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Adjustment of sperm cryopreservation laboratory management during the COVID-19 pandemic

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ABSTRACT

The COVID-19 pandemic has changed the order of life in all fields, particularly in medical services. Laboratory services in sperm cryopreservation that part of andrology or *in vitro* fertilization (IVF) laboratory cannot be separated with handling sperm from patients. Although the presence of viruses in semen has been known through various studies, there is scarce evidence regarding whether sperm fluid can be a medium of COVID-19 transmission. This raised the question of whether some adjustments to manage patient sperm should be made. This review described an overview of the various efforts that can be made to reduce the risk of disease transmission to health workers who handle patient sperm fluid.

ABSTRAK

Pandemi COVID-19 telah mengubah tatanan kehidupan di semua bidang, khususnya dalam layanan medis. Layanan laboratorium dalam kriopreservasi sperma yang merupakan bagian dari andrologi atau laboratorium *in vitro fertilization* (IVF) tidak dapat dipisahkan dengan penanganan sperma dari pasien. Meskipun keberadaan virus dalam semen telah diketahui melalui berbagai penelitian, belum banyak bukti apakah cairan sperma dapat menjadi media penularan COVID-19. Ini menimbulkan pertanyaan apakah beberapa penyesuaian untuk mengelola sperma pasien harus dilakukan atau tidak. Ulasan ini menjelaskan gambaran berbagai upaya yang dapat dilakukan untuk mengurangi risiko penularan penyakit kepetugas kesehatan yang menangani cairan sperma pasien.

Keywords:
COVID-19;
sperm freezing & storage;
cryopreservation;
management;
disease transmission;

INTRODUCTION

COVID-19 caused by SARS-CoV-2 that occurs throughout the world is considered a pandemic by the WHO.[1] This pandemic has changed the order of life in all fields, including biology, especially inmedicine. These changes also have a significant impact on medical services, both clinical and laboratory. Clinical services are greatly affected by this pandemic considering that patients will become indirect contact with healthcare workers, particularly doctors and paramedics during a consultation or physical examination. Preparedness and awareness of health workers in the pandemic situation must become the priority to minimize the transmission of the disease.[2] Therefore, it may need a few adjustments, including special protocols, to reduce the risk of person-to-person transmission in daily medical activities.

Laboratory services are an integral part of the effort to establish a patient's diagnosis and play a significant role in the management of a disease.[3] Recently, various forms of laboratory services are available in medical management.

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Including andrology laboratories that have activities in male infertility management specialized in the service of material or samples in the form of sperm/semen. Numerous activities are performed in andrology laboratories, such as semen examination/analysis, washing/preparation for assisted reproductive programs, various function tests, and frozen storage/ cryopreservation services.[4] Specifically, the freezing sperm storage service is usually integrated with the *in vitro* fertilization (IVF) or embryology laboratory service in a clinic or infertility unit. The activity of storing frozen sperm is an important part and cannot be separated in IVF laboratories.[5]

Sperm freezing allows male patients to store their sperm before they undergo some treatments that may decrease their fertility, such as chemotherapy, radiotherapy or other treatments that can damage the testicular tissue and decrease sperm production.[6] Their sperm can be stored in a fertility clinic or a sperm bank whose activity is to store sperm with cryopreservation originating from sperm donors, both voluntarily or paid.

The freezing and storage of sperm is conducted in an andrology or embryology laboratory with a procedure using certain reagents followed by storage in a small tube or small straw into a tank containing liquid nitrogen.[7] Patient's data were recorded before sperm cryopreservation, including their history and the results of their sperm examination. A protocol during the frozen sperm process is needed to ensure worker safety, particularly when using liquid nitrogen, which can injure the body.

The existence of the COVID-19 pandemic raises the question of whether there are changes or management arrangements in the andrology laboratory or IVF unit, especially for sperm cryopreservation services. This paper provided an overview of the various efforts that can be made to reduce the risk of disease transmission to health workers who carry out the action of storing frozen sperm and patients who will take advantage of this service during COVID-19.

