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Recommendations for the use of the InVitroCare® products.

This document contains recommendations for the use of the InVitroCare® products. This information has been compiled from several institutes working successfully with the InVitroCare® media. Please consider this information as recommendation only, since there are possibilities for variations to allow the user to optimize the use of these products in their own setting and situation.



General:

CO₂ concentrations:

InVitroCare's Follicle Flushing/oocyte retrieval medium (Cat# 2002/2002-5), its Sperm Wash media products (Cat# 2003/2005/2005-5) as well as its IsoCare One-Step sperm isolation medium (Cat# 2207-12) are all formulated with HEPES buffer so that they may be used outside a CO₂ environment.

InVitroCare's fertilization and embryo culture media are bicarbonate based buffers which require incubation in the presence of a 5% CO₂ concentration to maintain proper pH. The best results were obtained when this concentration of CO₂ is not only used in the incubator but also in the Laminar Flow cabinet.

Aliquoting of Media.

Once a bottle of fertilization or embryo culture medium has been opened, it is recommended that aliquots containing the desired volume of medium be transferred under sterile conditions into sterile tubes, using sterile pipettes. Fill tubes containing the aliquots of medium with sterile 5% CO₂ gas and seal. The addition of 5% CO₂ gas will prevent pH drift of the aliquoted medium during storage. Store these tubes at 4°C. These tubes have a shelf life of 1 week. Prior to using the media aliquots, supplement the medium by adding the correct quantity of HSA. Once the HSA has been added the product should be used within 2-3 days, when correctly stored at 4°C. If HSA has been added using a sterile pipette, there is no need for extra filtration.

Equilibration.

InVitroCare® recommends that the aliquots of bicarbonate based media, which have been supplemented with protein, be equilibrated overnight in incubator containing 5% CO₂ prior to use. The overnight equilibration allows the pH to stabilize before the media are used for fertilization and embryo culture.

Follicle flushing:

InVitroCare's HTF- HEPES (Cat # 2002) is recommended for follicle flushing and oocyte retrieval. The HEPES buffer maintains a stable pH during exposure of the medium to ambient room conditions such as those encountered during prolonged oocyte retrieval and follicle flushing procedures. Immediately after retrieval of oocytes in HTF-HEPES, the oocytes should be transferred to a bicarbonate based fertilization medium such as InVitroCare's HTF (Cat# 2001) which has been supplemented with 7% Human Serum Albumin (Cat 2101).

While not recommended, bicarbonate based media such as HTF (Cat# 2001) or IVC-ONE (Cat # 2006) may also be used for oocyte retrievals in cases where the retrieval procedure is performed in such a manner that the bicarbonate based media is kept in a 5% CO₂ environment such as in an Isolette.

ICSI

General:

Prepare the semen sample using a method described under "Semen preparation" Prepare ICSI dishes using HTF-HEPES with addition of HSA. 100 uL HSA to 900 uL HTF-HEPES. Perform the ICSI using your current method. Wash the fertilized egg in InVitroCare's IVC-TWO medium which has been supplemented with 10% HSA.

Day 3 Transfers

Transfer the fertilized egg into fresh embryo culture medium IVC-TWO (product # 2008) which has been supplemented with 10% HSA. Maintain the developing embryo in culture in an incubator containing 5% CO₂ for a period of three days or until the eight cell stage. Based on other clinical factors such as patient endometrial receptivity,

the embryo may be transferred to the patient at this stage of development or maintained in extended culture until development of expanded blastocyst. The embryo may be transferred at day three using IVC-TWO which has been supplemented with up to 20-30% HSA as the transfer medium.

Day 5 (expanded blastocyst) Transfers

If election is made to maintain the embryo in extended embryo culture until development of expanded blastocyst, continue to culture the developing embryo in IVC-TWO (Cat# 2008) which has been supplemented with 10% HSA through late day three or early day four until the embryo begins to compact. Upon compaction, transfer the embryo to IVC-THREE (Cat# 2007) which has been supplemented with 12%-15% HSA. Maintain the compacted embryo in culture in an incubator containing 5% CO₂ until day 5 or until development of expanded blastocyst (usually day 5 or early day 6).

The expanded blastocyst may transferred using IVC-THREE which has been supplemented with 20-25% HSA as the transfer medium.



Additional ICSI reagents

PVP (product # 2210)

InVitroCare's PVP reagents is composed of HEPES Buffered HTF containing 5mg/ml HSA to which has been added Polyvinylpyrrolidone at concentration of 10%. The PVP reagent is ready to use and maybe added directly to the sperm sample to slow down sperm movement during sperm capture in an ICSI procedure.

