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Low-air-pressure clean room system: A flexible, high-quality model for assisted reproduction laboratories

Minh Tam Le[1,2], Trung Van Nguyen[1], Thai Thanh Thi Nguyen[1], Hong Nhan Thi Dang[1], Quoc Huy Vu Nguyen[2]

[1]Center for Reproductive Endocrinology and Infertility, [2]Department of Obstetrics and Gynecology, Hue University of Medicine and Pharmacy, Hue University, Hue, Vietnam

Objective: This study aimed to develop a configurable clean room paradigm with low air pressure for assisted reproductive technology (ART) clinics and demonstrate the concept's efficacy using *in vitro* fertilization (IVF) treatment.
Methods: A high-standard clean room system with positive pressure (13 Pa) was built using accessible materials and equipment for ART laboratories. Methods for controlling and evaluating the clean room's characteristics were developed and implemented for quality assessment and calibration to maximize efficiency. The feasibility of the flexible clean room concept was assessed by analyzing the key performance indicators of embryo culture and IVF treatment.
Results: After 3 weeks of testing, the concentration of particles ≥0.5 μm was 6.04 times lower than the International Organization for Standardization (ISO) class 5 standard (3,520 particles/m^3) in the IVF laboratory. Air pressure, noise, temperature, and humidity were controlled stably and appropriately. Five days after installation and handover, the volatile organic compound concentration dropped to 0.00 ppm. With blastocysts and a respectable blastocyst rate, embryonic culture with female patients younger than 40 matched the criteria (63.5% and 38.9%, respectively). After vitrified blastocysts were transferred, the pregnancy and implantation rates were 58.5% and 36.2%, respectively, demonstrating a high degree of treatment success.
Conclusion: Our customizable, high-quality, low-air-pressure clean room model can be implemented to achieve positive outcomes for infertility treatment.

Keywords: Assisted reproduction; Environment, controlled; Fertilization in vitro; Laboratory model

Introduction

In assisted reproductive technology (ART), embryos are created through *in vitro* fertilization (IVF) by manipulating and processing gametes outside the body [1]. To minimize adverse consequences, gametes and embryos should be maintained in an artificial environment, simulating the oviduct and uterus [2]. According to recommendations and practice guidelines, ART laboratory conditions, such as temperature, humidity, and air quality, must satisfy strict standards [3]. A positive pressure differential (30 to 35 Pa) is recommended between the IVF laboratory and adjacent rooms, including the operating room and cryopreservation laboratory [4,5]. Although this pressure differential helps prevent the spread of dust particles, the high volumes of moving air required to maintain it can pose challenges in regulating other air quality factors.

Temperature and air humidity are crucial variables that significantly impact the effectiveness of IVF therapy. The ambient temperature of the embryo culture droplets must be maintained at 37±0.2 °C [2,4,5] to prevent damage to the oocyte and embryo [6-8]. During the manipulation and cultivation of embryos and gametes, incuba-

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Corresponding author: **Minh Tam Le**
Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue, Vietnam
Tel: +84-989-228779 Fax: +84-234-3822173 E-mail: leminhtam@hueuni.edu.vn

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tors and surface heaters aid in maintaining this temperature level. To prevent overheating, these devices operate optimally between 22 and 24±2 ℃. Additionally, elevated air humidity and temperature increase the growth of bacteria and fungi. Consequently, specific temperature and humidity levels have been implemented to guarantee comfortable working conditions and high-quality air. For IVF procedures, room temperatures of approximately 26 ℃ and relative humidity levels of less than 50% are typically optimal [6]. An ART laboratory must ensure that dust levels are below the permissible range to achieve efficient embryo culture. The International Organization for Standardization (ISO) 14644-1 standard for clean rooms defines a clean room as an area where the concentration of particles in the air is controlled. The air intake and outlet must be handled to reduce the amount of dust in the room [9]. Accordingly, practical instructions for constructing and operating support laboratories recommend that the air standards fulfill ISO class 5 dust criteria, as specified in Table 1 [5,6,10]. Although certain principles apply to designing and implementing air systems for ART, they are challenging to apply in practice. A heating, ventilation, and air conditioning (HVAC) system paired with a high-efficiency particulate air (HEPA) filter is a crucial element for ensuring air quality [5,6]. However, these systems are highly costly and inflexible, posing barriers for application in ART centers in terms of budget and clean room size.

