A Comparison of Embryo Culture Incubators for the IVF Laboratory

77

Jason E. Swain

77.1 Introduction

Minimizing environmental stressors and reducing variability during embryo culture are required to achieve optimal embryo development and maximize assisted reproductive technology (ART) outcomes. Key environmental variables to consider include carbon dioxide levels/pH of the culture medium, temperature stability, oxygen concentration, media evaporation/osmolality, and air quality. Importantly, all of these potential environmental stressors and others can be impacted or regulated by the laboratory incubator, which house gametes/embryos for the majority of their time in vitro. As a result, incubators are likely the most important pieces of equipment within the IVF laboratory, maintaining environmental stability within the culture system. As a result, incubator selection and proper use/management are critical for success of an IVF program.

With advances in manufacturing and technology, several incubator models now exist with varying capacities and capabilities and differing methods of controlling their internal environment (Table 77.1). This results in an increasing complexity when attempting to select an appropriate culture incubator for the IVF laboratory.

77.2 Incubator Function

The primary function of an incubator within the IVF laboratory is to provide a stable environment to hold gametes and embryos during their culture and development in vitro. To achieve this goal, an incubator must regulate several environmental variables, including gas concentrations (oxygen and carbon dioxide), temperature, and humidity. This must be done in a clean environment free of contamination and volatile organic compounds (VOCs), which can impair development. Importantly, a variety of methods are utilized by different incubators to maintain this stability. Additionally, considerations exist before selection and implementation of an incubator into the IVF laboratory.

77.2.1 Gas Atmosphere and Sensors

A primary function of a laboratory incubator is to consistently and reliably provide the appropriate gas atmosphere. Specifically, regulation of the concentration of carbon dioxide (CO_2) is of paramount importance, as this gas helps regulate the pH of the culture medium. The pH of media is an important variable that can significantly impact gamete function and embryo development [1–4].

Modern embryo culture incubators must also be able to provide an environment with a reduced concentration of oxygen (O_2). While atmospheric O_2 concentration is ~21%, it has long been shown that a reduced incubator oxygen concentration of ~5% during preimplantation embryo development is beneficial for embryo development and live birth in a variety of animal species, as well as human [5–7], most notably when used throughout the entire culture period to the blastocyst stage [8–10]. Reduced O_2 concentration is most commonly achieved by supplying a balance of nitrogen gas to displace atmospheric O_2 to achieve the desired O_2 concentration within the incubator. Whether a further reduction in O_2 concentration <5% is beneficial for human embryos is unknown but an active area of ongoing research [11, 12].

Rapid and accurate measurement of CO_2 and O_2 concentrations by the incubator is required to achieve target set points in a timely fashion and ensure appropriate growth conditions are maintained. Paramount to this essential function is the type of sensor installed. The two primary methods used in IVF incubators to monitor CO_2 concentration include thermal conductivity (TC) or infrared (IR) sensors (Fig. 77.1).

TC sensors operate via measurement of resistance between two thermistors, with one enclosed within an impermeable chamber and the other exposed to the incubator

J. E. Swain (✉)
CCRM IVF Network, Lone Tree, CO, USA
e-mail: jswain@ccrmivf.com

G. N. Allahbadia et al. (eds.), *Textbook of Assisted Reproduction*, https://doi.org/10.1007/978-981-15-2377-9_77

Table 77.1 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	Volume[a,b]	Humidity	Contamination control[d,e]	Other
Options	– CO_2-only – Low O_2 mixer – Low O_2 premixed cylinder	– IR – TC	–Zirconium – Galvanic (Fuel cell)	– Air jacket – Water jacket – Direct heat	– Benchtop 2-chamber Multichamber Other (i.e., timelapse) – Small-box – Large-box	– Yes[c] – No	– Heat – Internal UV – H_2O_2 – Copper alloy – External/ internal HEPA – External/ Internal VOC filter	– Data logging – Cost – Patient capacity – Service – Technology integration (dynamic culture, time-lapse cameras, alarm connectivity, real-time pH sensors, etc.)

[a]Actual volumes will vary from unit to unit
[b]Other novel designs exist, but these are general terms to refer to the most commonly used incubators in the IVF lab
[c]Some units bubble gas through a water pan to expedite re-humidification
[d]Ease of removing inner parts and/or wiping interior also is important to consider
[e]Presence/absence of an internal fan or other features may influence

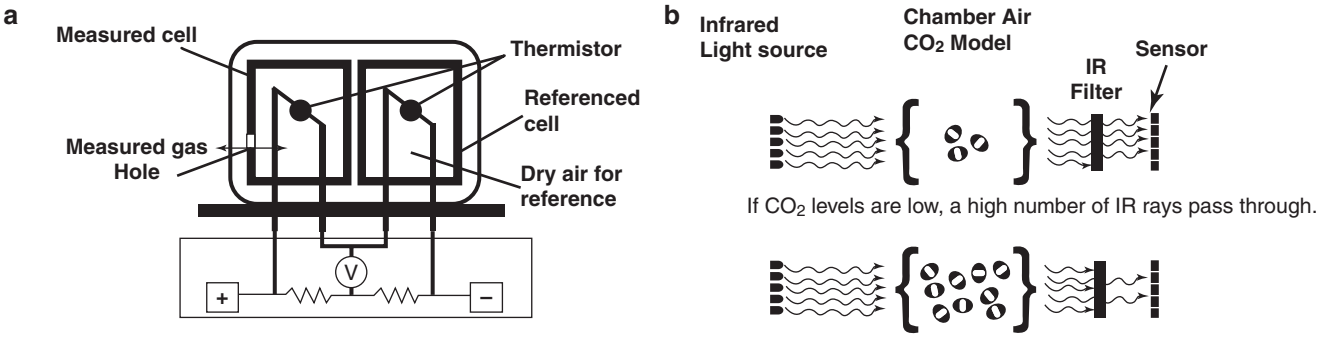

Fig. 77.1 Types of CO_2 sensors commonly used in culture incubators. (**a**) TC and (**b**) IR sensors

chamber [13]. The presence of CO_2 in the incubator chamber changes the resistance between the two thermistors and permits measurement of gas concentrations. Importantly, temperature and humidity impact the resistance of TC sensors and impact their measurements.

In contrast to TC sensor, IR sensors are largely independent of both humidity and temperature. IR sensors emit light and utilize specialized optics to detect IR light absorbance, which is relative to the levels of CO_2 inside the incubator chamber [14]. Thus, compared to IR sensors, incubators utilizing TC sensors tend to take a longer period of time to measure and therefore stabilize CO_2 levels following door openings since the gas concentrations cannot be fully determined and subsequently adjusted until both temperature and humidity stabilize. Due to reduction in cost and improvements to IR sensor lifespan, many embryo specific/modern IVF laboratory incubators utilize IR sensors and have become the preferred option for use.

Similar to incubator CO_2 sensors, two primary types of gas sensor are used to assess incubator O_2 concentration. These two types of O_2 sensors are galvanic/fuel cell or zirconium sensors [15] (Fig. 77.2). Though modern galvanic sensors have improved the rapidness of their responsiveness, they still tend to have slower response times compared to zirconium sensors. Additionally, compared to zirconium sensors, galvanic sensors tend to require more frequent replacement to ensure proper function.

