Chapter 1

Facilities for Embryo Culture

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1.1 Background

Cell culture laboratories in general require facilities with very high standards of hygiene and air quality. Maintaining a Grade-A environment (ISO 4 or above) in these laboratories is usually necessary with respect to particle counts and airborne microbial colony forming units. This minimizes the risk of contamination and permits cell culture for prolonged periods of time.

In vitro fertilization (IVF) laboratories also require high standards of cleanliness, but in addition, special requirements add an element of increased complexity. For some aspects, compromises must be made between the most desirable standards and what is practically possible. This refers particularly to the necessity for close proximity to the operating room (OR) where oocyte retrievals and embryo transfers are performed, as well as other outpatient surgery such as testicular biopsies. The sensitive nature of the oocytes and embryos places certain restrictions on ventilation and disinfection. The relatively short culture period in IVF on the other hand allows for somewhat greater flexibility while other aspects place certain restrictions on attainment of Grade-A hygienic standards. This pertains particularly to temperature sensitivity and very low tolerance to toxic compounds, such as volatile organic compounds (VOCs) which, for example, places restrictions on the use of disinfectants such as alcohol (Morbeck 2015; Mortimer 2005).

The aim of this chapter is to describe the facilities and layout of an IVF laboratory and how the requisite quality and hygiene standards are met while conforming to applicable regulations.

The chapter will be organized according to the following sub-headings:

- General aspects of air quality
- Hygiene
- Layout of the laboratory and communication with the OR
- Laboratory storage
- Safety in the IVF laboratory
- Security for the IVF laboratory
- Gas and electrical power supply.

1.2 General Aspects of Air Quality in the IVF Laboratory

Human embryo culture is highly sensitive to airborne contaminants – particulate, microbial and organic – and requires high standards of air purity and quality. Therefore, it is necessary to place high demands on facilities for assisted reproductive technology (ART) laboratories to ensure optimal culture conditions. Some countries place strict demands through legislation and formal regulation whereas others work through guidelines. For example, since 2004 the European Union has had a directive in force to ensure high standards in ART laboratories (Hreinsson and Kovačič, 2019). There is also a wide consensus within the ART community that air quality in the IVF laboratory needs to meet certain criteria and that contaminants must be kept to a minimum (Esteves & Bento 2016; Mortimer et al., 2018). For a discussion on ISO classes of air quality vs. the GMP A–D classes, see Guns and Janssens (2019).

In general, the ART laboratory can be compared to an OR in terms of air quality and should be supplied with HEPA (high-efficiency particulate absorption) filtered air to achieve a comparable level of cleanliness. This requires at least ISO class 7 for air quality (see Table 1.1). It is recommended that the laboratory should be located in the middle of the facilities so that the laboratory does not lie adjacent to the outside walls or windows of the building. In essence, this means maintaining the “room within the

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Table 1.1 Main criteria which must be met when designing and operating an IVF laboratory

Parameter	Details
Particulates	Maximum of 352,000 particles $\geq$0.5 µm
Airborne microorganisms	Maximum of 10 colony forming units per m^3 Maximum of 5 colony forming units per 90 mm settle plate
VOCs	<60 ppb
Air changes	15–20 per hour
Overpressure	Minimum of +30 Pa
Temperature	22–24°C
Humidity	40–50%
Air filtration	HEPA
Carbon and potassium permanganate filtration	Built into the HVAC system

For further details, see Mortimer et al. (2018).

room" principle, facilitating the maintenance of air purity and decreasing the risk for contamination.

It is well established that VOCs can have a detrimental effect on human embryo culture (Esteves & Bento, 2016). Materials used when building the laboratory, including flooring and wall covering as well as furniture and equipment, should have low off-gassing rates, and the entire laboratory in new or renovated facilities should be allowed a burn-off period of several weeks before being taken into use.

The HVAC system (heating, ventilation and air conditioning) is highly important and usually the laboratory and OR run on a separate, isolated system. Typically the HVAC system is installed during construction since most buildings do not have sufficient capacity in the regular systems. By including carbon and potassium permanganate filters in the ventilation system, toxic VOCs can be absorbed and removed from the air flowing into the laboratory. All filters in the HVAC system must be monitored and maintained regularly with replacements scheduled as necessary. To reduce the burden on these filters, the location of the air intake for the HVAC system must also be considered. Usually these are placed on the top of the building to avoid car exhaust and other contaminants which can be a problem in large cities. In hospitals, helicopter platforms on the roof of the building may also create complications and need to be taken into consideration.

