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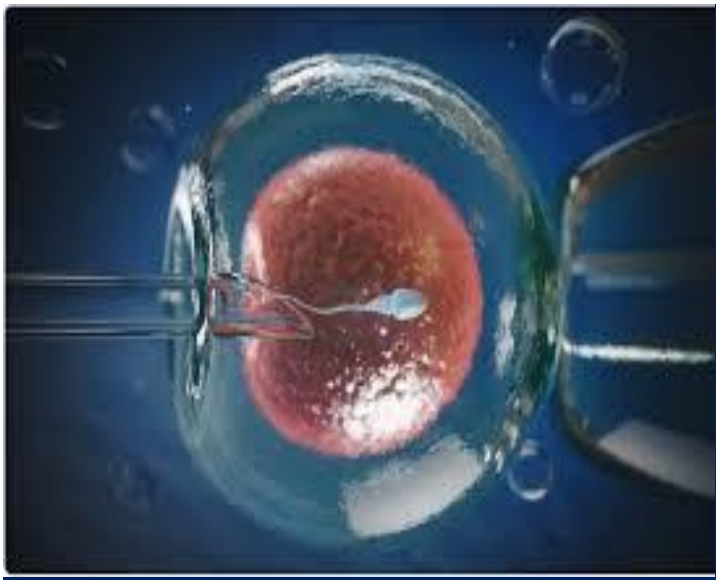

IVF UNIT MANUAL

SAIDEEP
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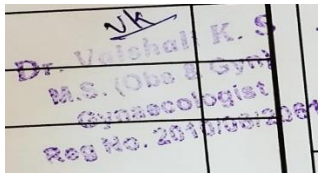
Annual Documents adequacy & Change Requirements Review

Sr.No	SOP /Doc No	Documents Name	Issue. No	Rev.No	Review Date	Change	Rev No	Revision Date	Reason for Change	Amendment
1	SDH/IVF/01	contents	1	1	20-Nov-22	No Any Change Review Completed	1	20-Nov-23	No Any Change Review Completed	No Any Amendment History
2	SDH/IVF/02	Department Structure	1	1	20-Nov-22		1	20-Nov-23		
3	SDH/IVF/04	Scope of Services	1	1	20-Nov-22		1	20-Nov-23		
4	SDH/IVF/5.1	Media	1	1	20-Nov-22		1	20-Nov-23		
5	SDH/IVF/5.2	SOP ICSI	1	1	20-Nov-22		1	20-Nov-23		
6	SDH/IVF/5.3	SOP IMSCI	1	1	20-Nov-22		1	20-Nov-23		
7	SDH/IVF/5.4	SOP-Assisted hatching of human embryos	1	0	20-Nov-22		1	20-Nov-23		
8	SDH/IVF/5.5	SOP-Invitro Maturation Of Immature Human Oocytes	1	1	20-Nov-22		1	20-Nov-23		
9	SDH/IVF/5.6	SOP-Vitrification and Warming of Human Oocytes	1	1	20-Nov-22		1	20-Nov-23		
10	SDH/IVF/5.7	SOP-Virtification And Hatching Of Human Embryos	1	0	20-Nov-22		1	20-Nov-23		
11	SDH/IVF/5.8	Laboratory Maintenanace	1	1	20-Nov-22		1	20-Nov-23		
12	SDH/IVF/5.9	Safe practices in Lab	1	1	20-Nov-22		1	20-Nov-23		
13	SDH/IVF/5.10	Identification, Handling and Transportation of samples	1	1	20-Nov-22		1	20-Nov-23		
14	SDH/IVF/5.11	Storage of Specimen	1	1	20-Nov-22		1	20-Nov-23		
		Original Date	**Effective Date**		**Next date of**		**Issue NO**			

				revision				
		01 November 2021	**20 November 2023**	**20 November 2024**		1		

Reviewed & Prepared By		**Recommended By**	**Approved By**
Dr.Vaishali Kiran	Mrs.Shraddha suryavanshi	Dr.H.Kalgaonkar	Dr.S.S.Deepak
Gynecologist (IVF HOD)	Quality Co-ordinator	Chief Medical Administartor	Chairman & Managing Director
			

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Sr.No	SOP /Doc No	Documents Name	Issue. No	Rev.No	Review Date	Change	Rev No	Revision Date	Reason for Change	Amendm
1	SDH/IVF/01	contents	1	1	05-Mar-21		1	20-Nov-22		
2	SDH/IVF/02	Department Structure	1	1	01-Nov-21		1	20-Nov-22		
3	SDH/IVF/04	Scope of Services	1	1	01-Nov-21		1	20-Nov-22		
4	SDH/IVF/5.1	Media	1	1	01-Oct-19		1	20-Nov-22		
5	SDH/IVF/5.2	SOP ICSI	1	1	01-Nov-21		1	20-Nov-22		
6	SDH/IVF/5.3	SOP IMSCI	1	1	01-Nov-21		1	20-Nov-22		
7	SDH/IVF/5.4	SOP-Assisted hatching of human embryos	1	0	01-Oct-19		1	20-Nov-22		
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12	SDH/IVF/5.9	Safe practices in Lab	1	1	01-Nov-21		1	20-Nov-22		
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14	SDH/IVF/5.11	Storage of Specimen	1	1	01-Nov-21		1	20-Nov-22		
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		01 November 2021	**20 November 2022**	**20 November 2023**		1		

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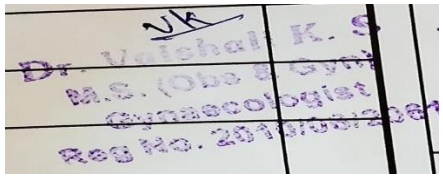
Dr.Vaishali Kiran	Mrs.Shraddha suryavanshi	Dr.H.Kalgaonkar	Dr.S.S.Deepak
Gynecologist (IVF HOD)	Quality Co-ordinator	Chief Medical Administartor	Chairman & Managing Director
			

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/00
		Issue No	01
		Rev No.	01
		Date	1 Nov 21
		Page	1
	Amendment Sheet		

Sr.No	Page No	Clause No	Date of Amendment	Amendment Made	Reasons	Signature of Approval Authority
01.						