Importantly, for both incubator O_2 and CO_2 readings, external incubator digital displays should not be solely relied upon to indicate rapidness of atmospheric recovery times during re-equilibration. Some incubator models are programmed to display their programmed set points prior to achieving re-equilibration of internal gas concentrations. If trying to assess environmental stability or speed of atmospheric recovery in IVF incubator chambers, the use of an independent measuring device placed within the incubator chamber is recommended for a more accurate assessment or comparison. These independent measuring devices may include independent gas sensors like fyrite or as part of an alarm system. Additionally, real-time pH meters may offer accurate assessment of CO_2 gas recovery (Fig. 77.3).

It should also be mentioned that accurate gas levels can be achieved in the absence of gas sensors or without gas mixers inside the incubator through use of cylinders of medical grade premixed gas. These premixed gases can be supplied directly to an incubator or to a sealed modular chamber placed inside the incubator, rather than requiring the incubator to have an internal gas mixer to adjust the gases into the proper ratios. Using this premixed gas approach, appropriate CO_2/O_2 concentrations are quickly achieved as soon as the

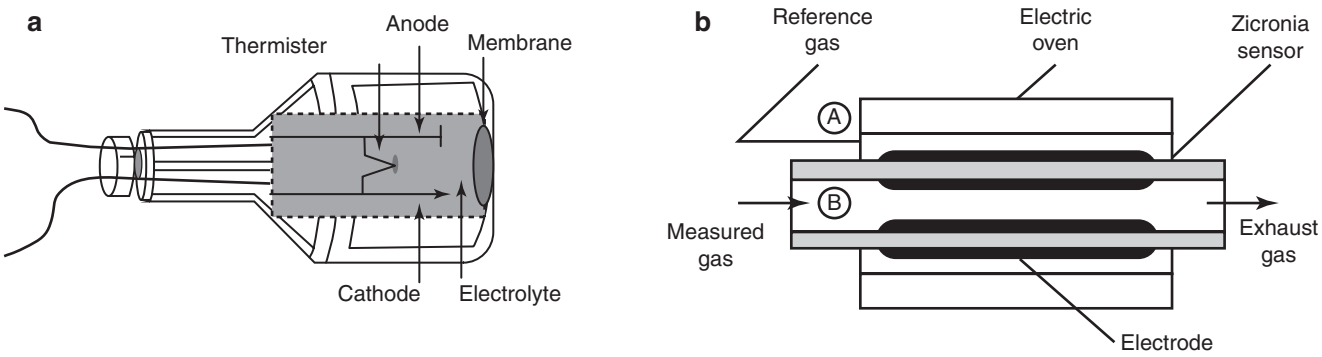

Fig. 77.2 Types of O_2 sensors commonly used in culture incubators. (**a**) Galvanic/fuel cells and (**b**) zirconium

Fig. 77.3 Type of incubators commonly found in IVF laboratories. (**a**) Large-box incubators can vary in size but are generally ~150 L. (**b**) Small-box incubators generally range in size from around 30–55 L

incubator volume has been filled with the premixed gas. Importantly, implementation of proper quality control is essential to ensure that the premixed gas concentrations/ratios inside the gas cylinder yield the appropriate/desired pH and growth conditions in the culture medium required by the particular laboratory. Factors such as the media used, protein type and concentration, and laboratory elevation above sea level will dictate what CO_2 concentration is required to obtain the desired media pH.

77.2.2 Incubator Chamber Size and Number

Chamber volume and the number of chambers are important factors to consider for incubator function and selection. Regardless of the gas sensor used or method of gas supply, incubator chamber volume influences gas equilibration and recovery timing. With door openings, traditional "large-box" incubators (~150–200 L) may require an extended time to refill with CO_2 and/or nitrogen gases. "Small-box" incubators (~14–50 L) have received increased use in IVF laboratories. Depending on the laboratory workflow, these smaller incubators may help improve gas recovery and reduce environmental stress, leading to improved outcomes compared to large-box incubators [16]. In cases of "box-type" incubators, often one patient is placed per shelf, and these units are useful for equilibration of media and holding test tubes for process like sperm preparation.

New incubators now being commonly used include benchtop/top load units of varying sizes/configurations. These incubators are designed specifically for clinical IVF and have extremely small chambers (~0.3–0.5 L), further improving atmospheric/environmental recovery time (Table 77.2). These modern benchtop incubators often provide several individual chambers for single patient use (Fig. 77.4).

Table 77.2 Types of modern benchtop IVF incubators and humidity options

Make/model	Type	Controlled humidity
K-systems G210	Multichamber	No
K-systems G185	Multichamber	No
Astec EC-6S	Multichamber	No
Astec EC-9	Multichamber	No
ESCO Miri Multiroom	Multichamber	No
IKS DS-1	Multichamber	Option yes
Synvivo CNC-I091	Multichamber	No
ESCO Miri TL	Timelapse	No
Vitrolife Embryoscope	Timelapase	No
Vitrolife Embryoscope plus	Timelapse	No
Genea Biomedx Geri	Timelapse	Option yes
Cook K-MINC	Dual chamber	Yes
Planer BT-37/INC-A20	Dual chamber	Yes
Labotect Labo C-Top	Dual chamber	Yes
Astec IVF Cube	Multichamber removeable	Yes
Planer CT37stax	Multichamber removeable	Yes

Fig. 77.4 A variety of modern benchtop incubators commercially available for use in IVF laboratories. Size, number of chambers, and various accessories vary between systems. (**a**) multichamber systems, (**b**) multichamber systems with removable incubator boxes, and (**c**) dual-chamber systems

A mixture of incubator types is often useful within the IVF laboratory, and, as will be discussed, incubator management is a key component for optimized incubator function regardless of chamber number or size.

77.2.3 Air Filtration and Quality

Another variable relevant to incubator gas atmosphere that impacts functional capability is air quality. Air quality, specifically the presence and amount of volatile organic compounds (VOCs), may negatively impact preimplantation embryo development [17–20], though relevant levels of VOCs 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 [20, 21]. However, while air quality inside the main IVF laboratory is important, the quality of the air/atmosphere inside the incubator chamber itself is of greater concern.

Background laboratory air quality will impact on the 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 levels to ~5%. VOCs have been detected in gas supply tanks used for IVF incubators [18]. In these cases, filtering the supply gases through inline filters prior to incubator entry may be an effective approach to improving incubator atmosphere. These inline filters contain HEPA (high-efficiency particulate air) filtration to reduce particle counts. Furthermore, additional filter methods to reduce VOCs include activated charcoal or potassium permanganate. At least one preliminary study showed improvement in embryo development following implementation of inline gas VOC filters [22].

Placement of specialized VOC filtration units inside incubators may also improve air quality and outcomes [19, 23, 24], though this is not always the case [25–27]. Their effectiveness depends on their size, and fitting into smaller incubators may be problematic. An emerging approach to improve air quality that is now being added to some incubators includes recirculating atmosphere via an ultraviolet light source to reduce possible microbials and to photocatalytically breakdown VOCs [28, 29].

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 entering the incubator chamber. By contrast, incubators that mix the gases themselves, such as either CO_2-only or low O_2 incubators, have at least some portion of room air present, though if room air is of high quality this likely poses little problem. Also, it is important to note that the plasticware or internal incubator components may "off-gas" inside the elevated temperatures of the incubator chamber [17]. Thus, despite having acceptable outside air quality or a prefiltered gas supply, VOCs may still be present inside an incubator. In these cases, proper initial cleaning of incubators and off-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.

77.2.4 Temperature Regulation and Stability

It is well-known that temperature can impact various aspects of gamete and embryo function, most notably meiotic spindle stability in the oocyte [30–32], possibly embryo metabolism [33] and mitotic cell division timings [34]. However, data indicate that temperature gradients may exist in the female reproductive tract [35–37]. Thus, while the optimal target temperature for IVF incubators that contain varying cell types and embryos at different developmental stages is still unknown [27, 38], maintaining a controlled/stable temperature inside the incubator while cells are inside is mandatory for reducing harmful environmental stress.