In this study, we developed an innovative, cost-effective clean room solution with lower pressure that meets the rigorous criteria for ART laboratories and is highly adaptable in terms of installation, operation, and maintenance. This system aimed to achieve a temperature range of 24 to 26 ℃, a dust level of ISO class 5 in the embryo culture area and ISO class 6 in the operator area, and low levels of microbiological contamination and volatile organic compounds (VOCs) at 0 ppm [3]. Embryo culture results and pregnancy rates following embryo transfer were used to assess the effectiveness of IVF treatment.

Methods

In 2020, the Hue Center for Reproductive Endocrinology and Infertility at Hue University of Medicine and Pharmacy constructed, implemented, and installed a new clean room laboratory. This model was built using locally accessible materials to meet the ART laboratory's requirements for clean room air quality.

This study was approved by the Ethics Board of Hue University of Medicine and Pharmacy. Informed consent was waived by the board because the study's design was based on the key performance indicators (KPIs) of the laboratory's methodologies.

1. Organization of functional regions

The embryo culture laboratory was located at the center's deepest area. It was isolated from the operation room to avoid risks while maintaining connectivity with other functional spaces, including the room for oocyte retrieval and embryo transfer (operation room), the andrology laboratory, and the cryopreservation laboratory. We designed a path through progressively cleaner rooms to reach the IVF laboratory. The entrance order was as follows: surgical dressing room, andrology laboratory, cryopreservation laboratory, and IVF laboratory. Similarly, the entrance to the operation area included the surgical dressing room and the room for oocyte retrieval and embryo transfer.

The rooms were constructed within the existing metal structure. Rather than painting the walls, they were covered with polyvinyl chloride wall panel sheets to minimize dust, VOC emissions, and construction time. Steel-framed partitions were employed to divide the available space into smaller rooms, based on the purpose and size of each room. A Vinh Tuong skeleton and Duraflex panels (60×60 cm) were used in ceiling systems, while antibacterial, dust-resistant vinyl sheets covered the floor.

Our project was designed to handle approximately 500 IVF cycles

Table 1. Clean room classifications according to ISO 14644-1 [10]

Classification	Particles (/m^3)					
	≥ 0.1 μm	≥ 0.2 μm	≥ 0.3 μm	≥ 0.5 μm	≥ 1 μm	≥ 5 μm
ISO 1	10					
ISO 2	100	24	10			
ISO 3	1,000	237	102	35		
ISO 4	10,000	2,370	1,020	352	83	
ISO 5	100,000	23,700	10,200	3,520	832	
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7				352,000	83,200	2,930
ISO 8				3,520,000	832,000	29,300
ISO 9				35,200,000	8,320,000	293,000

ISO, International Organization for Standardization.

each year. The IVF laboratory measures 22 m^2, the oocyte retrieval and embryo transfer room is 20 m^2, the andrology laboratory is 12 m^2, and the cryopreservation laboratory is 10 m^2.

2. Improved air distribution in the ceiling

A filtration unit (type 6/6; ADS Laminaire) was modified to convert the horizontal airflow into a top-down air stream supply. Air filtration units were housed in an aluminum box (dimensions 70×70×200 cm) and supplied to the IVF and cryopreservation laboratories via a polyester insulation flexible duct D250 (Se Han Flex Corp.). This flexible duct was connected to the HEPA filter unit to provide clean air to the rooms. These devices had a maximum airflow capacity of 1,200 m^3/hour. The equipment was mounted on the ceiling of the cryopreservation laboratory.

The recovered air vents were positioned 40 cm above the floor in three corners of the room, with an additional vent on the ceiling near the entrance. The ceiling-mounted ventilation fan, model BPT10-15 (Nanyoo), which has a capacity of 21 W and a flow rate of 150 m^3/hr, was used to circulate air in the IVF laboratory and direct it toward the outlet located just in front of the filtration system.

The filtration system comprised a G4 pre-filter and a HEPA filter. The G4 pre-filter, measuring 405×617×20 mm and demonstrating 90% to 95% effectiveness in filtering dust particles larger than 5 μm, was tailored and fitted into the type 6/6 filtration unit. The HEPA H14 filter, sized 610×610×69 mm with an inlet differential of 150 to 250 Pa, captures 99.995% of dust particles that are 0.3 μm or larger.

This technique was employed to maintain a positive pressure within the IVF laboratory. Filtration units operated in conjunction with the recovered air vents, delivering a 170-Pa positive pre-pressure to the HEPA H14 filter. This airflow ensured a 13-Pa positive pressure relative to the ceiling area of the IVF laboratory. Compared to the adjacent cryopreservation laboratory and the oocyte retrieval and embryo transfer laboratory, the positive pressure in the IVF laboratory was 10 Pa higher.