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar		Dr. S. S. Deepak	
Chief Medical Administrator		Chairman & Managing Director	

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/01
		Issue No	01
		Rev No.	01
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		Page	1
	Document Title : Contents		

Sl no	Particulars
1	Contents
2	Organisational Chart
3	Roles and Responsibilities
4	Scope of Services
5	Standard Operating Procedures 1.Media used for ICSI and IVF 2.Intra cytoplasmic sperm injection 3.IMSI 4.Assisted hatching of human embryos 5.Invitro maturation of immature human oocytes 6. Virtification and warming of human oocytes. 7. Virtification and warming of human embryo 8. Laboratory maintenance 9. Safe practices in lab 10. Identification, Handling and Transport of Samples 12. Storage of Specimens

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

Departmental Hierarchy

Scientific Director

↓

Lab In –charge

↓

Lab technician

The IVF laboratory provides the following services:

- Intra cytolpasmic sperm injection
- IMSI
- IVF
- Assisted hatching of human embryos
- Invitro maturation of immature human oocytes
- Virtification and warming of human oocytes.
- Virtification and warming of human embryo

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/5.1
		Issue No	01
		Rev No.	01
		Date of Issue	1 Oct 19
		Pages	1
	Document Title : Handling of Media		

PURPOSE AND SCOPE

This policy and procedure aims to provide guidance on handling of various types of media used for IVF procedures

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Laboratory

Is responsible to ensure the compliances in terms of ensuring that guidelines for handling of the media are followed by IVF unit staff

POLICIES& PROECURES

Media used for ICSI and IVF

1. Flushing Media
2. Sperm wash Media
3. Cleavage Media
4. Fertilisation Media
5. Hyaluronidase Media
6. PVP
7. Culture Media
8. Gradient Media.

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/5.1
		Issue No	01
		Rev No.	01
		Date of Issue	1 Oct 19
		Pages	2
	Document Title : Handling of Media		

All media should be kept at 2 to 8° c.

1. Preparation of Media

1. Flushing Media

Flushing media is prepared at least one hour before oocyte retrieval.It is appropriate to prepare 3 ml under mineral oil in a centre well and is kept on warm stage at 37^0 c.

2. Sperm wash media

Sperm wash media is prepared at least one hour before sperm preparation procedure and is kept on a warm stage.

3. Cleavage media

Cleavage media is prepared 24 hours before ICSI and is kept at 37^0c in an incubator with 6% CO_2.

4. Fertilisation Media

Fertilisation media is prepared at least 24 hours before ICSI and kept is at 37^0c in an incubator with 6% CO_2(For one pick up 2 centre well dish with fertilization media and one small dish with fertilization media drops under mineral oil.)

5. Hyaluronidase Enzyme

Hyaluronidase enzyme is prepared in centre well half an hour before denudation and kept on a warm stage.

6. PVP

PVP kept on a warm stage half an hour before adding sperm to ICSI dish

7. Culture oil

Culture oil is prepared and kept in an incubator at least one day before oocyte retrieval.

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

8.Gradient Medium

Gradient media is prepared at least 1 hour before sperm preparation and keep at 37^0c on a warm stage.

REFERENCES

Nil

PURPOSE AND SCOPE

This policy and procedure aims to guide the IVF Lab staff on protocol to be followed for ICSI

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Lab

Is responsible to ensure that the protocols are followed

PROTOCOLS

Introduction:

Intra cytoplasmic sperm injection is the most successful micromanipulation technique used for the treatment of male factor infertility. The procedure entails the mechanical insertion of single spermatozoa directly into the cytoplasm of an oocyte. One of the most important aspects of ICSI is finding and injecting the centre of the oocyte. Cytoplasmic leakage and latter degeneration is caused by inserting the injection pipette into an area other than the centre of the oocyte.

Procedure:

1. Locate the sperm. Immobilise and score its tail. Aspirate it up into the injection pipette, stabilising it at the tip.

2. Lift the pipette up slightly so that it does not drag on the bottom of the dish and move to a drop which contains the oocyte.

3. Rotate the oocyte so that the polar body is at the desired position and attach it on to the holding pipette.

4. Focus on the oolemma until the membrane is outlined by a distinct dark line.

5. Bring the injection pipette in to the same focus as the oolemma by using the z axis control knob on the joystick lever.

6. Adjust the position of the sperm so that it is at the tip of the injection pipette.

7. Advance the injection pipette in to the oocyte and note that the oolemma will evanginate as a distinct line.

8. If found the centre of the oolemma, inject the oocyte.

9. Immediately aspirate the cytoplasm in to the injection pipette using small movements on the control knob on the micro injector. The cytoplasm encased within the oolemma will creep up the shank of the injection pipette until the oolemma finally breaks. Stop aspirating further and immediately unravel the sperm with the cytoplasm back in to the oocyte. Only insert the minimum amount of medium/PVP back in to the oocyte.

10. When the sperm has been injected in to the centre of the cytoplasm, exit the oocyte using a slow, smooth movement to the right.

11. Decrease the suction holding the oocyte and with the aid of the injection pipette, move the oocyte off the pipette.

12. Move the holding pipette out of the drop making sure not to drag the oocyte along with it.

13. Lift the injection pipette off the bottom of the dish and enter the central drop of sperm and PVP to select another sperm.

14. Transfer the injected oocytes in to the flour well containing cleavage medium and keep it in the incubator at 37 ^{0}c and 6 % CO_2.

IDENTIFICATION OF FERTILIZATION

16 to 18 hours after ICSI, fertilization of the oocytes is checked under a microscope for the appearance of two distinct pro nuclei (2PN) and two polar bodies .It is not necessary to transfer the fertilized oocytes into another medium for continued culture.