Three primary methods of heating are utilized in IVF incubators. Two 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 method used by primarily newer IVF-specific benchtop/top load units entails contact of the warmed incubator surface and direct heat transfer to the culture dish and enclosed medium. Some incubators may warm the base of the chamber, while others may warm the tops and bases. Importantly, each of the three warming methods utilized in culture incubators has benefits and limitations.

Water-jacketed incubators retain heat for longer during incubator openings or power failure. However, these units are heavy, tend to have a higher power consumption, and may burden emergency power supplies. There are also concerns that contamination may originate from inside the water jacket. Conversely, air-jacketed incubators warm up quickly but do not retain heat for long periods with interrupted power supply. The third heating approach, utilizing direct heat/contact, results in very rapid heat recovery following opening of the incubator, but similar to air-jacketed units, maintaining this temperature for any period of time during power interruption can be problematic.

Importantly, temperature gradients can exist inside any type of incubator, regardless of the type of warming approach employed. Such gradients are most common in box-type incubators utilizing water or air jackets. A preliminary report indicated slight temperature variations when culture dishes were placed in various locations within a large-box water-jacketed incubator, with measurements varying between 36.97, 37.17, and 37.23 °C [39]. Whether such minor fluc-

tuations are detrimental is unknown, but independent temperature measurement between shelves on box-type units, known as temperature-mapping, is recommended. Furthermore, verifying the temperature between individual culture chambers or across warmed surfaces of various benchtop/top load unit configurations can give critical information on temperature accuracy and stability that could impact gamete and embryo development and function.

77.2.5 Humidity and Evaporation

Many incubators provide an elevated humidity in order to reduce media evaporation from the higher incubation temperature during culture. This helps avoid detrimental rises in medium osmolality that can compromise preimplantation embryo development [40, 41]. Humidification inside the incubator is usually achieved in a passive fashion, via evaporation or bubbling inlet gases through a water reservoir placed in the bottom of the incubator chamber. Importantly, the presence of a water reservoir for humidity is also a potential source of contamination and should be monitored with and water exchanged/replaced regularly.

It should be noted that humidity inside the incubator is not necessarily required to culture embryos. Many new IVF-specific benchtop incubators do not provide humidification (Table 77.2). If sufficient amounts of oil overlay are used and media is exchanged/replenished appropriately, high-quality embryo development in a non-humidified incubator is achievable. Importantly, evaporation of media can occur despite use of mineral oil overlay in non-humidified incubators [42]. Thus, variables such as volume of media and amount of oil and number of days of continuous culture should be considered and osmolality measured to confirm appropriateness of culture conditions. This is likely even more important with the increased use of single-step culture media and uninterrupted culture, where evaporation is more likely to occur over time.

Interestingly, a recent study indicated that humidification of a dry benchtop incubator resulted in improved embryo development compared to the totally dry incubator [43]. While evaporation of media was not assessed in this study, an increase in osmolality, or possibly even media pH, was assumed to be a possible cause. However, placement of water into a normally dry incubator can be problematic, due to condensation within the chamber and possible issues with internal electrical components that were not developed for use in a humidified environment.

77.2.6 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 an antimicrobial and antifungal agent [44, 45]. However, at least one study suggested that oxidized copper particles from incubator walls may have detrimental effects on bovine embryo development [16], though the experimental design utilized prevented any conclusive correlation and several copper-containing incubators are used successfully for human embryo culture.

As an alternative for contamination control, some air-jacketed incubators feature heat decontamination cycling capability. Other incubator types 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 on some units, though this feature is often turned off to avoid possible damage to cells cultured inside the incubator. Most incubators can be sanitized 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. Incubators with fewer removable parts or lacking internal circulation fans are easier to clean and may help reduce the risk of contamination.

Daily monitoring for quality control/assurance is another consideration when selecting a laboratory incubator. When dealing with multiple chambers in a benchtop incubator, daily measurement of gas levels or temperatures in each chamber can be time-consuming. However, newer technologies are starting to address these issues, with the availability of small real-time temperature sensors for each chamber [46] or real-time pH sensors.

Incubator selection criteria include other practical items as well. These include the space occupied, the manner in which chamber doors open and close and latch, how gas concentrations may be measured, ability for incubators or chambers to be connected to the current alarm system, and availability of preventative maintenance and service. It is recommended that "demo" units of incubators be trialed or careful examination of units at exhibit halls or in other laboratories be conducted, prior to purchasing and clinical implementation.

77.3 Comparative Studies and Clinical Outcomes

Very few comparative studies examining environmental stability and recovery of particular incubator units exist in the peer reviewed literature, and even fewer studies exist comparing outcomes of embryo development or assisted reproductive outcomes. Furthermore, careful examination of the existing literature is required to understand why any reported

differences may exist, and this scrutiny often points out limitations in study design that need to be considered when interpreting results.

A comparison of a small two chamber benchtop/top load units (~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/top load, direct heat unit [47]. Temperature in the benchtop/top load unit recovered to 37 °C within 5.5–6.5 minutes, dependent upon the volume of medium tested, while the large-box incubator failed to reach the set point following a 20-min recovery (36.2 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, the use of milled aluminum blocks to hold culture dishes within box-type incubators was able to help maintain stable temperature within the culture dish [47]. These data demonstrate the importance of proper incubator management in optimizing incubator stability and performance. Whether this would translate to differences in embryo development or clinical outcomes is unknown.

When comparing a small benchtop incubator unit with two top load chambers (~0.43 L) and a small-box incubator (~32 L), it was found that after a 5 s opening that the benchtop/top load unit had improved temperature recovery (5 min vs. 30 min) and O_2 recovery (3 min vs. 8 min), improved "good" early embryo development (40% vs. 38%), and improved "good" blastocyst formation (15% vs. 8%) [48]. Interestingly, this study 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 box unit that 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 if measuring CO_2, which is likely more important. Furthermore, in this case, the small-box unit was outfitted with outdated technology and 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-jacket heated unit is unknown. Importantly, no oil overlay was used in this study, and overall blastocyst conversion rates in both incubators were low. It is possible that the use of oil overlay would have stabilized pH and temperature and perhaps improved the suboptimal growth conditions. Thus, while the benchtop/top load unit likely recovered atmosphere and temperature more rapidly, a more thorough examination of the study design reveals that the discrepancies between the two incubators may not be as pronounced if using more modern/optimized approaches.

In another study, a box-type incubator and a small two-chambered benchtop/top load units were compared, examining the recovery of temperature, CO_2, and humidity. In addition, fertilization rate, embryo quality, clinical pregnancy, and implantation rates were compared between the incubator types [49]. Following a 10-s incubator opening, it was found that there was a significant difference in temperature recovery (1 min vs. 180 min), CO_2 recovery (8 min vs. 120 min), and humidity recovery (12 min vs. 180 min), with faster recovery occurring in the benchtop/top load unit. Of note, the large-box incubator was outfitted with non-airtight inner doors which may not provide a stable gas environment as newer incubator units which employ this stabilization measure. Additionally, large-box incubators used in the study utilized the slower TC sensor and were water-jacketed. Finally, the benchtop/top load unit utilized low O_2 culture via premixed gas, while the large-box incubator used CO_2 only. As previously mentioned, low O_2 appears to produce improved preimplantation embryo development and clinical outcomes compared to high oxygen culture [5–7]. Furthermore, the use of premixed medical gas in the benchtop/top load unit may provide improved air quality over use of ~94% room air in the large-box incubator. Support for this theory can be found in a preliminary study that compared the same type of large-box and a small benchtop/top load incubator. In this study, results indicated that indeed 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 to the large-box incubator. Interestingly, it is unknown why the benefit was not observed in the all the media types, although other culture system variables may have existed between the incubators [50]. These same confounding variables in the culture system exist in another study that compared the same type of large-box and benchtop unit [51] and make it impossible to precisely assess the impact of the incubator as the sole factor. 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 [49].

