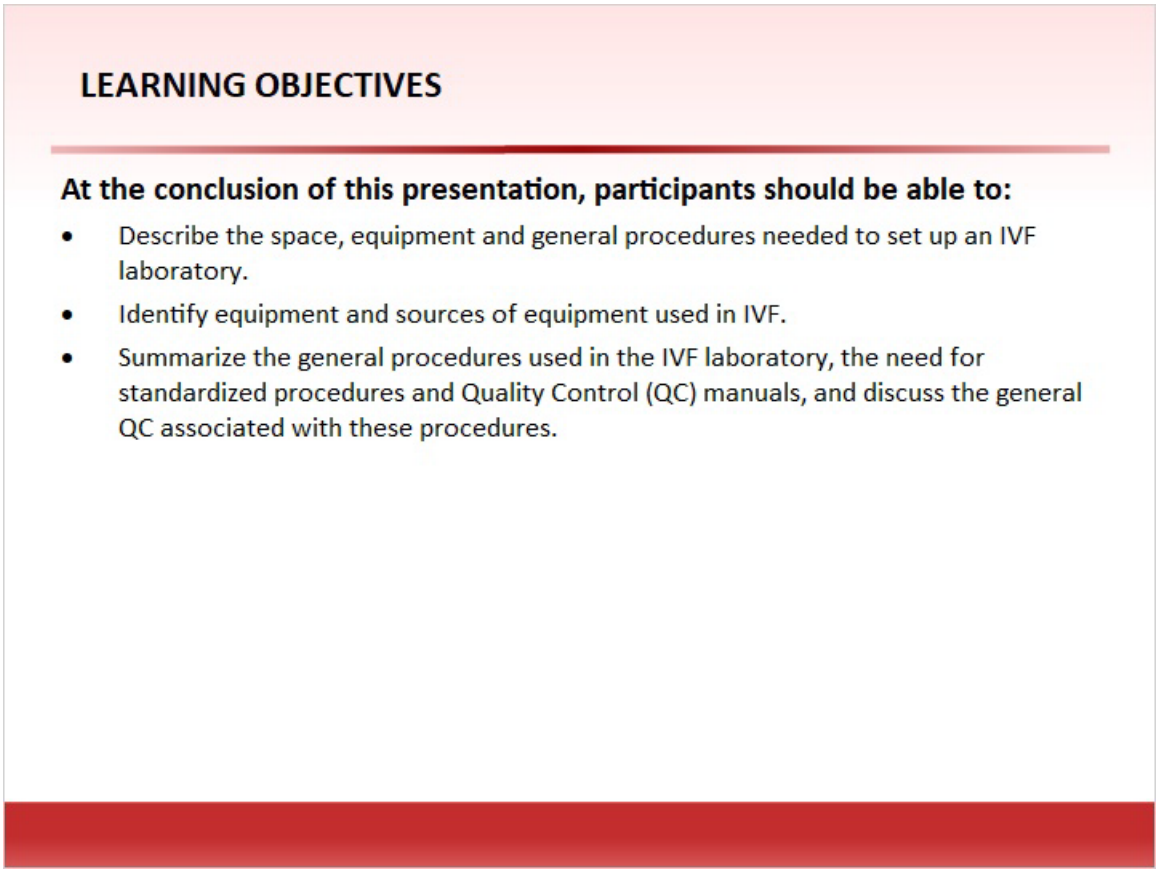
LABCC100 Lesson 33

1.1 The IVF Laboratory

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. This module addresses the setup of IVF laboratories in terms of physical space and equipment.

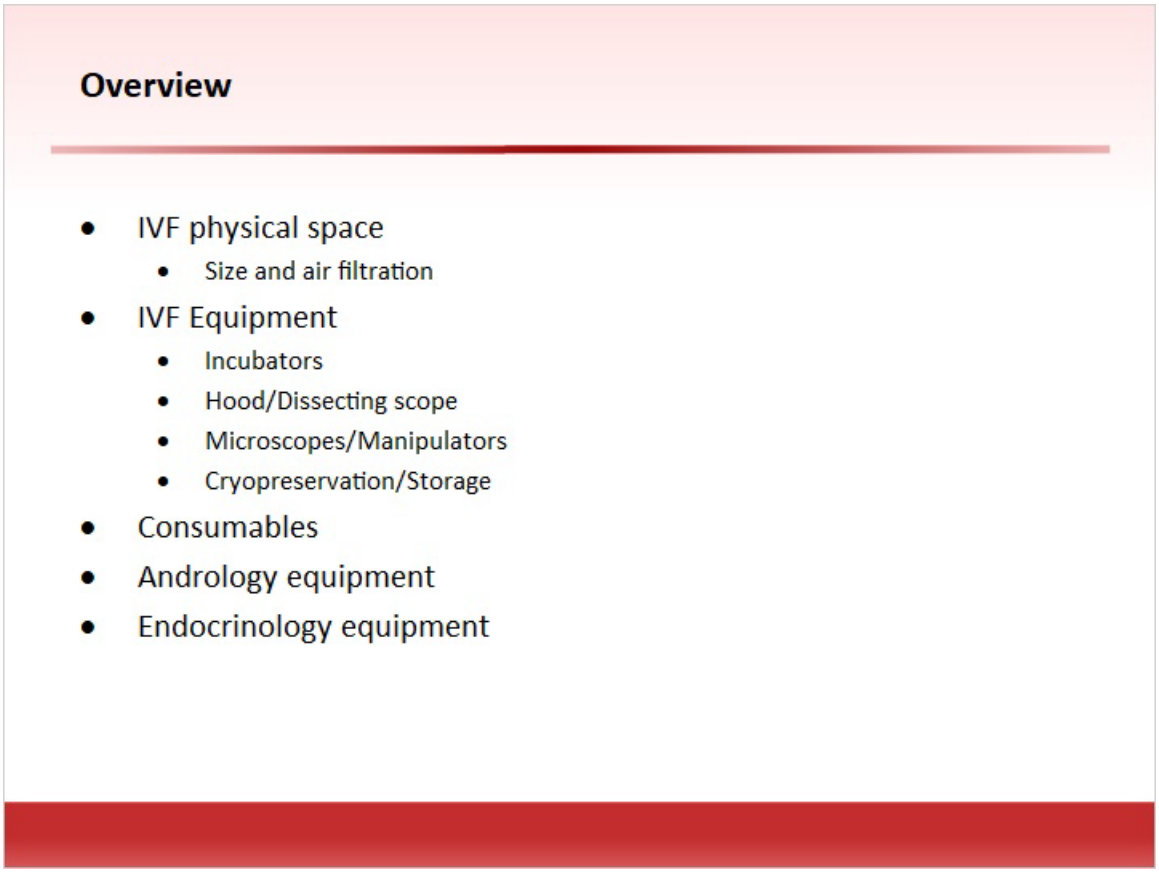
1.2 LEARNING OBJECTIVES



Notes:

At the conclusion of this presentation, participants should be able to: describe the space and equipment needed to set up and run an IVF laboratory including embryology, andrology, and endocrinology labs; identify the types of equipment needed and some sources to begin investigating the purchase of this equipment; summarize the general procedures used in the IVF laboratory, the need for procedure and Quality Control (QC) manuals, and discuss the general QC associated with these procedures.

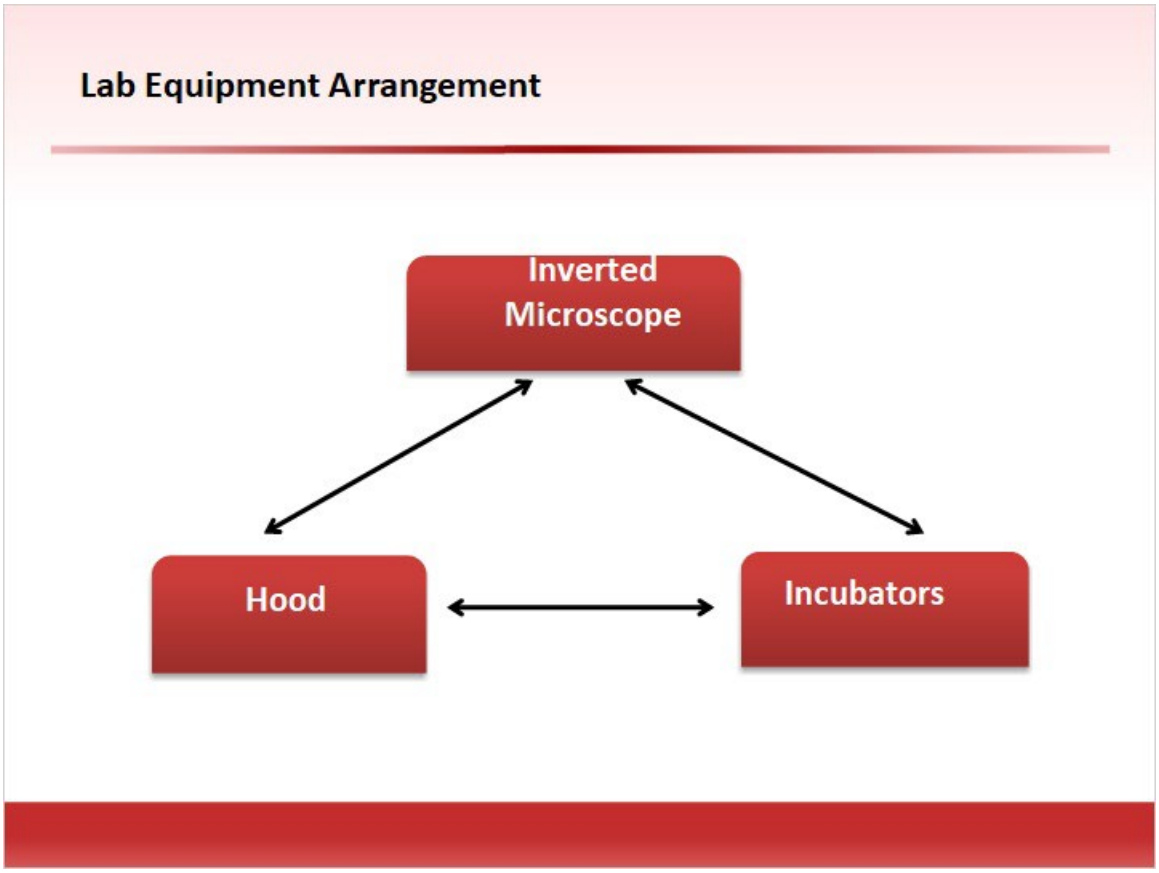
1.3 Overview



Notes:

This module will provide an overview of the space requirements for the IVF laboratory, including recommendations for air handling. It will address the basic equipment required for setting up a laboratory and what QC should be implemented for all equipment, and what to consider when purchasing consumables such as media, culture dishes, and micropipettes used in intracytoplasmic sperm injection (ICSI), assisted hatching, and embryo biopsy. General equipment for the andrology and endocrinology laboratories will also be discussed briefly.

1.4 Lab Equipment Arrangement



Notes:

Planning an IVF laboratory is often constrained by current physical space. Ultimately, however, it pays to consider the workflow for the embryologist when setting up the laboratory. Workflow within the laboratory can be looked at as being similar to the flow within a kitchen, represented by the classical "triangular" concept of design. An embryologist will spend much of the day moving from the hood or portable incubator when finding oocytes, checking embryo development, or doing cryopreservation, to the inverted microscope for pronuclear checks, cell counts, ICSI or biopsy, back to the hood or portable incubator to move oocytes or embryos, and then to the incubator for culture. Keeping all these types of equipment within close proximity reduces steps for the embryologist, lessens the time out of a controlled environment for the cells, and lowers the risk for accidents. These 3 pieces of essential equipment should all be contained within the same room even if room configurations do not allow for immediate proximity.

1.5 Physical Space

Notes:

IVF laboratories around the world have been as small as the size of a converted closet to as large as occupying one or more floors of a facility with multiple laboratory rooms and spaces. Lab size is driven by the projected patient volume and available space within a given facility.

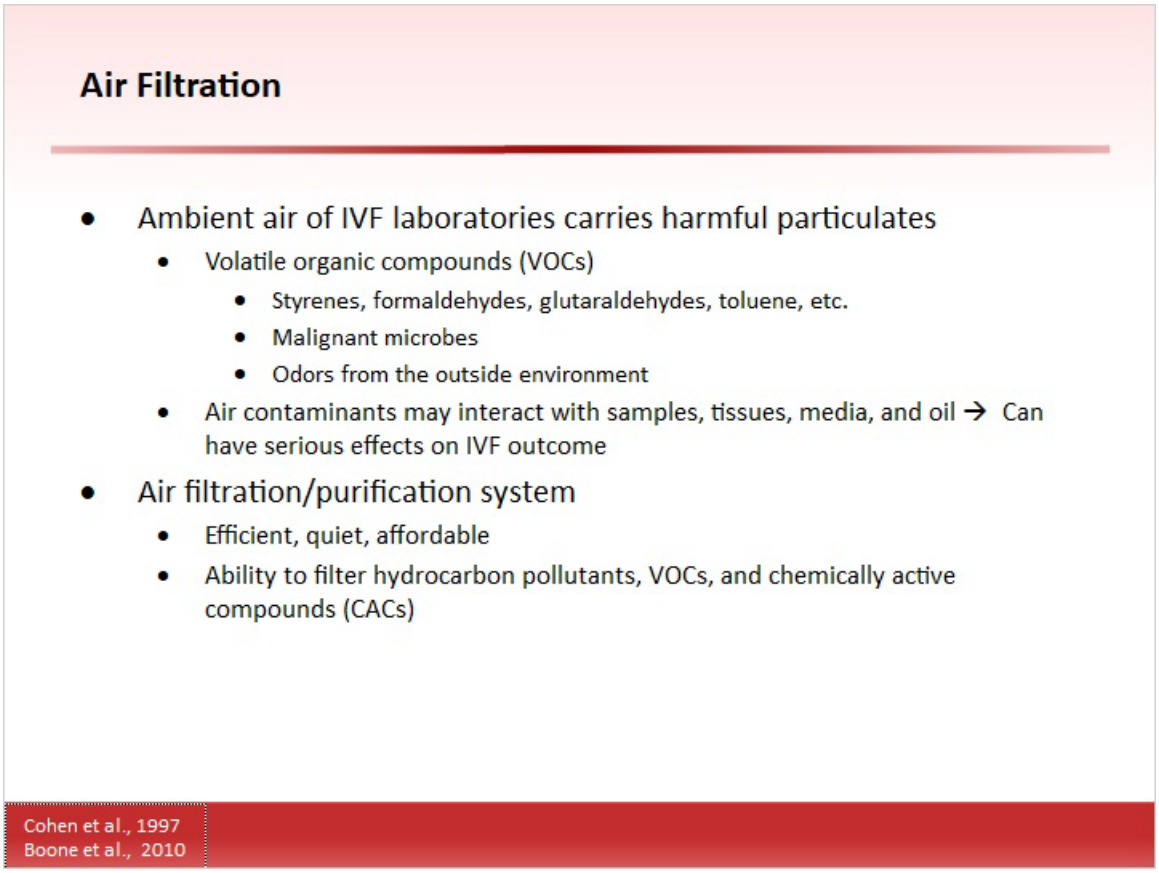
Whatever the size, it is generally recommended that the laboratory be treated as a clean area. It should be located in a secure, low traffic area, and should be isolated from other laboratory activities. In other words, the IVF lab space should not be located in a corner of another laboratory, but rather should have its own contained space. It should be located close to the procedure room to allow short distances of travel when delivering oocytes from the procedure room to the lab or embryos from the lab to the procedure room for transfer. It should also be viewed as a non-public space with access denied to nonessential personnel and patients. This room should have controlled access to only those who need access to the lab.

In an effort to create a cleaner environment, most IVF labs are designed as positive pressure environments such that the air pressure inside the lab is greater than the air

pressure outside. This ensures that when the laboratory door is opened, air moves from inside to outside the lab reducing the risk of inner air contamination. An easy way to determine if the air pressure is higher inside than outside is to place a small piece of paper on the floor near the door. When the door is opened the paper should move out the door rather than being pushed further into the room by the door opening.

Generally, the embryology laboratory should be the center of the filtered air environment. Clean air should then move out from the embryology laboratory through the procedure rooms.

1.6 Air Filtration



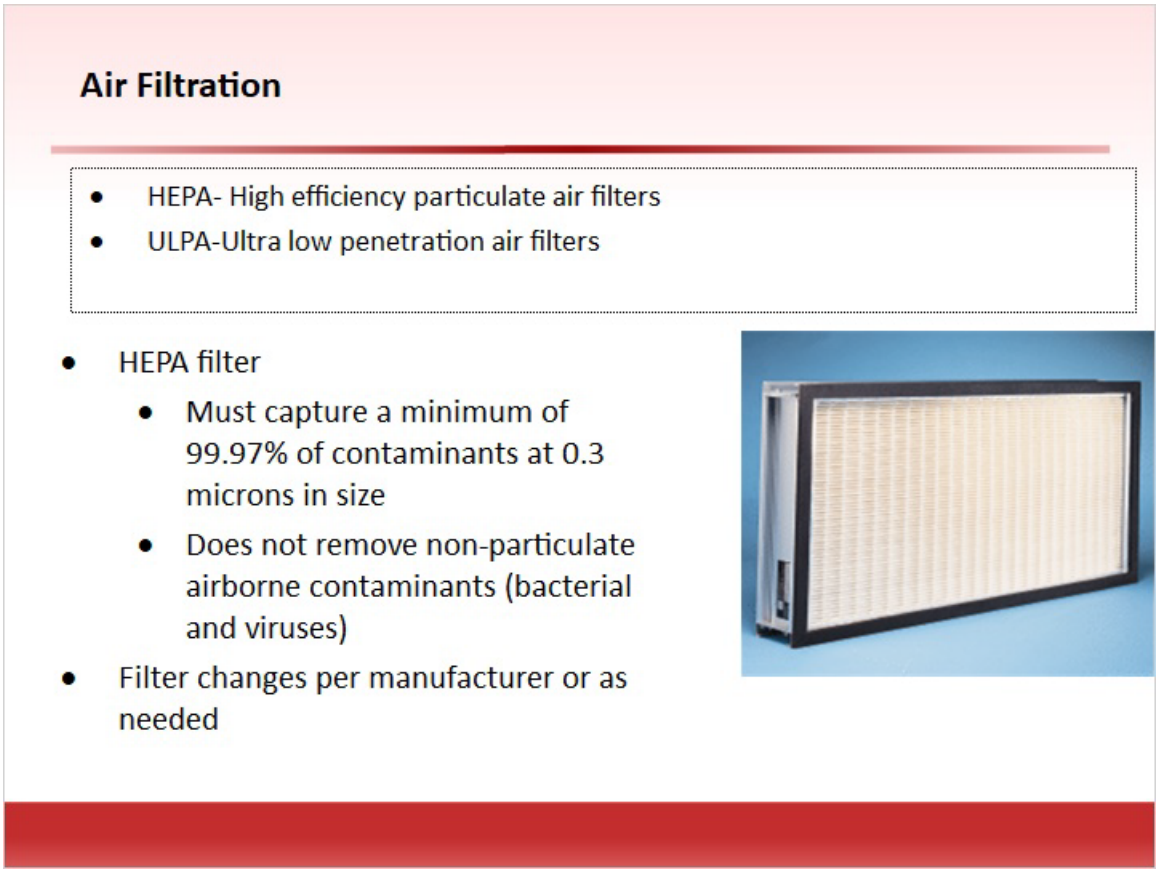
Notes:

It has been well documented that the ambient air of IVF laboratories carries harmful particulates; volatile organic compounds (VOCs) such as styrenes, formaldehydes, glutaraldehydes, toluene, etc.; malignant microbes; and even odors from the outside environment, which can affect embryonic development. Air contaminants, such as chemical air contaminants (CACs) and VOCs, which are introduced from various sources,

may interact with samples, tissues, media, and oil, and consequently can have serious effects on IVF outcome. Thus, it is essential to set-up an air filtration/purification system that is efficient, quiet, affordable, and has the ability to filter hydrocarbon pollutants, VOCs, and chemically active compounds, and thereby eliminate airborne pathogens.

The study by Boone et al. suggests that a significant yet delicate balance may exist between the changing organic chemistry of the laboratory ambient air and the potential effects it may exert on successful embryogenesis and implantation rate. Although particulate levels were negligible and met all specifications as a Class 100 CR, a slight increase in toluene was concomitant with a significant decrease in implantation rate. The subtle increase in toluene did not, however, affect fertilization rates or zygote and embryo morphology. These data suggest that control of VOC levels within the ambient air is critical for successful conception in vitro. This study is the first of several ongoing studies that may serve to broaden our understanding of the critical nature of the laboratory ambient air and the role it plays in pre-implantation toxicology.

1.7 Air Filtration



Notes:

High efficiency particulate air (HEPA), or ultra-low penetration air (ULPA) filters, and activated carbon filters, ultraviolet-C (UVC) irradiation positive pressure, and general sterility precautions can prevent contamination.