INVITROFERTILIZATION

1. Pick up the oocyte cumulus complex from the follicular fluid and keep it in the incubator.

2. After 30 minutes, transfer the oocyte cumulus complex in to the four well dish containing fertilization medium.

3. To this add 200 µl of sperms by using pipette.

4. Incubate it at 37^0 c and 6 % CO_2 for overnight culture.

5. Next day denude the oocyte complex by using 135µm diameter pipette.

6. Transfer the denuded oocytes in to the four well dish containing cleavage medium.

7. Observe under microscope for fertilization check up.

8. Keep the embryos in the incubator at 37 ^{0}c and 6 % CO_2

EMBRYO TRANSFER

In natural cycle, the embryo reaches the uterus at the morula –blastocyst stage. It would be logical to replace IVF embryo in to the uterus at this stage, however the limitation of the culture media has favoured embryo transfers on the second or third day. The number of embryos chosen for transfer must be considered for the

patient sake. Most units have come down to two embryos only increases the chance of multiple pregnancy. When deciding on the number of embryo transfer, take in toconsideration the patient's age, the number of embryo transfers the patient has had before and quality of the embryo. The day 2 transfer occurs when the embryo is between the 2 to 6 cell stages

REFERENCES: Nil

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/5.3
		Issue No	01
		Rev No.	01
		Date of issue	1 Nov 21
		Page	1
	Document Title : **Intra Cytoplasmic Morphologically Selected Sperm Injection (IMSI) Protocol**		

PURPOSE AND SCOPE

This policy and procedure aims to guide the IVF Laboratory staff on IMCI

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the protocol.

HOD – IVF

Is responsible to ensure staff training and adherence to protocol

POLICIES

Intra cytoplasmic Morphologically selected Sperm Injection (IMSI) is the latest technology in the field of assisted reproductive techniques. Currently, the sperm is magnified only 600 times. IMSI enables us to magnify the sperm as 7,000 to 10,000 times. This high magnification allows the embryologist to select a morphologically normal sperm thereby improving the pregnancy and reducing abortion rates. IMSI will prove to be beneficial to the couples with male factor infertility and unexplained infertility. IMSI resulted in a higher clinical pregnancy rate than ICSI when applied to unexplained infertility.

SPERM PREPARATION

Only freshly ejaculated semen was used for IMSI. The preparation of the semen was performed on the basis of a two layer (low density & high density) density gradient method.

GRADIENT METHOD

a) Take 1ml 80% lower phase gradient media and 1 ml 40% upper phase gradient in to a centrifuge tube.

b) Add semen sample on the top of the upper phase gradient without mixing.

c) Centrifuge at 2000 rpm for 20 minutes.

d) Remove supernatant, add 3 ml of sperm wash media and mix it well.

e) Again centrifuge at 1500 rpm for 15 minutes.

f) Remove the supernatant and add 300µl of sperm wash media drop by drop and incubate at 37˚c.

IMSI DISH PREPARATION

Most IVF units use sterile glass bottomed dish (Willco Wells BV) for IMSI. Dishes is made at least one hour prior to the actual injection. When writing on the bottom of the dish, always write on the edge as not to obscure the selection. Always take time to check the name on the dish and correlate it to the name on the sperm sample before loading the sperm.

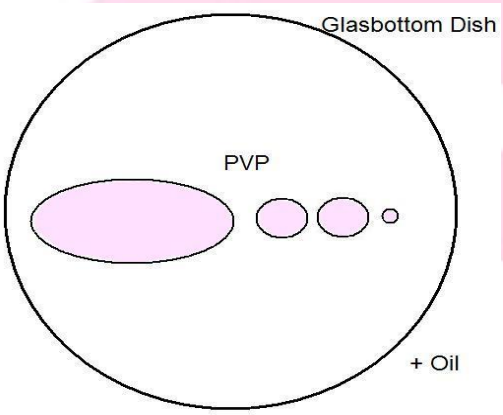
a) Write patients name on the outer edge of the bottom of the dish.

b) 200µl of observation macro-droplet was placed on the left side of the glass bottomed dish followed by two 10µl cleaning micro-droplet and a 5µl collection micro-dorplet made up with polvinylpyrrolidone under 3 ml of mineral oil.

c) Place these dishes in an incubator untile needed

Observation droplet

Collection droplet Cleaning droplet



Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

IMSI- SPERM SELECTION

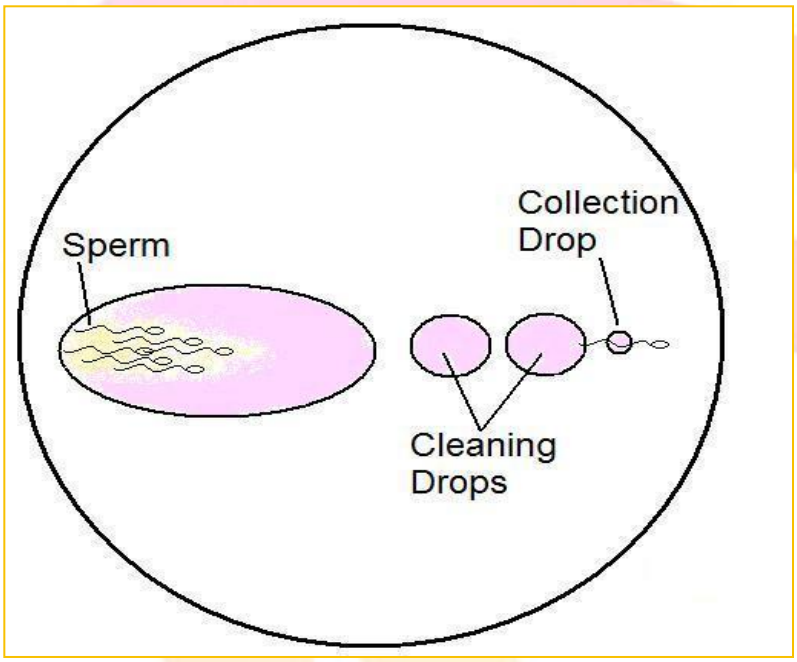
The sperm cell suspension obtained after sperm preparation is used for real-time high magnification that was performed on the observation droplets containing PVP solution. In order to perform a correct sperm evaluation, the embryologist have to follow each apparently suitable single sperm cell by moving the microscopic stage in the x, y and z direction for atleast 20 seconds until they observe even the smallest details. Therefore, the PVP solution was necessary to slow the sperm speed and thereby prevent the highly motile spermatozoa disappearing from the monitor screen during measurement. To reduce PVP toxicity, the concentration of the PVP was adjusted to a minimum (range 0-8%) which still enables image analysis.