LITERATURE REVIEW

Frozen Sperm Storage and Viral Transmission

Laboratory services perform a variety of examinations and actions of various human specimens consisting of blood, urine, feces, cerebrospinal fluid, and semen. Similar to other specimens, semen is categorized as specimens that can be intermediaries for the growth of pathogens in the form of bacteria,[8] viruses,[9] parasites,[10] or fungi (candida).[11] This is related to the involvement of sperm ducts in the process of transport and secretions from the accessory glands, namely, the prostate and seminal vesicles, which can become inflamed by various causes. Prostatitis, vesiculitis, or urinary tract infections are characterized by the presence of white blood cells in the sperm fluid or prostatic secretion, which can be a sign of an infection.[12]

The presence of viruses in sperm fluid is well known through various studies. Various kinds of viruses of more or less 27 types can be found alive in sperm fluid, including Zika virus and HIV.[13] The possibility of sperm as a medium of transmission for COVID-19 has been debated by experts. Reports on sperm-related COVID-19 were first reported by Song *et al.*[14] reported that no corona virus was found in the sperm fluid of COVID-19 patients. Subsequent research has instead suggested that the SARS CoV-2 virus was found in sperm fluid, causing the need to consider specific steps to prevent transmission of the virus when managing semen samples.[15]

SARS-CoV-2 was also found in the testis. This shows the possibility of

the presence of this virus in semen or sperm.[16] ACE2, the receptor of SARS-CoV-2, is highly expressed in testis tissue, including Leydig cells and seminiferous tubules.[17] Recent research on the corona virus in sperm fluid again shows the same thing as the first report that mentions the absence of corona virus in the sperm fluid of patients with COVID-19.[18] The impact of the presence of the virus in sperm fluid is still not widely known, especially regarding whether the virus can damage sperm, particularly its quality. However, several studies reported that HIV can cause damage or decrease sperm function.[19]

The process of storing frozen sperm is currently using several methods, including slow cooling and cryopreservation. The cryopreservation method is a technique of freezing cells by exposing them to very low temperatures, which results in the formation of ice. This method is widely used in frozen storage services, both gamete cells and embryos, because the use of liquid nitrogen is practical and inexpensive. Sperm cryopreservation also uses a cryoprotective agent known as cryoprotectant to prevent cell damage after a very low temperature environment.[20] Liquid nitrogen used in the cryopreservation method also has the potential risk of becoming a place for living pathogens, including viruses.[21] Research by Bielanski *et al.*[22] showed that virus transmission can occur in embryos that are frozen by the cryopreservation method using liquid nitrogen.

FIGURE 1. Sperm cryopreservation process.

FIGURE 1 shows the simple steps of sperm cryopreservation. Sperm collection followed by sperm analysis from the patient was added cryoprotectantly to the tube. It was vapored for a certain minute before entering the liquid nitrogen at -196ºC.

Focus Attention of Adjustment

Screening the patient

Tracking patient data related to various things that can inform the possibility of a patient contracting COVID-19 or at risk of carrying the virus is very important as a first step whether a special treatment needs to be conducted. Some matters related to screening patients according to the instructions issued by the Ministry of Health of Republic of Indonesia[23] include the following: Special officer at the clinic's entrance checks the patient's body temperature and requires the prospective patient to carry out a hand washing procedure. If the patient's temperature is below 38°C, the patient can continue the interview process with administration officers. Here are the suggestions during the step of patient screening: a). The interview process is conducted in a special room that has been provided previously while maintaining physical distances of approximately 1-2 meters; b). Administrative officers ask for COVID-19 related things in the COVID-19 Epidemiological Investigation from the Ministry of Health of Republic of Indonesia, among others: history of fever, cough, cold or sore throat, history of traveling to areas categorized as local transmission COVID-19; c). Administrative staff registers the patient

and inform the andrology laboratory or IVF laboratory staff who carry out the process of sperm freezing.