As defined by the Institute of Environmental Sciences and Technology, a HEPA filter must capture a minimum of 99.97% of contaminants at 0.3 microns in size. Use of a HEPA filter on the incoming air supply is essential to reduce the number of particulates in the laboratory. However, while this reduces the amount of particulates in the lab, HEPA filtration does not remove airborne contaminants that are not particulates, such as smaller bacteria and viruses. However, HEPA filters do remove particulate matter present in air supplies in many industrial cities. Filters should be changed at least once a year, or as recommended by the manufacturer. Filter changes will depend on air quality entering the system.

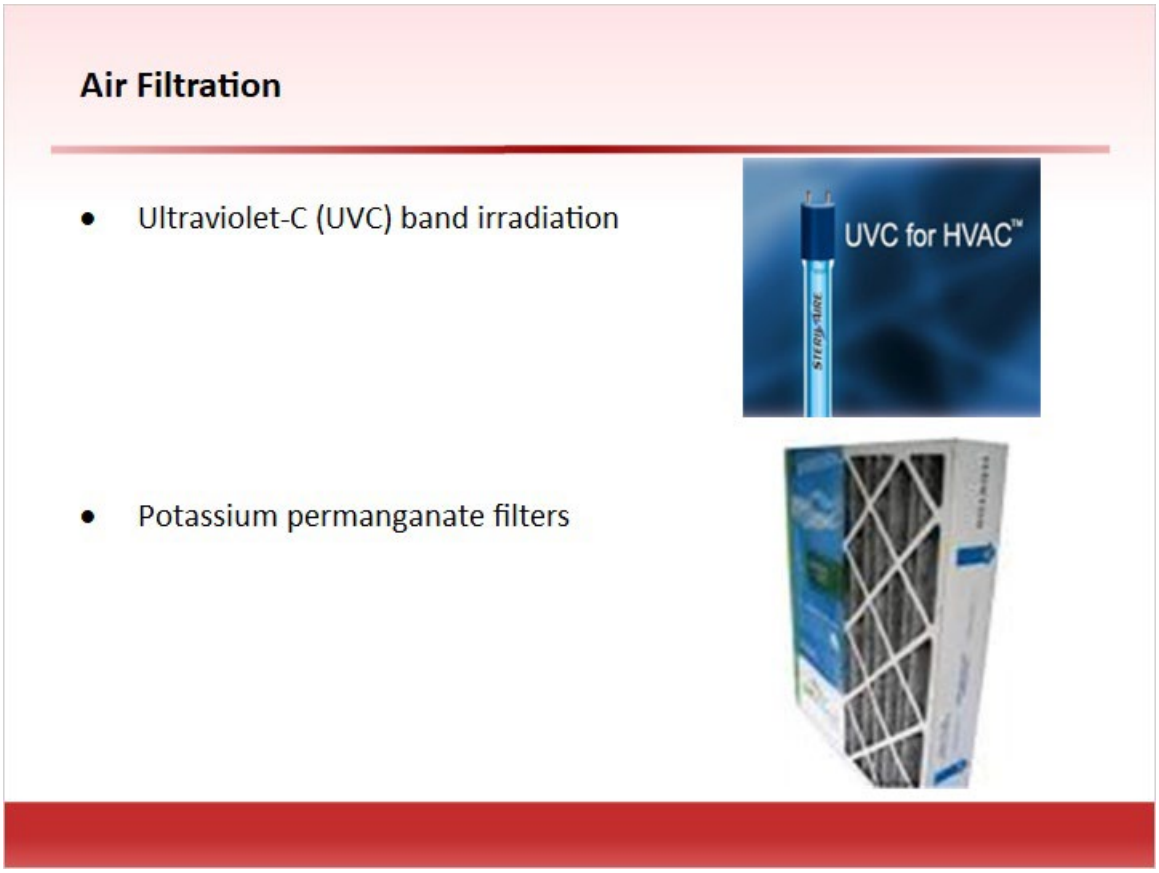
1.8 Air Filtration

Notes:

As an alternative, carbon-activated air filtration systems or comparable devices can be used within the laboratory if retrofitting the incoming air is untenable. Activated carbon filters contain charcoal that has been treated with oxygen to open up millions of tiny pores between the carbon atoms. The use of special manufacturing techniques results in highly porous charcoals that have surface areas of 300-2,000 square meters per gram. These so-called active, or activated, charcoals are widely used to adsorb odors.
When certain chemicals pass next to the carbon surface, they attach to the surface and are trapped.
While activated charcoal is good at trapping carbon-based impurities, many other chemicals such as sodium, nitrates, etc. are not attracted to carbon at all and thus pass right through. This means that an activated charcoal filter will remove certain impurities while ignoring others. It also means that, once all of the bonding sites are filled, an activated charcoal filter stops working. At that point the filter must be replaced. Activated carbon filters should be changed annually if in the ceiling or as recommended by the manufacturer if using free-standing lab filters.

A carbon-activated (charcoal) filtration system consists of an incubator filtration unit, which is used within the incubator, a CO_2 and tri-gas inline filter, which is used in the incoming gas lines, and the tower, which filters the air in the laboratory, procedure rooms, and working environment. Numerous studies have demonstrated improvements in pregnancy and implantation rates after using such a system or other similar filtration setups that can reduce VOCs and other toxicants in the laboratory. Activated carbon filters can be placed inline with HEPA filters to further filter incoming laboratory air.

1.9 Air Filtration



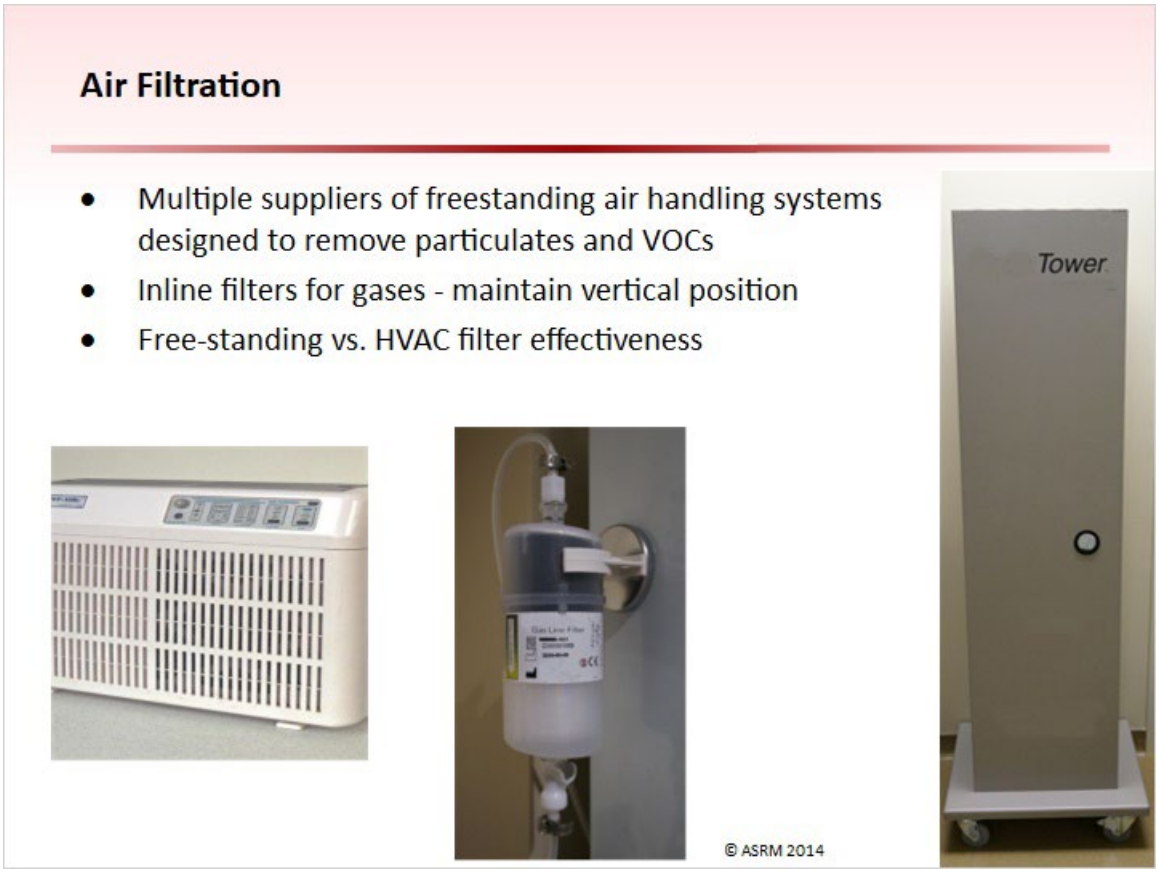
Notes:

Ultraviolet irradiation can also be added to the HVAC system as a means of reducing airborne microbial contamination.

Potassium permanganate filters oxidize and thus detoxify some compounds such as alcohols and ketones not easily trapped by carbon.

All of these filters may be added to the air handling system to filter and treat air as it enters the IVF lab. Numerous companies exist to provide assistance and recommendations for setting up this type of air handling system.

1.10 Air Filtration



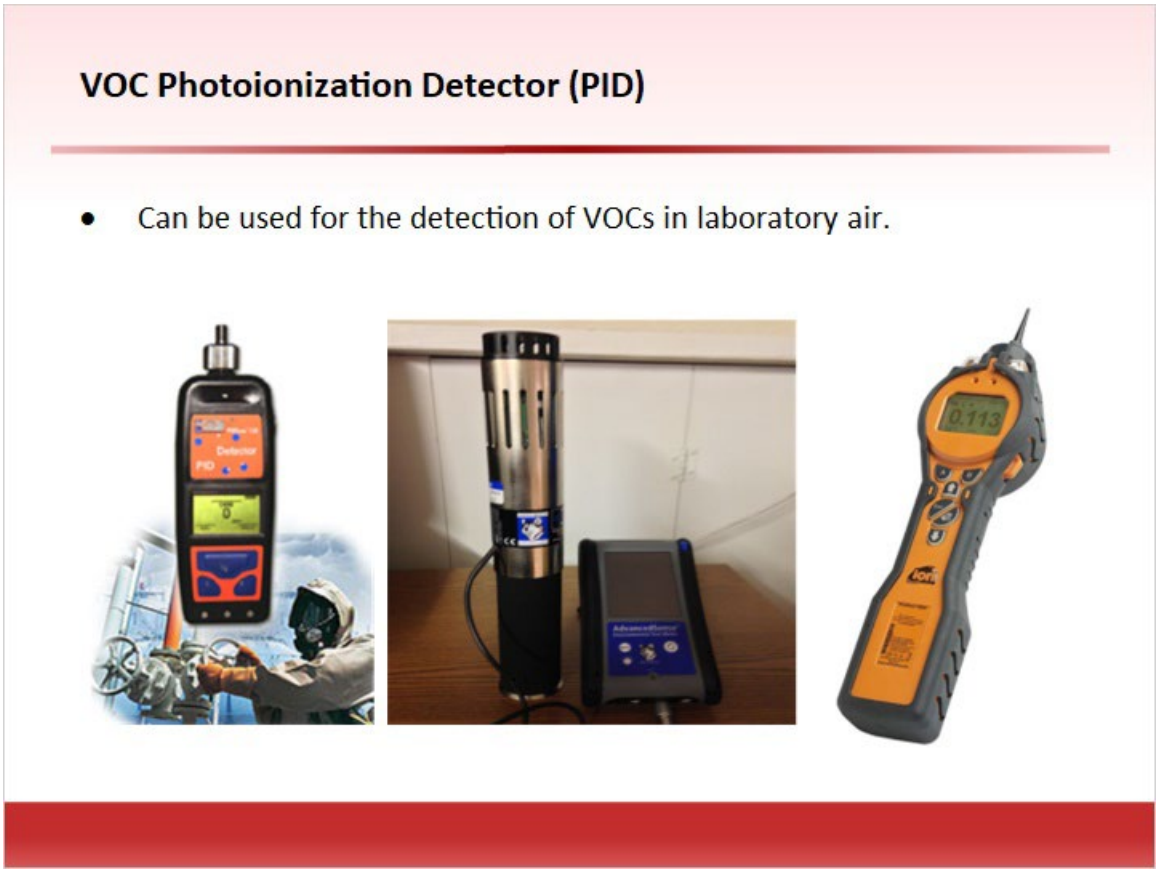
Notes:

In addition to installed air pressure systems, many companies provide freestanding air purification devices that can be used to reduce particulate counts and remove VOCs.

Devices can also be attached to air lines to treat gases in line with incubators. Activated carbon filters are commonly used for inline filtration and will remove a high percentage of the VOC contaminants in CO_2. Note that inline filters attached to gas lines should be kept vertical, not laid horizontally on the benchtop, in order to maximize filtering capabilities.

Not all laboratories are able to adapt their air supply to accommodate multiple inline filters; therefore in-lab filtration units are available. However, there seems to be some argument regarding the effectiveness of in-room air handling as compared with systems installed in the HVAC system.

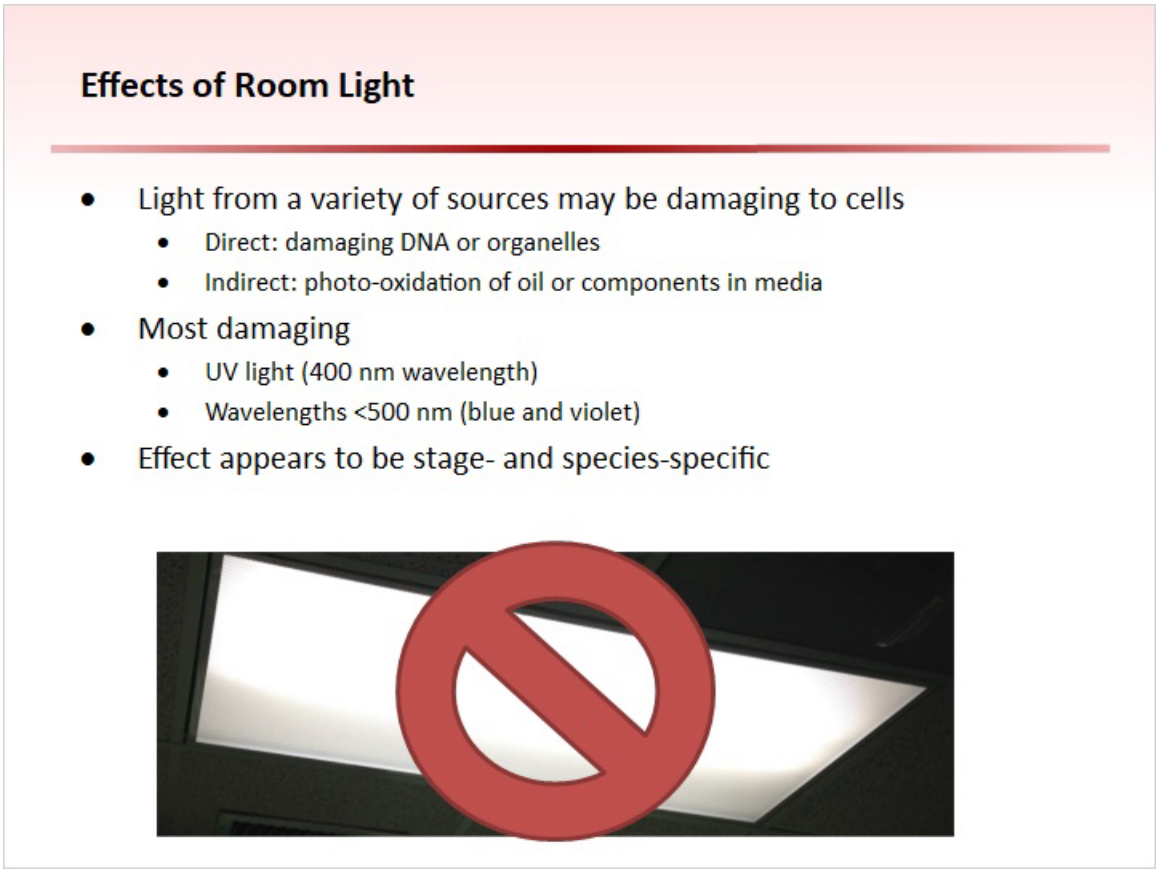
1.11 VOC Photoionization Detector (PID)



Notes:

Hand-held photoionization detectors can be used in the laboratory to detect the presence or absence of VOCs in the laboratory. While most will not identify the exact nature of the VOC, they can identify trends in air quality, are rapid and repeatable, and can be used to identify sources of VOCs in laboratory air. Some are capable of interfacing with a computer and can be used as a routine QC tool for monitoring specific sources that contribute to laboratory VOC concentration such as incubators, gases, or incoming air. Filters should be changed when VOCs spike.

1.12 Effects of Room Light



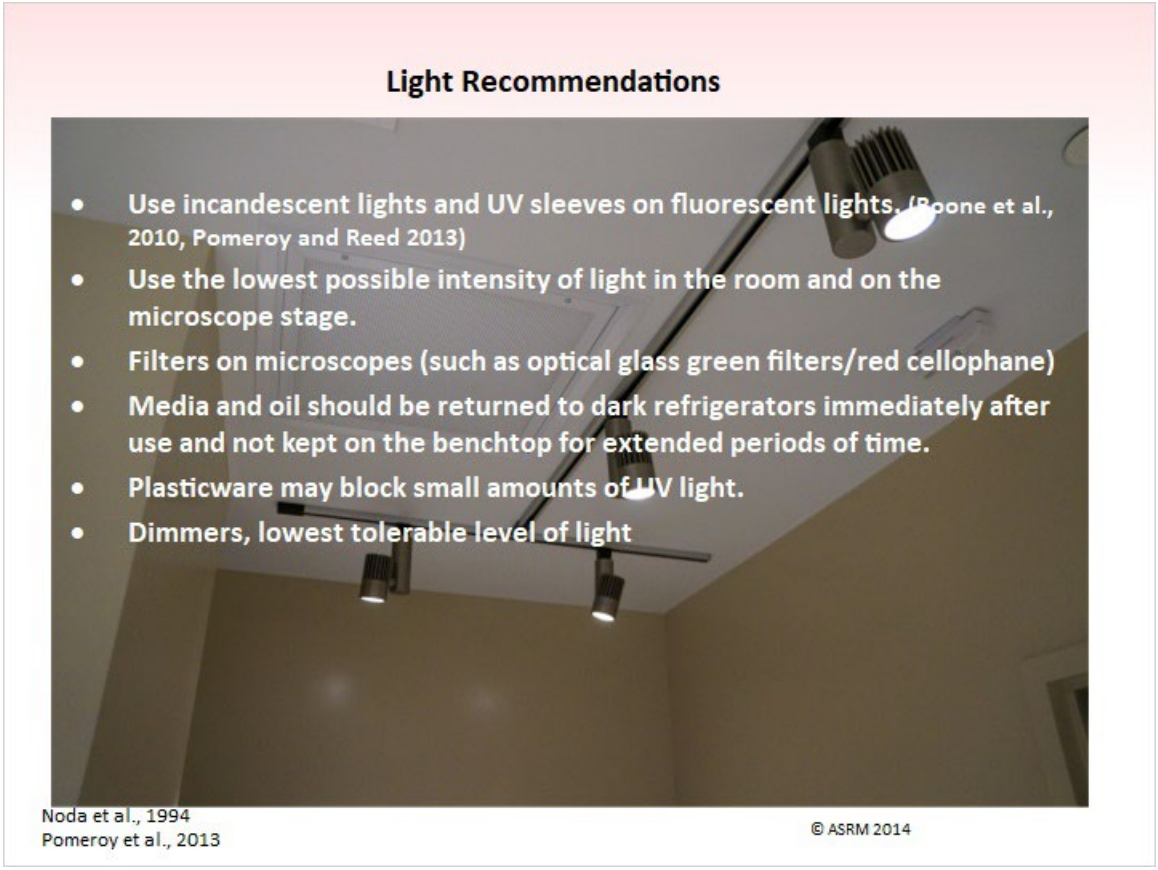
Notes:

The literature on the effects of light on human embryos is somewhat ambiguous; however there is sufficient literature on the effects of light on other species to suggest that there may be an impact on embryo development. Note that there is no standard or consensus as to an "ideal" laboratory environment for human embryos.

Light may directly impact oocytes and embryos by damage to DNA or organelles, or the effect may be indirect as light may initiate changes in the culture media or oil used for embryo culture. Light, in particular, appears to have an effect on oil by initiating peroxidation. General recommendations by at least one oil manufacturer include refrigerating IVF oil; keeping the oil cold and out of the light may reduce the peroxidation rate.

UV light at wavelengths around 400 nm and light in the blue and violet range of visible white light appear to be the most damaging of the visible wavelengths. Studies on the effects of light are controversial and have focused only on light from the visible spectrum. Little data are available on light from the non-visible spectrum.

1.13 Light Recommendations



Notes:

One of the easiest ways to reduce the introduction of low wavelength light is to avoid the use of fluorescent bulbs in the lab. Fluorescent bulbs emit wavelength spectra across 540-590 nm and 620-640 nm. On the other hand, incandescent bulbs emit wavelengths across 620-640 nm. Use of incandescent bulbs avoids the potential danger zone of 500 and below. If lights cannot be replaced, then an alternative is to use UV sleeves to cover the fluorescent lights.

One study by Noda in 1994 suggested that the use of low levels of light both at the microscope and in the room (100 and 20 lux respectively) improved embryo development. Reducing the amount of ambient light in the room to the lowest level workable by the embryologists and reducing the amount of light used at the microscope may improve embryo quality.

