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ANALYSIS OF ENVIRONMENTAL FACTORS ON HUMAN SPERM VIABILITY IN MODERN CLINICAL REPRODUCTION LABORATORY

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ABSTRACT

This study discusses the main influence of environmental factors on sperm viability in modern clinical reproductive laboratories, which is a crucial component in successful fertilization because only live and good quality sperm are capable of fertilizing an egg. Sperm are known to be very sensitive to environmental disturbances. Based on a review of the latest scientific literature (2020–2025), this study emphasizes the importance of maintaining the incubation temperature at 37°C, the pH of the culture medium between 7.2–7.4, and regulating the concentration of CO_2 (5–7%) and O_2 (5%) gases in order to create optimal culture conditions. Imbalances such as temperature fluctuations, excessively high pH, the presence of volatile organic compounds (VOCs), and exposure to light—particularly blue light—have been shown to increase oxidative stress that adversely affects sperm viability. Therefore, laboratory optimization strategies such as the use of compartment incubators, automated CO_2 regulation systems, fast and gentle sample handling, and protection from excessive light exposure are essential to enhance the success of assisted reproductive (ART) technologies such as IVF and ICSI.

A. INTRODUCTION

Viability indicates how much sperm is still alive in the semen sample. The way to find out is usually by using a special dye such as eosin-nigrosin. Sperms that are still alive will not absorb the dye, so it looks clear or colorless (Klau, 2024). A study by Shari (2024), says that sperm quality, such as viability, motility, shape, and intact DNA, is essential for successful fertilization, as only live sperm can fertilize an egg.

The laboratory environment is very important in handling sperm because sperm is easily damaged if conditions outside the body are not right. The temperature must remain stable at around 37°C for sperm to stay alive and moving properly. If the temperature is too cold or hot, the sperm cell membrane can be damaged and sperm function decreases dramatically. In addition, the time between sampling and examination should be as short as possible, ideally less than 60 minutes, so that sperm quality is maintained (Fitri, 2020). The Wibowo study (2021), also said that sperm are susceptible to osmotic stress and damage due to free radicals that increase oxidative

stress, damage membranes and DNA when processed time. Therefore, the diluting medium is usually equipped with antioxidants to protect sperm. Nugroho's (2021) study on sperm susceptibility in reproductive laboratories is important because sperm quality greatly determines the success of ART such as IVF and ICSI. Sub-optimal laboratory conditions—unstable temperatures, long handling, oxidative stress, and microbial contamination—can degrade sperm quality and affect fertilization and embryo development.

This study aims to analyze the main environmental factors that affect the viability of sperm in modern reproductive laboratories. Through a systematic literature review, this article examines the impact of temperature, pH, osmolality, media, air quality, and light on sperm, and formulates practical recommendations for optimizing the laboratory environment to support the success of ART.

B. METHOD

This research was compiled based on a systematic literature review to support a case study entitled Environmental Factor Analysis on Human Sperm Viability in Modern Clinical Reproduction Laboratories. The literature studied comes from scientific publications in the range of 2020 to 2025. Literature searches were conducted through PubMed, ScienceDirect, and Google Scholar databases using keywords such as sperm viability, laboratory environment, ART, IVF, and other related terms. The selection is carried out in stages from the review of titles and abstracts to the evaluation of the full text, resulting in a minimum of 15 articles that meet the inclusion criteria for in-depth analysis.

C. RESULTS AND DISCUSSION

Table 1. Summary of Findings from 16 Articles Related to Environmental Factors on Human Sperm Viability

Authors	*Year*	*Key Findings and Relevance*
Choudhary	2020	Incubator temperature fluctuations of ±0.5°C can occur due to frequent door opening
Matsuura	2021	Cold shock damages sperm membranes, decreases motility and viability, and increases reactive oxygen species (ROS) levels.
Organization	2021	The ideal pH for sperm culture is between 7.2 and 7.4
Fitri	2020	pH media >7,4 menurunkan motilitas dan viabilitas sperma, merusak membran
Maharajan	2021	Osmolalitas stabil (290 mOsm/kg), pH naik menurunkan motilitas meski osmolaritas normal
Guo	2020	Exposure of sperm to pH/osmolality different from physiological levels is detrimental; CO_2 loss increases pH.