The sperms were put in the observation droplet in the IMSI dish. Spermatozoa with severe malformations, such as pin, amorphous, tapered, round or multinucleated head, which can be identified clearly by low magnification (20X to 60X) are avoided. The normal looking sperms at low magnification are selected into the injection pipette and observed under the high magnification eye piece (60X) along with the cytoscreen software which further magnifies the sperm to 6000 to 7200 times . The sperms were observed while it is present in the injection pipette itself, so that the sperm does not go out of the view at such high magnification and its movement in the pipette can be controlled.

Out of six sperm sub cellular organelles (neck, tail, mitochondria, acrosome, post-acrosomal lamina, nucleus) the morphological normalcy of the sperm nucleus (shape, chromatin content) was positively associated with both fertilization rate and pregnancy outcome. Spermatozoa with doubtful determination were excluded from selection. Finding normal looking spermatozoa took a minimum of 60 minutes and upto 210 minutes, depending on the quality of the semen sample in each case.

Only motile spermatozoa with normal morphology which are selected from the observation droplets are then transferred into the collection droplet one by one as shown in the below figure. But care is taken to wash away unselected sperms which gets stuck to the injection pipette

while selecting sperms from the observation droplet and this is done by dipping the pipette in the two cleaning droplets.



Once enough number of sperms are selected in the collection drop, they are again aspirated into a injection pipette and then glass bottom dish is removed and an ICSI dish is kept. The selected sperms are then expelled to the sperm droplet in the ICSI dish and the these sperms are immobilized and injected into the oocytes by classical ICSI. This procedure was performed using an eppendorf micromanipulation system.

REFERENCES

Standards

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

PURPOSE AND SCOPE

This protocol aims to provide guidance to IVF staff on Assisted Hatching of Human Embryo

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the protocol rests with the MD of the hospital.

HOD – IVF Unit

Is responsible to for ensuring training and competency of staff on protocol

POLICIES

The inability of blastocysts to hatch from the zone pellucid has been blamed for the high implantation failure in Human IVF. After fertilization, the zona becomes more brittle, less elastic and possible hardens in culture as a result of in vivo aging .Embryos with a thick zone on day two had a low implantation rate when compared with thick zona which had assisted hatching performed prior to transfer.

Assisted hatching is recommended for patients exhibiting one or more of the following indications:

- Elevated basal FSH levels
- Brown coloured zona
- Thick Zona
- Zona difficult to pierce during ICSI
- Patient's>37 years old
- Repeated implantation failure

- Poor embryo quality

Assisted hatching is performed with a 1.48um diode laser where a few milliseconds of laser irradiation instantly make a hole in the zona. The whole size can be controlled very accurately within a 3-25 µm range.

Assisted hatching involves the slow, controlled release of acidified culture medium in order to progressively thin the zona by dissolving the protein content of the zona pellucid. The acid eventually opens up a hole within the zona about 20 µm in diameter.

REFERENCES

Standards

PURPOSE AND SCOPE

This policy and procedure aims to provide guidance on Invitro Maturation Of Immature Human Oocytes

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Laboratory

Is responsible to ensure the compliances in terms of ensuring that guidelines are followed by IVF unit staff

POLICIES& PROECURES

Introduction

In IVM immature eggs or oocyte are retrieved from the ovary, then matured in the laboratory before being fertilised and replaced to the womb.

CULTURE MEDIA INVOLVED IN IVM TREATMENT

1.OOCYTE WASHING MEDIUM:-

This medium is used for washing immature oocytes, cumulus oocyte complexes, collected from the follicles before maturation in culture . This medium is buffered with HEPES. This medium is ready for use following pre incubation for atleast min at 37^0c.

2.OOCYTE MATURATION MEDIUM :-

This medium is used for maturation in culture of immature occytes in an incubator at 37^0c & 5% CO_2

3.EMBRYO MAINTENANCE MEDIUM:-

This medium is used for fertilization and embryonic culture following insemination by ICSI.

ADVANTAGES OF IVM

The major advantages of IVM treatment include.

1. Avoidance of the side effects resulting from gonadotropin stimulation including the risk of OHSS.
2. Reduced cost
3. Simplified treatment.

FLUSHING MEDIUM

COC$_S$ are collected in10 ml culture tubes containing approximately 2 to3 ml of heparinised warm flushing medium. Make sure the medium used contains heparin to prevent clouding of the aspirates.

PREPARATION OF OOCYTE WASING MEDIUM

Oocyte washing medium must be prepared at least 1hr before oocyte collection and kept at 37^0c .Briefly 3 petridishes each containing approximately 2-2.5ml of oocyte washing medium under mineral oil are prepared for every patient.

PREPATION OF OOCYTE MATURATION MEDIUM

The immature COC_S are incubated in an organ culture dish containing1 ml oocyte maturation medium supplemented with a final concentration of 75 mlU/ml FSH and 75 mlU/ml LH at 37^0c and 5% CO_2 . Oocyte maturation medium should be prepared for equilibration at least 2 hrs before immature oocyte retrieval.

1. Place 10 ml of oocytes maturation medium in to a test tube.
2. Dissolve completely 1 ampoule of 75IU FSH and 75 IU LH in to (1)
3. Place 9.90ml of fresh oocyte maturation medium in to a test tube.
4. Take 100µl FSH and LH dissolved as per item (1) and transfer in to (3)
5. Prepare 3 organ tissue culture dishes for each patient. in each dish, the inner well contains ml of (3) and the outer well 2ml of (3)
6. Cover the organ culture dish with the dish cover and place it in the incubator

PREPARATION OF EMBRYO MAINTANANCE MEDIUM

Embryo maintenance medium must be prepared at least hr before ICSI and kept at 37^0c in an incubator with 5% CO_2 and 100% humidity. It is appropriate to prepare 20µl of droplets under mineral oil in a petridish.