Some laboratories choose to add filters to their microscopes that will reduce the wavelengths that are transmitted. Optical glass green filters are an example of the type

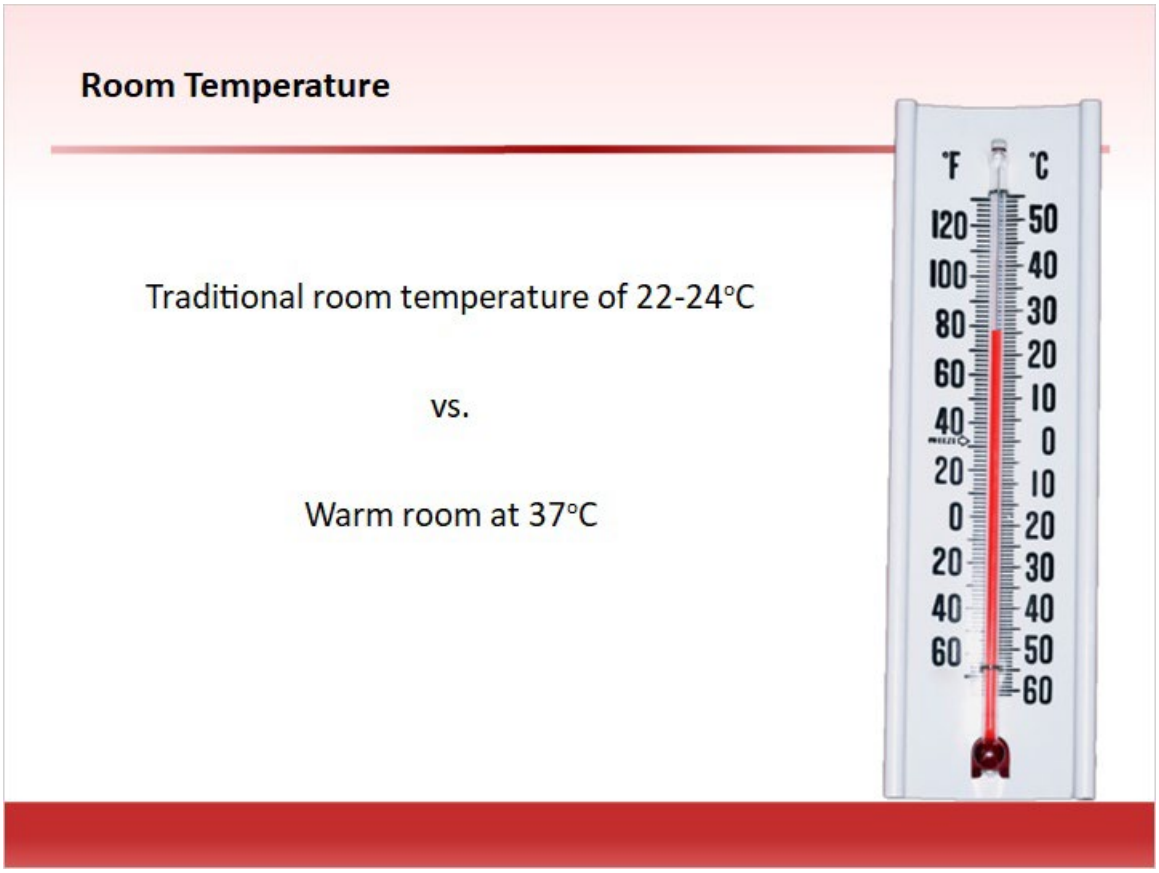
of filter that may be added to a microscope. A simple and efficient way to filter light is to place a piece of cellophane over the light source of the microscope. Red cellophane is often used..

Since light may also induce changes in culture media or oil an easy way to reduce the impact of light on these products is to limit the exposure of culture media or oil to ambient light. Media and oil should be kept in the refrigerator and removed only when in use and then returned immediately to the refrigerator.

Plastic-ware, rather than glass may have a protective function by filtering small amounts of UV wavelength light.

A common-sense approach is to put all overhead lights on dimmers and maintain the lowest tolerable level of light in the embryology lab.

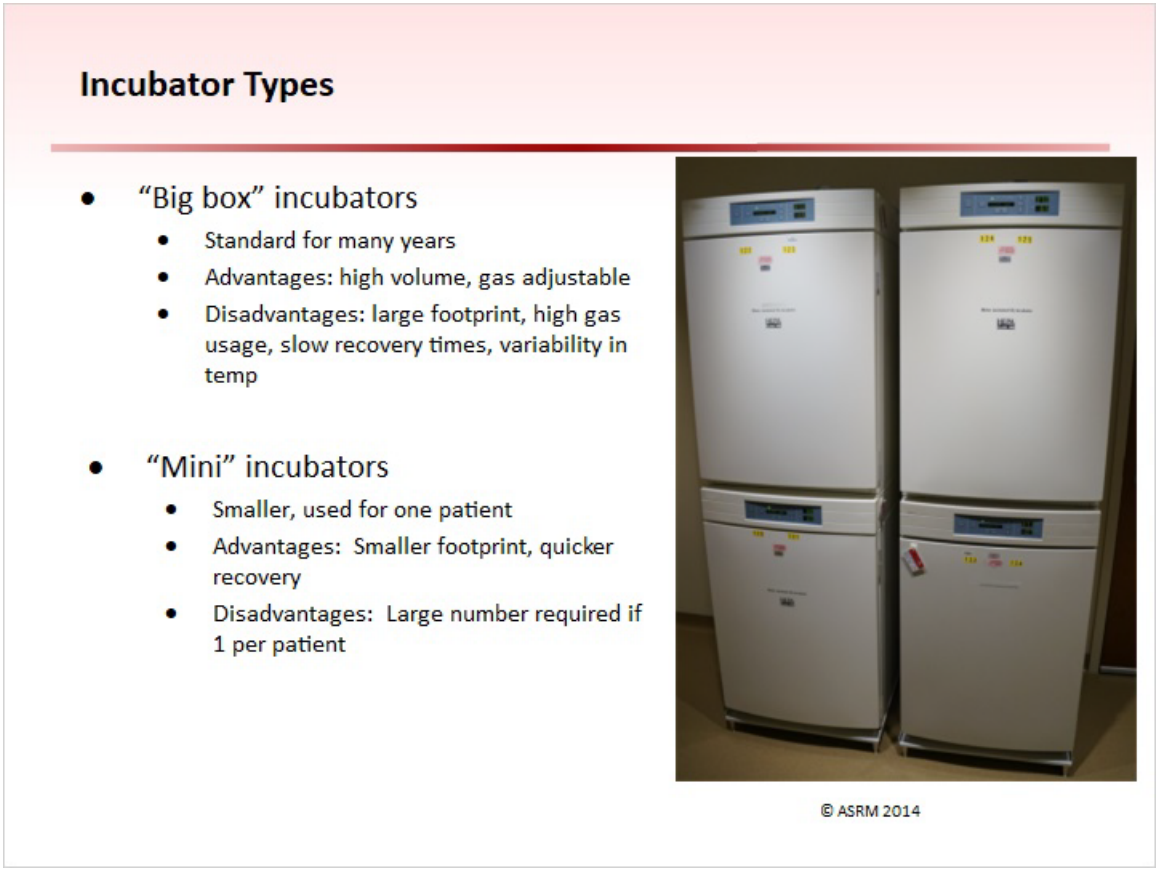
1.14 Room Temperature



Notes:

No good data exist documenting whether room temperature has a beneficial or adverse effect on IVF outcome. Although potentially uncomfortable for the embryology staff, many laboratories choose to have their labs at body temperature as an adjuvant to reducing embryo stress through cooling when moving embryos in and out of incubators and on and off heated surfaces. Certainly there are tolerance limits for equipment and many devices are designed to be used at traditional room temperatures. Incubators, for example, have systems in place to increase their temperatures, but have nothing that is designed to actively cool, except for passively turning off the heating elements when the designated temperature is met. Thus, equipment continually used in hotter environments may have a shorter life span then equipment used at the temperature for which it was created and tested.
However, any discussion of this nature is speculative.

1.15 Incubator Types



Notes:

All IVF laboratories culture oocytes and embryos for an extended time, ranging from day 2 to as long as day 7 in some instances. This culture is done in some kind of incubator. A

variety of incubators are now available and all have advantages and disadvantages. Generally, incubators fall into one of four categories.

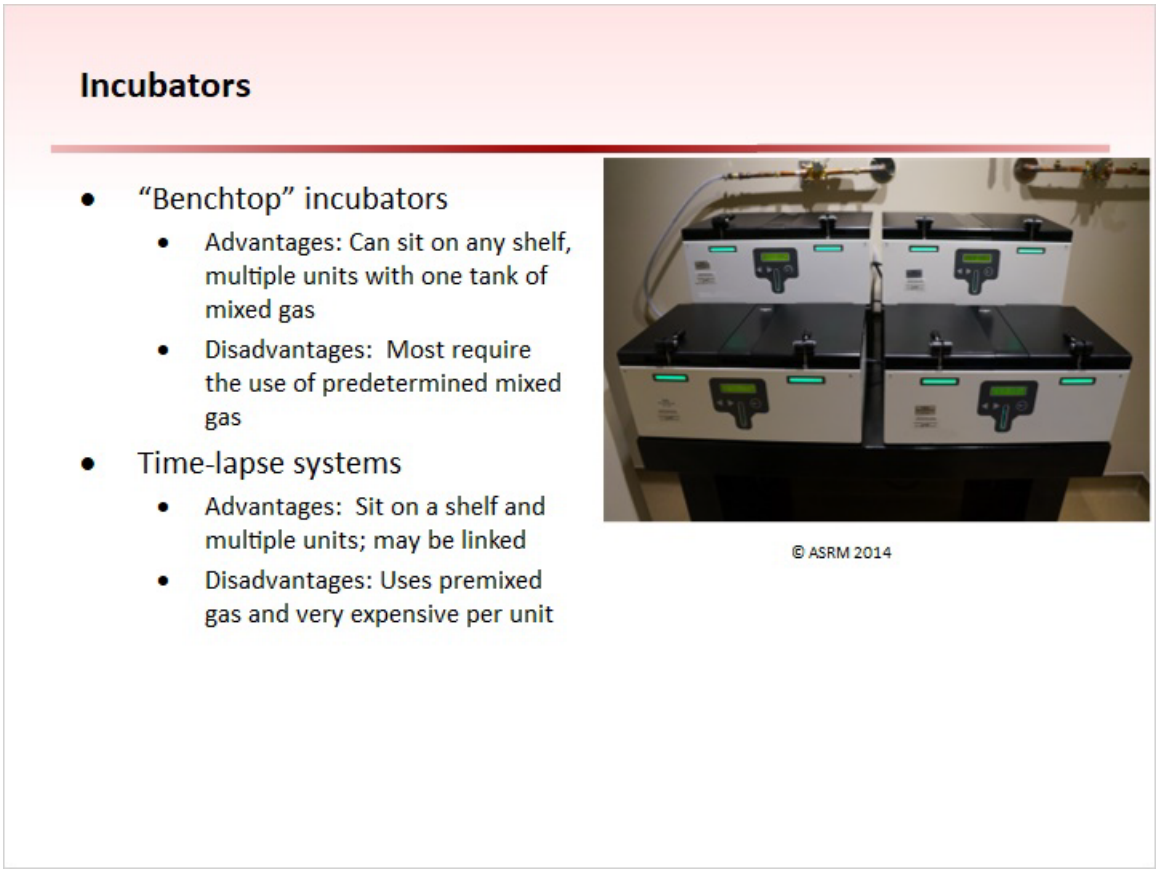
So-called “big box” incubators were the standard for years in IVF laboratories throughout the world. Their use followed directly from the experience of many IVF laboratory scientists who came to the IVF world in the early years from the cattle or cell culture world.
The major advantages to these types of incubators have been their large holding capacity, their flexibility in adjusting the gas concentrations, and their availability. Unfortunately, these incubators also pose a number of problems for the IVF lab including relatively large gas usage due to the large size of the incubator and the detrimental effect of door openings on gas exchange, leading to slow recovery times of temperature and CO_2 concentrations after opening, as well as highly variable temperatures from shelf to shelf, or even across one shelf.

These limitations drove the development of smaller versions of traditional incubators, generally referred to as mini incubators. Most programs using these types of incubators place only one, or at the most two, patients per incubator. While this necessitates a considerable number of incubators for a high volume program, because of the small footprint, multiple incubators can be stacked and placed within the space previously occupied by the big box style incubators. Many manufacturers who produce big box incubators now produce smaller versions too.

One of the overall advantages to either type of these incubators is that they provide the ability to adjust CO_2 and/or oxygen mixtures (although usually adjustment of oxygen is done by piping in nitrogen that displaces the oxygen, thus lowering the amount of oxygen delivered to the incubator). This allows the laboratory a certain amount of flexibility in adjusting the CO_2 or pH of different media from different manufacturers, or when changing the pH for different stages of embryo culture as is the practice for some laboratories.

The inherent disadvantage to this system, especially with the bigger versions of the incubators, is that large amounts of nitrogen are used in the course of maintaining the incubators in the face of multiple door openings. This can be compensated for by using large tanks of liquid nitrogen where the nitrogen vapor is captured and controlled by a manifold system. However, this also requires a large room for storage and management of gases if large numbers of incubators are in use.

1.16 Incubators



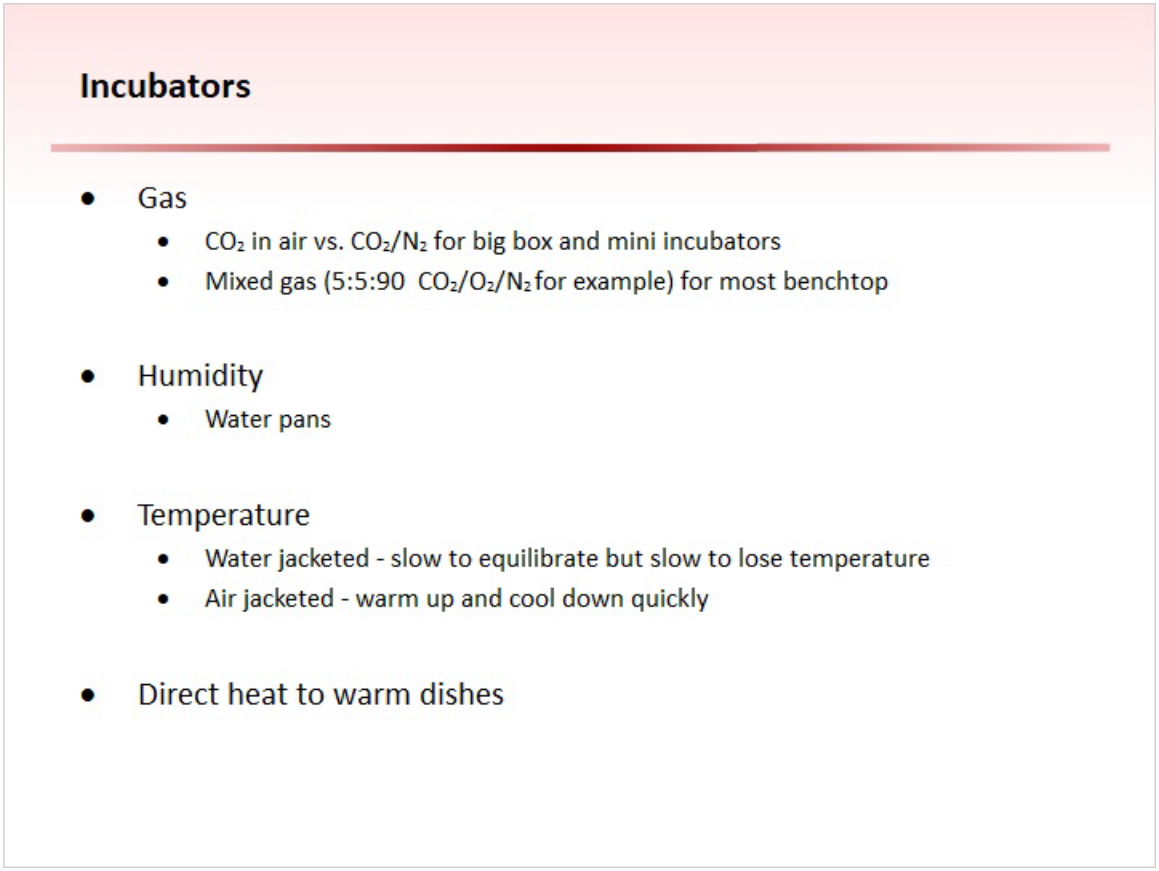
Notes:

Another newer form of incubator is the benchtop style of incubators. Multiple incubators can be placed on a counter and linked together using one tank of mixed gas to feed several incubators. The very small footprint allows many benchtop incubators to be used in a relatively small space.

One of the advantages to the big box or mini types of incubators is that they provide the ability to adjust CO2 and/or oxygen mixtures. Benchtop incubators require the use of premixed triple gas as most of the current versions do not have gas mixing capabilities- although newer versions appear to be adding this capability. However, until this is standard, considerable forethought must go into establishing the gas mixtures that are used in an effort to drive the appropriate pH of whatever culture media is chosen for use. This is a problem that requires some time and consideration, but is not an overwhelming obstacle to the use of benchtop incubators. However, once the system is calibrated and controlled for use with a particular medium, users should be careful when changing vendors or types of media as different vendors use different formulations of bicarbonate. Therefore, new media placed into use without testing pH may not provide an ideal environment for embryo culture.

Another type of benchtop incubator is a system that allows for constant time-lapse videography of embryos in culture. These time-lapse systems fall into two categories, either systems that can be placed in big box incubators, or self-contained systems that sit on the benchtop. They function much like traditional benchtop incubators in that the region that holds the embryo culture dish has a mixed gas delivery system, and is temperature controlled. These systems offer most of the advantages of other benchtop types of incubators, as well as the disadvantage of using premixed gas. The other major disadvantage of these benchtop time-lapse systems is the cost. These are often more than $100,000 per unit. Therefore they provide a very expensive, but apparently very effective incubation system even if the time-lapse function is not considered.

1.17 Incubators



Notes:

Most incubator manufacturers produce incubators that can mix CO_2 with room air at a designated concentration of CO_2, or incubators that can mix CO_2 and nitrogen, often captured from large liquid nitrogen tanks or a nitrogen generator, to create an internal

environment acceptable for embryo culture. The purpose of pumping nitrogen into the incubator from an external source is to displace the oxygen within the incubator, resulting in an environment that has a lower oxygen concentration than the laboratory environment.

Humidity control within the incubator is essential in incubators with thermoconductivity detection of CO_2. It has also been used to prevent dehydration of media during culture and subsequent rises in osmolarity of the culture media. Humidity is usually generated passively by placing water pans in the bottom of the incubator. However these water pans can create a source of contamination within the incubator and humidity may not be essential if all dishes are used with a sufficient oil overlay.

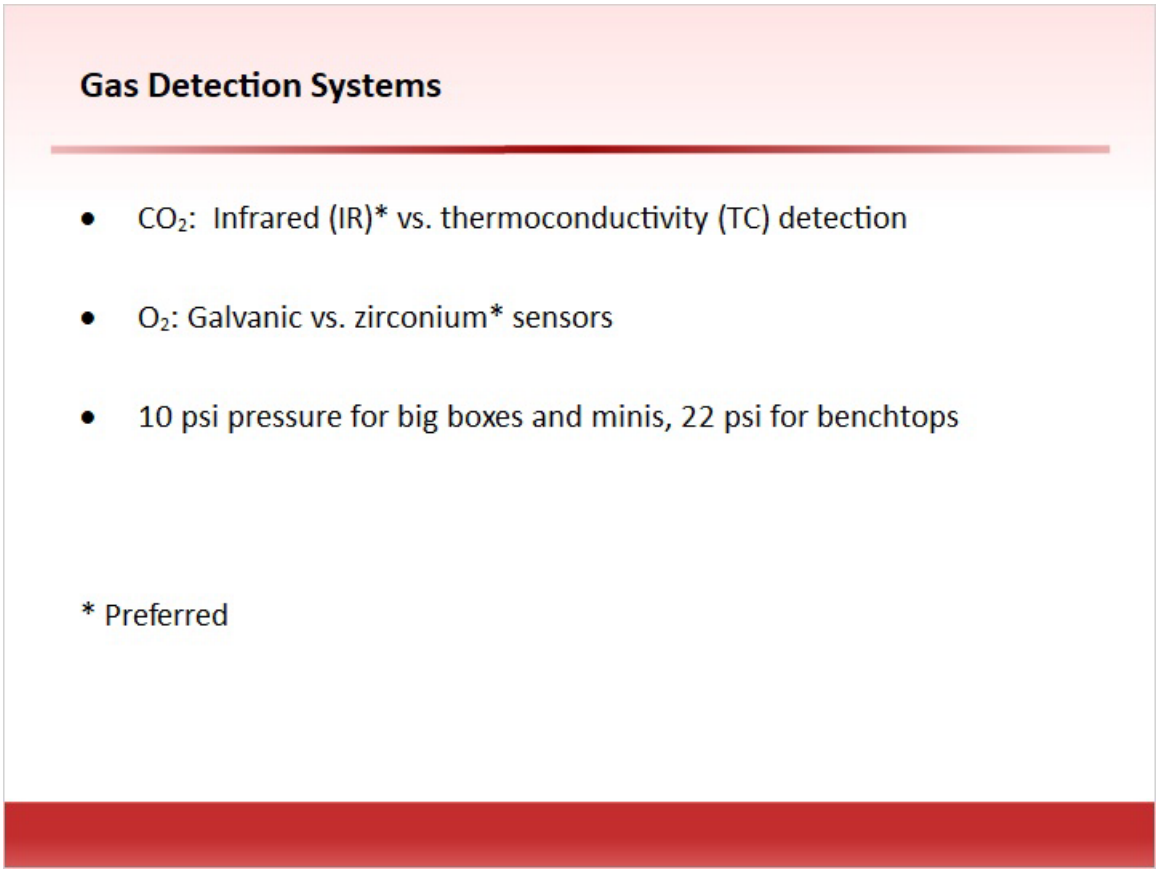
Traditionally incubators were set up based on cell culture methods with the CO_2 percentage set to 5%. At this setting, the pH of a 25 mM bicarbonate solution should be approximately 7.4. However, as will be discussed, many variables have an impact on pH including elevation, humidity, and media composition. Many labs choose to lower the pH of their culture systems and thus increase the CO_2 concentration during culture.