In a comparative study examining culture incubators using human donor oocytes, clinical outcomes between a benchtop/time-lapse incubator and a large-box incubator were assessed (large-box incubator size confirmed via personnel communication M. Cruz). Despite significant differences in embryo handling approaches, including an uninterrupted embryo culture paradigm in the benchtop incubator while handling/removing embryos at least twice from the large-box incubator, as well as use of low O_2 in the benchtop unit but high O_2 the large-box, no difference in blastocyst formation, blastocyst score/quality, or ongoing clinical pregnancy was reported [52]. Additionally, embryos were cultured individually in microdrops in the large-box incubator while being placed into individual microwells for the benchtop (pers. comm. M. Cruz). This difference in culture dishes is important to note because the type of culture

Fig. 77.5 Three modern incubators incorporating time-lapse imaging (TLI) that utilize individual chambers for each patient to provide environmental stability. Patient capacity and accessories available vary between systems

platform used can create unique microenvironments and differentially impact embryo development [53]. While no significant difference between the numbers of day 3 or day 5 transfers based on a particular incubator was reported (benchtop/time-lapse vs. box incubator) [52], upon reanalysis of the reported data using different statistical software, it appears that more day 5 transfers were performed from the larger box-incubator (34/58) compared to the smaller time-lapse incubator (19/50). It is a common practice for day 5 transfer to be dictated by superior quality or quantity of available embryos though no differences in clinical outcomes were reported. Thus, the use of smaller benchtop incubators does not necessarily equate to better embryo quality, as several other culture system variables are can impact development (Fig. 77.5).

Another published report compared a benchtop/time-lapse incubator versus a standard large-box unit (large-box incubator size confirmed via pers. comm. J. Hindkjær) using the key performance indicators of embryo development, clinical pregnancy, and implantation rates. Even with several confounding variables between the two incubator treatments, such as the use of different culture dishes (Embryoslide™ vs. Nunc 4-well) and embryo culture density (single vs. group), no statistically significant differences in any examined endpoint were noted [54]. While neither incubator used low O_2 (pers.comm. K. Kirkegaard), other conditions used in the incubators, such as humidity or pH similarities/differences, were not reported. Failure to properly control all these sort of impactful culture system variables between incubators makes it impossible to truly determine "superiority" of a particular incubator over another. Thus, while these published reports help demonstrate safety of time-lapse imaging (TLI) systems for embryos, the use of a smaller model incubator does not guarantee superior clinical outcomes. The same data could be used to defend an alternate viewpoint and to demonstrate that a large-box incubator, with proper management, can yield similar outcomes to a TLI benchtop unit.

A more recent retrospective observational multicenter cohort study compared clinical pregnancies following transfer of embryos cultured in a TLI incubator compared to a large-box CO_2 incubator with a TC sensor. The study demonstrated a 20.1% increase in clinical pregnancy per oocyte retrieval or 15.7% per embryo transfer [55]. However, as pointed out in the paper, this could be due to a variety of factors including, but not limited to, improved embryo selection via TLI and from the uninterrupted culture approach utilized in the TLI unit. Importantly, the medical gas supply of the TLI incubator was extensively filtered via HEPA, active carbon, and UV, while the large-box incubator was not. An improved approach to isolate the impact of the incubators may include comparison of outcomes using TLI inside a large-box incubator with similar air quality to those from a benchtop TLI incubator.

An additional retrospective matched-pair analysis of a TLI system to a large-box incubator was performed [56]. Approximately half of the patients cultured in the large-box incubator used high O_2 culture, while the TLI incubator utilized low O_2 culture. Using four-well dishes in the box incubator compared to a culture slide in the TLI incubator, clinical outcomes were compared. There was no comparison of preimplantation embryo development. Higher clinical pregnancy, implantation, and live birth rates were associated with the TLI system compared to the large-box incubator. Importantly, several variables in the culture system differed between incubator treatments due to the retrospective nature of the study (method of embryo selection, lot numbers of various culture items, oxygen tension, etc.). Thus, it is difficult to determine if one incubator was truly more efficient than another in terms of improved embryo quality or if the culture system as a whole was primarily responsible for reported differences

A prospective study using patient randomization examined outcomes following culture of embryos in a TLI incubator after 2 days to that of embryos cultured in a standard box incubator [57]. Both systems utilized low O_2 and the same

media. The box incubator had embryos cultured in 20 μL microdrops, while the TLI system utilized the proprietary TLI culture slide. Embryos cultured in the TLI system were not disturbed, while those cultured in the box incubator were removed for observations at three time points. No difference in the number of good quality embryos between the incubators was observed on day 2. No differences in pregnancy or implantation were noted, but patients with transfers from embryos cultured in the TLI system had higher rates of miscarriage. Whether similar results would be observed following extended culture to the blastocyst stage is unknown.

Another prospective comparison of a box incubator to a TLI system was performed using a poor prognosis patient population. Using 20 μL drops in a standard petri dish in a box incubator or use of 25 μL of media in a conical culture slide in a TLI incubator, patients were randomized and outcomes compared after 3 days of culture [58]. Both incubator systems utilized low O_2 and the same media. Embryos in the box incubator were removed and examined three times, while those in the TLI incubator were left undisturbed and imaged using the TLI monitoring system. Looking at 16 patients (44 zygotes) in the TLI incubator and 15 patients (42 zygotes) in the box incubator, no differences in embryos quality were noted on day 3, and no differences in pregnancy rates were identified (18.8 vs. 20.0%). The authors noted significantly that more time was required to utilize the TLI incubator compared to the standard culture system. Seven oocyte donors were also randomly assigned to the two culture systems. It was noted that with 36 embryos cultured in the TLI system, lower levels of Grade A embryos were available, though no differences in Grade A+B embryos were noted between the two systems. Low numbers and lack of blastocyst culture should be noted.

To illustrate the importance of other factors regulating embryo development other than the actual incubator type or chamber size, a comparison of two identical ten-chamber benchtop units was performed, creating humidity in one incubator through addition of a water dish while leaving the other incubator non-humidified. Patient randomization was utilized, and no differences between patient populations were noted. All other conditions were similar. The authors reported that embryos cultured in the non-humidified incubator had impaired development on day 3 and day 5, and transfers yielded lower pregnancy rates [43].

While new incubator technology should be beneficial, it should be noted that "more physiologic" approaches, which are less technologically advance, can also potentially lend themselves to improved embryo development. A comparison between a vaginal culture capsule and a box-type low $O_{2\ incubator}$ was performed following patient randomization [59]. While more cleaved embryos (88 vs. 69%) and more overall blastocysts >2BB (51 vs. 31%) were present in the box incubator, the authors noted no difference in the number of high-quality blastocysts available for transfer, and only the in vivo cultured embryos yielded fully hatched blastocysts. No differences in pregnancy or implantation were observed following transfer. Thus, while preimplantation embryos would never normally see the vagina and variations may exist between patients in terms of the environmental conditions present during vaginal culture, this approach does appear to be able to provide good quality embryos for use. Whether the same findings would hold if compared to a modern benchtop incubator is unknown.