Sunde	2021	Cultural media maintains a stable pH against external changes.
Culture Collections	2023	CO_2 and bicarbonate keep the pH of the medium stable.
Ergun	2022	CO_2 fluctuations due to the open incubator door increase pH and disrupt fertilization.
Swain	2020	Ideal CO_2 5–7%, O_2 5%; the frequency of opening the incubator needs to be reduced.
Rahman	2020	Long-term exposure to VOCs increases oxidative stress in cells/gametes.
Santoso	2021	VOC exposure increases during floor cleaning; repeated occurrences can disrupt cell culture.
Mauchart	2021	White/blue light triggers ROS, damaging the membrane and DNA of sperm.
Boitrelle	2021	Laminar flow light/ROS trigger room reduces sperm DNA viability and integrity.
Wang	2025	Light triggers ROS, disrupts mitochondria, decreases sperm quality; light protection is important.
Bodis	2020	Light protection enhances fertilization, blastocyst formation, and pregnancy in the ICSI cycle.

Temperature

The study of Choudhary (2020), said that the results of the data analysis of the incubator logger showed that the temperature of 37°C was generally stable. However, there are small fluctuations of around ±0.5°C, especially when doors are often opened during rush hour. Although it seems trivial, this sudden change in temperature can cause cold shock. According to Matsuura (2021), cold shock can damage sperm membranes, lowering motility, viability, and fertilization. This damage occurs because the sperm membrane is rich in unsaturated fats that are sensitive to temperature, as well as an increase in free radicals due to thermal stress.

pH and Osmolality of Culture Media

The results of random pH measurements of culture media from incubation cups showed that the pH value was slightly higher than normal, in the range of 7.6–7.8, whereas the ideal pH for human sperm culture was 7.2–7.4 (Organization, 2021). The Fitri study (2020), said that when the pH of the culture medium rose above 7.4 to 7.6 -- 7.8, sperm motility and viability decreased significantly after 2 hours of incubation. This overly alkaline pH damages the sperm membrane and interferes with enzymes important for movement, thereby degrading the sperm's ability to fertilize the egg. A media environment with improper pH can increase osmotic stress and free radical production (ROS), thereby degrading the viability of sperm during in vitro storage or manipulation. Maharajan's study (2021), said that the osmolality

of the media remained in the normal range of around 290 mOsm/kg, so osmotic pressure was not an issue.

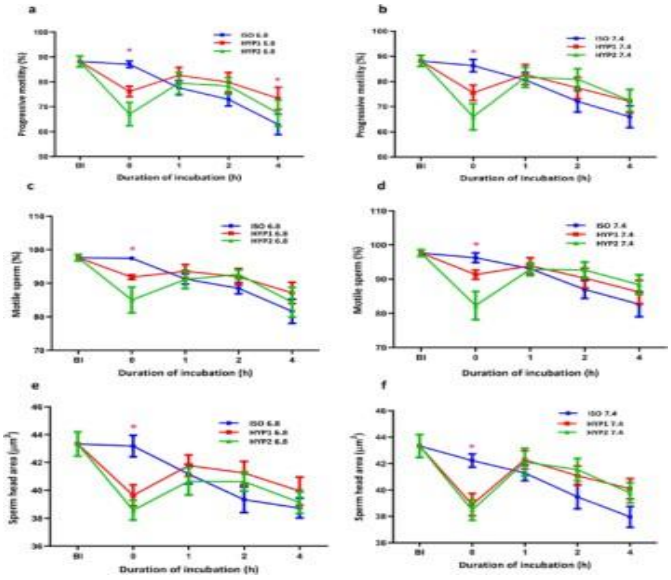

Figure 1. Buffering Diagram (Maharajan, 2021)

Figure 1 above shows that the total and progressive motility of cow sperm decreases as the pH increases, although osmolality remains stable at about 290 mOsm/kg. Guo's study (2020), also said that after being expelled from the body, spermatozoa can be exposed to pH and osmolality that are much different from physiological conditions, which can harm their survival after ejaculation. The pH of fresh ejacate is normally almost neutral, which is between 7.2 and 8.2, but it will increase in a short period of time due to the loss of CO_2 when it is outside the body.