An alternative to using gas that is mixed with laboratory air within the incubator is to use gas that has been pre-mixed in tanks prior to delivery to the laboratory. Use of pre-mixed gases may be impractical with large incubators because of the large volume of gas used and the cost, but more practical for using in small benchtop incubators. Most, but not all, benchtop incubators require the use of pre-mixed gas because the incubator is not equipped with mixing capability.

Maintaining the temperature of samples inside the incubator can be achieved one of three ways. The inside may be heated by heating the outer jacket of the incubator. Outer jackets may be filled with water or filled only with air. Each has its advantages and disadvantages. Water-jacketed incubators are slow to come to equilibration, but also slow to lose their temperature in the event of power failure. They are heavy and consume more power and there is concern that the water reservoirs may provide an active source of contamination. Air-jacketed incubators warm up but also cool down, much more quickly. They can accommodate heat-sterilization decontamination procedures which may help with contamination concerns.

The final method used in many benchtop incubators is the application of direct heat to culture surfaces and lids that warm dishes placed inside by being in contact with the top and bottom of the dishes.

1.18 Gas Detection Systems



Notes:

Two types of gas sensors are used to detect CO_2 concentration within incubators. Thermoconductivity sensors function by measuring resistance differences between two thermistors-one enclosed and one exposed to the environment within the chamber. The presence of CO_2 in the chamber changes the resistance between the two thermistors. Resistance is affected by humidity as well as temperature.

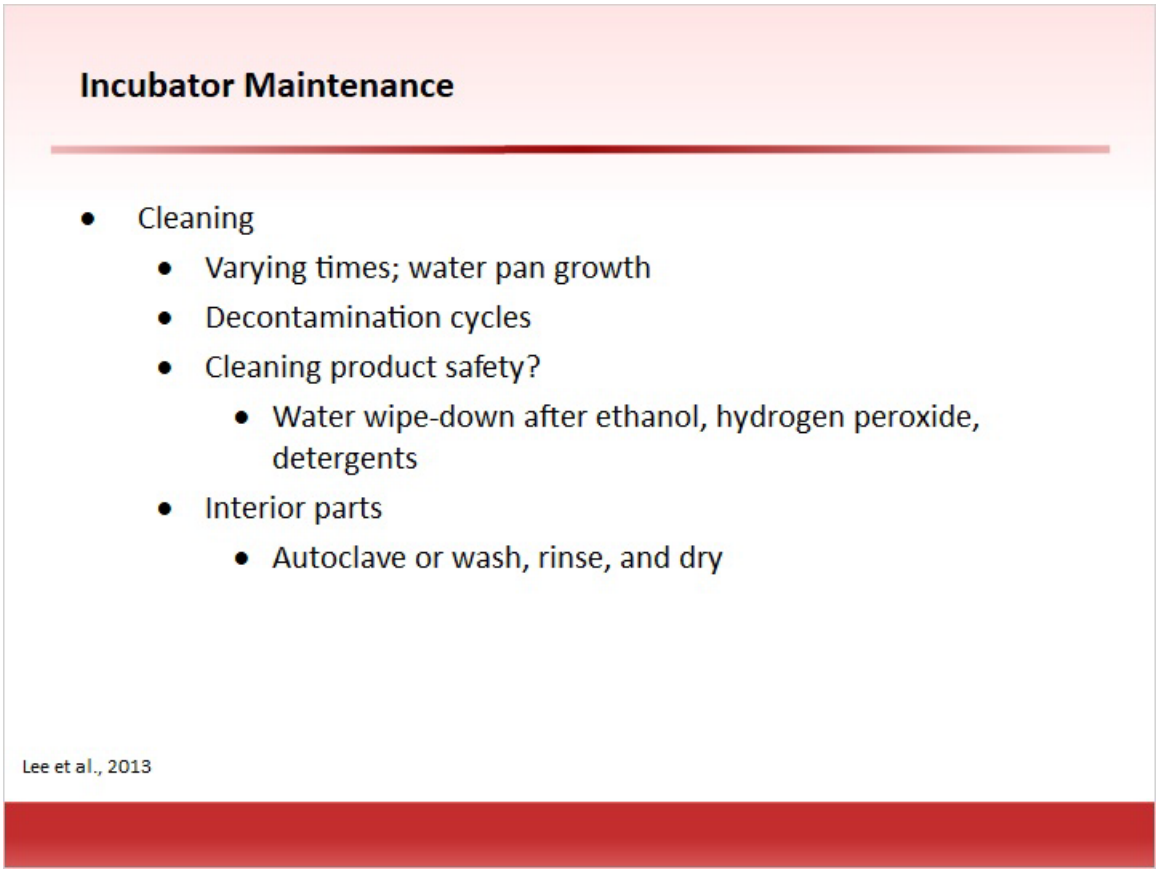
Infrared sensors are temperature and humidity independent. Instead they use a light and optics to determine IR absorbance that is CO_2-dependent. Therefore, gas control in incubators equipped with infrared detection tends to be more tightly controlled and less responsive to changes in temperature and relative humidity.

O_2 detection can also be done two ways. A galvanic sensor is a diffusion-limited metal/air battery. Oxygen diffuses through 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 O_2 consumption, is generated when the cathode-anode circuit is complete. A zirconium sensor is an impervious tube with a zirconia element with a closed end that 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 pressure of the O_2 in the sample and the O_2 in a reference gas.

Galvanic sensors tend to yield slower response times compared with zirconium sensors and require changing more frequently.

1.19 Incubator Maintenance



Notes:

Incubators must be cleaned at varying times. The timing between cleanings can be determined by the usage of the incubator and set by lab policy as there are no hard and fast rules regarding the maximum or minimum time between cleanings. One of the most obvious indicators that an incubator needs to be cleaned is the presence of growth in the water pan.

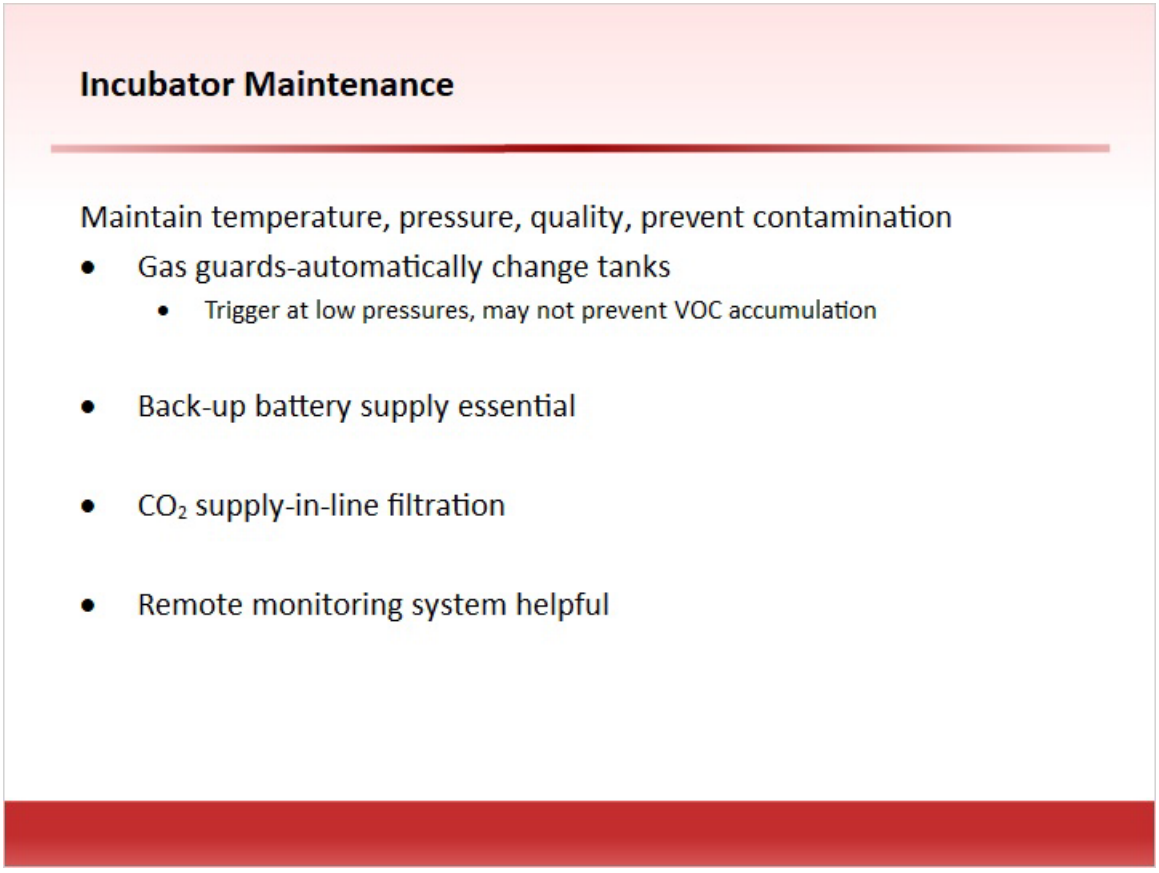
Many incubators manufactured in recent years incorporate a decontamination cycle

into their programs for air-jacketed incubators. A decontamination cycle usually entails running the temperature of the incubator up until it is hot enough to kill most bacteria and fungi. For example, one system's cycle runs for 25 hours and includes heating and maintenance of elevated temperatures up to 90 degrees Celsius for several hours.

The use of cleaning products such as ethanol and hydrogen peroxide has also been suggested; however there are reports that these products are embryotoxic and should be used with care. Incubators may be washed down with these products but all surfaces should be fully wiped down with water after the use of alcohols or hydrogen peroxide. The same is true concerning the use of detergents.

Most of the interior parts of an incubator can be sterilized by autoclaving, or they can simply be removed, washed, rinsed well, dried and returned to the incubator.

1.20 Incubator Maintenance



Notes:

One potential problem with any incubator is losing power and subsequently

temperature and CO_2 during a time when the embryologists are not in the laboratory. Some incubators have built-in gas guards that can be used to monitor CO_2 and nitrogen pressure in the incubator and automatically trigger the transfer from one gas-source tank to another. Unfortunately, these usually trigger at very low pressures (approximately 15 psi), which may be too late to prevent VOC accumulation in the air entering the incubator.

All incubators should be wired to battery or generator backup, to maintain temperature during the course of an electrical failure within the lab.

In addition, while most IVF laboratories are equipped with air filtration capabilities, the CO_2 generated from liquid CO_2 is only as good as the purity of the liquid CO_2 in the tank. If CO_2 contaminated with VOCs enters the incubator from the tank supply, no amount of filtration of the surrounding room air will ensure a VOC-free environment in the incubator. Addition of air filters on the gas lines will limit contamination from piped in gases if there is VOC contamination of gas lines, or in the tanks. However, proactively addressing this issue can reduce a significant production of VOCs generated in CO_2 tanks.

1.21 When to Change CO2 Tanks

When to Change CO_2 Tanks

- When pressure falls below 830 psi
- When weight of tank falls below about 15 pounds
- When VOC levels begin to rise

http://commons.wikimedia.org/wiki/File:Gas_regulator.jpg Public domain image

Notes:

A tank of CO_2 (but not pre-mixed gas) contains 50 pounds of liquid CO_2 in equilibrium with gas CO_2 (if you are using a tank that is approximately 5 feet tall or 200 cubic feet). As the gas is piped off the tank the level of the liquid CO_2 decreases. Eventually no liquid CO_2 remains within the tank. Any VOC contamination (and there has been shown to be some) that might be in the CO_2 will eventually vaporize and be delivered along with the CO_2. The vapor pressure of CO_2 is 838 psi. A gas pressure of 838 or above indicates that there is still liquid in the tank maintaining equilibrium between CO_2 in the liquid phase and in the gas phase. When the pressure in the tank falls below this level you know that no liquid nitrogen remains in the tank.

Most embryologists will have seen that gas pressure within CO_2 tanks will remain above this level for the majority of the use of the tank. Once the pressure starts to fall it will drop quickly. A safe policy to prevent potential increases in VOCs in the gas phase and to prevent the loss of gas overnight or during a time when an embryologist may not be in the office is to change the tanks before reaching the bottom of the liquid phase.

An alternative to watching the change in gas pressure in the tank is to monitor the

weight of the tank. A 200 cubic foot cylinder will hold 50 pounds of CO_2 and weigh around 150 pounds, 50 pounds of which are liquid CO_2 upon delivery. If the tank is weighed when it first arrives, it should be changed before it has lost 50 pounds.

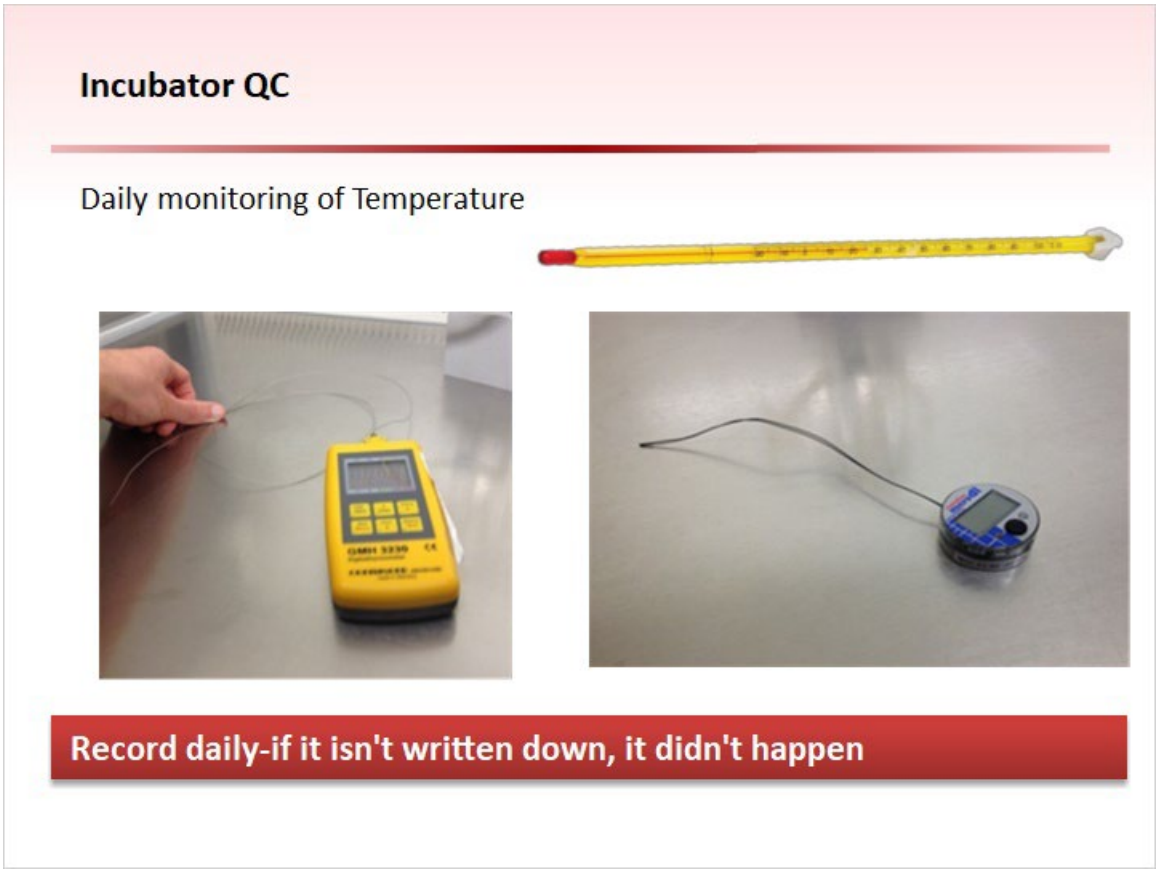
Each CO_2 tank has a tare (empty) weight stamped on it. When the gas company fills the tank it will add 50 pounds of liquid CO_2 to the cylinder and then stop. However, different sizes of cylinders will hold different volumes of liquid CO_2. The air-gas company a laboratory uses should be able to tell the embryologist exactly how many pounds of liquid CO_2 it is adding to the cylinders that the lab is receiving based on the size of the cylinder.

An alternative method would also be to use an inline VOC detector. VOC concentrations in the line can be monitored and as soon as the levels begin to rise the tanks could be changed.

Any method will work, as long as it allows the embryologist to anticipate the gas source running dry.

None of these methods will work for pre-mixed gases as the tanks are not filled based on weight, and the weight of the gas will be minimal compared to the weight of the cylinder.

1.22 Incubator QC



Notes:

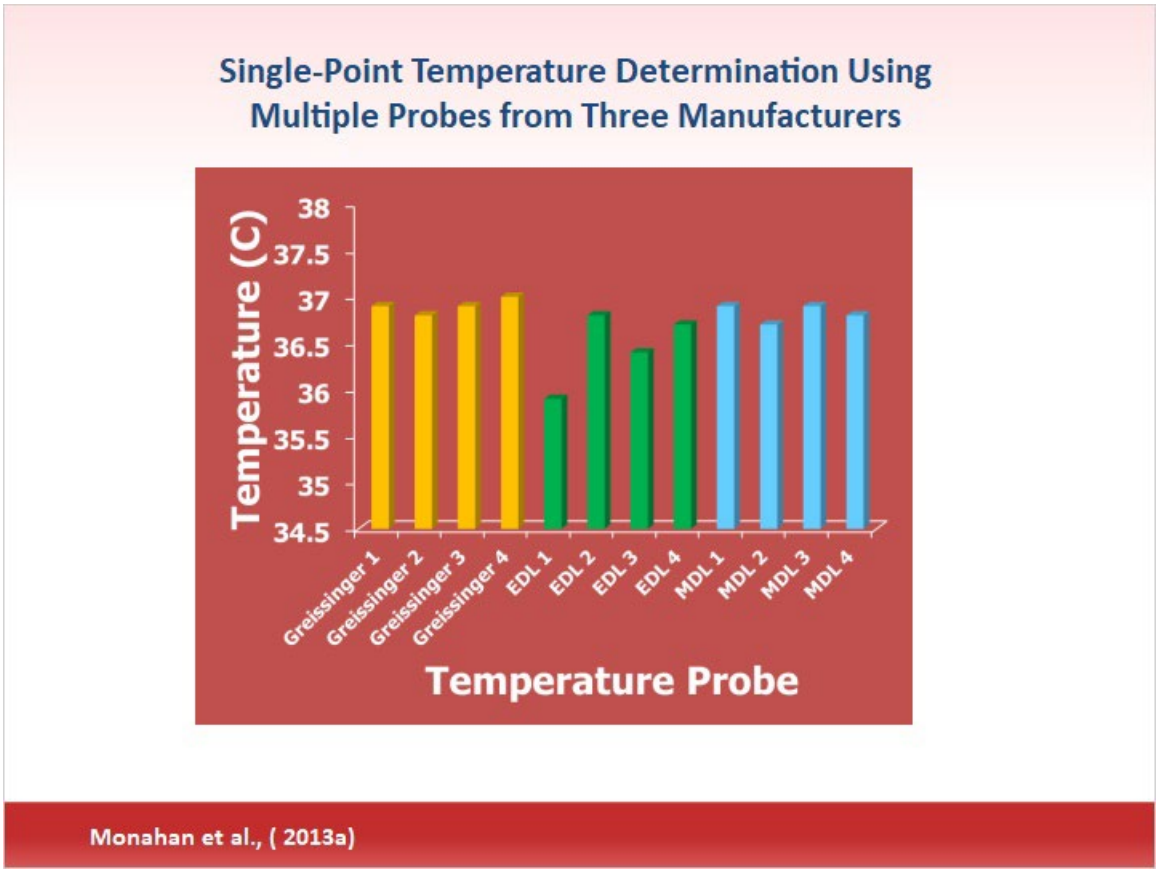
Each piece of equipment in the IVF laboratory should have a protocol identifying the vendor and source of the equipment, describing how to operate the equipment, and detailing what QC procedures are required for each device.