The number of air changes in a room of ISO class 7 should be 60–150 per hour. However, IVF laboratories usually aim at 15–20 air changes per hour with only part of the air flow coming directly from the air intake and the rest being recirculated. This is because too high air flow rates may impact temperature stability of heating zones and in culture dishes and also increase evaporation rates from culture droplets during culture media preparation. Here, a balance must be found between air quality and maintaining general physical parameters within acceptable ranges. Laminar flow hoods are to be used for preparation of the dishes to counteract this potential problem.

Maintaining humidity levels between 40% and 50% in the laboratory is optimal since lower levels will increase microdroplet evaporation whereas higher levels may induce formation of mold. There is also the issue of maintaining a healthy environment for staff. The same applies to temperature in the laboratory which should be kept at a level ensuring a comfortable environment. A high room temperature is not optimal for incubators which are designed to run at a temperature differential of approximately 13–15°C above ambient surroundings.

It is standard for all clean rooms to have a positive pressure differential from the laboratory to their surroundings. This will reduce the risk of contaminants entering the laboratory from the outside. This pertains to general air contaminants as above but is also relevant to the OR since the disinfectants and cleaning agents necessary for optimal patient care must not carry over into the laboratory. Each laboratory needs

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to be able to perform tests to determine particle counts, microbial contamination, and VOCs in the air in the facilities.

Typically, an ART clinic operates as an outpatient clinic with minor surgery under local anesthesia only, the great majority of patients being ambulatory. Although emergency provisions and access for persons with disabilities should be possible in the facilities, this is usually feasible in office buildings as well. If general anesthesia and more invasive surgery are to be performed in the facilities, this places additional demands on patient monitoring, emergency access and around the clock availability. When planning general anesthesia and more invasive surgery, separate demands must be met. This chapter does not address the issues raised in these cases

1.3 Hygiene

When operating a clean laboratory with adequate ventilation and filtration, it becomes clear that the staff is a major source of particle contamination in the IVF laboratory. Therefore the use of non-shedding clothing and hair covers is mandatory in the IVF laboratory. Gloves are used for personal protection when working with body fluids, such as sperm samples or follicle aspirates. In addition, the use of masks is recommended in certain instances, such as when performing embryo biopsy and tubing for preimplantation genetic testing. Hand hygiene must be observed at all times since use of gloves is often considered inconvenient and possibly risky when working with fine manipulation, as in the IVF laboratory setting. Rings, wrist watches, and jewelry should not be worn in the OR or in the laboratory, as is the general hospital standard. In addition to soap and water, hand disinfectants which do not emit VOCs should be used.

Use of alcohol for disinfection in the laboratory is normally discouraged because of the potential toxic effects associated with it. Although using alcohol on work surfaces in a well-ventilated laboratory after all cell culture work has been performed may be in order, especially when using incubators with closed gas circulation, it is generally avoided. Instead quaternary ammonium compounds which do not emit VOCs can be used, or hydrogen peroxide solutions which do not leave a residue after use. Each laboratory needs to verify and validate the cleaning methods used as well as establishing general hygienic standards, finding a balance between maintaining good hygiene and avoiding infections while minimizing any potential toxic effects to the gametes and embryos.

All work areas must be thoroughly cleaned both in terms of pathogens but also to avoid DNA-contamination in embryology laboratories performing preimplantation genetic testing. Supplies in cardboard boxes must never enter the laboratory (see 1.5 Laboratory Storage). Administrative work should be minimized in the laboratory and OR covers for computers should be considered.

To facilitate cleaning of the rooms, sealed mats with rounded corners should be used for the floor and the ceiling should be sealed with rounded corners as well. Lights should be built into the ceiling. Proper floor material, such as metal sheets, should be used where liquid nitrogen is handled. If windows are exposed to direct sunlight, dark shades need to be applied.

The laboratory and OR should be tested for airborne microorganisms using sedimentation plates or specialized measuring devices to evaluate the standard of the air quality in this respect.

The issue of lighting in the IVF-laboratory often comes up. As a general rule, low intensity lighting is recommended while maintaining a safe and secure working environment is paramount. When considering light sources in the IVF-laboratory, it is useful to consider that oocytes and embryos are kept in incubators most of the time and are not exposed to ambient light. The majority of light exposure to the ovum and embryo occurs during microscopic examination and micromanipulation. The light sources in the microscopes are typically halogen light bulbs from 30 watts up to 100 watts and the operator can minimize this exposure by using the lowest possible intensity required to perform the work (Ottosen et al., 2007). Use of colored light filters can be considered as well.