Composition of Culture Media

According to Sunde (2021), culture media is made to maintain a stable pH despite external changes, such as fluctuations in CO_2 levels, temperature, or cell activity. In the technical combination Culture Collections (2023), it is said that the presence of bicarbonate and CO_2 in the incubator plays an important role in maintaining pH stability and preventing the pH from becoming too alkaline.

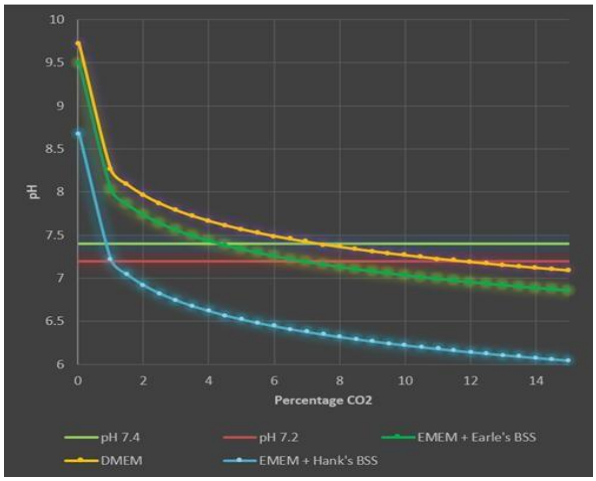

Figure 2. Different percentages of CO_2 in the incubator (Collections, 2023)

Figure 2 describes the theoretical pH range for various culture media under conditions of increased CO_2 levels in a humid incubator at 37°C shown in the graph, with the lower and upper limits of physiological pH marked with red and green lines (pH 7.2–7.4). The DMEM (with 44 mM $NaHCO_3$, orange line shown) is within the physiological pH if the CO_2 ranges from 7.5% to 11%. If DMEM is used in an environment with 5% CO_2, its pH becomes about 7.5. Although it is still acceptable for most cell cultures, this value is slightly outside the ideal physiological pH range.

Air and Gas Quality (CO_2, O_2)

According to Ergun (2022), fluctuations in CO_2 levels due to small leaks or frequent opening of the incubator door can increase the pH of the medium, which ultimately interferes with culture conditions and decreases fertilization success. Swain (2020) emphasizes that incubators used in ART procedures should be kept to have a CO_2 concentration between 5–7% and O_2 around 5%. The frequency of opening the incubator door needs to be minimized so that the pH of the culture medium remains stable and the risk of adverse environmental changes in gametes or embryos can be prevented. The study by Rahman (2020), said that although VOC levels in the culture chamber are still below the limit of direct harm, long-term exposure can increase oxidative stress in cells and gametes. The study of Santoso (2021), said that VOC levels increased during floor cleaning in the area around the laboratory. Although the levels are low, repeated exposure still has the potential to affect the health of cells cultured in the laboratory.

Light Exposure

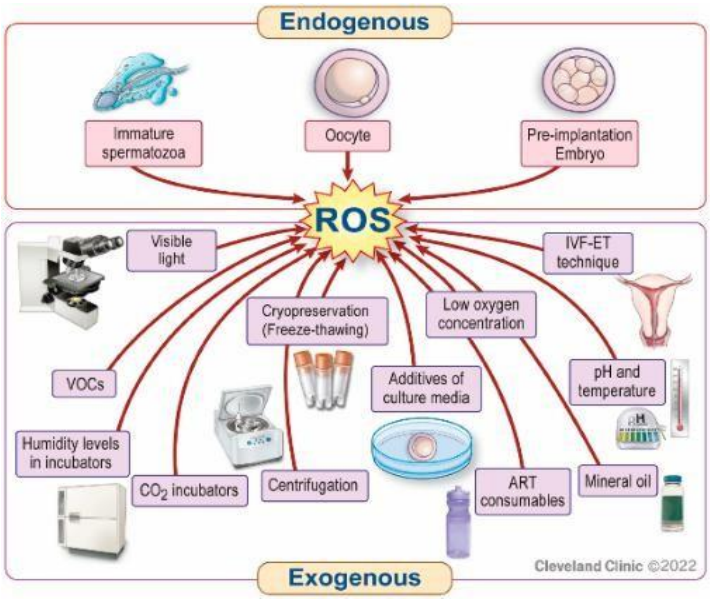

Figure 3. Illustrative diagram (Boitrelle, 2021)

Figure 3 shows that various external factors, such as exposure to visible light, can trigger the formation of ROS in sperm or embryos, which contributes to oxidative stress.