Temperature is one basic function of an incubator that should be measured each day of the incubator's operation, especially when there are embryos in the incubator. A thermometer or thermocouple should be used that, at the least, has been calibrated against a National Bureau of Standards (NBS)-approved device. If multiple thermocouples are used and switched out to be read by one electronic device, each thermocouple should be tested against the NBS thermometer. Currently there are a number of sources for wireless or constant monitoring devices that can be remotely accessed. While there is no requirement for using such devices, it may be worthwhile to consider these types of constant monitoring devices for remote assessment of temperature.

Temperature should be monitored daily and recorded on daily QC sheets. It is important to note that multiple door openings in big box incubators will have a

significant effect on temperature within an incubator.

1.23 Single-Point Temperature Determination Using

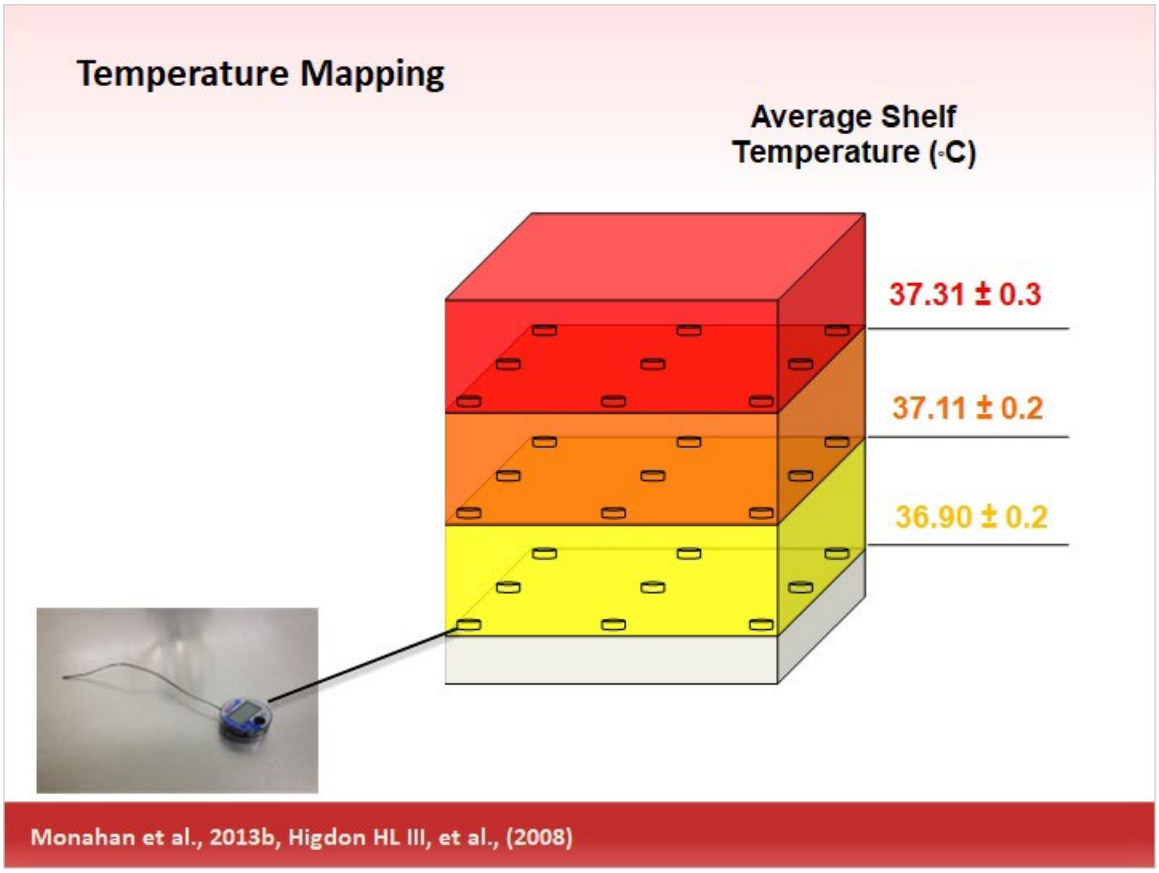


Notes:

Temperature within an incubator should always be monitored by an external device with little reliance on the readout on the outside of the incubator itself.

Most laboratories have multiple incubators and will therefore need multiple thermometers or digital temperature probes for the independent confirmation of temperature. As shown in this figure, multiple copies of a single type of temperature probe can be found to be different from one another, and different from devices manufactured by other companies. Each thermometer or digital “reader” or device should be standardized annually against an NBS standard, and each probe used with the reader should also be standardized against at least one tested probe that can be used as the standard for all the others.

1.24 Temperature Mapping



Notes:

Big box incubators have a great deal of variation within and between shelves as shown in this figure. Higdon and colleagues also showed a significant difference in pregnancy rates and implantation rates after culture of embryos in different incubators of the same model and between shelves used in the same incubator. Laboratories would be well advised to understand the variation within their incubators, both on and between shelves if using large or small box incubators with different shelves.

Similar differences have not been reported between benchtop incubators, but that may simply be because no one has asked the question to date.

Temperature within an incubator should always be monitored by a standardized external device.

1.25 Incubators

Notes:

The digital displays on the front of incubators should never be viewed as a definitive reflection of the actual environment within the incubator. Concentration of gases within the incubator, just as temperature within the incubator, should always be validated and monitored with an external, independent device.

A potassium hydroxide red dye chemical analyzer has been the traditional method for determining incubator CO_2 concentrations. However in this digital age, such measurements are considerably less accurate, specific and/or repeatable than using other types of detection devices and its use is not recommended. The use of electronic or digital detection of CO_2 is preferred. However, all CO_2 detection should go hand-in-hand with measurement and validation of the CO_2 concentration by pH measurements.

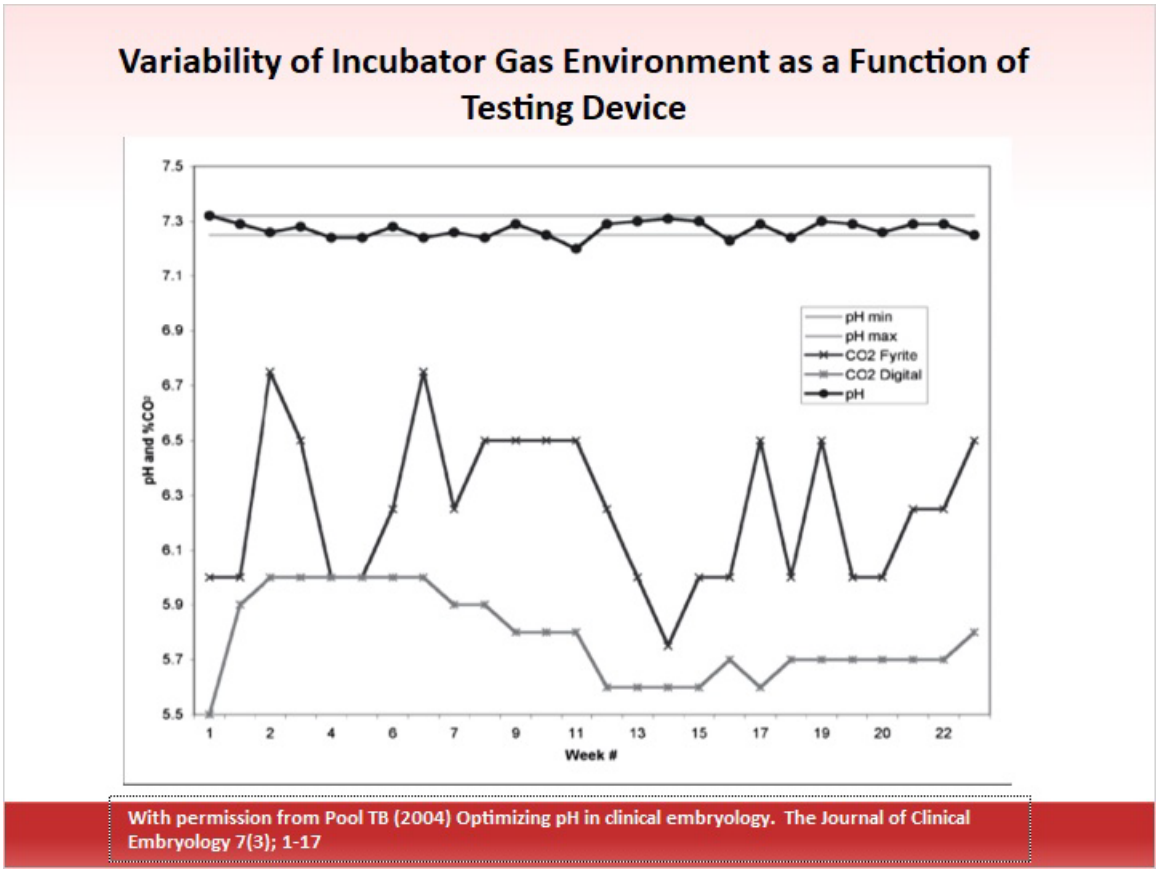
Oxygen concentration can also be measured by the use of a digital device. There are several manufacturers of devices suitable for measurement of the oxygen concentration within an incubator.

CO_2 and O_2 determination is not quite as easy in benchtop models, compared with big

box incubators. However, there are a number of smaller devices that can be used. This is where using premixed gas is an advantage. The College of American Pathologists (CAP) has stated in their checklists that certificates of analysis are satisfactory evidence of gas mixture identification, so the use of secondary measuring devices is not required when using premixed gases.

Since the precise concentration of O_2 required to produce maximal embryo development has not been quantitated, precise measurement of oxygen is probably not absolutely essential. However, numerous studies have shown that using lower oxygen concentrations (4%-6%) is advantageous compared with using room air concentrations (21%) in terms of pregnancy outcomes.

1.26 Variability of Incubator Gas Environment as a Function of Testing Device



Notes:

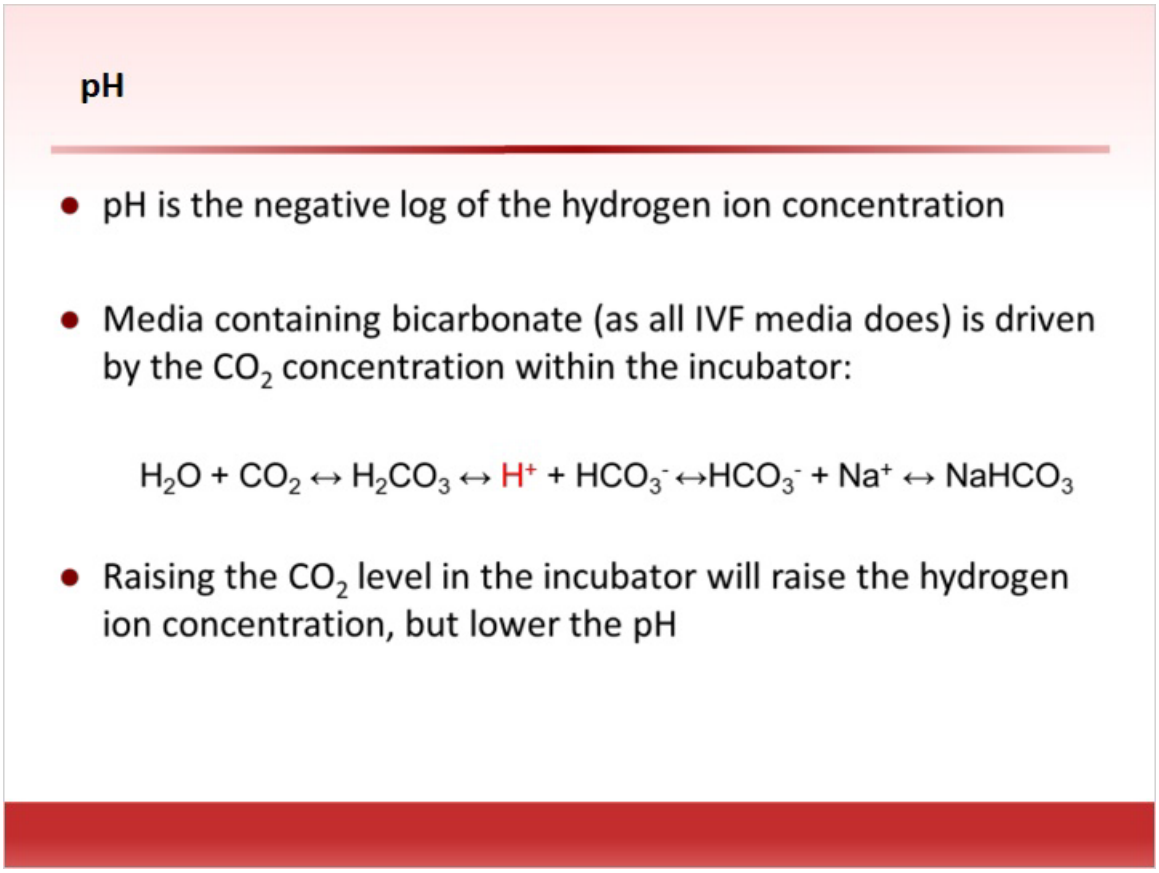
In 2004, Pool demonstrated the variability of both the digital readout on his incubator and the inaccuracies of a commercially available chemical measurement known as Fyrite® relative to his endpoint of interest, which was media pH. During the course of this study Fyrite® measurements indicated an entire percentage point of difference in the CO_2 concentration despite radical differences in pH determination. However, Pool did detect a trend in his pH drift. Initially the pH was more alkaline than he wished. Since he wanted the pH of his media to trend toward the center of the range, rather than to one side or the other, he adjusted his incubator setting on week 2. The pH trend during this time was downward. However, Fyrite® did not reflect these adjustments.

By week 11 the pH of the media in his incubator was trending generally downward too far, eventually going out of range. At that point he adjusted the CO_2 downwards, to drive pH up. Again, Fyrite® measurements did not reflect changes in pH.

Dr. Pool states emphatically in his article that in his opinion there is no reason to measure CO_2 levels at all since the interest is not in the environment within the incubator but rather how the environment affects culture conditions, and specifically how the environments affect the pH of the culture media that nurture the embryos.

While others argue that CO_2 and pH are so intimately connected that measurement of either one can provide sufficient insight, many continue to maintain that pH is the only measurement that is physiologically significant.

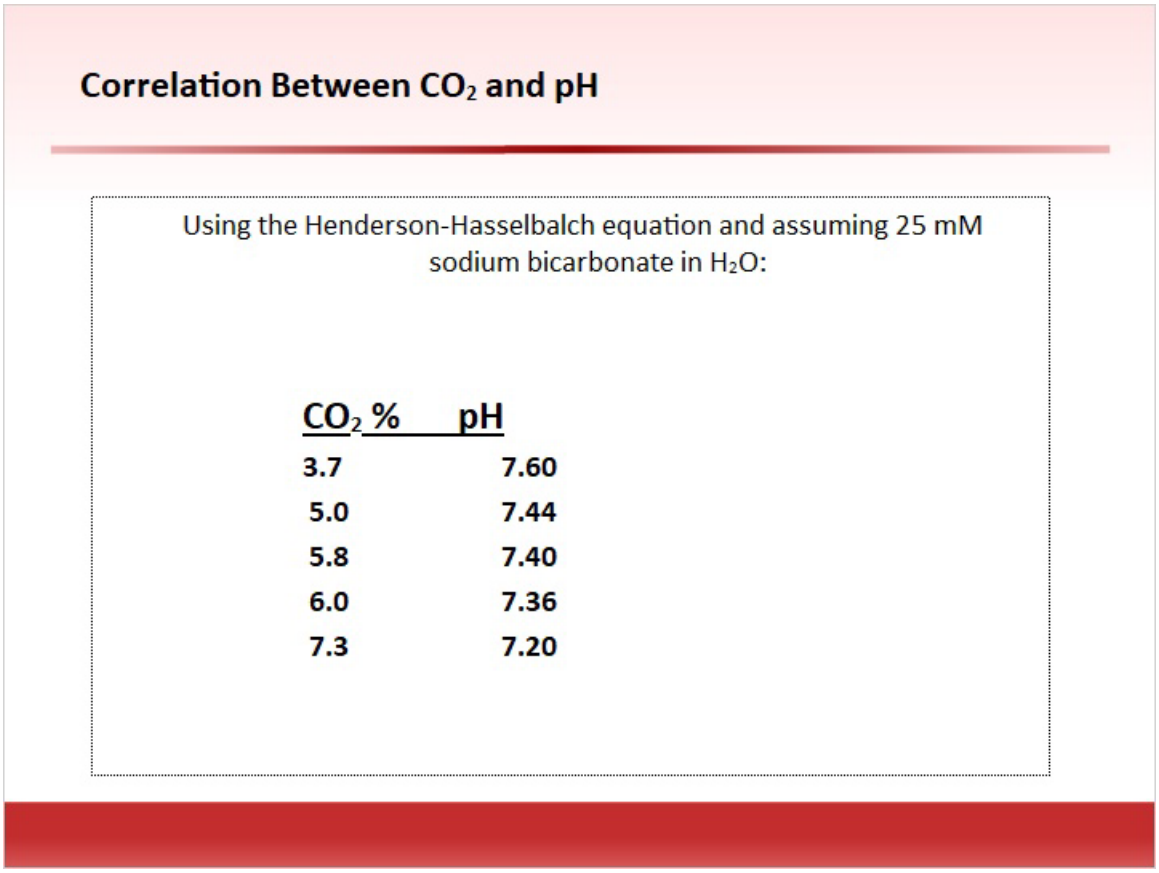
1.27 pH



Notes:

pH is derived as the negative log of the hydrogen ion concentration. Practically this means that in the IVF lab, when using media containing sodium bicarbonate as the pH buffer, to raise the hydrogen ion concentration the CO_2 level must be raised. However, when there are more hydrogen ions in solution, the measured pH will be lower. In other words, media with a pH of 7 has more hydrogen ions than a media with a pH of 8, therefore it is more acidic. A more acidic solution with a lower pH has more free H ions and thus a lower pH-again because pH is the negative log of the hydrogen ion concentration. So, to reduce the pH in media in the incubator, the CO_2 level must be raised. Conversely, to raise the pH of media in the incubator, the CO_2 must be lowered.

1.28 Correlation Between CO2 and pH



Notes:

Theoretically, as calculated by the Henderson-Hasselbalch equation, and assuming that the culture media contains 25 millimolar sodium bicarbonate, pH values at a given CO_2 concentration can be calculated. These calculations predict what pH should be seen in culture media at a given CO_2 concentration.

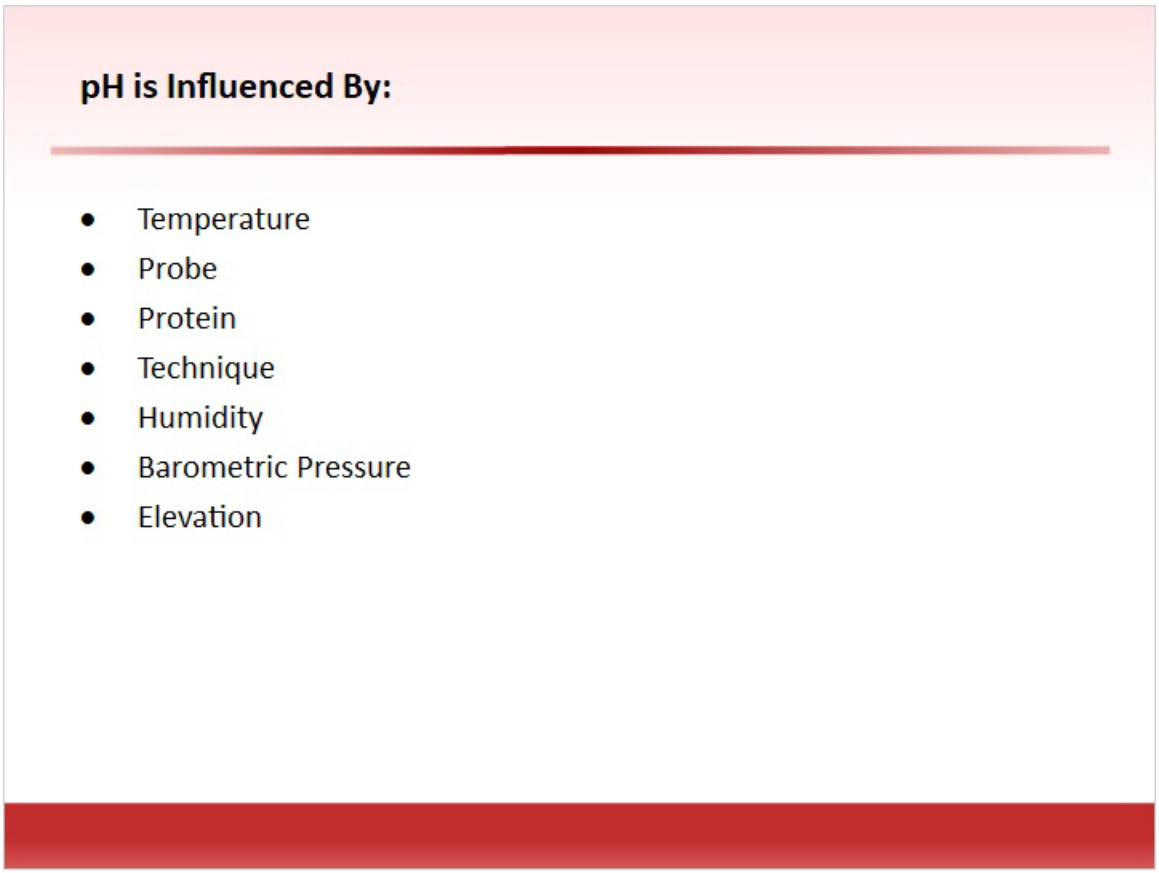
These numbers provide an excellent starting point for determining the approximate setting for CO_2 on any incubator. However once the CO_2 percentage is set, the actual pH of the medium after equilibration should be measured to validate that the correct CO_2 concentration is being used.

