Microbiological prepare culture medium should be virtually sterile

Microbiological safety includes the following: 1. Screening patient for pathogenicmicro-organisms: European Societyfor Human Reproduction and Embryology (ESHRE) recommends that the women who areundergoing IVF treatment should have undergone screening test for HIV, HBsAg, Antibodies to Rubella, Toxoplasma. Theirpartners should also undergo the screening test for these infections.

In Netherlands, screening is not indicatedunless case history indicates the possibility for any infection. Screening isnot indicated if the IVF treatment does not include cryopreservation of theembryos. If the patients serum is to be used in the medium and the embryos areto be cryopreserved then serum should comply with quality standards such asthose used for blood banks (HIV- negative, HBsAg- negative, HSV-negative &Syphilis -negative).

Screening of patients for sexually transmitted diseasessuch as gonorrhoea & chlamydia are generally carried on indications liketubal pathologies, Cervicitis, Leukospermia etc., if the results are positivetreatment for the infection has to be given in first place before starting theIVF treatment.
2. Contamination of the culture medium includesthe following a. Safety preparation of the culture medium: Erlenmeyer flask should be cleaned according to the cell and tissue cultureprocedures. Heat sterilization is preferred. Water that is used to prepareculture medium should be virtually sterile and the prepared medium should besterilized using membrane filtration before storage.

To secure complete safetymedium should be checked for the growth of bacterium after preparation andfiltration by incubating at 37oc for 48 hours.

Cloudiness of themedium indicates bacterial contamination. Routine examination for the endotoxinis not necessary.

Paraffin oil that is used to overlay the medium need not besterilised since the high standard company products are used. b. Contamination of medium from the serumcomponent: If patients own serum is used for preparing the culture medium, screening test for diseases transmissible via blood is necessary for thecryopreservation of the embryos. During cryopreservation it is very importantto avoid contamination of liquid nitrogen. Negative results are valid for oneyear after which the donor is tested again for further use of the embryos. Useof pooled sera must be avoided since the test that is used for the detection ofdiseases has its own sensitivity. c.

Contamination of the medium from follicularfluid: Follicular fluid can cause contamination during vaginal puncture. Itis not advisable to disinfect vagina as its highly embryotoxic especiallyiodine. d. Contamination of the semen: Mostcommon. e. Contamination of the medium from thelaboratory environment: It is important to maintain air quality in IVFlaboratory since it might affect the clinical outcome.

Laminar Air Flow (LAF)is used to avoid contamination of the culture medium from laboratoryenvironment. Laminar air flow is of two types vertical and horizontal. Verticalair flow is safer than horizontal air flow since it provides protection to theuser as well as the culture.. Air is drawn into the HEPA filter which flowsgentle and smooth towards the operator in horizontal air flow and

towards thetable in vertical air flow. There are three types of laminar hood. Class I –airflow characterise to chemical fume hood.

Provides significant protection forthe user but does not protect the culture. Class II-provides asepticenvironment . hence indicated for IVF laboratory. Class III-gas tight cabinetsand provides high protection for the user. HEPA filters can filter theparticulate size of upto 0. 3 microns whereas ULPA filters filter upto 0. 1 microparticle size. Floor and the instruments can be disinfected with phenoliccompounds or 0.

5%hypochlorite. UV light should be avoided since it can causeozone formation. Use of disposable materials of tissue culture quality isrecommended. To work with tissue cultures glass wares should be sterilised inaccordance with the procedure.

it is recommended to clean the incubators withopen water system for every four weeks with 70% ethanol. To prevent the developmentof fungi in the incubator copper sulphate or amphotericin can be used. f.

Contaminantsof the culture medium expected: E. coli, yeasts and fungi are the morecommon contaminants. Bacterial investigation has to be carried out to discoverpathogens. In case of infection refined semen sample used for insemination hasto be subjected to microbial investigation. If semen is the contaminant, gramnegative rod shaped bacilli will be found in the culture. Yeast will be seen ifcontamination is due to follicular fluid and fungal growth will be observed inthe culture if the contamination is from the incubator. Oocyte degenerates ifthe culture medium is contaminated by bacteria. In case of yeast and fungalcontamination vital embryos survive but the

transfers of the contaminatedembryos were not indicated due to lack of documentation.

g. Donor semen: Fresh semen sample is notadvisable for donor insemination. Minimum of six months quarantine period isnecessary for donor sample. h. Co-cultures: Co-culture of humanembryos with animal cell or the other human cell which is followed by transferis not advisable, since it might result in viral contamination.

i. Safetyof enzyme treatment: alpha amylase or hyaluronidase should be free ofviruses or prions. j. Cryopreservation: Contamination of the liquid nitrogen should be avoided to prevent the otherembryos from infection as decontamination of liquid nitrogen is not advisable.