According to Mauchart (2021), exposure to unfiltered light, especially blue light (400–500 nm) or white light, can trigger an increase in ROS such as H_2O_2 in sperm cells. These free radicals can damage sperm membranes and DNA through oxidative stress. The Boitrelle study (2021), said that exposure to light from laminar flow lamps or room lighting for long periods of time can trigger an increase in free radicals, which ultimately decreases viability and damages the integrity of sperm DNA due to oxidative stress. The explanation of the illustrative diagram is as follows.

Oxidative Stress (ROS and Its Impacts)

According to Wang (2025), excessive light exposure can increase the production of free radicals (ROS) in sperm, which leads to oxidative stress. This stress damages important parts of sperm such as membranes, proteins, and DNA, resulting in decreased sperm viability and movement.

Table 2. Impacts and prevention solutions

Challenges in In Vitro Research	Impact	Solutions/Precautions
Unstable temperature	Decreases sperm motility and viability	Use incubator and warming plate, temperature control 37°C
Long delay time	Decreased sperm quality and fertilization	Limit the time between ejaculation and analysis <60 minutes
Osmotic & enzymatic stress	Damage to sperm membranes and DNA	Use a suitable diluent that contains antioxidants
Osmotic & enzymatic stress	Disorders in oocytes and embryos	Use sterile media and antibiotics, avoid contamination
Poor laboratory environment	Fertilization failure due to pollution	Control CO_2, humidity, ventilation, and restrict personnel access

In addition, light can also interfere with the function of sperm mitochondria,
lowers the energy produced, and damages mitochondrial DNA, all of which have a detrimental effect on sperm quality and male fertility. In clinical reproduction laboratories, it is important to reduce direct light exposure to sperm to maintain their quality and viability, as light can increase ROS and oxidative stress. Steps that can be taken include:

1. Use a red light filter on the lamp to reduce blue light and UV.
2. Installing UV and infrared filters on the microscope.
3. Creates a dark environment when manipulating gametes.

This step is effective in increasing fertilization, blastocyst development, and pregnancy success in the ICSI cycle with light protection compared to conventional methods (Bodis, 2020).

CO_2 Leak Repair

Table 3. Comparison between Conventional Incubators and Compartment Incubators (Sciorio, 2023)

Aspects	Conventional Incubator	Compartment Incubator	Reference
Space Structure	One big space for all cultures	Separate chambers (compartments) for each sample	Swain (2020); Zhang et al. (2023)
Temperature Recovery (post-open	10–20 minutes to return to 37°C	<2 minutes	Zhang et al. (2023)
CO_2/O_2 Recovery	Disrupted entire space, slow recovery	Only the related space is disturbed, fast recovery	Swain (2020); Ergun et al. (2022)
Medium pH stability	Susceptible to fluctuations due to CO_2 changes	More stable, the pH of the medium remains within the p	Culture Collections (2023); Swain (2020)
Risk of Oxidative Stress	Higher due to repeated temperature/gas fluctuations	Lower due to stable environment	Mauchart (2021); Wang (2025)
Environmental Exposure	Height: the entire culture is exposed every time the door is op	Minimal: only certain compartments are exposed	Swain (2020); Zhang et al. (2023)
Sperm/Embryo Quality	More often have decreased motility and viability	Better quality, better blastocysts	Zhang et al. (2023); Bodis (2020)
Operational Efficiency	Low if a lot of culture manipulation in the near future	High: fast and localized access	Swain (2020); Zhang et al. (2023)
Cost & Complexity	Cheaper and simpler	More expensive, requires staff training	Swain (2020)

According to Dubey (2021), disruption of CO_2 supply can cause the pH of the culture medium to shift towards alkaline (alkaline). Intelligent fail-safe systems help stabilize conditions quickly to maintain pH stability. A lack of CO_2 supply can make the culture medium more alkaline, which can damage the cell's environmental conditions. Therefore, repairs using intelligent fail-safe systems can handle leaks so that they can restore the pH of the media to optimal physiological levels.