In summary, examination of comparative studies on embryo culture incubators indicates some differences are apparent between units in endpoints like environmental recovery, including gas atmosphere and temperature. These environmental recovery differences depend largely on the size of the incubator and the technology utilized in the unit, such as gas sensor type or temperature control approach. Importantly, careful attention must be paid to the use of optimal available technology/approaches for each incubator type to better assess comparisons between units. Many of the existing reports compare newer smaller benchtop units or TLI units to older outdated large-box incubators. 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 into impact of the incubator itself.

Additionally, it becomes apparent in examining prospective studies that while smaller incubator units recover gas atmosphere and temperature more rapidly, which undoubtedly reduces environmental stress, this may not necessarily equate to better clinical outcomes. Furthermore, published comparative studies fail to properly control confounding variables, such as gas environment, type of dish used, and embryo selection methods. This makes it very difficult to determine potential impact and or superiority of a particular incubator type.

77.4 Incubator Management

A critical review of existing comparative incubator studies makes it clear that it is not possible to determine the "best" incubator. Superiority of an incubator will vary for each laboratory based on particular use and needs. As previously mentioned, results can vary between incubators types for a variety of reasons [16, 27]. This reinforces the need for strict quality control as well as proper management of laboratory IVF incubators to optimize function and outcomes [27]. Insight into specific incubator units, both benchtop/top load and standard box-type, their functioning and potential drawbacks can be found elsewhere [60]. 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 involves steps aimed at maintaining environmental stability inside the unit. A critical approach to achieve this includes distribution of patient samples and proper workflow to avoid overuse of specific incubators. Not taking these measures results in "overcrowding" and an inability to maintain a stable culture environment due to repeated door openings/closing. Thus, incubator management requires a sufficient number of units 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 300 cycles spread over a 12-month period will have a different requirement for number of incubators than an IVF laboratory that performs the same 300 cycles batched at intervals throughout the year. The number of incubators needed will also differ for laboratories that perform blastocyst culture compared to those that do not.

In addition to considering the number of incubators required, the use or workflow between incubators must also be considered. Preferential use of a particular unit over others as a result of a more convenient location/proximity can compromise the environmental stability of the individual incubator due to increased openings/closing. It was demonstrated that reducing door opening from six to four times over a 6-day period on a small-box incubator utilizing a water jacket with TC CO_2 and galvanic O_2 sensors resulted in significant improved human blastocyst formation (53 vs. 43%) and "good" quality blastocysts (60% vs. 51%), though no differences in day 3 embryo quality, implantation, or clinical pregnancy rates were noted [61]. Further supporting the benefit of reduced incubator door openings and improved embryo development, the use of a gas-sealed modular chamber placed inside the incubator to stabilize gas atmosphere resulted in significantly improved mouse blastocyst development and increased cell number compared to embryos cultured in a standard box incubator opened approximately 11 times per day [62]. Similar improvements in mouse embryo development and clinical outcomes were observed with use of a large enclosed isolator-based culture system, likely due, in part, to improved environmental stability [63]. Thus, IVF cases should be distributed as evenly as possible between all available incubators to avoid overuse or excessive door openings, regardless of the size or format of the unit.

Another method to reduce incubator door opening includes the use of "holding" incubators that can be used for transient procedures, such as dish equilibration, sperm swim-up/capacitation, or even brief culture of thawed embryos prior to same-day/immediate transfer. Using older "outdated" incubators, like many large-box units, for these purposes may help reduce excessive use of incubators used primarily for extended embryo culture.

Finally, the use of various commercially available incubator adjuncts can help with incubator management and improve environmental stability. These approaches include use of gas or air filters to improve air quality. Additionally, the use of inner doors on box-type incubators can aid in reducing gas loss. Desiccator jars or modular chambers can maintain gas atmosphere within box-type incubators during repeated openings/closings, and specialized milled aluminum blocks designed to hold culture dishes can help maintain a stable temperature.

77.5 Incorporating New Technology and Future Directions

Another important consideration for incubator selection entails the ability to implement new technology. The field has already seen an increase in new incubators incorporating TLI with small individual chambers for each patient. These incubators often require fewer door openings and provide a more stable growth environment. However, future improvements may be achievable.

Recent advances in dynamic embryo culture include motorized tilting devices, vibrating platforms, or even piezo-actuated pin systems [53, 64, 65], all which require standard box-type (large or small) incubators for placement. While with proper management, these innovations are aimed at improving embryo development and/or selection and may be performed in a similar fashion as benchtop units. Indeed, perhaps novel dynamic culture devices can be scaled down to permit incorporation into small benchtop/top load incubators (Fig. 77.6).

For example, emerging TLI 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 or other similar brief intervals improved embryo development and outcomes [66–69], though rates of control samples are often poor and there is disagreement that the beneficial effects exist [70].

Furthermore, microfluidic capabilities could be incorporated to help with media exchange or novel dishware utilized where lyophilized media would be reconstituted automatically inside the incubator with an automated water pipetting system and equilibrated at the appropriate time. The embryos could then be moved along a microfluidic pathway to this new media, thereby achieving uninterrupted embryo culture but not being confined to using a single-step media while alleviating potential concerns about ammonia buildup. Additionally, more insightful noninvasive imaging technology could be implemented to supplement the normal

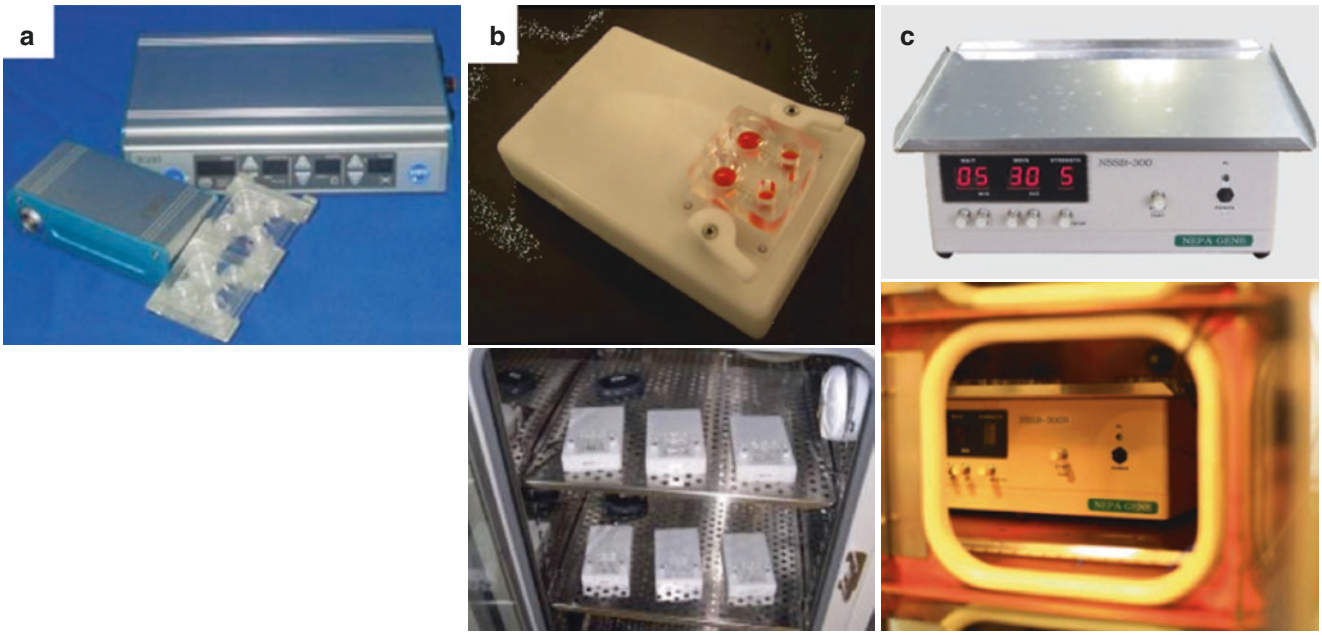

Fig. 77.6 Examples of novel, dynamic embryo culture platforms that may require specific incubator/space requirements for clinical use. (**a**) Motorized tilting device, (**b**) piezo-actuated microfluidic platform, and (**c**) vibrating platforms

dark field or bright field images commonly used currently [71].