ATS (product # 2211)

InVitroCare's Acidified Tyrodes buffer is applied to a localized section of the embryo using an assisted hatching pipette to facilitate the hatching process by creating a hole in the outer membrane of the maturing embryo.

Hyaluronidase (product # 2212)

InVitroCare's Hyaluronidase reagent, consists of HEPES buffered HTF containing 5mg/ml HSA to which has been added bovine testes derived Hyaluronidase at a concentration of 100IU/ml. The Hyaluronidase reagent is ready to use and may be applied directly to the oocyte to remove the surrounding cumulus cells and zona pelucida as part of the denuding process prior to ICSI.

IVF

Prepare the semen sample using a method described under "Semen preparation" Transfer the retrieved oocytes to the recommended fertilization medium HTF (Cat#2001) which has been supplemented with 7% HSA.

If using microdroplet culture under oil, transfer oocyte in 10-20ul HTF to a culture dish containing an appropriate amount of mineral oil. To the microdroplet add the sperm sample prepared above. Allow the sperm and oocytes to incubate in an incubator containing 5% CO₂ for 24 hours.

Using microscopic examination identify and isolate fertilized oocytes which now contain two pronuclei and place them in fresh IVC-TWO which has been supplemented with 10% HSA. Maintain in culture in an incubator containing 5% CO₂. Proceed with day three or day five culture as described above.

SEMEN PREPARATION I

IsoCare ONE-Step™ (product# 2207-12)

This procedure is intended to process sperm fast in one step. This medium can be used for processing semen for IUI directly or other procedures when followed by a wash step.

1. Bring the appropriate volume of IsoCare to 37°C before use. The temperature should never become higher than 39°C.
2. Transfer 1 ml of IsoCare One-Step to a sterile 15 ml centrifuge tube
3. Gently transfer 1 - 2 ml of fresh or frozen-thawed specimen on top of the IsoCare layer. Frozen-thawed samples should be thoroughly warmed to 37°C to maximize sperm motility. There should be no mixing of the sample and the IsoCare layer. If the semen volume is more than 2 ml, use more than one tube of IsoCare One-Step.
4. Centrifuge for 30 minutes at 500 x g (alternatively 40 minutes at 300 x g is possible) If the sample has a high viscosity or a low sperm count centrifuge for an extra 10 - 20 minutes. NB since the density of IsoCare is similar to the buoyant density of semen, no pellet will form. The processed sperm will remain suspended in the IsoCare layer.

5. Using a pipette or syringe, carefully remove the spent seminal fluid layer and interface layer by aspiration without disturbing the underlying IsoCare layer. Aspirate from the top downward by always keeping the pipette tip just below the fluid surface.
6. The sample is now ready for IUI applications. Transfer the processed semen into an approved IUI catheter and perform the insemination. If the semen is used for other procedures (IVF or ICSI continue with #7)
7. Using a syringe or pipette, add 2 - 3 ml InVitroCare® Sperm Wash Medium (product # 2003/2005) and mix thoroughly.
8. Centrifuge for 5 minutes at 500 x g. Now a pellet will be visible.
9. Carefully remove the supernatant and re-suspend the sperm pellet in a suitable volume of appropriate medium. (HTF-HEPES or IVC-One for use in IVF or ICSI)



SEMEN PREPARATION II

Swim up procedure

For laboratories wanting to use a swim up from semen the following procedure is recommended:

1. Place 1.5 ml of warm (37°C) InVitroCare® Sperm Wash Medium (product # 2005) in a sterile round bottom culture tube.
2. Using an appropriate syringe (like a 3 cc syringe with a 1.5" , 21 gauge needle) gently place up to 1 ml liquefied semen under the medium into the bottom of the tube and cap the tube. (If semen sample is greater than 1 ml, use multiple tubes).
3. Place the tubes in an incubator (water bath can be used as an alternative) at an incline of 30 - 45° and incubate for one hour. If a CO₂ incubator is used, the caps may be loosely in place since InVitroCare® SPERM WASH medium is carbonate buffered.

4. Using a pipette or syringe, carefully remove the upper 1.0 to 1.2 ml of the medium down to the interface between the medium and semen. (Combine the overlays from several tubes if more than 1 ml of semen was processed)
5. Place (the combined) overlay(s) into a sterile, 15 ml conical centrifuge tube. Add additional InVitroCare® SPERM WASH to make a total volume of 3 ml in the centrifuge tube.
6. Centrifuge for 5 minutes at 500 x g.
7. Carefully remove the supernatant and re-suspend the sperm pellet in a suitable volume of an appropriate medium. (e.g. for IUI use 0.4 - 0.5 ml InVitroCare® SPERM WASH for IVF, use InVitroCare® IVC-ONE diluting the sperm to an appropriate volume)

Human Serum Albumin

Product information:

InVitroCare® HSA contains 100 mg/ml total protein (weight/volume) in saline solution. Each lot of HSA is tested for pH (7.4 ± 0.2), osmolarity (280 ± 10 mOsm/Kg water), sterility (no detectable contamination) and biocompatibility (>80% mouse zygote development to blastocysts).