3. Temperature control system

Two air conditioners (Toshiba 2 HP RAS-H18U2KSG-V) installed above the ceiling regulated the temperature in the IVF laboratory. Each unit had a capacity of two horsepower (18,991 kJ). Alternating the use of each air conditioner promotes a longer service life and simplifies maintenance and repairs. A ceiling temperature of 21 ℃ supports the maintenance of the laboratory's temperature range of 24 to 27 ℃.

4. Sterilization and VOC reduction

The solution to these challenges involved using ultraviolet (UV) radiation for periodic disinfection. We installed seven UV lamps at various locations. Two UV lights were mounted on the ceiling of the IVF and cryopreservation laboratories, two additional lamps were positioned in the middle of the IVF laboratory, and one lamp was placed in the center of the cryopreservation, andrology, oocyte retrieval, and embryo transfer laboratory. The UV lights were activated daily using a timer controller from 6:00 PM to 8:00 PM. Additionally, a G4 pre-filter was combined with an activated charcoal filter to absorb some of the VOCs.

5. Measurement and control of the environment and air quality of the IVF lab

Differential pressure gauges (Magnehelic 2300-60PA; Dwyer Instruments) were used to measure airflow under positive pressure. A range from −30 to 30 Pa was selected to monitor the pressure in these regions. We conducted a temperature survey of the areas using a 2110T meter (RI), which has an accuracy of ±0.2 ℃ and a measuring range from −25 to 40 ℃. Humidity levels were monitored using the FujiE HM-620EB dehumidifier (Fuji). This unit has a daily dehumidification capacity of 20 L and features a 6-L wastewater tank. It was consistently set to maintain 50% relative humidity. The level of dust was measured using an eHandiLaz Mini particle counter (Particle Measuring Systems). This system quantified particles across three size channels—0.3, 0.5, and 5.0 μm—employing a laser reader. It could detect a maximum dust concentration of 2,000,000 particles per cubic foot for particles measuring 0.3 μm. The MiniRAE Lite Portable Handheld VOC Monitor (Honeywell Analytics) was used to measure VOC concentrations ranging from 0 to 5,000 ppm. For concentrations between 0 and 999.9 ppm, the resolution is 0.1 ppm, and for concentrations from 1,000 to 5,000 ppm, the resolution increases to 1 ppm. Microbiological contamination testing was conducted quarterly at Hue University of Medicine and Pharmacy, following established protocols and utilizing the Koch procedure to acquire and isolate microorganisms. Results from the microorganism detection tests were collected from September 2020 through December 2023.

6. Quality control of embryo culture and treatment

To assess the effectiveness of the model, data on blastocyst culture and transfer outcomes at our center from September 2020 to December 2021 were compared with previously collected data from January 2019 to December 2019. This analysis included patients under 40 who underwent IVF and exhibited no signs of diminished ovarian reserve. Patients with diminished ovarian reserve, as determined by the retrieval of fewer than four metaphase II oocytes, were excluded. Data on embryo culture were recorded, including the fertilization rate and blastocyst formation rate, and evaluated on day 5 (116 to 118 hours post-injection) using the Gardner criteria in order to test the model's applicability. This analysis involved assessing the

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blastocyst cavity's expansion and the amount and integrity of trophectoderm cells and internal cell mass (ICM). Good embryos were defined as blastocysts with ICM, trophectoderm grade AA, and a blastocyst cavity volume greater than 70%.

Results

1. The air quality of the ART center

Positive pressure and a filtering device were employed to maintain air quality before air was introduced into the critical area. The ceiling system functioned as an airlock, ensuring that the IVF laboratory consistently maintained a pressure greater than 10 Pa relative to adjacent rooms. The cleanliness level increased progressively before entering the IVF laboratory, and the particle index remained below the thresholds set by the ISO class 5 standard. After 3 weeks of testing, the concentration of airborne particles measuring 0.5 μm was recorded at 582 particles/m^3 in the IVF laboratory. Specific data can be found in Table 2.

When the embryo culture area was operational, the temperature and humidity were consistently maintained at 24.3 to 26.2 ℃ and 50%, respectively. This range of temperature and humidity is considered optimal for IVF laboratory operations. Five days after the handover, the VOC level in the IVF laboratory was measured at 0.00 ppm. The IVF laboratory exhibited a comparatively low microbiological contamination index of 19.2±17.3 colony-forming units (CFU)/m^3, in contrast to the operating room (83.3±78.0 CFU/m^3) and cryopreservation laboratory (47.8±17.3 CFU/m^3). Furthermore, no documentation of microorganisms within the incubator was detected. The data are detailed in Table 3.