77.6 Conclusion

Incubator selection is perhaps one of the most important decisions for an IVF laboratory, as these pieces of equipment control the growth environment of the preimplantation embryo via regulation of several environmental variables. While newer and more novel culture approaches may reduce the need for traditional incubators [63, 72–81], for the time being, these laboratory workhorses 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, are important considerations. Smaller incubator units, especially benchtop/top load devices, result in faster temperature and gas recovery. However, no published studies have demonstrated a clear benefit of any particular incubator type in terms of human preimplantation embryo development or clinical outcomes. Regardless of the incubator type, low O_2 capability should be utilized, and an IR CO_2 sensor is preferable for those units that mix the gases internally to permit the quickest gas recovery. Practical issues, such as cost and space requirements, must also be considered. The appropriate number and type of incubators are needed to adequately support the patient caseload, and this requirement must be determined on a lab-by-lab basis based on workflow. A combination of different incubator types, including large and small-box as well as benchtop/top load within a lab, helps cover multiple scenarios and offers several options for utilization, including implementation of emerging technologies.

Importantly, to improve incubator function and help optimize performance, proper incubator management is essential. Regardless of the size of the incubator or the technology incorporated/utilized, failure to implement proper management of case workflow or failure to perform proper daily quality assurance/control can compromise the culture conditions provided by any incubator. Proper incubator management should consider the daily caseload, rather than annual cycle number to avoid unnecessarily high incubator door openings/closings and maintain a stable internal growth environment. As technology continues to advance and new culture platforms and embryo selection technologies become available, incubators will undoubtedly need to continue to evolve to meet the changing needs of the field.

References

1. Swain JE. Is there an optimal pH for culture media used in clinical IVF? Hum Reprod Update. 2012;18(3):333–9. PubMed PMID: 22311857. Epub 2012/02/09.eng
2. Swain JE. Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality. Reprod Biomed Online. 2010;21(1):6–16. PubMed PMID: 20570214. Epub 2010/06/24.eng
3. Swain JE. Media composition: pH and buffers. Methods Mol Biol. 2012;912:161–75. PubMed PMID: 22829374
4. Swain JE, Pool TB. New pH-buffering system for media utilized during gamete and embryo manipulations for assisted reproduction.

Reprod Biomed Online. 2009;18(6):799–810. PubMed PMID: 19490784. Epub 2009/06/06.eng
5. Bavister B. Oxygen concentration and preimplantation development. Reprod Biomed Online. 2004;9(5):484–6. PubMed PMID: 15588462. Epub 2004/12/14.eng
6. Bontekoe S, Mantikou E, van Wely M, Seshadri S, Repping S, Mastenbroek S. Low oxygen concentrations for embryo culture in assisted reproductive technologies. Cochrane Database Syst Rev. 2012;7:CD008950. PubMed PMID: 22786519. Epub 2012/07/13. eng
7. Mantikou E, Bontekoe S, van Wely M, Seshadri S, Repping S, Mastenbroek S. Low oxygen concentrations for embryo culture in assisted reproductive technologies. Hum Reprod Update 2013;19(3):209. PubMed PMID: 23377864. Epub 2013/02/05.eng.
8. Kovacic B, Vlaisavljevic V. Influence of atmospheric versus reduced oxygen concentration on development of human blastocysts in vitro: a prospective study on sibling oocytes. Reprod Biomed Online. 2008;17(2):229–36. PubMed PMID: 18681997. Epub 2008/08/07.eng
9. Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, et al. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. Hum Reprod. 2009;24(2):300–7. PubMed PMID: 18927130. Epub 2008/10/18. eng.
10. Waldenstrom U, Engstrom AB, Hellberg D, Nilsson S. Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. Fertil Steril. 2009;91(6):2461–5. PubMed PMID: 18554591. Epub 2008/06/17.eng
11. Morin SJ. Oxygen tension in embryo culture: does a shift to 2% O2 in extended culture represent the most physiologic system? Journal of assisted reproduction and genetics. 2017;34(3):309–14. PubMed PMID: 28181051. Pubmed Central PMCID: 5360691.
12. Bolnick A, Awonuga AO, Yang Y, Abdulhasan M, Xie Y, Zhou S, et al. Using stem cell oxygen physiology to optimize blastocyst culture while minimizing hypoxic stress. J Assist Reprod Genet. 2017; 34(10):1251–9. PubMed PMID: 28647787.
13. Chou J. Electrochemical sensors. Hazardous gas monitors: a practical guide to selection, operation and applications. New York: McGraw Hill; 1999. p. 27–35.
14. Chou J. Infrared sensors. Hazardous gas monitors: a practical guide to selection, operation and applications. New York: McGraw Hill; 1999. p. 55–72.
15. Instruments H. Oxygen sensors: theory and application. http://www.hitech-instcouk/pdfs/technical/oxygen_sensorspdf [Internet].
16. Avery B, Greve T. Impact of incubator type on the yield of in vitro produced bovine blastocysts. Acta Vet Scand. 1992;33(4):341–8. PubMed PMID: 1488949. Epub 1992/01/01.eng
17. Cohen J, Gilligan A, Esposito W, Schimmel T, Dale B. Ambient air and its potential effects on conception in vitro. Hum Reprod. 1997;12(8):1742–9. PubMed PMID: 9308805. Epub 1997/08/01. eng
18. Hall J, Gilligan A, Schimmel T, Cecchi M, Cohen J. The origin, effects and control of air pollution in laboratories used for human embryo culture. Human reproduction. 1998; 13(Suppl 4):146–55 PubMed PMID: 10091065. Epub 1999/03/26.eng.
19. Merton JS, Vermeulen ZL, Otter T, Mullaart E, de Ruigh L, Hasler JF. Carbon-activated gas filtration during in vitro culture increased pregnancy rate following transfer of in vitro-produced bovine embryos. Theriogenology 2007;67(7):1233-1238. PubMed PMID: 17331571. Epub 2007/03/03.eng.
20. Khoudja RY, Xu Y, Li T, Zhou C. Better IVF outcomes following improvements in laboratory air quality. J Assist Reprod Genet. 2013; 30(1):69–76. PubMed PMID: 23242648. Pubmed Central PMCID: 3553352. Epub 2012/12/18.eng.
21. Boone WR, Johnson JE, Locke AJ, Crane MM, Price TM. Control of air quality in an assisted reproductive technology laboratory. Fertil Steril. 1999;71(1):150–4. PubMed PMID: 9935133. Epub 1999/02/06.eng
22. Esteves SC, Gomes AP, Verza Jr S. Control of air pollution in assisted reproductive technology laboratory and adjacent areas improves embryo formation, cleavage and pregnancy rates and decreases abortion rate: comparison between a class 100 (ISO 5) and a class 1.000 (ISO 6) cleanroom for micromanipulation and embryo culture. Fertil Steril 2004;82(Suppl 2):S259–S260.
23. Schimmel T, Gilligan A, Garrisi G, Espositi B, Cecchi M, Dale B, et al. Removal of volatile organic compounds from incbuators used for gamete and embryo culture. Fertil Steril. 1997;68(Suppl 1):s165.
24. Mayer J, Nechchiri F, Weedon V, Jones E, Kalin H, Ochniger S, et al. Prospective randomized crossover analysis of the impact of an IVF incubator air filtration system (coda, genx) on clinical pregnancy rates. Fertil Steril. 1999;72(Suppl 1):s42.
25. McLellan S, Panagoulopoulos C, Dickinson K, Wright D, Toght T, Lanzendorf S. Effect of incubator air filtration system on IVF outcomes. Fertil Steril. 2001;76(Suppl 1):s103.
26. Battaglia D, Khabani A, Rainer C, Moore D. Prospective randomized trial of incubator CODA filtration unites revealed no effect on outcome. Fertil Steril. 2001;75(Suppl 1):s6.
27. Higdon HL 3rd, Blackhurst DW, Boone WR. Incubator management in an assisted reproductive technology laboratory. Fertil Steril. 2008;89(3):703–10. PubMed PMID: 17524397. Epub 2007/05/26. eng
28. Chapuis Y, Klvana D, Guy C, Kirchnerova J. Photocatalytic oxidation of volatile organic compounds using fluorescent visible light. J Air Waste Manag Assoc. 2002;52(7):845–54. PubMed PMID: 12139349. Epub 2002/07/26.eng
29. Sharmin R, Ray MB. Application of ultraviolet light-emitting diode photocatalysis to remove volatile organic compounds from indoor air. J Air Waste Manag Assoc. 2012;62(9):1032–9. PubMed PMID: 23019817. Epub 2012/10/02.eng
30. Wang WH, Meng L, Hackett RJ, Oldenbourg R, Keefe DL. Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates. Fertil Steril. 2002;77(6):1274–7. PubMed PMID: 12057740. Epub 2002/06/12.eng
31. Wang WH, Meng L, Hackett RJ, Odenbourg R, Keefe DL. Limited recovery of meiotic spindles in living human oocytes after cooling-rewarming observed using polarized light microscopy. Hum Reprod. 2001;16(11):2374–8. PubMed PMID: 11679523. Epub 2001/10/27.eng
32. Sun XF, Wang WH, Keefe DL. Overheating is detrimental to meiotic spindles within in vitro matured human oocytes. Zygote. 2004;12(1):65–70. PubMed PMID: 15214582. Epub 2004/06/25. eng
33. Leese HJ, Baumann CG, Brison DR, McEvoy TG, Sturmey RG. Metabolism of the viable mammalian embryo: quietness revisited. Mol Hum Reprod. 2008;14(12):667–72. PubMed PMID: 19019836. Pubmed Central PMCID: 2639445. Epub 2008/11/21.eng.
34. Walters EA, Brown JL, Krisher R, Voelkel S, Swain JE. Impact of a controlled culture temperature gradient on mouse embryo development and morphokinetics. Reprod Biomed Online. 2020; 40(4):494–9. PubMed PMID: 32171708.
35. Hunter RH, Einer-Jensen N. Pre-ovulatory temperature gradients within mammalian ovaries: a review. J Anim Physiol Anim Nutr (Berl). 2005;89(7-8):240–3. PubMed PMID: 15972073. Epub 2005/06/24.eng
36. Hunter RH, Einer-Jensen N, Greve T. Presence and significance of temperature gradients among different ovarian tissues. Microsc Res Tech. 2006;69(6):501–7. PubMed PMID: 16703612. Epub 2006/05/17.eng