IDENTIFICATION OF IMMATURE OOCYTES

There are two ways to look for and collect cocs from follicular aspirates.

1.Dish Research:-

The follicular aspirates are poured directly in to petridish and examined for coc_sunder a stereomicroscope.

2.Cell strainer:-

The follicular aspirates are filtered through a cell strainer (Falcon,70µm Nylon). After filtering, the collected aspirates can be rinsed with prewarmed IVM washing medium and transferred to a petridish to search for COC_S under a stereomicroscope. All handling procedure is conducted on warm stages at 37^0c.

PROCEDURE

INDITIFICATION OF COCS FROM FOLLICULAR FLUID

To use a cell strainer, each tube of follicular aspirate is poured through the cell strainer immediately after the collection. The cell strainer can be placed in a petridish containing 3-5 ml of oocyte washing medium on a warm stage or plate in order to prevent the COCS from drying in the strainer between the time of follicular aspiration and tube collection. Once follicular aspiration has been completed, the COCS contained in the cell strainer are collected with a pipette,then immediately transferred in to petridishes for scanning of the COCS under a stereomicroscope. COCS are transferred IN to oocyte WASHING MEDIUM maturation medium for maturation in culture.

WASHING IMMATURE OOCYTES

COCs are picked up with a sterile cotton plugged Pasteur pipette fitted to a pipette pump and transferred in to the pre-warmed oocyte washing medium for washing several times (at least 3) before maturation in culture.

SPERM PREPARATION

Semen can be collected and prepared for insemination on the day of oocyte retrieval if a mature oocyte has been from the dominant follicle. Otherwise, semen collection and preparation should be performed the day after oocyte retrieval. If possible, a fresh sperm sample should be obtained, which can then be prepared for the insemination.

STRIPPING OOCYTES 24 HOURS AFTER CULTURE

The immature COCs are cultured in the oocyte maturation medium in the incubator and allowed to begin the maturation process for 24-48 hrs.24 hours after maturation in culture, all of the COCs are stripped for identification of oocyte maturity. COCs will be denuded using a finely drawn glass pipette following minute of exposure to a commercially available hyaluronidase solution. The mature oocytes are then subjected to insemination by either IVF or ICSI after stripping .The remaining immature oocytes will continue to mature in culture for another 24hrs.

IDENTIFICATION OF MATURE OOCYTES 48HOURS AFTER CULTURE

48hrs after oocyte retrieval, the remaining stripped oocytes are re-examined and if any have matured at this point, they are inseminated immediately by either IVF or ICSI

INSEMINATION BY ICSI

Commercially available ICSI medium and PVP solution is used to prepare the ICSI dish. It is also appropriate to use oocyte washing medium for preparation of the ICSI droplets because the PH of the oocytes washing medium is quite stable at room temperature and atmosphere. However, it is important to note that the ICSI dish is prepared at least 1hr before ICSI and kept at 370c in the incubator or on warm stage or plate for equilibration. It is preferable to prepare sperm freshly before ICSI. After ICSI, the individual oocyte is transferred in to a droplet (2µl) of embryo maintenance medium in a petridish for culture in the incubator.

IDENTIFICATION OF FERTILIZATION

16 to 18 hrs after ICSI, fertilization of the oocytes is checked under microscope for the appearance of two distinct pro nuclei (2PN) and two polar bodies. It is not necessary to transfer the fertilized oocytes in to another medium for continued culture .

EMBRYO CULTURE

The fertilized oocytes are to be cultured in the droplets under mineral oil for 1 or 2 additional days, depending upon the number and quality of embryos achieved. If blastocyst culture is requested, the cleaved embryo is transferred to new droplets in a petridish containing the same embryo maintenance medium under mineral oil for 2 days after ICSI.

REFERENCES

Standards

PURPOSE AND SCOPE

This policy and procedure aims to guide the the IVF lab staff on **Vitrification and Warming of Human Oocytes**

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Unit

Is responsible to ensure that the staff is trained and competent on the process

POLICIES

Introduction

Virification of human oocyets

vitrification protocol (using surelife media)

1. Prepare a loop using steel wire on the cap of a 1.5 ml sterile cryo vial and sterilize the loop by showing on the gas flame inside the hood .

2. Take liquid nitrogen in a cryo bath and bring to the lab.

3. Label 1.5ml cryo vial containing the loop with patients name and date .Put the labelled cryo vial into the liquid nitrogen in the cryo bath.

4. Take out a 60mm dish and dispense 0.15-0.20ml droplets of solution 1,2 and two droplets of 0.20ml solution 3 on the lid .

5. Leave at room temperature for about 5 minutes for equilibration.

6. Select the eggs for vitrification , usually 2-3 eggs are placed in the device.

7. Place the eggs in solution 1 for 4 minutes and then into solution 2 for 2 minutes.

8. Take a cryo loop, touch a droplet of solution 3 to make a thin film of solution in the loop and remove any excess solution by touching the loop on the surface of the dish.(Do this before transferring eggs into solution 3) .

9. Transfer the eggs into the first droplet of solution 3 and rinse for 20-30 seconds.

10. Transfer the eggs into second droplet of solution 3 and rinse for 20-30 seconds.

11. Using a flexi pipette (135µm diameter), quickly pickup the eggs after 30 seconds from sec ond droplet of solution 3 and place in the thin film of solution 3 already prepared on the loop.

12. Make sure the eggs are in the loop.

13. Close the cryo vial tightly and immediately plunge the vial into the liquid nitrogen in the cryo bath.

14.Take the cryo bath along with the cryo vial containing vitrified eggs to the egg storage tank containing liquid nitrogen and store inside the tank. Record the location of the cryo vial in the tank for future retrieval.

THAWING PROTOCOL (USING SURELIFE MEDIA)

1. Take out the required cryo vial from the egg storage tank and carry to the lab in a cryo bath containing liquid nitrogen.