Ambient light should be reduced and direct sunlight cannot be allowed in the IVF laboratory.

1.4 Layout of the Laboratory

As mentioned previously, there are many advantages if the laboratory is designed as a “room within the room,” i.e., having a corridor or other rooms between the laboratory and the outside walls. The laboratory should also not open up directly to the surrounding clinic but should be entered through an air lock or

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vestibule used for hand washing, change of footwear, donning masks, and hair covers etc. A changing room for staff should be located in the vicinity of the laboratory and laboratory coats should be available for guests. As a rule, sinks for hand washing and for cleaning instruments should not be located within the clean room laboratory. These can be placed in the vestibule.

The embryology laboratory should be located next to the OR to facilitate safe and swift transfer of oocytes and embryos. Although the OR should be accessible from the laboratory by a door, transfer of follicular aspirates and embryo transfer catheters, should preferentially be achieved through a window or hatch, which can be closed between operations. By locating the workstation by the window on the laboratory side, the embryologist and physician can work close to each other and tubes with follicular aspirates or embryo transfer catheters can easily and quickly be transferred between the OR and laboratory. This minimizes staffing and also maximizes patient integrity during the procedure while improving quality and ease of operations.

Each workstation in the laboratory should allow for a computer station to facilitate registration in the Electronic Medical Records system (EMR) and an electronic witnessing system should also be incorporated in the work station to minimize risk for identification errors (see details in Chapter 7).

It is often considered practical to place the incubators in the center of the laboratory with the gas lines coming from the ceiling. Separate work stations can then be located around the periphery of the laboratory allowing for easy access to the long-term culture incubators. The laboratory should be planned in such a way that the distance from any given working station to the long-term culture incubator is as short as possible to minimize walking with dishes containing oocytes or embryos.

Each work station in the laboratory should be equipped with a laminar flow hood and a zoom stereo microscope. The specifications for these microscopes may vary, as typically low magnification is needed for oocyte retrieval and embryo transfer whereas higher magnification may be required for more advanced manipulation and evaluation. All embryo evaluation is performed at the inverted microscope (or through time lapse monitoring) at 200× magnification (ESHRE Guideline Group et al., 2016). Also the work stations should be separated with respect to working on warm vs. room temperature surfaces. For example it is preferable that vitrification work stations are located closer to liquid nitrogen supply than the intracytoplasmic sperm injection (ICSI) or embryo biopsy workstations.

While vitrification and cryopreservation are performed in the embryology and andrology laboratories, long-term storage of samples may be better achieved in a separate facility. This becomes particularly pertinent if a large number of samples are stored, as in larger laboratories with gamete banks or those performing fertility preservation banking for oncology patients. Here, ease of access should be considered for filling the nitrogen supply tanks because of the large volumes of liquid nitrogen required. Storing numerous large dewars in the IVF laboratory itself is not recommended.

The andrology laboratory should be located in the vicinity of the embryology laboratory. The general principles as specified above also apply there. It is useful to have direct access between embryology and andrology and in smaller facilities an "andrology corner" can be a part of the embryology laboratory. The work stations in andrology are by necessity different with Class II flow hoods being preferable and with a small centrifuge included in the hood. It is highly recommended to plan andrology and the number of work stations in such a way that one sample can be processed at a time in a dedicated flow hood and with a dedicated andrologist while using electronic witnessing systems.

1.5 Laboratory Storage

Every IVF laboratory requires a dedicated storage area for supplies. In order to maintain a high standard, supplies must be unpacked outside the laboratory itself and cartons must not be brought into the IVF laboratory. Ideally, two storage areas should be used – the first for supplies coming in and the second for batches which have been tested and/or approved for use. This setup allows for optimal supply management ensuring that the first-come-first-use principle is adhered to and also that only approved materials are in use in the laboratory.

1.6 Safety in the IVF Laboratory

The IVF laboratory uses liquid nitrogen for cryopreservation and cryostorage in vacuum isolated dewars. In many cases large volumes of liquid

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nitrogen are handled by the laboratory, often hundreds of liters. This raises two safety issues.

