aubriot, F.X., Dubuisson, J.B., Cardone, V., Foulot, H., Poirot, C., Chevallier, O., 1988. A new in vitro fertilization technique: intravaginal culture. Fertil. Steril. 49, 654—657.
Schimmel, T., Gilligan, A., Garrisi, G., Espositi, B., Cecchi, M., Dale, B., Cohen, J., 1997. Removal of volatile organic compounds from incubators used for gamete and embryo culture. Fertil. Steril. 68, s165.
Sharmin, R., Ray, M.B., 2012. Application of ultraviolet light-emitting diode photocatalysis to remove volatile organic compounds from indoor air. J. Air Waste Manag. Assoc. 62, 1032—1039.
Smith, G.D., Takayama, S., Swain, J.E., 2012. Rethinking in vitro embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies. Biol. Reprod. 86, 62.
Stoddart, N., Rho, H., Maisel, H., Zhao, T., Tarantino, S., Fleming, S., 2003. Influence of the position of the culture dish within the incubator chamber and temperature of culture media on clinical outcome following Assisted Reproductive Technology (ART) treatment. Fertil. Steril. 80, s164—s165.
Sun, X.F., Wang, W.H., Keefe, D.L., 2004. Overheating is detrimental to meiotic spindles within in vitro matured human oocytes. Zygote 12, 65—70.
Suzuki, T., Sumantri, C., Khan, N.H., Murakami, M., Saha, S., 1999. Development of a simple, portable carbon dioxide incubator for in vitro production of bovine embryos. Anim. Reprod. Sci. 54, 149—157.
Swain, J., 2010. A self-contained culture platform utilizing chemically generated carbon dioxide supports mouse blastocyst development in vitro. Hum. Reprod. 25, i42.
Swain, J.E., 2011. A self-contained culture platform using carbon dioxide produced from a chemical reaction supports mouse blastocyst development in vitro. J Reprod Dev 57, 551—555.
Swain, J.E., 2012. Is there an optimal pH for culture media used in clinical IVF? Hum. Reprod. Update 18, 333—339.
Swain, J.E., Smith, G.D., 2011. Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment. Hum. Reprod. Update 17, 541—557.
Swain, J.E., Cabrera, L., Xu, X., Smith, G.D., 2012. Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development. Reprod. Biomed. Online 24, 142—147.
Swain, J.E., Lai, D., Takayama, S., Smith, G.D., 2013. Thinking big by thinking small: application of microfluidic technology to improve ART. Lab Chip 13, 1213—1224.
Taymor, M.L., Ranoux, C.J., Gross, G.L., 1992. Natural oocyte retrieval with intravaginal fertilization: a simplified approach to in vitro fertilization. Obstet. Gynecol. 80, 888—891.
Vajta, G., Holm, P., Greve, T., Callesen, H., 1997. The submarine incubation system, a new tool for in vitro embryo culture: a technique report. Theriogenology 48, 1379—1385.
Vajta, G., Bartels, P., Joubert, J., de la Rey, M., Treadwell, R., Callesen, H., 2004. Production of a healthy calf by somatic cell nuclear transfer without micromanipulators and carbon dioxide incubators using the Handmade Cloning (HMC) and the Submarine Incubation System (SIS). Theriogenology 62, 1465—1472.

Van Blerkom, J., Ombelet, W., Klerkx, E., Janssen, M., Dhont, N., Nargund, G., Campo, R., 2013. First births with a simplified culture system for clinical IVF and embryo transfer. Reprod. Biomed. Online 28, 310—320.

Varisanga, M.D., Dong, Y.J., Mtango, N.R., Fahrudin, M., Ni Wayan, K.K., Suzuki, T., 2000. Bovine blastocysts obtained from reconstructed cytoplast and karyoplasts using a simple portable CO_2 incubator. Cloning 2, 167—173.

Waldenstrom, U., Engstrom, A.B., Hellberg, D., Nilsson, S., 2009. Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. Fertil. Steril. 91, 2461—2465.

Walker, M.W., Butler, J.M., Higdon, H.L., Boone, W.R., 2013. Temperature variations within and between incubators-a prospective, observational study. J. Assist. Reprod. Genet. 30, 1583—1585.

Wang, W.H., Meng, L., Hackett, R.J., Odenbourg, R., Keefe, D.L., 2001. Limited recovery of meiotic spindles in living human oocytes after cooling-rewarming observed using polarized light microscopy. Hum. Reprod. 16, 2374—2378.

Wang, W.H., Meng, L., Hackett, R.J., Oldenbourg, R., Keefe, D.L., 2002. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertil. Steril. 77, 1274—1277.

Wong, C.C., Loewke, K.E., Bossert, N.L., Behr, B., De Jonge, C.J., Baer, T.M., Reijo Pera, R.A., 2010. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat. Biotechnol. 28, 1115—1121.

Zhang, J.Q., Li, X.L., Peng, Y., Guo, X., Heng, B.C., Tong, G.Q., 2010. Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate. Reprod. Biomed. Online 20, 510—515.

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