37. Hunter RH. Temperature gradients in female reproductive tissues. Reprod Biomed Online. 2012;24(4):377–80. PubMed PMID: 22369989. Epub 2012/03/01.eng
38. Hong K, Forman E, Lee H, Ferry K, Treff N, Scott R. Optimizing the temperature for embryo culture in IVF: a randomized controlled trial (RCT) comparing standard culture temperature of 37C to the reduced more physiologic temperature of 36C. Fertil Steril. 2012;98(3):s167.
39. Stoddart N, Rho H, Maisel H, Zhao T, Tarantino S, Fleming S. 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. 2003;80.(suppl(3):s164–5.
40. Swain JE, Cabrera L, Xu X, Smith GD. Microdrop preparation factors influence culture-media osmolality, which can impair mouse embryo preimplantation development. Reprod Biomed Online. 2012;24(2):142–7. PubMed PMID: 22197605. Epub 2011/12/27.eng
41. Lane M, Mitchell M, Cashman KS, Feil D, Wakefield S, Zander-Fox DL. To QC or not to QC: the key to a consistent laboratory? Reprod Fertil Dev. 2008;20(1):23–32. PubMed PMID: 18154695. Epub 2007/12/25.eng
42. Swain J, Schoolcraft W, Bossert N, Batcheller A. Media osmolality changes over 7 days following culture in a non-humidified benchtop incubator. Fertil Steril 2016;106(3):e362.
43. Fawzy M, AbdelRahman MY, Zidan MH, Abdel Hafez FF, Abdelghafar H, Al-Inany H, et al. Humid versus dry incubator: a prospective, randomized, controlled trial. Fertil Steril. 2017;108(2):277–83. PubMed PMID: 28651960.
44. Borkow G, Gabbay J. Putting copper into action: copper-impregnated products with potent biocidal activities. FASEB J. 2004;18(14):1728–30. PubMed PMID: 15345689. Epub 2004/09/04.eng
45. Grass G, Rensing C, Solioz M. Metallic copper as an antimicrobial surface. Appl Environ Microbiol. 2011;77(5):1541–7. PubMed PMID: 21193661. Pubmed Central PMCID: 3067274. Epub 2011/01/05.eng.
46. Jarmuz P, Barrett D, Sakkas D. Continual temperature and humidity measurement allows more efficient and accurate monitoring of incubator performance. Fertil Steril. 2014;102(35 suppl):e133.
47. Cooke S, Tyler JP, Driscoll G. Objective assessments of temperature maintenance using in vitro culture techniques. J Assist Reprod Genet. 2002;19(8):368–75. PubMed PMID: 12182443. Pubmed Central PMCID: 3455576. Epub 2002/08/17.eng.
48. Fujiwara M, Takahashi K, Izuno M, Duan YR, Kazono M, Kimura F, et al. 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(1):5–9. PubMed PMID: 17160731. Pubmed Central PMCID: 3455086. Epub 2006/12/13.eng.
49. Lee M, Grazi R, Seifer D. Incorporation of the Cook K-Minc incubator and media system into the IVF lab: the future of IVF. J Clin Embryol. 2013;13(3):21–32.
50. Morbeck D, Fredrickson J, Walker D, Daftary G. Culture in a benchtop incubator reduces in vitro stress in a sensitive mouse embryo QC assay: potential role of air quality. Fertil Steril. 2011;96(3):s249.
51. Mortimer S, Fluker M, Yuzpe A. Effect of culture system upon fertilization rate and embryo development for patients 40 years and older. Hum Reprod. 2003;80(suppl 3):s168.
52. Cruz M, Gadea B, Garrido N, Pedersen KS, Martinez M, Perez-Cano I, et al. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J Assist Reprod Genet. 2011;28(7):569–73. PubMed PMID: 21394522. Pubmed Central PMCID: 3162049. Epub 2011/03/12.eng.
53. Swain JE, Smith GD. Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment. Hum Reprod Update. 2011;17(4):541–57. PubMed PMID: 21454356. Epub 2011/04/02.eng
54. Kirkegaard K, Hindkjaer JJ, Grondahl ML, Kesmodel US, Ingerslev HJ. A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. J Assist Reprod Genet. 2012;29(6):565–72. PubMed PMID: 22460082. Pubmed Central PMCID: 3370049. Epub 2012/03/31.eng.
55. Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A. Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. Fertil Steril. 2012;98(6):1481–9.e10. PubMed PMID: 22975113
56. Barrie A, Homburg R, McDowell G, Brown J, Kingsland C, Troup S. Embryos cultured in a time-lapse system result in superior treatment outcomes: a strict matched pair analysis. Hum Fertil. 2017;20(3):179–85. PubMed PMID: 27884061
57. Park H, Bergh C, Selleskog U, Thurin-Kjellberg A, Lundin K. No benefit of culturing embryos in a closed system compared with a conventional incubator in terms of number of good quality embryos: results from an RCT. Hum Reprod. 2015;30(2):268–75. PubMed PMID: 25432920
58. Wu L, Han W, Wang J, Zhang X, Liu W, Xiong S, et al. Embryo culture using a time-lapse monitoring system improves live birth rates compared with a conventional culture system: a prospective cohort study. Hum Fertil. 2017;09:1–8. PubMed PMID: 28597711
59. Doody KJ, Broome EJ, Doody KM. Comparing blastocyst quality and live birth rates of intravaginal culture using INVOcell to traditional in vitro incubation in a randomized open-label prospective controlled trial. J Assist Reprod Genet. 2016;33(4):495–500. PubMed PMID: 26843390. Pubmed Central PMCID: 4818641.
60. Meintjes M. CO2 and Low-O2 Incubators. In: Nagy Z, Varghese A, Agarwal A, editors. Practical manual of in vitro fertilization. New York: Springer; 2012. p. 61–70.
61. Zhang JQ, Li XL, Peng Y, Guo X, Heng BC, Tong GQ. Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate. Reprod Biomed Online. 2010;20(4):510–5. PubMed PMID: 20129824. Epub 2010/02/05.eng
62. Gardner DK, Lane M. 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. 1996;11(12):2703–12. PubMed PMID: 9021376
63. Hyslop L, Prathalingam N, Nowak L, Fenwick J, Harbottle S, Byerley S, et al. A novel isolator-based system promotes viability of human embryos during laboratory processing. PLoS One. 2012;7(2):e31010. PubMed PMID: 22393356. Pubmed Central PMCID: 3290619. Epub 2012/03/07.eng.
64. Smith GD, Takayama S, Swain JE. Rethinking in vitro embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies. Biol Reprod. 2012;86(3):62. PubMed PMID: 21998170. Pubmed Central PMCID: 3316262. Epub 2011/10/15.eng.
65. Swain JE, Lai D, Takayama S, Smith GD. Thinking big by thinking small: application of microfluidic technology to improve ART. Lab Chip. 2013;13(7):1213–24. PubMed PMID: 23400523. Epub 2013/02/13.eng
66. Isachenko E, Maettner R, Isachenko V, Roth S, Kreienberg R, Sterzik K. Mechanical agitation during the in vitro culture of human pre-implantation embryos drastically increases the pregnancy rate. Clin Lab. 2010;56(11–12):569–76. PubMed PMID: 21141442. Epub 2010/12/15.eng
67. Isachenko V, Maettner R, Sterzik K, Strehler E, Kreinberg R, Hancke K, et al. In-vitro culture of human embryos with mechanical micro-