2. Fertilization rate and blastocyst development rate

After intracytoplasmic sperm injection, the fertilization rate reached 70.3%, surpassing the competency threshold set by the key

Table 3. Changes in temperature, microbiological contamination, humidity, and VOCs

Variable	Temperature (℃, number of counts = 64)	
	Mean ± SD	Min to max
Location		
Outside	31.23 ± 24.2	27 to 36
Andrology lab	26.84 ± 0.86	24.5 to 29.5
Cryopreservation lab	24.85 ± 0.87	23.4 to 27.9
Ceiling	22.47 ± 1.18	20 to 25.3
IVF lab	26.70 ± 0.91	24.2 to 28.7
Δt_Andrology lab_outside	4.39 ± 2.84	−2.1 to 10.3
Δt_Cryopreservation lab_outside	6.38 ± 2.80	−0.9 to 12.5
Δt_Ceiling_outside	8.76 ± 2.75	3 to 15.5
Δt_IVF lab_outside	4.53 ± 2.34	−1.7 to 8.3
Δt_Ceiling_IVF lab	4.23 ± 1.22	1.2 to 7.9
Microbiological contamination		
Operating room (n = 6, CFU/m^3)	83.3 ± 78.0	16.0 to 234.0
Cryopreservation lab (n = 6, CFU/m^3)	47.8 ± 17.3	31.0 to 78.0
IVF lab (n = 6, CFU/m^3)	19.2 ± 17.3	0 to 47.0
Incubator (n = 24, CFU/m^3)	0	0
IVF lab surfaces (n = 37, CFU/m^2)	3.0 ± 10.5	0.0 to 60.0
Noise level (dB)		
Ceiling (n = 60)	81.9 ± 1.10	80 to 85
Cryopreservation lab (n = 60)	68.18 ± 2.61	67 to 70
IVF lab	64.75 ± 1.05	64 to 67
Humidity (%, no. counting = 15)		
IVF lab	47.53 ± 4.7	38 to 58
VOCs (ppm)		
Date 1 (n = 6)	74.33 ± 19.2	58 to 110
Date 2 (n = 6)	3.32 ± 0.57	2.5 to 4
Date 3 (n = 6)	0.22 ± 0.13	0.2 to 0.4
Date 4 (n = 6)	0.05 ± 0.05	0.0 to 0.1
Date 5 (n = 6)	0.00 ± 0.00	0 to 0

VOC, volatile organic compound; SD, standard deviation; IVF, *in vitro* fertilization; CFU, colony-forming unit.

Table 2. Air quality in the functional rooms after 3 weeks of test operation

Details	Particle concentration (particles/m^3)		
	≥ 0.3 μm	≥ 0.5 μm	≥ 5 μm
Change of the number of particles according to each location			
Outside	12,004,705 ± 3,798,169	4,385,882 ± 3,893,696	189,347 ± 211,512
Andrology lab	1,410,150 ± 708,812	479,095 ± 251,481	82,700 ± 66,884
Cryopreservation lab	51,435 ± 48,615	14,448 ± 14,835	3,491 ± 4,231
IVF lab	2,202 ± 3,453	582 ± 688	220 ± 565
No. of dust particles in the IVF laboratory according to the test operation time			
Week 1	50,397 ± 49,735	12,187 ± 11,387	4,945 ± 7,819
Week 2	14,740 ± 17,191	4,450 ± 5,667	1,558 ± 1,684
Week 3	2,202 ± 3,453	582 ± 688	220 ± 565

Values are presented as mean ± standard deviation.
IVF, *in vitro* fertilization.

performance indicators for fertilization. On day 5 of culture, the formation and quality of blastocysts served as the most accurate indicators of the quality of the assisted reproduction method. Our clean room model, once operational, provided optimal environmental control and yielded exceptionally successful embryo culture outcomes. The percentage of blastocyst formation was 63.8%, and the rate of good blastocyst development was 38.9%. These results showed significant improvement compared to the laboratory's performance before the model was implemented, which were 58.6% and 31.1%, respectively (p<0.001) (Table 4).