The Wardiansah study (2020), said that advanced incubators use an automated system that regulates CO_2 injection when the gas level drops below 5%, with adjustment of injection time and frequency based on internal

sensors. The study suggests the application of infrared sensors and automatic injection mechanisms to maintain CO_2 concentrations according to set points, as well as being the technical basis for the development of automatic alarm and control systems in reproductive laboratories.

Optimization of Incubator Protocols

Murayama's study (2022), said that in Applied Sciences showed that although an incubator can maintain a temperature of 37 °C with an accuracy of ±0.1 °C, opening the door for 10 seconds leads to a temperature drop of about 5 °C in an incubator with an air jacket and 0.7 °C in an incubator with a water jacket. The temperature takes between 3 to 30 minutes to return to stability. The study by Sciorio (2023), also said that a compartment incubator is an incubator designed with separate small spaces, each used to incubate different cultures such as sperm, oocytes, or embryos. The use of compartment incubators with local access is an optimal strategy in modern reproductive laboratories because it can minimize disturbances to the microenvironment, maintain the stability of the culture media, and increase the success of fertilization and embryo development.

Sample Handling Procedure

Clinical reproductive laboratory SOPs are important to maintain sperm quality in several ways that include gentle sample handling to prevent membrane damage, minimize sperm time outside the incubator to avoid environmental stress (Harada, 2021), avoid shocks that can damage sperm (J Maia, 2020), and protect sperm from direct light exposure that can increase oxidative stress and DNA damage (Sansone, 2021).

Staff Training and Quality Control

Table 4. Summary of Staff Training and Quality Control

Aspects	Explanation	Reference
Staff Training	Laboratory personnel must receive comprehensive and ongoing training on gamete maintenance, environmental influences, and laboratory best practices.	Rienzi et al., 2022
Quality Control	Internal and external quality control programs are mandatory to ensure consistency, detect errors, and improve the quality of laboratory services.	Rienzi et al., 2022; De Geyter et al., 2021
Standards and Guideline	Guidelines from ESHRE affirm the importance of staff training and quality control as the foundation of in vitro fertilization success and the	ESHRE Guideline Group, 2023

Laboratory personnel who handle gametes must receive in-depth and regular training on the basic principles of gamete maintenance, the impact of environmental factors such as temperature, pH, light, and contaminants, and procedures and best practices in gamete culture and manipulation (Rienzi, 2022).

This training is important to reduce errors, improve risk understanding, and fertilization outcomes (De Geyter, 2021). For quality control, internal and external programs are needed to monitor laboratory conditions (temperature, pH, CO_2, air cleanliness), ensure procedures are up to standard, detect and correct problems quickly, and conduct continuous evaluations to improve clinical reproductive quality (Levy, 2020).

D. CONCLUSION

The viability of sperm in the reproductive laboratory is greatly influenced by various microenvironmental conditions. The temperature of the incubator needs to be kept stable at around 37°C to avoid damage to the sperm membrane and decreased motility that can occur due to temperature fluctuations, especially when the incubator door is opened frequently. The use of an incubator with a special compartment helps maintain the stability of these microtemperatures. In addition, the pH of the ideal culture medium ranges from 7.2 to 7.4, because too high a pH or osmolality instability can damage the sperm membrane as well as reduce viability due to osmotic stress and increased free radicals. The composition of the media with bicarbonate and CO_2 content plays an important role in maintaining pH stability. Air quality and gas levels also have a significant effect, with CO_2 concentrations having to be maintained at 5–7% and O_2 around 5%. CO_2 leakage or exposure to volatile organic compounds (VOCs) has the potential to increase oxidative stress and decrease fertilization success rates. In addition, exposure to light, especially from the blue and white spectrum, can trigger the production of free radicals that damage sperm membranes and DNA. Therefore, the use of light filters and proper lighting settings during gamete manipulation is highly recommended to maintain sperm quality and support successful fertilization. The viability of human sperm in modern clinical reproductive laboratories is strongly influenced by microscopic environmental factors such as temperature, pH and osmolality of the media, composition of the culture media, air quality (including CO_2, O_2, and VOCs), and light exposure. Strict control of these factors is essential for maintaining sperm integrity and function, as negligence can lead to oxidative stress and cell damage that degrades viability. Therefore, optimizing the laboratory environment through the use of quality equipment, advanced monitoring systems, and trained and disciplined personnel in carrying out SOPs is the main requirement for the success of ART.

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