vibration increases implantation rates. Reprod Biomed Online. 2011;22(6):536–44.
68. Hur YS, Park JH, Ryu EK, Park SJ, Lee JH, Lee SH, et al. Effect of micro-vibration culture system on embryo development. J Assist Reprod Genet. 2013;30(6):835–41. PubMed PMID: 23657828. Pubmed Central PMCID: 3696450. Epub 2013/05/10.eng.
69. Isachenko V, Sterzik K, Maettner R, Isachenko E, Todorov P, Rahimi G, et al. In vitro microvibration increases implantation rate after embryonic cell transplantation. Cell Transplant. 2017;26(5):789–94. PubMed PMID: 27725062
70. Juneau CR, Franasiak JM, Morin SJ, Werner MD, Upham KM, Scott RT. EnMotion: embryos natural motion. blastulation is not different between static and dynamic culture systems. Fertil Steril. 2016;106(3):e358–9.
71. Jasensky J, Swain JE. Peering beneath the surface: novel imaging techniques to noninvasively select gametes and embryos for ART. Biol Reprod. 2013;89(4):105. PubMed PMID: 24068105
72. Ranoux C, Aubriot FX, Dubuisson JB, Cardone V, Foulot H, Poirot C, et al. A new in vitro fertilization technique: intravaginal culture. Fertility and sterility. 1988;49(4):654–7. PubMed PMID: 3350160. Epub 1988/04/01.eng.
73. Ranoux C, Seibel MM. New techniques in fertilization: intravaginal culture and microvolume straw. J In Vitro Fert Embryo Transf. 1990;7(1):6–8. PubMed PMID: 2187040. Epub 1990/02/01.eng
74. Taymor ML, Ranoux CJ, Gross GL. Natural oocyte retrieval with intravaginal fertilization: a simplified approach to in vitro fertilization. Obstet Gynecol. 1992;80(5):888–91. PubMed PMID: 1407935. Epub 1992/11/01.eng
75. Vajta G, Holm P, Greve T, Callesen H. The submarine incubation system, a new tool for in vitro embryo culture: a technique report. Theriogenology. 1997;48(8):1379–85.
76. Suzuki T, Sumantri C, Khan NH, Murakami M, Saha S. Development of a simple, portable carbon dioxide incubator for in vitro production of bovine embryos. Anim Reprod Sci. 1999;54(3):149–57. PubMed PMID: 10066102. Epub 1999/03/05.eng
77. Varisanga MD, Dong YJ, Mtango NR, Fahrudin M, Ni Wayan KK, Suzuki T. Bovine blastocysts obtained from reconstructed cytoplast and karyoplasts using a simple portable CO2 incubator. Cloning. 2000;2(4):167–73. PubMed PMID: 16218853. Epub 2005/10/13. eng
78. Vajta G, Bartels P, Joubert J, de la Rey M, Treadwell R, Callesen H. 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. 2004;62(8):1465–72. PubMed PMID: 15451255. Epub 2004/09/29.eng
79. Blockeel C, Mock P, Verheyen G, Bouche N, Le Goff P, Heyman Y, et al. An in vivo culture system for human embryos using an encapsulation technology: a pilot study. Hum Reprod. 2009;24(4):790–6. PubMed PMID: 19273881. Pubmed Central PMCID: 2656929. Epub 2009/03/11.eng
80. Swain JE. A self-contained culture platform using carbon dioxide produced from a chemical reaction supports mouse blastocyst development in vitro. J Reprod Dev. 2011;57(4):551–5. PubMed PMID: 21532256. Epub 2011/05/03.eng
81. Itoi F, Tokoro M, Terashita Y, Yamagata K, Fukunaga N, Asada Y, et al. Offspring from mouse embryos developed using a simple incubator-free culture system with a deoxidizing agent. PLoS One. 2012;7(10):e47512. PubMed PMID: 23056643. Pubmed Central PMCID: 3467247. Epub 2012/10/12.eng.