3. The outcome of vitrification-warming blastocyst transfer

The transfer of blastocyst embryos resulted in a β-human chorionic gonadotropin (β-hCG) positive rate of 58.5% and a clinical pregnancy rate of 49.8%. Additionally, our implantation rate was 36.2%. These outcomes are superior to those of previous blastocyst transfer cycles in our old laboratory by 50.8%, 44.9%, and 31.3%, respectively (Table 5). These ratios demonstrate that our flexible clean room model is adequate for IVF treatment.

Table 4. Results of blastocyst culture in female patients ≤40 years of age undergoing *in vitro* fertilization

Factor	Blastocyst culture cycles		*p*-value
	New model (n=153)	Control (n=144)	
Female age (yr)	31.73±4.05	31.71±3.93	0.971
No. of oocytes	18.22±9.02	17.46±8.51	0.454
No. of MII oocytes	14.79±7.79	13.65±6.52	0.170
No. of normal fertilization	10.39±6.77	10.27±5.65	0.862
No. of cleavage embryos	10.33±6.62	10.18±5.60	0.838
No. of blastocyst formation	6.63±4.96	5.97±4.17	0.216
No. of good blastocysts	4.05±3.72	3.16±2.93	0.023
Normal fertilization rate	70.3 (1,590/2,263)	74.7 (1,468/1,965)	<0.001
Cleavage rate	99.4 (1,580/1,590)	99.9 (1,466/1,468)	0.026
Blastocyst formation rate	63.8 (1,014/1,590)	58.6 (860/1,468)	<0.001
Good blastocyst rate	38.9 (619/1590)	31.0 (455/1,468)	<0.001

Values are presented as mean±standard deviation or percentage (number/total number).
MII, metaphase II.

Table 5. Results of blastocyst transfer cycles in female patients ≤40 years of age

Factor	Blastocyst transfer cycles		*p*-value
	New model (n=233)	Control (n=118)	
Female age (yr)	32.07±3.70	31.47±3.78	0.162
No. of blastocyst transfers	1.70±0.84	1.84±0.47	0.041
No. of good blastocyst transfers	1.15±0.64	1.04±0.60	0.122
β-hCG positive	58.5 (134/233)	50.8 (60/118)	0.248
Clinical pregnancy	49.8 (116/233)	44.9 (53/118)	0.396
Implantation rate	36.2 (143/395)	31.3 (68/217)	0.232
Multiple pregnancy	11.6 (27/134)	30.2 (16/53)	0.135
Miscarriage rate	7.7 (18/134)	5.7 (3/53)	0.135

Values are presented as mean±standard deviation or percentage (number/total number).
hCG, human chorionic gonadotropin.

Discussion

A positive pressure system must be maintained in a highly efficient manner [5]. Air pressure variations are produced by introducing air movement channels through clefts, leading to room leaks [5]. In this model, the generation of positive pressure was powered by the pressure differential between the air supplied by the filtration unit with the HEPA filter installed in the ceiling and the air provided by the ventilation fan [11]. The specific technology used for delivering clean air was an HVAC system, which required a distance from the IVF laboratory and a direct connection to the outside air supply. These characteristics make HVAC systems expensive and pose challenges in maintaining stable temperature, humidity, and air pressure, especially during significant changes in the external environment, such as harsh weather conditions [12]. While a range of 30 to 35 Pa was recommended in prior standards for constructing clean rooms, other published research has found that favorable outcomes can still be achieved even with a modest pressure differential. Our model of a positive pressure system was installed adjacent to the IVF laboratory on the ceiling of the cryopreservation laboratory. The model's supplied air flow diagram is presented in Figure 1. The airflow path was shortened, the pressure recovery time was minimal, and the pressure differential between rooms could remain over 10 Pa at all times. Specifically, a pressure differential of 13 Pa for the IVF laboratory was established for our model. The IVF laboratory had a minimum air pressure of 13 Pa, which enabled it to maintain a pressure differential with adjacent areas, including the cryopreservation laboratory (5 Pa) and operating room (10 Pa). This configuration is consistent with previously established clean room construction standards, which recommend that a differential of 5 to 10 Pa is sufficient to effectively prevent cross-contamination between contaminated and uncontaminated areas.

Instead of using an airlock as a buffer zone between two independent sections with uneven pressure [5], the ceiling system created a negative pressure zone between rooms with differing clean room levels. Such a layout helps to supply fresh air to the clean room that has been stabilized in the building, avoiding the influence of changing air conditions outside.