2.Arrange the lid of a 60mm dish and place 0.20 ml droplets of solution 1,2,3,and,4 .

3. Allow the solutions in the dish to equilibrate for about 5 minutes.

4. Unscrew the cap from the cryo vial and gently remove the cryo loop out of the vial without touching the sides of the vial.

5. Plunge the loop along with the eggs quickly in solution 1 and find all the eggs in the loop.

6. Leave the eggs in solution 1 for 3 minutes and transfer into solution 2 for 3 minutes and then solution 3 for 3 minutes

7. Place the eggs in solution 4 for 3 minutes.

8. Finally transfer the eggs into equilibrated fertilization medium and incubate in the co_2 incubator at 37^{o}c .Check the quality of the eggs under microscope

VITRIFICATION PROTOCOL (USING QUINN'S ADVANTAGE MEDIA)

1. Fill the liquid nitrogen reservoir with liquid nitrogen to a sufficient depth to submerge a cryotube or goblet on a cryocane. Attach a cryotube or goblet to the bottom of the cryocane and submerge in the liquid nitrogen in preparation for storage of the vitrified specimen.

2. Take liquid nitrogen in a cryo bath and bring to the lab.

3. Determine the number of eggs to BE vitrified.

4. Label 4 well dish and cryo tube or goblet with patients name and date. put the labelled cryotube or goblet in to the liquid nitrogen in the cryobath.

5. Make sure the contents of each vial of Equilibration Solutions (ES) and vitrification solution (VS) are well mixed by gentle inversion several times before use.

6. Prepare a 4 well dish by aseptically dispensing 0.5ml of ES into well -1 and 0.5ml of VS into well-2.

7. Select the eggs for vitrification , no more than 2 in one procedure.

8.Using a flex pipette (135µm diameter), carefully transfer the eggs with a minimal volume of culture medium to the top of well -1 containing ES and start the timer.

9. Allow the eggs to equilibrate in ES for 7-11 minute. The eggs will shrink and then gradually re-expand to its original size, indicating that equilibration is complete.

10. After equilibration in ES is complete, draw up some ES in to the transfer pipette and transfer the eggs with minimal volume from the ES well in to the centreof VS well .

11. Gently swirl the eggs in the VS for 20 seconds to thoroughly mix it with the VS solution.

12. Transfer the eggs in <1µl of VS solution into the labelled cryotube or goblet attached to a cryocane with another goblet inverted over the top to act as a cap. Immediately plunge the cryotube or goblet containing the carrier device into the liquid nitrogen in the cryo bath.

13. Transfer the cryocane to an egg storage tank containing liquid nitrogen . Record the location of the cryotube or goblet in the tank for future retrieval.

THAWING POTOCOL (USING QUINN'S ADVANTAGE MEDIA)

1. Take out the required cryotube or goblet containing the carrier device from the egg storage tank and carry to the lab in a cryobath containing liquid nitrogen. Keep the carrier device under liquid nitrogen.
2. Take 4 well plates; add 0.5ml of 1 m sucrose solution in well 1 and 0.5ml of 0.5 m sucrose solution in well 2.
3. Also add 0.5 ml of mops solutions into each of well 3 and well 4.
4. Using tweezers, locate the carrier device on the cane in the liquid nitrogen reservoir. Only one carrier device at a time is to be processed.
5. Carefully remove the straw or other carrier device from the cane,keeping the lower part containing the eggs under the surface of the liquid nitrogen.

6. Immediately immerse the device (within 2 seconds) after it has been extracted from its protective covering into well 1 containing 0.5ml of IM sucrose solution. Leave the egg in this solution for 1 minute. The egg will shrink and float to the top of the well.

7.Using a flexi pipette (135 µm diameter), draw up some 0.5M sucrose solution and transfer the eggs from well 1 to well 2 containing 0.5M sucrose solution for 3 minutes. The egg will remain shrunken during exposure to 0.5M sucrose solution.

8. Then transfer the eggs to the top of well 3 containing 0.5ml Mops solution for 5 minutes.

9. Next, transfer the eggs to the top of well 4 containing 0.5ml Mops solution for 5 minutes.

10. Finally, transfer the eggs to a dish of pre-equilibrated fertilization medium and incubate in the CO_2 incubator at 37^0C for 3 to 4 hours. Check the quality of the eggs under microscope.

REFERENCES

Standards

PURPOSE AND SCOPE

This policy and procedure aims to guide IVF staff on Virtification and Warming Of Human Embryos

RESPONSIBILITIES

Medical Director

The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Unit

Is responsible to ensure that the staff is trained and competent on the procol.

POLICIES

VITRIFICATION OF HUMAN EMBROYS

Introduction

Cryopreservation (vitrification) means preservation in the frozen state bring the embryos to a zero metabolism or no dividing state by reducing the temperature in the presence of cytoprotectant.

PREPARATION OF VITRIFICATION MEDIA

1.Solution A:-10% Ethylene glycol

To make 10ml solution A:

1. Take 9ml of Quinn's Advantage Protein Plus Fertilization medium in a 15ml blue capped tube and add 1ml of ethylene glycol to it.

2. Close the tube tightly and mix well by inverting the tube a few times.

3. Sterile filter the solution A in to a 15ml blue capped tube using a Millipore filter and a 10ml syringe.

4. Keep in refrigerator by properly labelling the name and date of preparation.

2.Solution B:-40%Ethylene glycol +0.6M Sucrose

To make 10ml solution B:

1. weigh 2.054g of sucrose and put in a 15ml blue capped tube.

2. Add 6ml of Quinn's Advantage Protein Plus Fertilization medium in to the tube.

3. Close the tube and vortex until all the sucrose dissolves. Then add 4ml of ethylene glycol to the solution and mix well by inverting the tube a few times.

4. Sterile filter the solution B in to a 15ml blue capped tube using a Millipore micro filter and a 10ml syringe.

5. Keep in refrigerator by properly labelling the name and date of preparation.

VITRIFICATION PROTOCOL

1. Prepare a loop using steel wire on the cap of a 1.5ml sterile cryo vial and sterilize the loop by showing on the gas flame inside the hood.