The disinfection activity in the sperm cryopreservation laboratory

Disinfection is expected to kill viruses that are thought to be able to live for several hours on the surface, so they will not become as medium for virus transmission. Disinfection should be carried out using disinfectants that can effectively kill viruses and are human-friendly. World Health Organization (WHO) recommended the local production of hand rubs with safety criteria.[24] With regard to skin reactions, hand rubbing with alcohol-based solutions is better tolerated than hand washing with soap and water. The use of UV light can be recommended for the prevention of viral growth.[25] It can be used when the laboratory room is empty or there are no laboratory staffs inside the laboratory because of harmful effects on the body. Here are some recommendations related to disinfection: a). The disinfection routine must be performed in several facilities, including rooms, office equipment, computers, desks and chairs; b). Disinfection of routine laboratory equipment, such as microscope, safety cabinet, laminar flow, centrifuge; c). Disinfection of the routine room used for sperm collection of patients, especially after being used by patients in the framework of the sperm collection process; d). Disinfection of liquid nitrogen storage tanks including surfaces for all the tanks that were placed in the room; e). Turn on the UV lamp after laboratory activities are finished and staff leave the room; f). All disinfection actions should be carried out regularly, and it is recommended to use disinfecting materials that are human-friendly.

Personal protective equipment as an effort to prevent transmission of infection

Personal protective equipment must be an absolute concern of both laboratory workers storing frozen sperm and patients who aim to prevent the transmission of the virus from the patient to the patient or vice versa. Personal protective equipment must also be worn by supplier employees while filling the liquid nitrogen in nitrogen tanks. Although the presence of SARS Cov-2 virus in sperm is still being debated, standard procedures for preventing transmission must have been made. In general, standard tools to prevent the transmission of infection are gloves, masks, gowns and face shields. Additional protection against injury is carried out due to contact with liquid nitrogen, which is at risk of causing injury. This moment can occur during the process of sperm freezing or routine liquid nitrogen filling. The suggestions are as follows: a). Administrative officers use masks and face shields; b). Patients who will carry out the process of storing frozen are requested to wear masks, special dresses/gown and face shields that are provided in the laboratory; c). Laboratory personnel receiving and examining sperm samples must always use gloves, masks, special gowns and face shields; d). Patients are required to wash their hands before and after sperm removal; e). Always using disposable material for handling sperm, such as pipettes and pots; f). Users who carry out the storage process must be more careful with regard to injuries due to liquid nitrogen vapor, so it is mandatory to use protective goggles and special gloves resistant to liquid nitrogen; g). Perform disinfection of containers used before the thawing process; h). Liquid nitrogen tank fillers are required to use masks,

gowns and face covers during the filling action; i). The waste was separated from non-infectious garbage and treated with special treatments.

Laboratory logistics

Andrology or IVF laboratories that provide sperm cryopreservation services during the COVID-19 pandemic must maintain stock or supplies of consumables such as pipettes, straw, object glass, sperm collection pots, reagents or liquid nitrogen. This can be caused by territorial restrictions or lock downs carried out by several countries that have been producing such tools or reagents, making shipping more difficult or even not possible at all. The result is a vacuum or lack of stuffs lab/consumable or reagents that can cause service cessation or stop. Some adjustments that are considered to be made by the laboratory are a). Make contact with consumable product suppliers to ensure the stock availability and fluency of the delivery process; b). Identifying items that are difficult to find with other similar products that are easier. Try to get with the same quality of stuffs; c). Make contact with liquid nitrogen supplier companies to ensure the liquid nitrogen always available during the pandemic; d). Look for alternative suppliers of liquid nitrogen as a substitute if the main supplier is unable to provide liquid nitrogen.

DISCUSSION

Adjustments to the management or laboratory management of sperm cryopreservation services during the COVID-19 pandemic are needed. Various protocol changes in many fields of life, including in the field of laboratory services to cryopreserve sperm. At this time, aiming to prevent the process of disease transmissionis urgently needed. The policy of the government leaders of several countries to implement lockdown by stopping transportation services, laying off employees and stopping business activities greatly affects activities in the medical laboratory. Sperm cryopreservation services laboratories that are usually integrated with andrology or IVF laboratories need to adjust to the various limitations that arise during the COVID-19 pandemic that has been going on for several months.