Instead of using a fully enclosed ceiling, we simply placed Duraflex panels on the skeleton of the ceiling. The installation of the Duraflex

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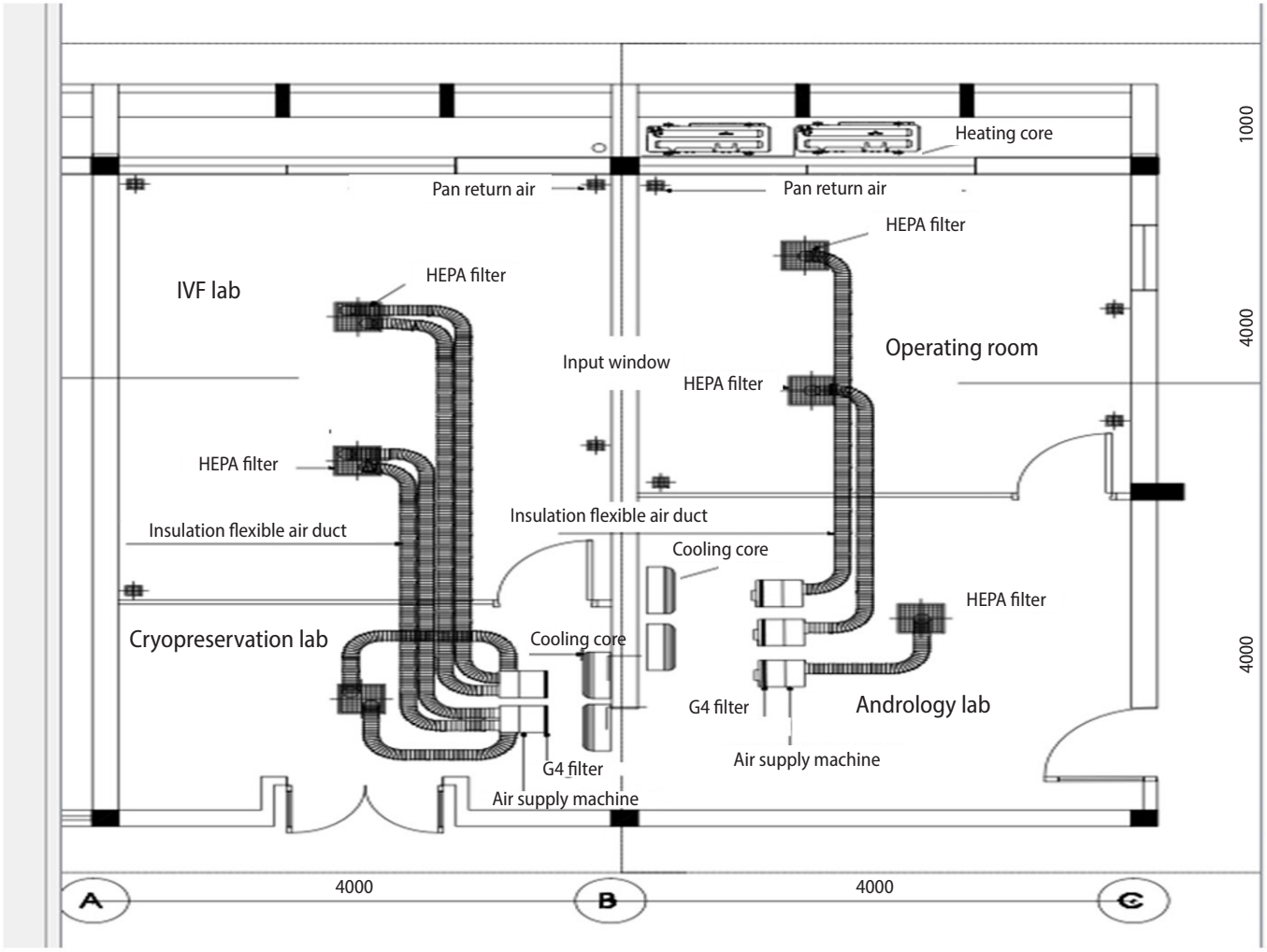

Figure 1. Layout of functional rooms in the clean zone. IVF, *in vitro* fertilization; HEPA, high-efficiency particulate air.

