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Culture Media Preparation for Embryos

Post-Vitrification and Warming

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

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

INTRODUCTION

Vitrification solutions contain 20% (v/v) Dextran Substitute Supplement (DSS), while culture media typically contains 10% or less depending on the protein supplement. Protein is a potent osmolyte and is thought to protect embryos from osmotic stress when exposed to cryoprotectants during the vitrification process. It is recommended to supplement embryo culture media with additional protein up to 10% (v/v) of HSA (P/N 9988) or 20% (v/v) of SSS (P/N 99193) or DSS (P/N 9301) for oocytes and embryos post-warming.

PREPARING A PROTEIN SOLUTION FOR A CULTURE MEDIUM

When using a culture medium that is not pre-supplemented with protein from the manufacturer, SSS, DSS, or HSA should be added according to Table 1 or Table 2, respectively.

TABLE 1

Serum Substitute Serum (SSS) or Dextran Substitute Supplement (DSS)

Volume of medium	Volume of protein solution to add	Final volume of protein solution (v/v)
8 mL	2 mL	20%

TABLE 2

Human Serum Albumin (HSA)

Volume of medium	Volume of protein solution to add	Final volume of protein solution (v/v)
9 mL	1 mL	10%

10% (v/v) HSA is equivalent to 20% (v/v) SSS or DSS

To increase the protein concentration in a pre-supplemented culture medium, protein should be added according to Table 3 and Table 4, respectively.

TABLE 3

Serum Substitute Supplement (SSS) or Dextran Substitute Supplement (DSS) (Initial volume of medium containing 10% (v/v) protein)

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 4

Human Serum Albumin (HSA) (Initial volume of medium containing 5% (v/v) protein)

Volume of medium	Volume of HSA to add	Final volume of 10% HSA (v/v)
1 mL	0.056 mL	1.056 mL
5 mL	0.28 mL	5.28 mL
10 mL	0.56 mL	10.56 mL

CULTURE MEDIA DISH PREPARATION FOR EMBRYOS POST-WARMING

It is recommended that culture media dishes are prepared at least 12 hours prior to warming embryos to allow for equilibration in a gas phase of 5–7% CO_2, depending on the particular culture conditions and target pH.

PLACING THE EMBRYOS IN CULTURE

Once the embryos have been warmed, they should be rinsed through the pre-equilibrated dish of culture medium that has been prepared according to the tables above. 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.

If the embryos will be transferred on the same day, culture in increased protein solution is appropriate. If the embryos are to be cultured overnight, they can be placed back in pre-equilibrated medium with 10% (v/v) SSS/DSS or 5% (v/v) HSA after 4 hours. Oocytes can be placed in culture medium with a normal protein solution concentration after ICSI (usually 4 hours post warming).

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