In the clinical IVF laboratory, most use media manufactured and QC tested by various companies. Not every company uses 25 millimolar sodium bicarbonate in their media. The addition of protein supplements such as human serum albumin will change the pH.

Therefore, it is imperative that laboratories measure pH in incubators using the media they will culture their embryos in, rather than rely on the pH as indicated by media manufacturers. Furthermore, pH in the bottles as they arrive from the manufacturer is

clinically irrelevant as no media should be used without equilibration. Therefore, the only clinically relevant number is the pH of the media after equilibration.

1.29 pH is Influenced By:



Notes:

The same media placed into an identical model of incubator that is set at the same CO_2 level will have a different pH depending on a number of different environmental influences. Some of these are subtle, some are not.

Therefore, it behooves each laboratory to determine exactly what is happening in their particular circumstances.

1.30 Measurement of pH

Measurement of pH

- pH Measurement (pH meter)
  - Calomel or double junction probe may be best.
  - Update standards often. Standardize every use.
  - Use 2-point determination with warm probe, and standards
  - Clean probe properly or change often. Change probe if pH drifts or time for stabilization increases.
  - Store properly-in storage solution, not water (storage solution is an acid solution, pH=3). Always rinse the probe well prior to use.
  - Place at least 4 tubes of media in the incubator overnight. Use smallest amount of medium the probe will reliably measure. Use same amount of medium in all tubes (pH will be different in 5 vs. 10 mL).
  - Tighten all lids immediately prior to reading pH. Remove one tube one at a time and place the pH probe into the tube. Wait for the reading to stabilize. Remove second tube, wait for the reading to stabilize. These are only equilibration tubes.
  - Use the next two tubes to take actual pH readings. Average the two.

Notes:

Most laboratories have pH meters in them, and yet very little time or energy is taken to consider the correct use of a pH meter. The following are recommendations collected from various pH meter and probe manufacturers.

It is important to match the pH probe to the application. Manufacturers of pH meters make probes that will function in lipid as opposed to aqueous solutions, at ultracold as opposed to room or body temperatures, etc. A reasonable probe to use for IVF applications is one that can be used in an aqueous environment at 37 degrees Celsius. Calomel or silver double-junction probes are acceptable for this application.

Standards should be used to calibrate the probe and it is best to use two of them to get a two-point determination. Standards are usually pH of 4, 7, or 10. A combination of 4 and 7, or 7 and 10 is appropriate.

Manufacturers disagree as to whether probes and standards should be warmed prior to use, but generally, warming is recommended. Secondary temperature probes can also be used. The use of two probes allows for correction of pH due to temperature drift. If

using two probes, both should be placed into the same tube at the time of pH determination.

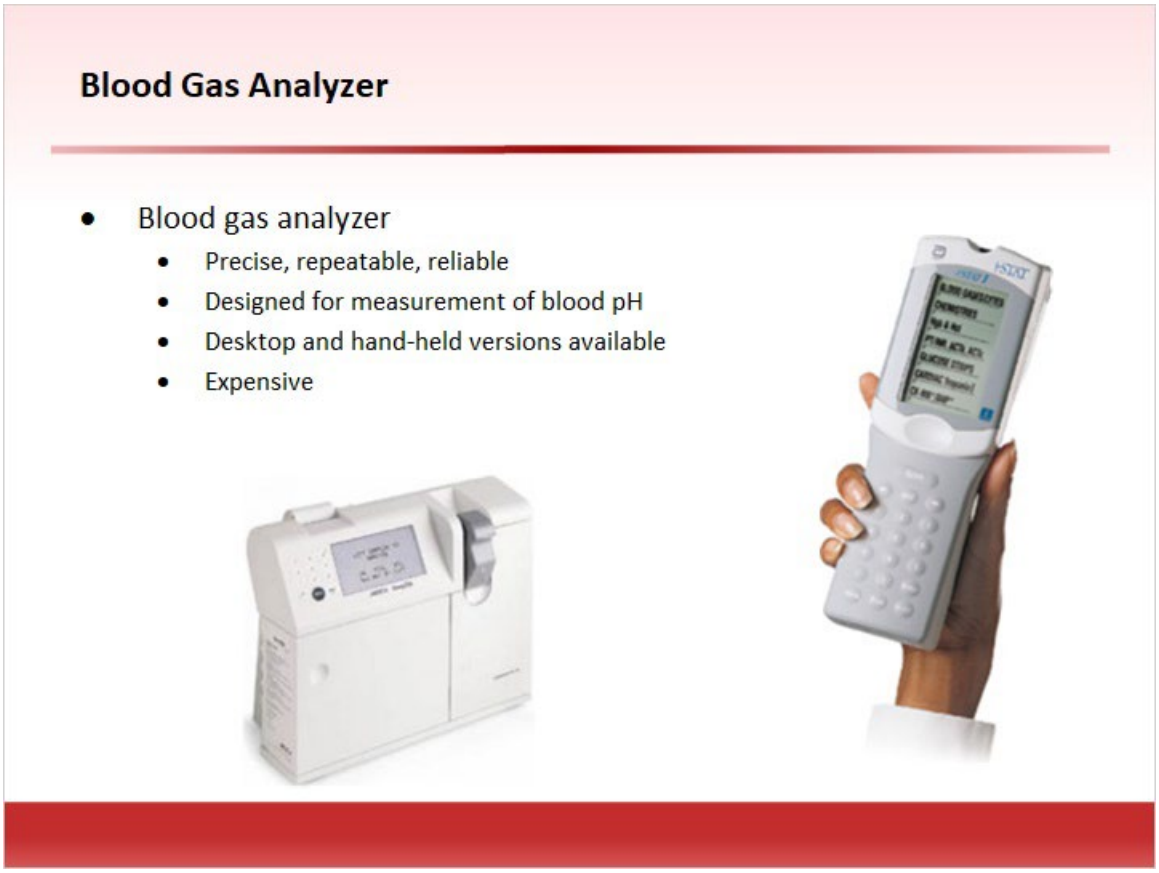
If the pH readings drift, or take a longer than usual time to stabilize, a new probe should be purchased, or the probe should be cleaned. Probes may be cleaned in solutions provided by the manufacturer, but these are usually acid solutions used to dissolve precipitated proteins that collect at the junctions of the probe. Cleaning of the pH probe would best be done outside of the IVF lab. As an alternative, probes can be changed regularly and replaced with new ones, rather than bringing caustic chemicals into the laboratory area.

Between readings the probes should be kept in storage solution provided by the manufacturer. This solution is a very dilute acid, at a low pH. It is not water and probes should not be stored in water alone. Before using, remove the probe from the storage solution and rinse well, ideally in the media that will be used with the probe.

When setting up to measure pH, the first few tubes that are used should be considered equilibration tubes. Therefore, at least 4 tubes should be set up for the first test. All four tubes should contain the same volume of medium and equilibrated overnight in the incubator. At the initial opening of the incubator door, close all lids tightly. Remove the first tube and remove its lid. Place the probe into the media and watch the meter until the pH stabilizes. Discard this tube and immediately place the probe into a second tube. Wait until the pH stabilizes. Discard this tube and ignore the pH reading. Place probe into a third tube, record the pH. Place the probe into a fourth tube and record the pH. Average the readings from the third and fourth tube to get the pH of that incubator. Tubes from additional incubators can then be read immediately after the first four tubes, using two tubes per incubator and averaging the two readings from each incubator.

A couple of cautionary notes are in order here. First, the user needs to understand that the pH in 5 mL of media in a tube will be different then the pH of a 30 microliter droplet of media under an oil overlay. Volume in the tube, surface area of the media, the presence or absence of an oil overlay, and the time from removal of the tube to insertion of the pH probe will all affect the measured pH. Therefore, measuring pH by this method will only give an approximation of the pH that will occur in the actual culture dish if microdrops are being used.

1.31 Blood Gas Analyzer



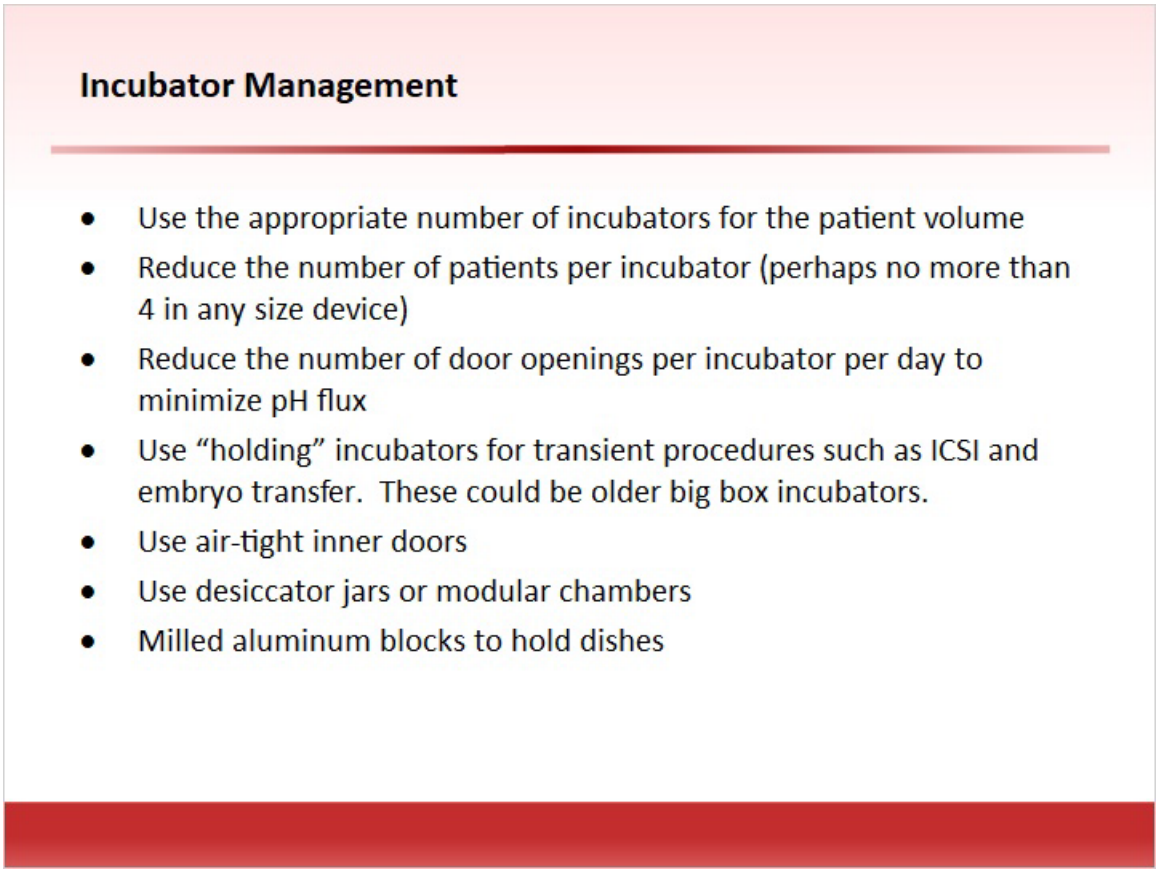
Notes:

Perhaps a more accurate alternative to the pH meter is the blood gas analyzer.

These devices are more accurate, precise, repeatable, and reliable across multiple users than pH meters. Many of them can be used to measure pH in very small volumes (100 microliters). This provides an opportunity to measure pH in the environment in which it is being used. Both desktop and hand-held devices are available, but these can be expensive.

Using a blood gas analyzer to measure pH in culture media is technically an off-label use compared with the intent and use for which the analyzers are tested by the manufacturer. However, since they are designed to measure pH in blood, the measurement of pH in culture media should be acceptable.

1.32 Incubator Management



Notes:

In summary, providing a stable incubator environment is instrumental in creating healthy embryos. Since embryos in the laboratory spend the majority of their time in the incubator, attention to incubator function will go a long way to producing healthy embryos.

Every door opening to an incubator changes the environment inside the incubator. Limiting door opening to a clean, well-maintained incubator can reduce environmental fluctuations that can have an impact on culture conditions.

To stabilize the incubator environment a lab may want to consider investing in additional incubators, thereby reducing the number of incursions into any one incubator in a day. This will also allow the laboratory to reduce the number of patients cultured per incubator, thereby helping to stabilize the culture environment.

It is potentially better to open the door longer to remove more dishes at one time, then to open and close the door of an incubator multiple times. Using a holding incubator for transient procedures and going in and out of that incubator more frequently, then

returning all dishes to the potentially more stable environment of the less-opened culture incubator may help prevent long delays in recovery in larger incubators.

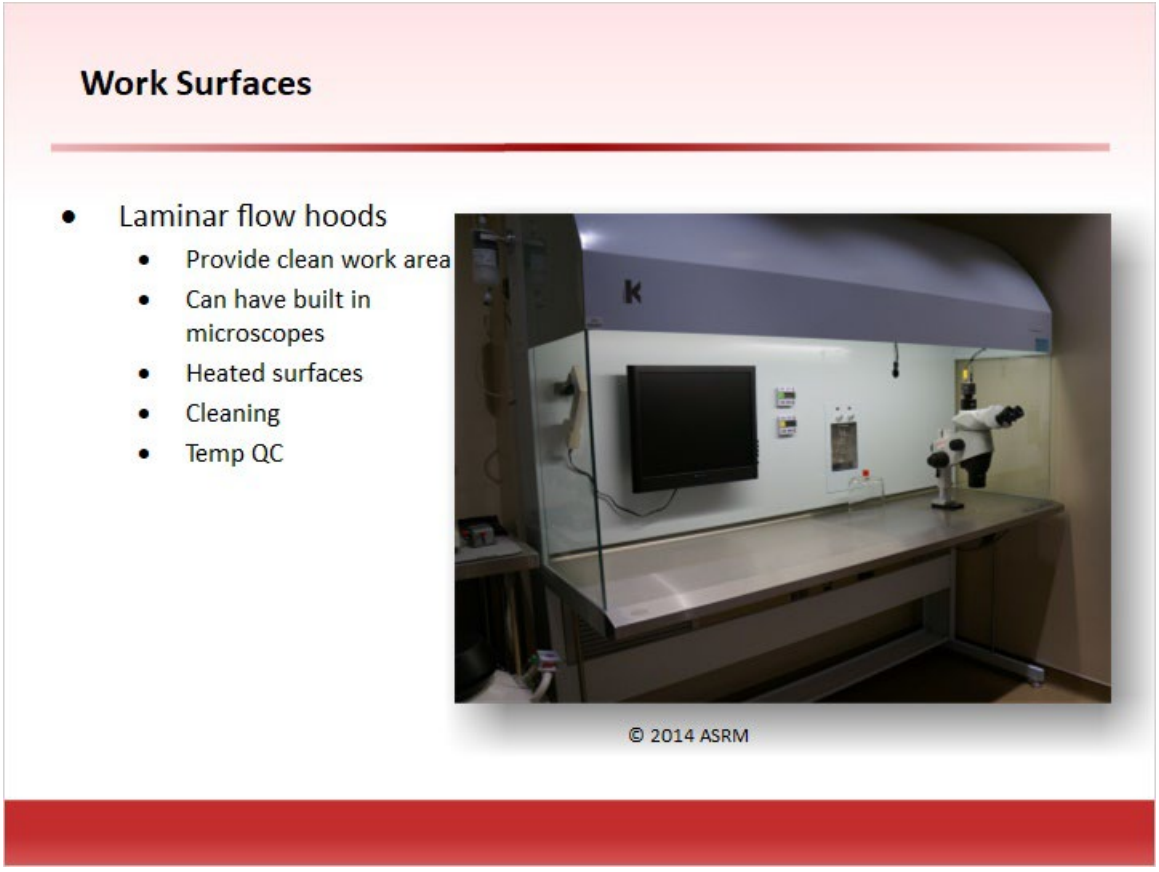
The use of air-tight inner doors also allows access within the incubator to one portion of one shelf, but doesn't require opening the entire front of the incubator. This potentially reduces the influx of colder air from the room, which lowers temperature, and helps maintain a more stable internal CO_2 level which will reduce fluctuations in pH.

Small air-tight, gassed chambers within the incubator have been used in many labs. These include desiccators and modular chambers.

Aluminum blocks for holding dishes within the big incubators have also been used to help stabilize temperature fluxes.

All of these procedures can be used to improve embryo development.

1.33 Work Surfaces



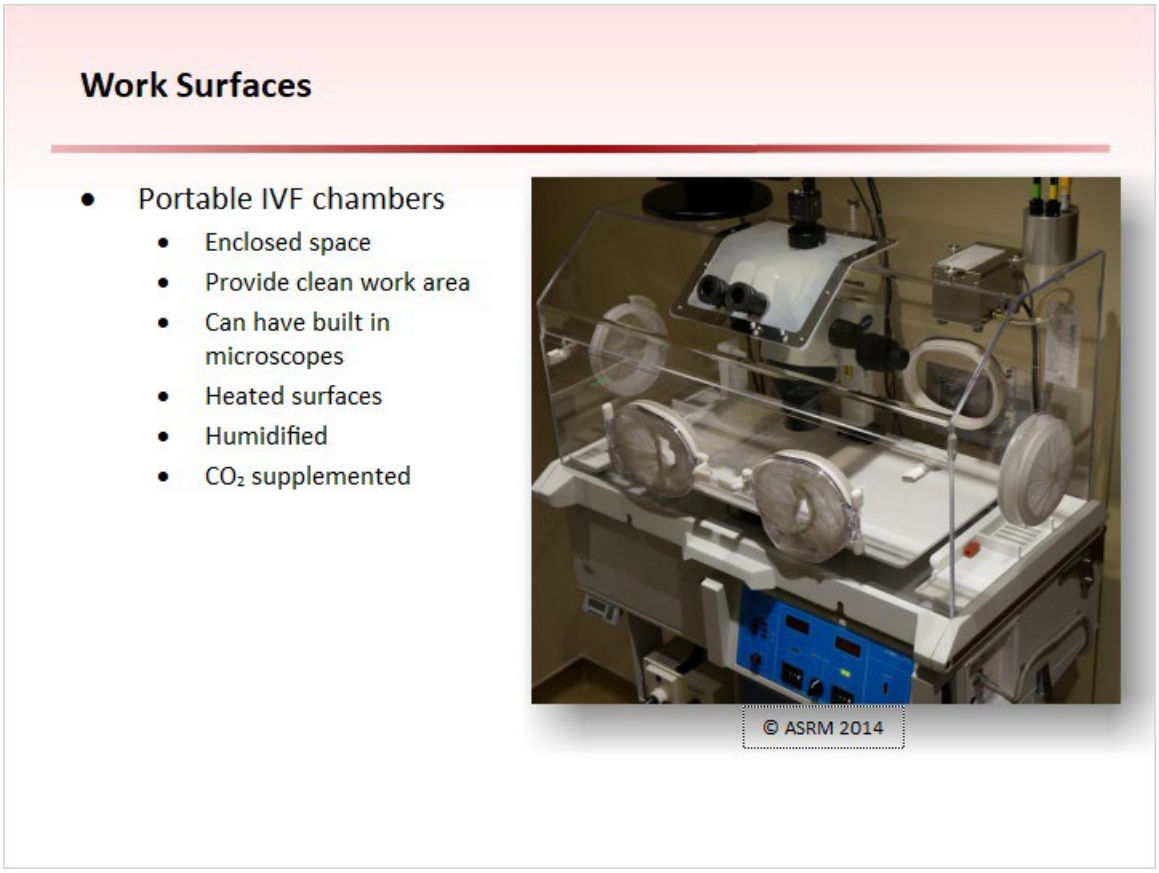
Notes:

Perhaps the second most commonly used piece of equipment in the IVF lab after the incubator is the laminar flow hood. These hoods have been modified to have warm water circulating beneath the surface. Built-in microscopes allow oocytes and embryos to be examined on a warm surface to maintain constant temperature environments in an effort to reduce embryo stress.

Hoods are easily wiped down at the end of the day thus making them easy to maintain. However, temperature across the warm surface can be variable and should be monitored. In addition, dishes placed on the surface tend to run slightly cooler, usually due to a small air space under the bottom of the dish. Therefore, both the temperature of the actual surface of the hood and the temperature in dishes placed on the surface should be monitored. The temperature of the circulating water should be adjusted to maintain the dish at an appropriate temperature.