Why add protein to culture media?

A protein source is added to culture media because it is thought to maintain the cell membrane stability and to remove by chelation, traces of toxic contaminants which may be present in the media components (including culture water) and/or culture dishes. This protein is usually in the form albumin, which makes up the bulk of the protein present in blood serum and reproductive tract fluid. Protein, in the form of patient's serum or albumin, HSA been used extensively in the ART procedures of sperm washing for IUI, IVF, GIFT, ICSI, embryo transfer and cryopreservation.

Medical safety of product.

The parent HSA product was derived from blood donors who were individually tested and found to be non-reactive for Hepatitis B Surface Antigen (HbsAg) and antibodies to Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV) by approved testing methods. The product was heated to 60°C for ten hours and the HSA prepared by cold alcohol fractionation. Experimental evidence (Transfusion 26:210, 1986) indicates that the cold ethanol fractionation process effectively inactivates the HIV virus. Although no positive assertions can be made, the heat treatment probably destroys the causative agents of viral hepatitis. After this alcohol treatment, the fraction is washed several times to remove any remaining alcohol traces.

Directions for use

For sperm preparation and embryo culture:
use at 5 mg/ml. For 10 ml of medium, add 0.5 ml of HSA solution to 9.5 ml
of IVC-ONE (Cat# 2006).

NOTE: For washed sperm samples for IUI, use
SPERM WASH MEDIUM (Cat# 2003/2005)
that already contains 5mg/ml HSA.

For embryo transfer:
use at 30 mg/ml. For 10 ml of medium, add 3 ml of HSA solution to
7 ml of HTF HEPES (Cat# 2002).

For embryo cryopreservation:
use at 10 mg/ml. For 10 ml of medium, add 1 ml of HSA solution to
9 ml of HTF HEPES (Cat# 2002).

For micromanipulation (ICSI and Assisted Hatching):
use at 5 mg/ml. For 10ml of medium, add 0.5 ml of HSA solution
to 9.5 ml HTF HEPES (Cat# 2002).



Human Serum Albumin HSA vs other protein supplements In IVF Culture Media

Why add protein to IVF culture media?

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Comparison of HSA & other protein supplements.

Initial comparisons of the commercially available plasma expander, Plasmatein resulted in enhanced embryo development in both mouse and human embryos when compared to Human Serum Albumin (HSA). However, not all lots of Plasmatein exhibited the same level of enhanced performance. This lot-to-lot variation may be due to the presence of chemical stabilizers sodium caprylate and sodium acetyltryptophanate, which are known to inhibit sperm motility and also have an embryo toxic effect.

Synthetic Serum Substitute (SSS).

Similar in composition to Plasmatein, is composed of 84% Human Serum Albumin (HSA) and 16% alpha globulin. The material is formulated in saline using pharmaceutical grade HSA which contains the stabilizers sodium caprylate and sodium acetyltryptophanate, and a crude preparation of alpha globulin which has been heat treated at 65° for 10 hours, prior to use in the formulation. SSS has exhibited the same lot-to-lot variation in mouse and human embryo culture systems, due again, to the embryo toxic stabilizers, as well as, the variations in concentration of the alpha globulin fraction resulting from the manufacturing process. More recent clinical studies report no significant difference in human embryo quality when HSA or SSS is used. Although the fertilization rate may be higher in SSS-supplemented medium, more zygotes develop to good quality embryos in HSA- supplemented medium.(presented at the 1996 ESHRE Meeting by Andre Van Steirteghem's group in Brussels).

InVitroCare Product Features

- One-Cell mouse embryo tested.
- Cytotoxicity tested with sperm hyper activation assay.
- pH range 7.25 – 7.45
- Osmolarity 270 – 290 mOsm/Kg
- Packaged under CO₂ blanket

Shelf life:

- HTF-HEPES 12 months
- Sperm Wash Medium 12 months
- Human Serum Albumin 12 months
- IsoCare One-Step 12 months
- HTF 4 months
- IVC-One 4 months
- IVC-Two 4 months
- IVC-Three 4 months

Increased shelf life due to:

- Use of Gentamycin as antibiotic
- Alanylglutamine as nitrogen source
- Sealed under CO₂ environment.