2. Take liquid nitrogen in a cryo bath and bring to the lab.

3. Label 1.5ml cryo vial containing the loop with patients name and date. Put the labelled cryo vials in to the liquid nitrogen in the cryo bath.

4. Take a 4 well plate, add 700µl of Quinn's HTF medium in a well 1,700µl of solution A in well 2 and 700µl of solution B in well 3.

5. Take out the embryos from the co2 incubator and put it in well 1 using a micropipette for 1 minute. Place a maximum of 4 to 5 embryo at a time.

6. After 1 minute equilibration in medium in well 1, transfer the embryos to solution A in well 2 and incubate for 5 minutes. During this time take out the cryo vial from cryo bath and dip the loop in solution B in well 3 to make a thin film on the loop. Close the vial and put it in the cryobath. Also prepare a drop of solution B near well 3.

7. After 5 minutes in incubation in solution A transfer the embryos to the drop of solution B near well 3 for 40 seconds. Quickly pick up the embryos after 30 seconds and place them on the thin film of solution B already prepared on the loop.

8. Close the cryo vial tightly and immediately plunge the vial in to the liquid nitrogen in the cryo bath.

9. Take the cryo bath along with the cryo vials containing vitrified embryos to the embryo storage tank containing liquid nitrogen and store inside the tank. Record the location of the cryo vial in the tank for future retrieval.

PREPARATION OF THAWING MEDIA

Make a stock solution of 1M sucrose in Quinn's medium and at the time of thawing the embryos, make serial dilutions of 0.5 M,0.25 M AMD 0.125 m solutions in a 4 well plate.

To make 10 ml of 1m sucrose solution

1. weigh 3.42 g of sucrose and put in a 15 ml blue capped tube.

2. Add 8 ml of quinn'shtf medium and vortex the tube until all the sucrose dissolves.

3. Check the volume in the tube and make up to 10 ml if necessary with quinn'shtf medium.

Sterile filter the solution in to a 15 ml blue capped tube using a Millipore micro filter and 10 ml syringe. Keep in refrigerator by properly labelling the name and date of preparation.

THAWING PROTOCOL

1. Take out the required cryo vial from the embryo storage tank and carry to the lab in a cryo bath containing liquid nitrogen.

2.Take a 4 well plate ,add 50 µl of 1m sucrose stock solution in well 1 and well 2.also add 500 µl of quinn's medium in well 2,well 3 and well 4.

3. Serially dilute 1M sucrose solution in well 2 to make 0.5M, 0.25M and 0.125M.Mix the contents in well 2 by pipetting up and down 4 times using a 1ml micropipette and transfer 500µl of the solution to well 3l.Similarly mix the contents in well 3 and transfer 500µl to well 4,mix well and discard 500µl from well 4.now wells 1-4 have 500µl of solution and contains 1M,0.5M,0.25M and 0.125M sucrose concentrations respectively.

4. Open the cryo vial and immediately plunge the loop along with the embryos in to well 1 containing 1M sucrose solution. Keep it for 2.5 minutes.

5. Transfer the embryos in to well 2 using a micropipette and keep for 2.5 minutes.

6. Transfer the embryos in to well 3 and keep for 2.5 minutes.

7. Transfer the embryos to well 4 and keep for 2.5 minutes.

8. Finally transfer the embryos to a culture dish containing cleavage medium and incubate in the co2 incubator at 37°c for 3-4 hrs. Check the quality of the embryos under microscope and do ET.

REFERENCES

Standards

PURPOSE AND SCOPE

To guide IVF Lab staff on the periodic maintenance activities and checklist for the IVF laboratory

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Laboratory

Is responsible to ensure that the protocol for maintenance is implemented

POLICIES

The daily check should include the following

1. Check the incubator temperature and co2 readings.
2. Check the refrigerator temperatures.
3. Check the temperature of the heated stages and blocks.
4. Check the water level in the incubator.
5. Check the gas level in the gas cylinders.
6. Check the availability of liquid nitrogen
7. Check the level of liquid nitrogen in the embryo dewars.
8. Check the availability of lab profroma sheets.
9. Check that the dishes with media have been made for next day patient.
10. Check the media supply.
11. Check that the embryo freezing and inseminations have been done.
12. Re-stocking the bench tops.
13. Turn of all unnecessary equipments.
14. Enter patient's records in to the data base.

THE WEEKLY LIST SHOULD INCLUDE THE FOLLOWING.

1. The bench tops and shelves should be swabbed down with 15 solution of 7x.
2. Consumable stock-take and ordering
3. Ordering liquid nitrogen and gases.
4. General lab cleaning including the floor.
5. Refill all water and alcohol squirt bottles.

The following should be performed every month

The incubators, centrifuges and humid cribs should be thoroughly cleaned.

- The accuracy of the incubator temperature readings should be tested against a reference thermometer. Laboratory staticis.

The yearly duties should include:

- Emptying the incubator water jacket and replacing it with fresh tap water.
- Statistics for the whole yea.
- Total dismantling of the benches and shelves for cleaning.
- Servicing of all equipment including microscopes and centrifuges.

All incubators will need cleaning at least every month. This has to occur on a day where the incubator is free and it is important to have a second incubator as back up to shift cultures. The inside surfaces of the incubator should be cleaned with 70 % ethanol. The micro pipettes need monthly calibration to ensure accurate volumes of liquids are delivered.

REFERENCES

Standards

PURPOSE AND SCOPE

To specify the practices of safety and disinfection of IVF Laboratory

RESPONSIBILITIES

Medical Director

The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – IVF Laboratory

Is responsible to ensure that the protocols of safety and disinfection are implemented

POLICIES

1. GENERAL PRACTICES

1. Wear lab uniform and chappals in the lab
2. Do not use lab chappals outside the lab.
3. Use appropriate personal protective equipment.