ceiling system was simple to perform, maintain, and upgrade, and it features an effective dust filter, sound solid absorption, and reduced vibrations. An actual image of the air handling system model is presented in Figure 2. Typically, an IVF laboratory has a complete ceiling with inlets to collect minute suspended dust particles. This design was intended to minimize tiny joints, resulting in a flat, easy-to-clean surface, and easy maintenance of high air pressure [13]. In our clean room model, a dust trap system includes the Duraflex panels and ceiling frame system, along with the positive pressure of the room. Although the air pressure does not remain as high as the 20 to 30 Pa recommended for the IVF laboratory, this setup passively collects tiny dust particles in the crevices due to the pressure difference between the clean room and the room ceiling, which are then processed by a filter air system. Consequently, the level of particulate matter in the embryo culture area and adjacent rooms rapidly decreased. After 3 weeks of testing, the average number of airborne particulates 0.5 μm in our IVF laboratory was 582 particles/m^3, 6.04 times lower than the required threshold in ISO Class 5 (3,520 particles/m^3) [11]. Our tests revealed that the noise level in the IVF laboratory was between 58 and 64 dB. This level provided comfortable working conditions for laboratory personnel, and no vibration was recorded.

A clean environment is vital for an IVF laboratory. Air in an IVF laboratory has no harmful impact on embryo growth [13], but successful infertility treatment outcomes depend on the rigorous control of airborne particles and microbiological contamination [14]. Sperm, oocytes, and embryos are sensitive and intolerant to a poor environment, particularly the number of airborne particles [15,16]. Each time the door is opened, the pressure in the room drops quickly, and conversely, it increases rapidly when the door is closed. This fluctuation in air pressure exacerbates the disturbance of airborne particles already present in the clean room, leading to an increase in particle count. Previous research on clean rooms has shown that a high-pressure differential does not effectively prevent cross-contamination

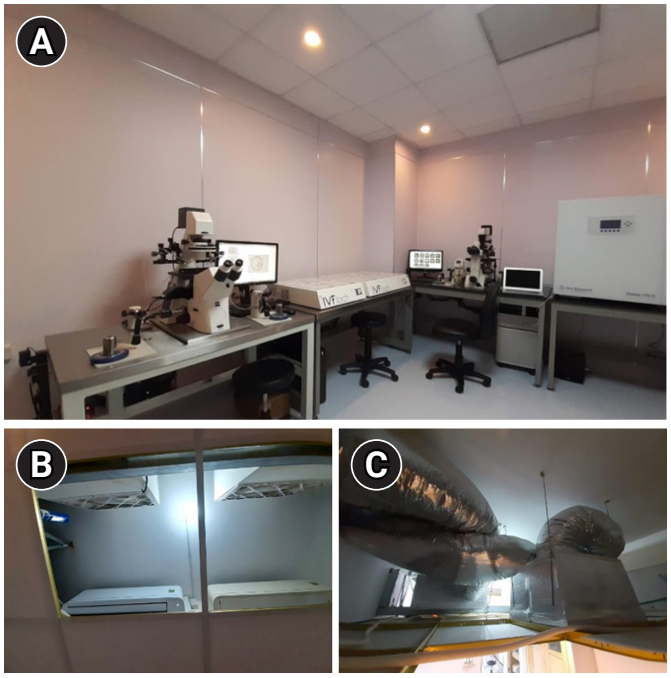

Figure 2. Clean air supply for the *in vitro* fertilization (IVF) laboratory. (A) Clean zone in the IVF laboratory. (B) In-ceiling air conditioning and filtration system. (C) Air vents that provide clean air.

when doors are opened or closed, due to inadequate pressure maintenance during these actions. In our newly designed clean room model, we implemented lower air pressure and differential pressure areas with effective airflow recovery systems to accommodate personnel access and movement. This setup significantly reduced coiled airflow. However, due to the high concentration of airborne particles sized ≥0.5 μm (4,385,882 particles/m^3), the clean room failed to meet the ISO class 8 standards when construction activities were ongoing outside. Using our model, the concentration of airborne particles sized 0.5 μm was significantly reduced, well below the threshold for ISO class 5. After implementing this clean room model, extremely low levels of microorganisms were detected in the IVF laboratory, and the incubators did not detect any microorganisms during testing. The ambient temperature and humidity were crucial to equipment operation, making embryologists comfortable and preventing the growth of microorganisms [14]. Our microbiological testing results also indicated the presence of microorganisms at low levels when implementing this model (refer to the attached appendix). The use of air conditioning in an IVF laboratory could disrupt airflow and compromise the cleanliness of the room. Consequently, we developed a ceiling air cooling system that integrates with the air filtration system. A single air conditioner with a capacity of 2 HP and 18,991 kJ proved adequate to maintain the ceiling temperature between 20 and 25.3 °C. The cooled air was channeled through an air filter unit and distributed to the IVF and cryopreservation laboratories just below the ceiling. The design of the ceiling as a cooling chamber, coupled with the continuous circulation of air, ensured that the rooms below maintained a constant temperature and appropriate dehumidification levels. During our survey, we noted that while the ambient temperature ranged from 27 to 36 °C, the temperatures in the cryopreservation and IVF laboratories ranged from 23.4–27.9 °C and 24.2–28.2 °C, respectively, with humidity levels within the recommended 50% range. This design effectively met the airflow, temperature, and humidity specifications required for the clean room in the assisted reproduction laboratory [14].