Firstly, liquid nitrogen expands 696 times when it evaporates and atmospheric oxygen may be depleted if the facilities are not well ventilated. Large volumes of nitrogen gas may be emitted in cases of tank failure. Therefore all facilities with nitrogen storage must be equipped with an oxygen depletion alarm for safety. The alarm should have both visual and audible signals and should be sent through the general alarm channels (see Section 1.7 Security in the IVF Laboratory). In large storage facilities, for safety, access is permitted only for at least two persons simultaneously.

Secondly, because of the extremely low temperature of the liquid (−196°C), working with liquid nitrogen confers certain risks. Thermoisolated gloves, goggles, and isolation aprons as well as shoes which completely cover the operators feet should be available and used.

Most IVF laboratories are from time to time required to handle samples from patients with known viral infections. Personal protection in the form of gloves and visor should be considered and processing of these samples should be performed separately from other samples. At least, these samples should be separated in time from other samples and through cleaning performed afterwards. Good laboratory practice includes using gloves when processing sperm samples, identifying oocytes from follicle fluid and during embryo transfer.

It is advisable to consider ergonomics when planning the IVF laboratory. Work stations with adjustable height allow for varying working positions and also for adapting to optimal working positions for each individual. Ergonomically adjustable microscopes and other equipment allow for long-term working in the IVF laboratory while maintaining good posture and health (Hreinsson & Borg, 2019).

1.7 Security in the IVF Laboratory

All areas where cells and tissues are handled and/or stored need to maintain strict access control. This is easy to achieve using modern systems where access is coupled with an individual's identification card. This affords the added advantage of complete traceability of who has entered the laboratory and when. All visitors must sign confidentiality agreements and must be accompanied by a designated supervisor for the visit. Facilities should be equipped with an intruder alarm after hours.

IVF culture and cryostorage is maintained around the clock, whereas clinical procedures are usually performed during daytime. Therefore it is essential that all critical equipment is linked to an external alarm system with separate monitoring of critical parameters such as temperature and gas levels. This includes incubators, nitrogen tanks, refrigerators, gas manifolds, and others. Calibration of sensors is performed according to international standards. Staff may take turns carrying a portable alarm or a single person may take responsibility of monitoring alarms. Alarms must be monitored and tested regularly and trends in critical parameters, such as temperature, should be analyzed. Laboratories with large volumes of samples in cryostorage do well to have reserve tanks in case of tank failure as such an event only allows for only a few hours response time before samples are at risk of being compromised (Pomeroy & Marcon, 2018). Typically the alarm systems monitor temperature, both in incubators and also in the nitrogen dewars. However, a weight-based monitoring system may also have advantages and can be considered (Michaelson et al., 2019).

The issue of gas phase vs. liquid phase storage at nitrogen temperatures often comes up. Typically samples of larger volume, such as sperm samples, are stored in the gas phase as this is considered safer regarding possible contamination. Smaller volume samples, such as vitrified oocytes and embryos, can be stored in the gas phase of liquid nitrogen and this is the rule during transport. However, many embryologists are concerned that this will increase risk of premature warming during handling so long-term liquid nitrogen storage is often recommended for these samples.

1.8 Gas and Electrical Power

The gas manifold and nitrogen supply are usually located outside the IVF laboratory and on the ground floor to allow for cleanliness as well as to ensure easy access and delivery.

The IVF laboratory typically requires three types of gas which should be supplied through stainless steel tubing, not copper. These are CO_2, N_2 and a gas mixture for culture (6% CO_2, 5% O_2 and 89% N_2, for example). The gas manifold should be included in the alarm system and located outside of the laboratory itself. The gas lines do not require high pressure but if the laboratory is located several levels above the

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ground floor, this must of course be taken into consideration. Redundancy and automatic switching to full gas tanks is necessary.

In addition to the laboratory gas supply, the OR requires medical gases, such as oxygen.

Any IVF laboratory will experience power outages from time to time. Therefore it is important that critical equipment is connected to an uninterrupted power supply such as a UPS battery backup supply. A local generator may be in place for long-term power shortages, but the battery backup must be in place for uninterrupted power. It should be considered that some equipment in the IVF laboratory, such as flow hoods, are sensitive to power surges and may be negatively affected by power outages even though they are not part of the culture system itself.

1.9 Conclusions

Facilities for the IVF laboratory and adjacent ORs need to be carefully planned and designed to allow for safe and efficient workflow. One of the main areas of focus is ensuring high standards of air quality to allow for a safe culture environment. Provisions for technical, safety and security measures for continuous monitoring, culture, and storage of gametes and embryos need to be made and upheld in all ART facilities.

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