2. DISINFECTION

a) Sweeping and mopping the floor is done daily with and with diluted 7X solution.

b) Wipe the table, slab, equipments, etc.., with 70% ethanol daily.

c) Laminar Air Flow chamber is wiped with 70% ethanol daily

d) Wash hands with soap solution as mentioned in the hand wash protocol and dry with tissue paper.

Before every processing wear face mask and

REFERENCES

Standards

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	

	SAIDEEP HOSPITAL IVF LABORATORY	Doc No	SDH/IVF/5.10
		Issue No	01
		Rev No.	01
		Date of issue	1 Nov 21
		Pages	1
	Document Title : Protocols for Identification, Handling and Transportation of samples:		

PURPOSE AND SCOPE

To specify the protocol for Identification, Handling and Transportation of samples:

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

HOD – Emergency Care Unit

Is responsible to ensure that the protocol is ensured by staff in all cases

POLICIES

Identification, Handling and Transportation of samples:

1. Labelling all the samples and tissue catheter were with patient's name, Hospital No., and date and recheck during procedure. Work with one sample at a time.

2**.** Perform double identification checks (i.e. two people) before oocyte pick up, insemination, embryo transfer, cryopreservation and embryo thawing.

3. Staff responsible for handling of samples is required to wear appropriate personal protective equipment which includes gloves, mask, cap.

I. Storage of specimen

Embryos cryopreserved in liquid nitrogen.

REFERANCES: Standards

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar		Dr. S.S. Deepak	
Chief Medical Administrator		Chairman & Managing Director	

PURPOSE AND SCOPE

To specify the protocol for storage of specimen in IVF Unit

RESPONSIBILITIES

Medical Director
The overall responsibility of implementing the policy rests with the MD of the hospital.

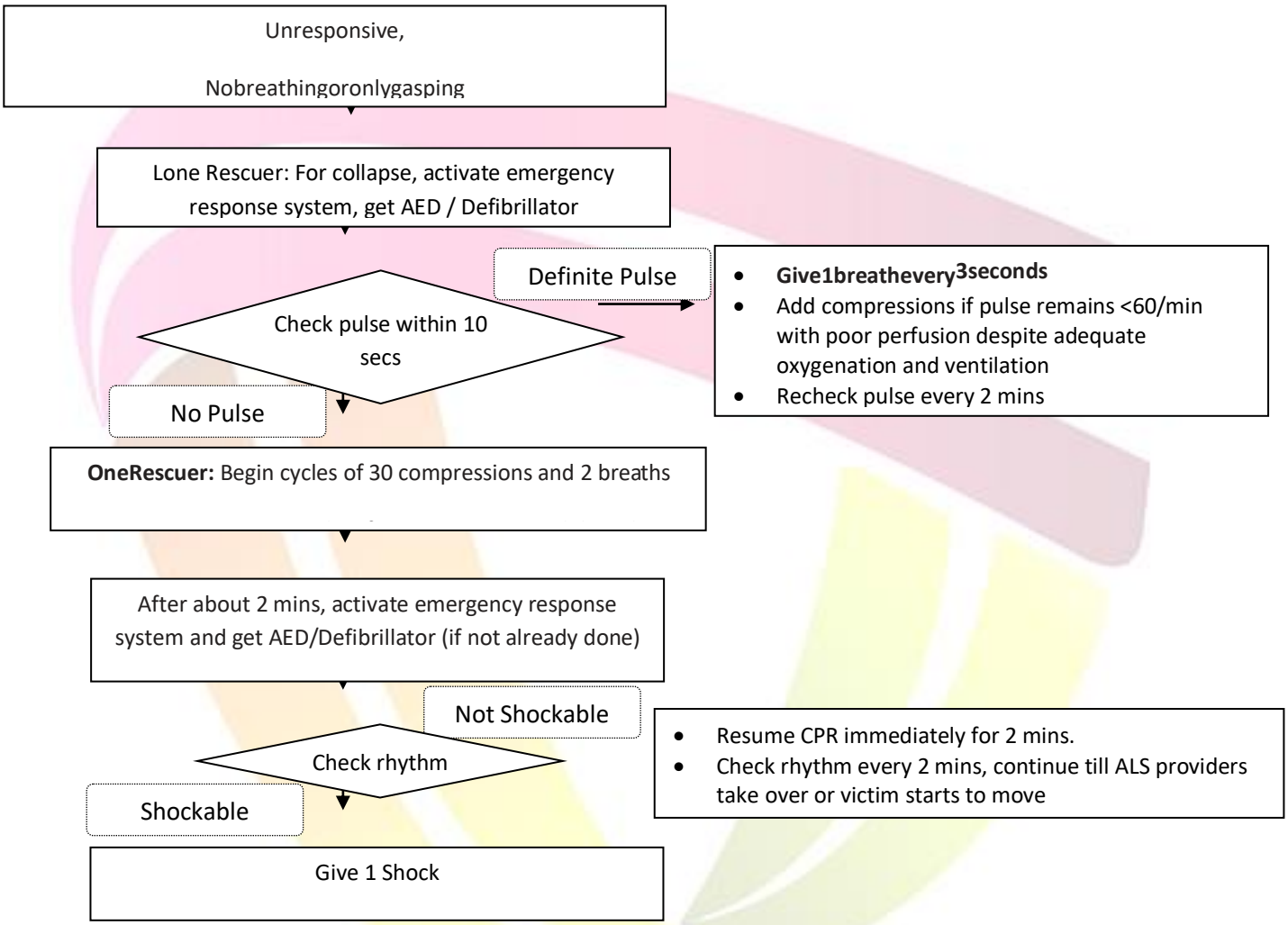
HOD – Emergency Care Unit

Is responsible to ensure that the protocol is ensured by staff in all cases

POLICIES
Embryos cryopreserved in liquid nitrogen

Needs detailing

PEDIATRIC CARDIO PULMONARY RESUSCITATION (CPR)



REFERENCES

Standards

Recommended By	Signature	Approved By	Signature
Dr. Hrishikesh Kalgaonkar Chief Medical Administrator		Dr. S.S. Deepak Chairman & Managing Director	