

CULTURE OF EMBRYOS PROTOCOL

Post - Vitrification and Warming

ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22 - 27°C)

Have all necessary materials, tools and equipment ready and handy before starting procedure.

INTRODUCTION

Vitrification solutions contain 20% (v/v) protein solution, while culture media typically contains 10% or less. Protein is a potent osmolyte and is thought to protect embryos from osmotic stress when exposed to cryoprotectants during the vitrification process. It is strongly suggested that embryo culture media are supplemented with 20% (v/v) protein solution for oocytes and embryos post-warming.

PREPARING A MEDIUM CONTAINING 20% PROTEIN V/V SOLUTION

For those laboratories that purchase culture media without added protein, making a 20% (v/v) solution is relatively easy. Typically, 2 mL of protein solution would be gently mixed with 8 mL of culture medium. However, many facilities purchase medium with protein already added, either at a concentration of 5% (v/v) when pre-supplemented with HSA or 10% (v/v) when pre-supplemented with SSS™.

To create a 20% (v/v) protein solution or equivalent from pre-supplemented solution, protein should be added according to Table 1 when starting with 10% (v/v) SSS or DSS solution, or Table 2 when starting with 5% (v/v) HSA solution.

PREPARING DISHES FOR CULTURE EMBRYOS POST-WARMING

It is recommended that culture media dishes are made up at least 12 hours prior to warming any embryos to allow for equilibration in a gas phase of 5-7% CO₂, depending on your particular culture conditions and target pH.

PLACING THE EMBRYOS IN CULTURE

Once the embryos have been warmed, they should be washed into the pre-equilibrated dish of culture medium with 20% protein solution. The additional protein solution will have a mildly acidic

effect on the medium, but the pH should not be more than 0.02 below what would be seen with 10% protein solution.

For short-term culture, where the embryos are transferred on the same day, culture in 20% protein solution is appropriate. If the embryos are to be cultured overnight, they can be placed back in pre-equilibrated medium with 10% protein solution after 4 hours. For oocytes, these can be placed in medium with a normal protein solution concentration after ICSI (usually 4 hours post warming).

TABLE 1

Serum Substitute Supplement (SSS) or Dextran Substitute Supplement (DSS) (Initial volume of medium containing 10% (v/v) protein (mL))		
Volume of medium	Volume of protein solution to add	Final volume of 20% protein (v/v) solution
1 mL	0.125 mL	1.125 mL
5 mL	0.625 mL	5.625 mL
10 mL	1.25 mL	11.25 mL

TABLE 2

Human Serum Albumin (HSA) (Initial volume of medium containing 5% (v/v) protein (mL))		
Volume of medium	Volume of protein solution to add	Final volume of 10% protein (v/v) solution
1 mL	0.056 mL	1.056 mL
5 mL	0.28 mL	5.28 mL
10 mL	0.56 mL	10.56 mL



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