Sperm processing ideally is done in a separate lab from the embryology lab since ejaculated sperm are not "sterile." When processing sperm that are positive for HIV, Hepatitis B or Hepatitis C, it is advisable to process them in a biological safety hood, but not in a laminar flow hood. Laminar flow hoods are designed to protect the specimens within the hood by pulling air in through the HEPA filters and then across the samples, thus potentially exposing the user to contaminants from within the hood. Biological safety hoods pull air in from the room, across the samples within the hood and are then vented out the top of the hood, and into the outside environment. Processing sperm should be done in a way that limits exposure of the technician to aerosolized products. Infectious samples should always be processed in an environment that minimizes exposure.

1.34 Work Surfaces



Notes:

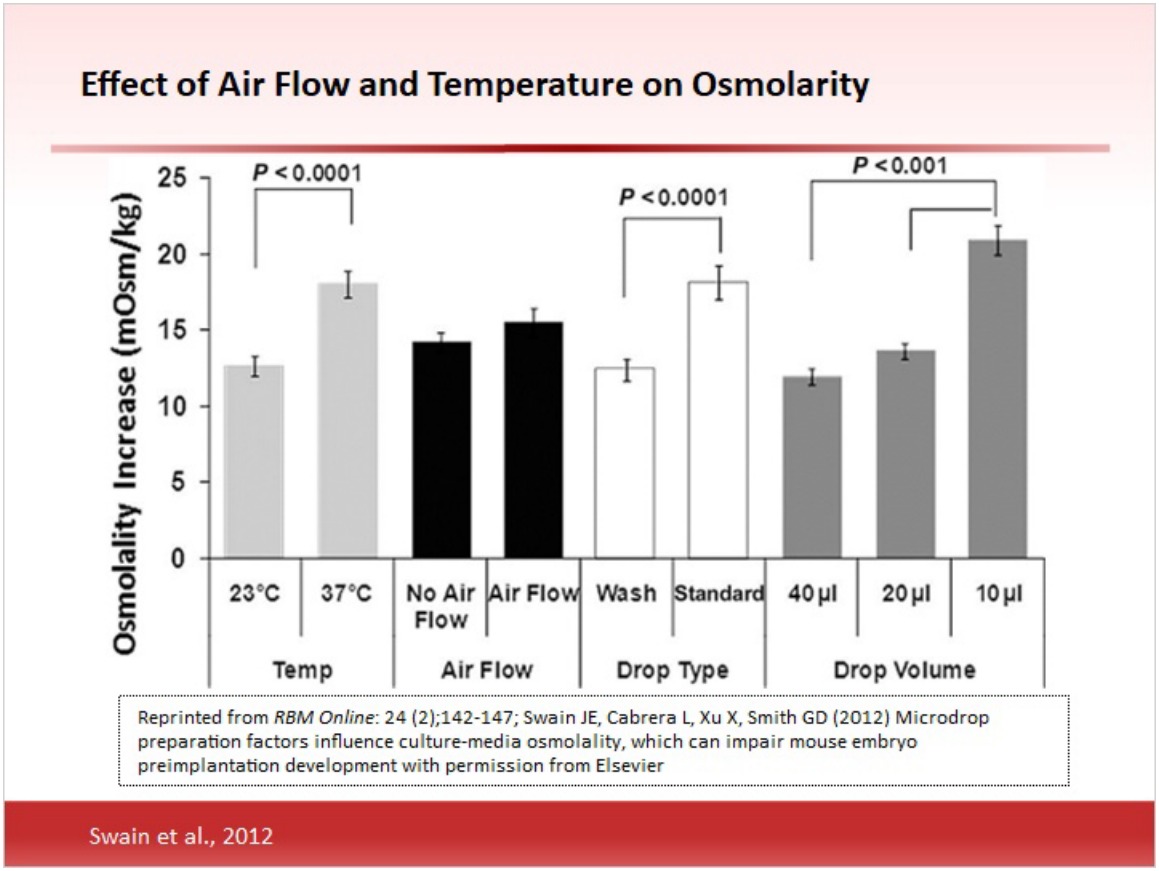
Portable IVF chambers can also provide a work space that is similar to that of laminar flow hoods. The advantages to these are that the entire interior is warm, not just the surface under the dish, CO_2 is pumped through the chamber creating an environment that can be comparable to that of the incubator environment, and the entire system is humidified. This chamber is like an incubator with a microscope inside. Temperatures and humidity are not constant within the various microenvirons of the chamber, but all parameters can be adjusted to make the environment around the viewing stage consistent and as close to that found within the incubator itself as possible.

The major disadvantage to these is the cost. Usually a hood of some sort is needed in addition to the chamber, so having these does not preclude the need for a hood for setting up dishes, doing freezing, or other similar processes.

Use of time-lapse devices has the potential to significantly reduce the need or amount of time one would use a hood or chamber for handling embryos. All warming surfaces should be carefully monitored for temperature, and surface temperatures adjusted to drive the temperature of media in dishes. Many dishes have small air spaces between the bottom of the dish and the counter. Therefore temperature in the dish may be different then temperature on

the surface of the hood. In addition, temperature at various locations around the heated surface may vary. All of these factors should be examined as part of the QC for a piece of equipment. If cold or hot spots are identified they should be indicated so all users know where to avoid placing dishes and embryos for long periods of time.

1.35 Effect of Air Flow and Temperature on Osmolarity

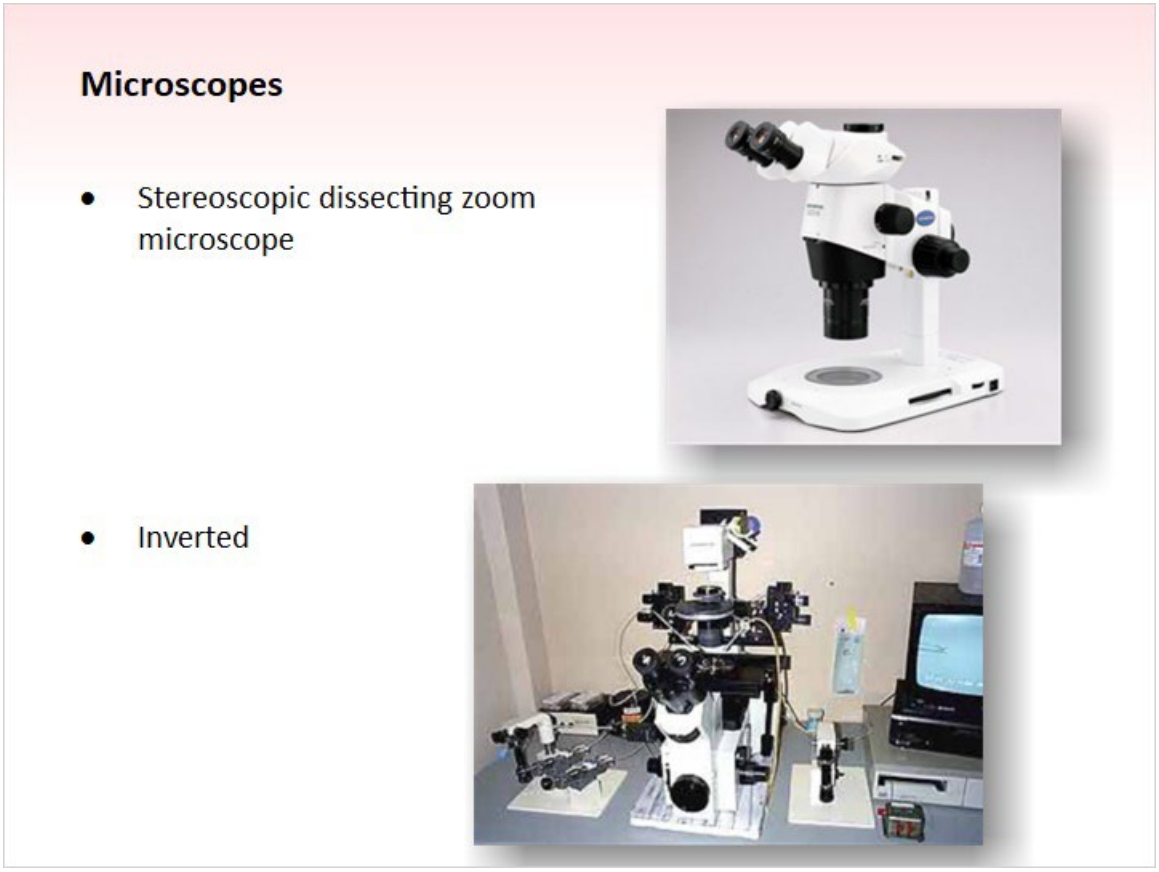


Notes:

Laminar flow hoods are used as a clean place to prepare dishes for use in IVF. There are always questions about whether to have the heated surface and/or the air turned on when preparing dishes. A report from Swain and colleagues in 2012 showed that preparing dishes on a heated surface and with the laminar flow turned on in the hood at the time of dish preparation resulted in higher osmolarity in the droplets of media. Higher osmolarity translated into poorer embryo development in a mouse model. The researchers found that one way to prevent the elevation in osmolarity was to lay down the droplets of media first, cover them with oil, and then remove the droplets and replace with fresh media.

Therefore, while laminar flow hoods provide a clean surface for use with human embryos, care should be taken to ensure that use of the hood does not cause other problems.

1.36 Microscopes



Notes:

The workhorse in the IVF laboratory is the stereoscopic zoom microscope. It is used during egg retrievals to find oocytes, during ICSI, fertilization checks, cleavage assessment, and any time oocytes or embryos are moved with embryo transfers, embryo biopsy, and cryopreservation.

Most dissecting scopes do not have a heated stage and most come with a sintered or frosted glass cover plate on the base. Heated clear cover plates can be purchased separately. The sintered or frosted glass cover plate must be replaced with a clear one in order to visualize human embryos. Stereoscopes should be chosen that have a generous working distance between the stage and the objective to accommodate multiple kinds of dishes.

Work stations built into hoods that have heated surfaces and IVF chambers can accommodate a variety of different types of stereoscopes.

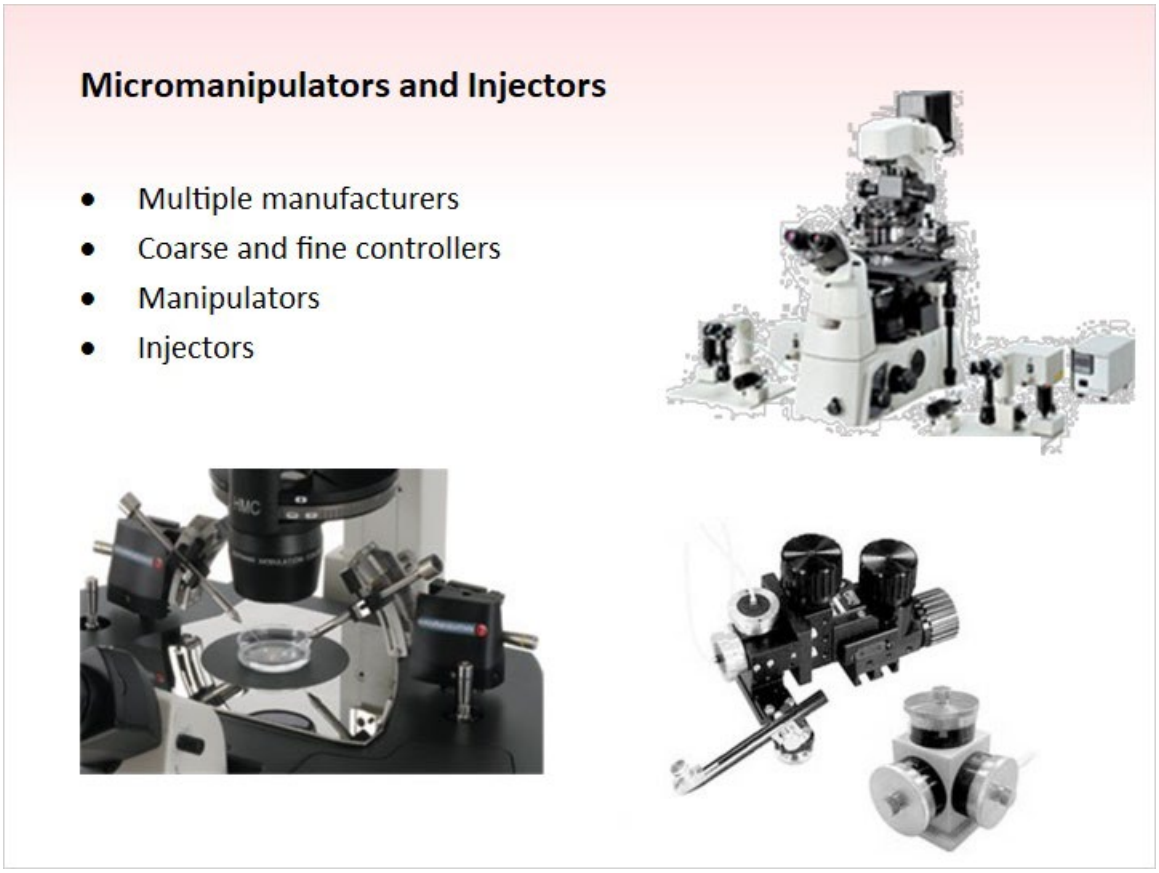
Inverted microscopes provide much greater magnification compared with stereoscopes. Inverted microscopes outfitted with microinjection equipment allow the introduction of ICSI and embryo biopsy and are standard in virtually all IVF laboratories.

The use of various contrast techniques improves the visualization with inverted scopes, allowing increased distinction and visualization of the interior of the oocyte, sperm head abnormalities, etc. The most common kinds of contrast optics include differential interference contrast (DIC) and Hoffman modulation contrast (HMC).

Inverted scopes may be retrofitted with additional devices such as a laser for performing assisted hatching and embryo biopsy, or a polarized light microscope for visualization of the spindles inside the egg during ICSI.

All of these microscopes may be fitted with cameras for remote visualization of what is on the stage or for photographs. Laser-assisted hatching requires the addition of a camera and computer.

1.37 Micromanipulators and Injectors



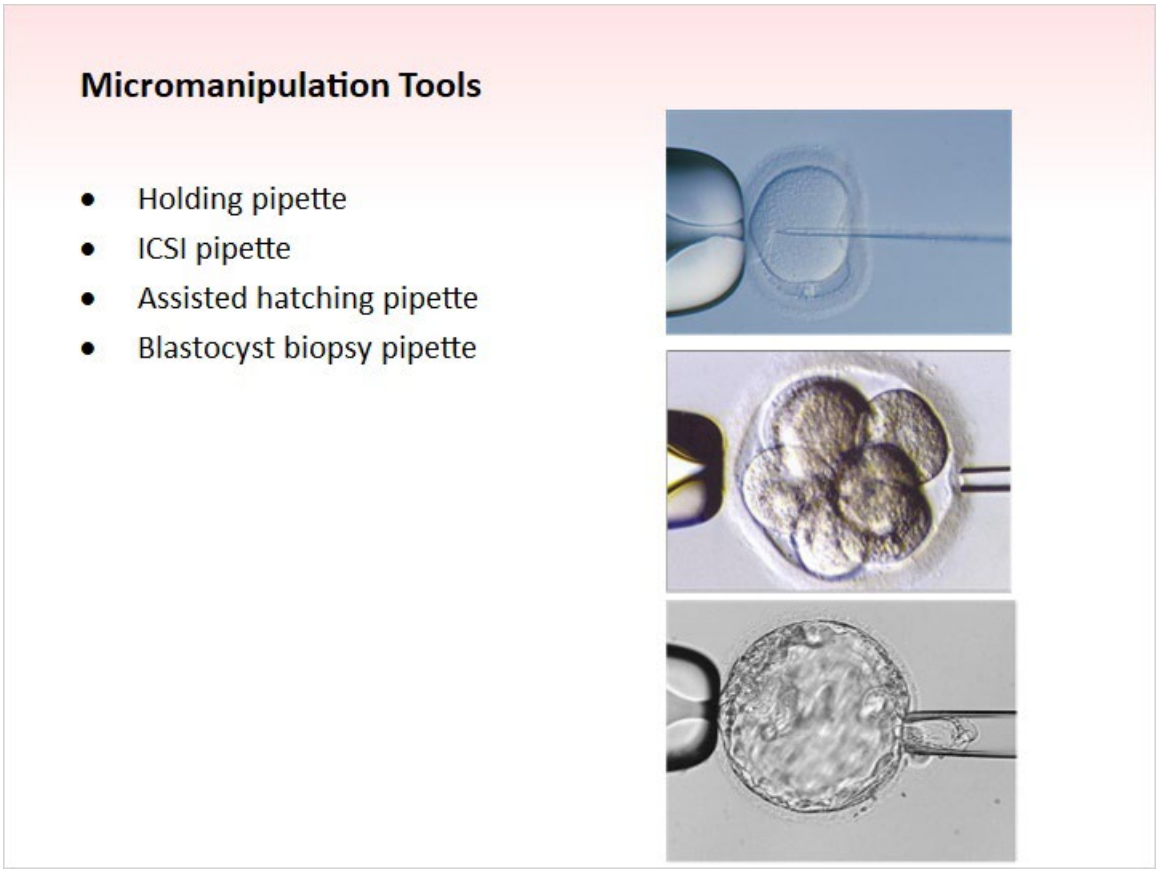
Notes:

Micromanipulation setups allow the embryologist to perform microscopic procedures on oocytes and embryos such as ICSI and embryo biopsy using robotically controlled tools.

Several companies produce microinjection systems. Microinjection systems utilize micromanipulators attached to the base of the inverted microscope for controlled movement up and down, in and out horizontally, and in some cases in and out at an adjustable, predetermined angle. Microtools are attached to the micromanipulators and controlled remotely by maneuvering coarse and fine controllers.

Microinjectors provide tight control of very small volumes of media for use during ICSI. Microinjectors may be controlled by adjusting the pressure on oil, water, or air lines through the use of screw-type controlled syringes.

1.38 Micromanipulation Tools

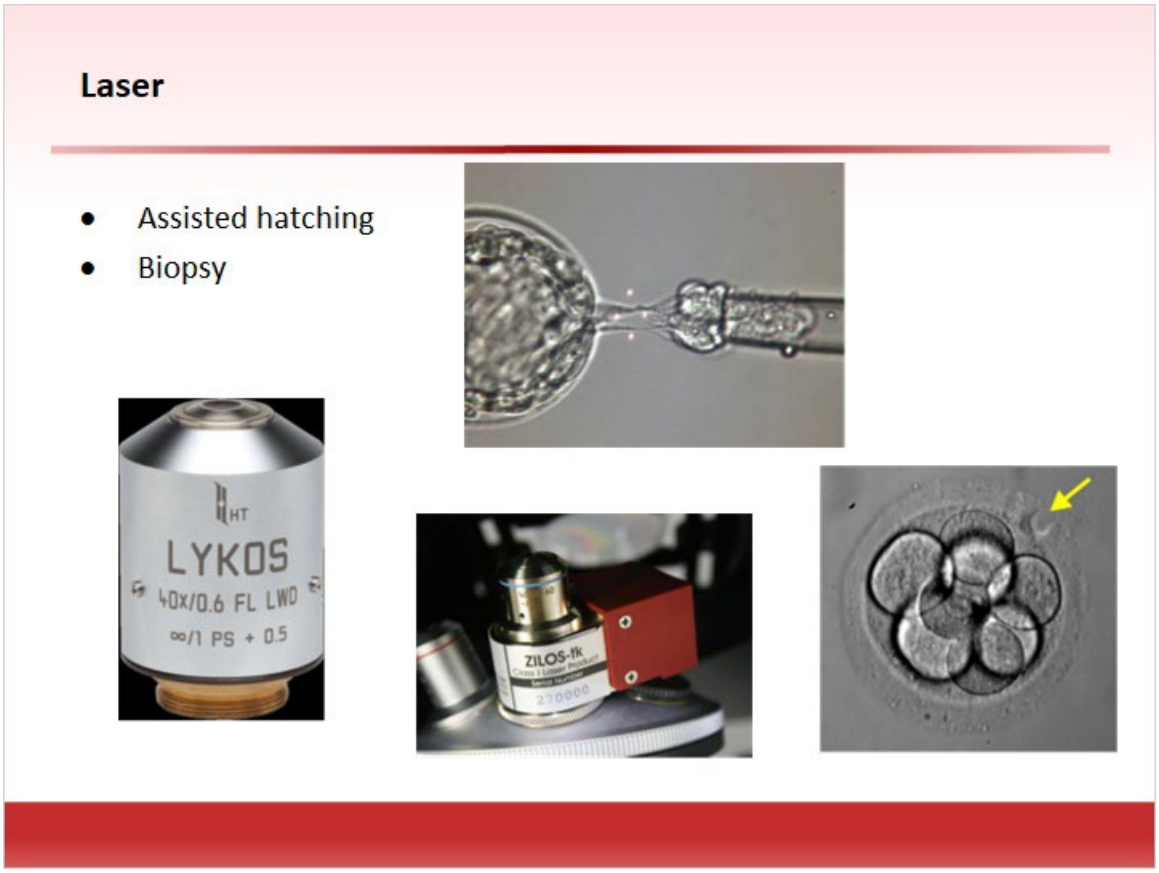


Notes:

Each micromanipulation procedure requires specialized tools and multiple companies produce and distribute microtools for each specialized procedure. Holding pipettes can be obtained with different external diameters and internal hole size, ICSI pipettes can be produced both with and without a spike on the end of the bevel to assist in penetrating the zona and plasma membrane. Some programs prefer tools with a particular angle, others prefer no bend associated with the tools. It is up to the laboratory to determine what works best in their hands with their equipment.