Exposure to VOCs poses significant risks to embryo development and the outcomes of IVF. While embryos can develop into blastocysts at VOC concentrations as low as 0.5 ppm, the risk of miscarriage at this level increases. At a concentration of 1 ppm, VOCs are considered directly toxic to the embryo [3]. Therefore, eliminating and reducing VOCs are of the utmost importance for IVF laboratories [14]. After construction, an IVF laboratory requires a 'burn-in' period of 10 to 28 days to effectively reduce the concentration of VOCs in the air. During the 'burn-in' period, the laboratory is kept inactive while maintaining a temperature range of 30 to 35 °C and enhancing air circulation [7]. Our model necessitated the use of VOC-emitting adhesives for wall and floor installations. Employing a burn-in process that includes an enclosed blower and a UV lamp periodically can significantly reduce the levels of VOCs in the air [1,16]. Furthermore, maintaining high air circulation, low air pressure, and low humidity enables the activated charcoal filter to function optimally, removing VOCs from the air. Consequently, handheld VOC detectors were unable to detect any VOCs after 5 days.

It is essential to recognize that the operational method played a crucial role in managing the air quality parameters within the IVF laboratory. The embryo culture outcomes reached the optimal level necessary for an IVF treatment cycle according to Alpha Scientists in Reproductive Medicine 2017 [4]. During the test operation, the air quality parameters, including dust particle index, temperature, humidity, and VOC levels, remained very stable. Thus, there was minimal need to adjust the parameter settings from the original, even when external weather conditions changed.

When our clean room model was applied to initial treatment, the blastocyst culture results for 153 patients under 40 were above the competency value. Specifically, the average fertilization rate with two pronuclei was 70.3%, exceeding the competency criterion of 65% [7]. The other criteria assessed were the blastocyst formation rate and the rate of good-quality blastocysts on day 5, which met the benchmark values for high-quality IVF laboratories at 63.8% and 38.9%, compared to the standards of 60% and 40%, respectively. These data suggest that the efficiency of blastocyst culture was im-

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proved with the implementation of this clean room approach compared to previous treatment cycles.

For the therapeutic impact of IVF cycles, we analyzed 233 vitrified blastocyst transfer cycles in women aged 40 years or younger. On average, each cycle involved the transfer of 1.70 blastocysts, with 1.15 being of good quality. The rates of β-hCG positivity and clinical pregnancy following these transfers were 58.5% and 49.8%, respectively, while the implantation rate stood at 36.2%. When compared to data from 118 blastocyst transfer cycles previously conducted in our old laboratory, where the β-hCG-positive, clinical pregnancy, and implantation rates were 50.8%, 44.9%, and 31.3%, respectively, these outcomes suggest a trend of improvement. This improvement demonstrates that the air quality, regulated by a model for a flexible, clean room, was superior and highly efficient.

In conclusion, applying our clean room model, which utilizes a lower air pressure approach, in an ART center proved to be simple and effective. The installation, operation, and maintenance processes were straightforward. Furthermore, the efficacy of the application was evident when the clean room standards met the requirements for IVF laboratories and other adjacent rooms. The results of clinical application highlighted the model's superior quality. The key performance indicators for embryo culture and treatment quality surpassed critical thresholds. Modifications to the model identified key expectations for the quality of laboratories specializing in assisted reproduction.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Minh Tam Le, https://orcid.org/0000-0001-6225-3108

Author contributions

Conceptualization: MTL, TVN, TTTN, HNTD. Methodology: MTL, TVN, TTTN, HNTD, QHVN. Formal analysis: MTL, TVN, TTTN, HNTD. Data curation: TVN, TTTN, HNTD. Funding acquisition: MTL. Project administration: MTL, QHVN. Visualization: MTL, TVN. Validation: MTL, TVN, TTTN, HNTD, QHVN. Investigation: MTL, TVN, TTTN, HNTD, QHVN. Writing-original draft: MTL, TVN. Writing-review & editing: MTL, TVN, TTTN, HNTD, QHVN. Approval of final manuscript: MTL, TVN, TTTN, HNTD, QHVN.

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