Screening process or patients selection who will take the sperm cryopreservation program is the biggest adjustment. Provisions on a patient who may or can do sperm cryopreservation are not always based on medical indications but for now also consider whether the patient has a history of COVID-19 or the risk of transmission from the outside environment.

As a part of the IVF program, ESHRE[26] has release guidance for patients undergoing the IVF program. The important thing is the triage phase that can be conducted 2 weeks before the program. Patients also need to test for SARS-CoV-2 antibodies (IgM and IgG) if the triage is potentially positive.This protocol can be adopted for patients undergoing sperm cryopreservation programs. The patient should contact the clinic by phone or email to obtain the test result.

Laboratory staffs are also concerned about safety protocols for handling special materials, such as infectious samples. There are certain protocols to treat sperm samples safely to prevent the transmission of diseases. The WHO published instructions for safely handling sperm for analysis in several editions, considering that sperm material can be a source of transmission of several diseases, such as HIV, hepatitis B or chlamydia.[27]

Adjustments in patient selection may cause decreasing number of patients during the pandemic for several reasons, including: a) patients who are indicated

to be positively affected by COVID-19, b) there are restrictions on the area where patients live so they cannot be free to travel or c) just afraid to come to the clinic because they do not want to be infected. Decreasing patient numbers is expected not permanently related to pandemic, and hoping tends to increase after the discovery of the treatment to combat this corona virus.

Education about the possibility of liquid nitrogen as aviral transmission media is very important for employees of the company and health workers/ staff. The protocol of wearing personal protection equipment during tank filling must be implemented to prevent the spread of infections. The sterility of liquid nitrogen during transportation from the company to the lab must also be considered because of the transmission risks associated with the use of straws and cryovials and liquid nitrogen dewars.[28] In several studies, some viruses, such as hepatitis B virus (HBV), herpes simplex, adenovirus, and papillomavirus, showed possible the transmission throughout the dermatologic practices of direct exposure to liquid nitrogen.[29,30]

The container should be disinfected with an alcohol swab on the tank surface. Alcohol was chosen as a disinfectant because it works well for pathogens and is safe for health workers compared with bleach. Using bleach can also cause corrosive effects on metal materials.[31] To minimize the contamination of liquid nitrogen, Pamergiani *et al.*[32] showed that UV irradiation can be used for the sterilization method.

An adequate supply of liquid nitrogen in the laboratory is needed in the current pandemic conditions, because some sperm have been stored for a long time before the COVID-19 pandemic. The contents of liquid nitrogen decrease and even run out over time, which can damage the sperm that have been stored. This need to be considered about having sufficient liquid nitrogen reserved tanks for several months adjusted to the number of patient's sperm straw. Contamination of sperm/ gamet from microorganism pathogens may occur from many factors. The freezing method, loading and sealing of freezing containers, liquid nitrogen and tank sterility are known to have direct impacts on the contamination process.[28]

Regarding the prevention of transmission, disinfection of the nitrogen tank storage room and the surface outside of the tank must be routinely performed. It was performedrarely before the COVID-19 pandemic, so this can be categorized as a new protocol. The use of chemical disinfectants containing chlorine and alcohol must be considered for killing microorganisms.[33] It was also important, but never conducted before the COVID-19 period, to provide information for liquid nitrogen company employees. They must know the minimal requirements of personal protection, such as wearing masks and facing shields when filling liquid nitrogen tanks. It will minimize the transmission of virus to the laboratory environment either through existing tools or directly when talking with other laboratory staff. It is considered using a separated liquid nitrogen tank for patients who underwent sperm cryopreservation before and during the COVID-19 pandemic period.

CONCLUSION

The existence of the COVID-19 pandemic has changed the management of various fields, including governance in laboratories that serve sperm cryopreservation in an IVF unit as apart of the andrology laboratory in a hospital. Adjustments in several cases need to be conducted to prevent or minimize the process of viral transmissionin the laboratory environment from health workers to patients or *vice versa*. Adjustments can

be conducted in several ways, including screening patients who will store frozen sperm, disinfection activities in the laboratory, use of personal protective equipment and consideration of several steps in logistics.

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