A few laboratories still choose to manufacture their own microtools, but the majority of labs find that it is easier to purchase premade tools due to the volume of use since the advent of ICSI and biopsy.

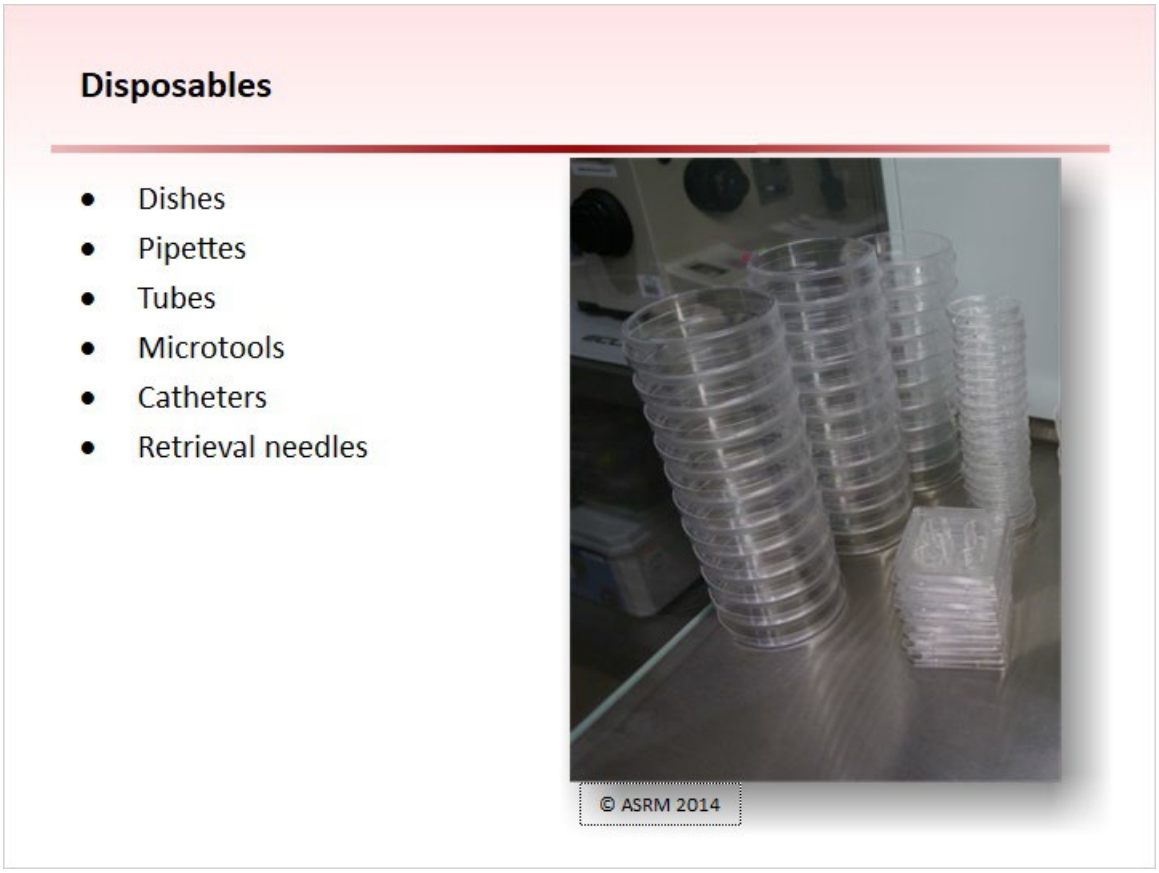
1.39 Laser



Notes:

Recent developments in the area of embryo biopsy have been advanced by the introduction of laser optics that can be added to inverted microscopes. Lasers are an easy, but expensive, addition to the microscope allowing very tightly controlled administration of a beam of high energy light to open holes in the zona pellucida or sever the connections between cells. Most lasers can be remotely controlled by a foot pedal allowing use of both hands, which is advantageous, particularly during biopsy. Newer versions of the laser also allow visualization of the target while looking through the eyepieces. Older versions require visualization on the computer screen.

1.40 Disposables



Notes:

An embryologist has a wide range of sources for all disposable items used in the laboratory. However, it is in their best interest to choose items that have been produced within and for the IVF industry. Most of these products have been mouse-embryo tested prior to release as part of the manufacturing quality control process. Dishes, in particular, are items that can be difficult to QC test in-house since dishes from multiple production runs are packaged into one sleeve, sterilized, and then given a lot number based on sterilization. Thus, one lot number may contain dishes from several production runs. Therefore, testing of only one or two dishes from a lot may not fully test for potential toxicity from all the various runs incorporated into one lot number. Use of dishes and other plasticware that have been tested by the manufacturer using some form of test for embryotoxicity will reduce the risk of this type of variability within a lot number.

Most items from this supply list that have direct human contact such as catheters and retrieval needles also have to meet FDA requirements and have received FDA clearance. The use of catheters that have been approved for veterinary use, such as Tomcat catheters, are subject to different quality control standards and should not be used in

humans, although they tend to be much more affordable than items that have had to pass FDA review.

1.41 Additional equipment

Notes:

There are numerous additional items that are critical additions to the IVF laboratory. Although many laboratories use standard household refrigerators, laboratory versions are probably a better idea. If using a standard household refrigerator, the freezer section should not be frost-free as this utilizes small, repeated warming cycles to keep the freezing section frost-free. This may have an adverse effect on items stored in the freezer, especially blood or serum products.

Laboratories can select media from among a variety of manufacturers. Few laboratories now manufacture their own media.

Disposable pipettes are used for pipetting large volumes of media and oil, while small volume pipettes can be used with disposable tips for pipetting very small volumes and

handling oocytes and embryos.

Every IVF lab needs to have some type of dewar for long-term storage of gametes and embryos, even those laboratories that ship regularly to long-term storage facilities and do not maintain large quantities of embryos on site.

Finally, some system for remotely monitoring incubators and cryotanks is an important safeguard against power failures and tank failures.

1.42 Andrology Equipment

Notes:

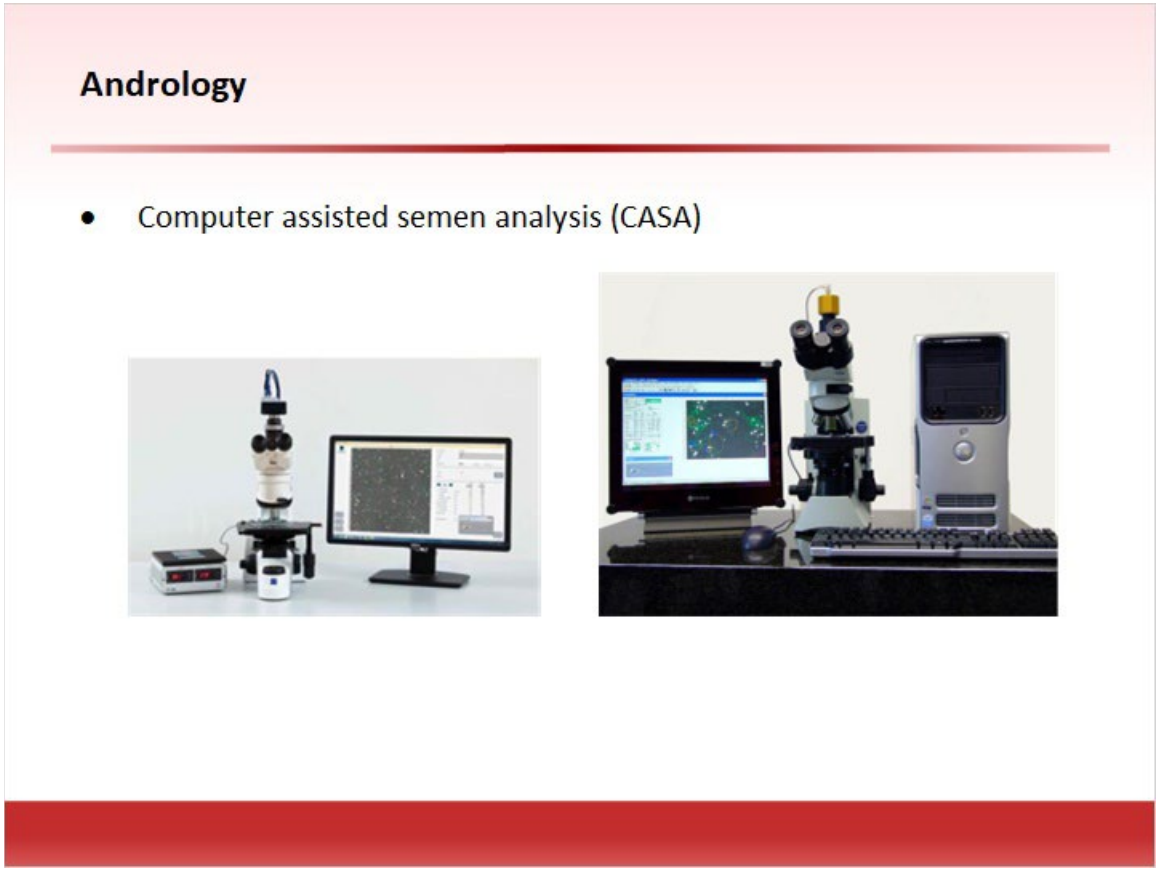
Most sperm preparation techniques require the use of a centrifuge to spin sperm over a gradient of some sort. A wide variety of centrifuges are available that can be used for this purpose.

Some manufacturers of gradient solutions for sperm isolation recommend the use of swinging buckets in the centrifuges for optimal recovery during sperm preparation.

However, this does not seem to be a consistent recommendation by all manufacturers.

In addition, the use of sealed buckets may also provide some safety advantages during centrifugation in the event of a cracked tube, or when processing infectious material to reduce aerosolization.

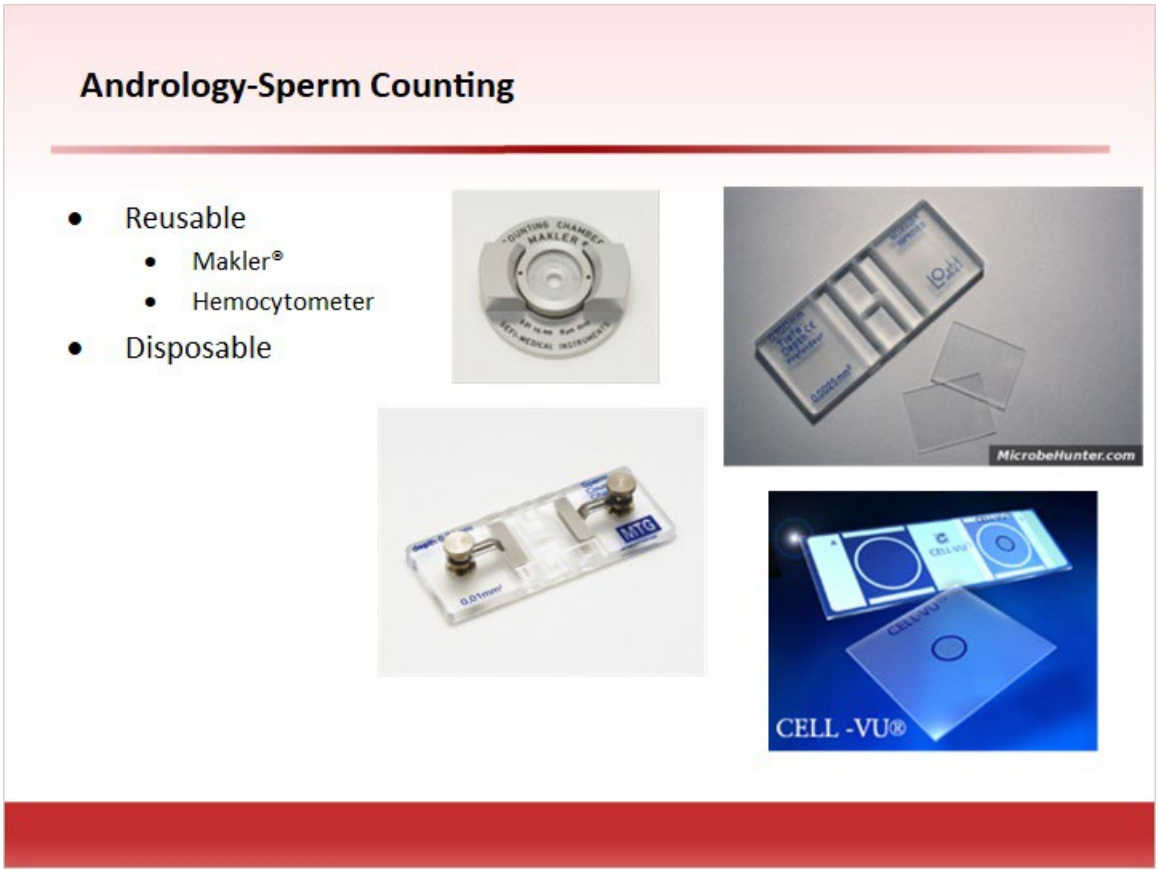
1.43 Andrology



Notes:

Several somewhat costly systems are available to automatically count semen using digital recognition software. Despite the popularity of computer assisted counting, it is always advisable for laboratory personnel to be familiar with the procedure of counting sperm manually.

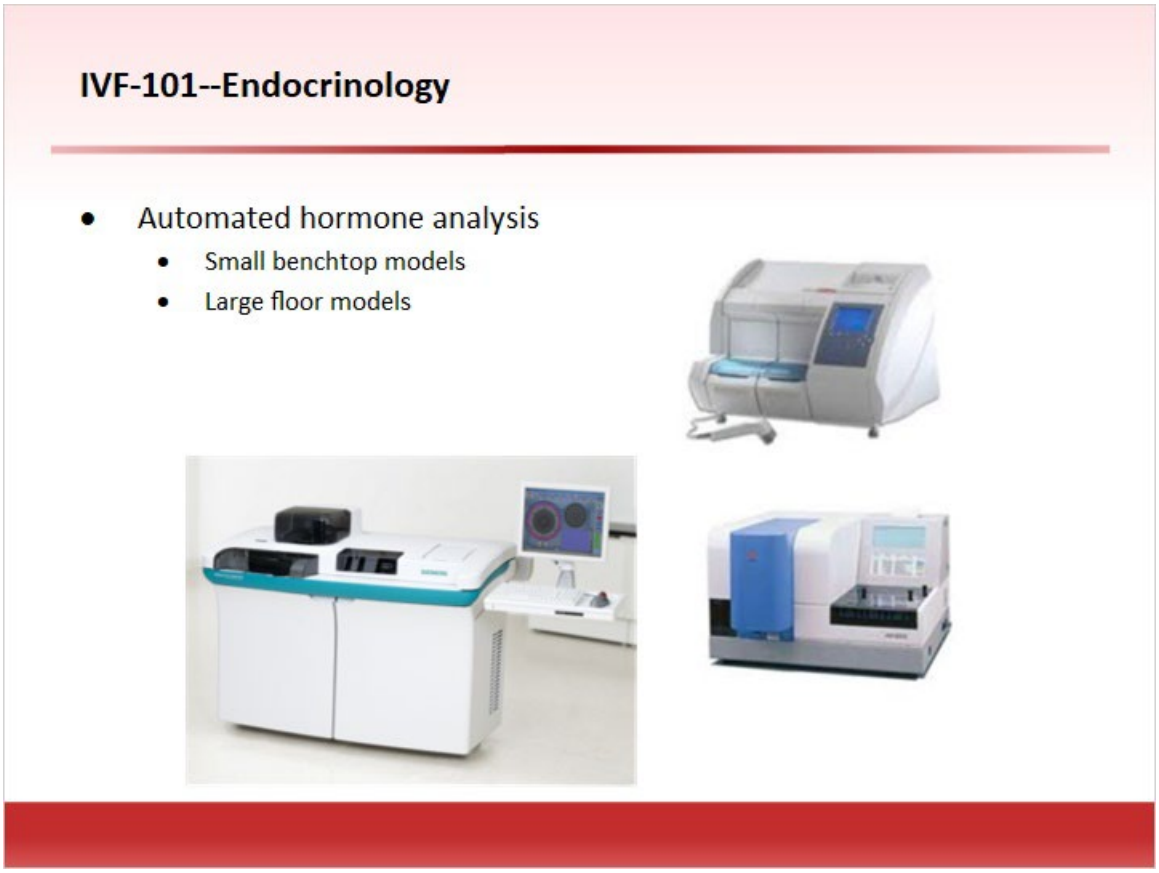
1.44 Andrology—Sperm Counting



Notes:

Numerous types of sperm counting chambers are also available. The use of a counting chamber often depends on whether the lab is using some type of automated counting chamber, in which case disposable devices are often used, or if the lab is manually counting the sperm, in which case reusable devices are often the choice. All devices seem to have their advantages and disadvantages and all seem to work reasonably well for sperm counts.

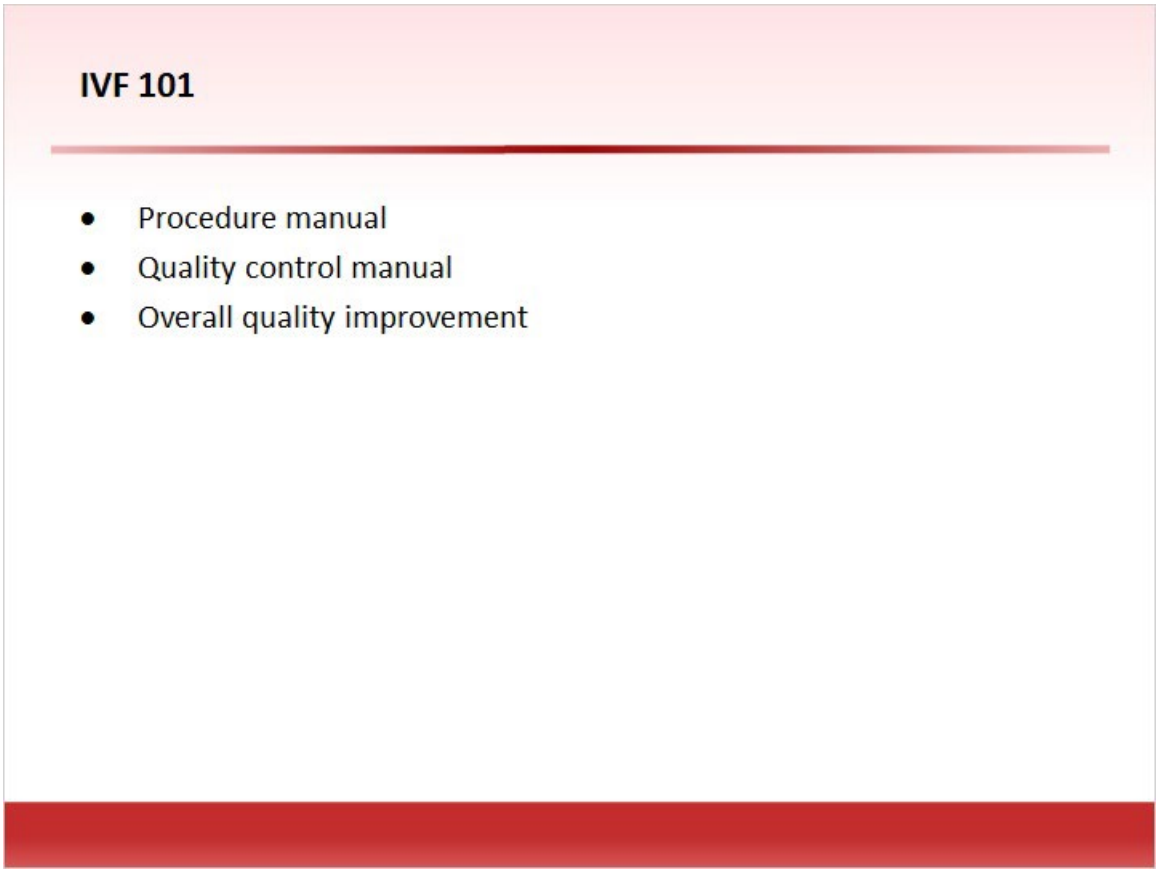
1.45 IVF—101--Endocrinology



Notes:

IVF laboratories located in hospitals and near medical centers often send patients' blood samples to the affiliated clinical reference laboratories to be analyzed. Other centers, particularly private centers, may choose to perform hormone analyses in-house. However, labs choosing to do in-house analyses of hormones should maintain all appropriate QC and standards and proficiency testing to ensure correct result reporting.

1.46 IVF 101



Notes:

All equipment in the IVF laboratory should include a Procedure Manual that details how to use the piece of equipment, including troubleshooting and all maintenance details. The laboratory should also detail all QC testing for each piece of equipment and regular review of all QC testing results.

In addition, the laboratory should have a plan for reviewing all QC data and proposing improvements in procedures and outcomes that are monitored by personnel.

The bottom line for all quality control and maintenance for all equipment is that if the process and data are not written down, then it didn't happen. Review of all testing, data, processes, and equipment is ultimately the laboratory director's responsibility.

1.47 Thank you!

Notes:

Thank you for participating in this educational activity.