PREPARATION AND USE OF SAGE MEDIA IN A TYPICAL ART CYCLE: PREPARATION OF DISHES

Media should be supplemented with protein as follows:

Medium	Use	mL medium	mL Protein
QA Medium with HEPES	Rinsing of oocyte-cumulus complexes (OCC)	10 mL in a 60 mm diameter dish	0
QA Medium with HEPES + HSA	1. Storage OCCs during the aspiration procedure. 2. Fertilization assessment of oocytes on D1	9.5 mL Use a Falcon Organ Culture dish with 1 mL of medium covered with oil in the center well and 5 mL of QA HEPES with no protein in the outer moat.	0.5 mL HSA
QA Fertilization Medium + HSA ***(SEE NOTE 2. BELOW)***	1. Incubation of oocytes before conventional insemination or ICSI. 2. Conventional insemination of oocytes	9.5 mL Use 60 mm diameter dish with 10 x 30 uL drops covered with oil.	0.5 mL HSA
QA Cleavage Medium + SPS	Culture of embryos from D1 to D3	9.0 mL Use 60 mm diameter dish with 10 x 30 uL drops covered with oil.	1.0 mL SPS
QA Blastocyst Medium + SPS	Culture of embryos from D3 to D 5/6	9.0 mL Use 60 mm diameter dish with 10 x 30 uL drops covered with oil.	1.0 mL SPS
QA Medium with HEPES + HSA or SPS	For embryo transfer (ET)	with HSA: 7.0 mL with SPS: 5.0 mL Use a Falcon Organ Culture dish with 1 mL of medium covered with oil in the center well and 5 mL of medium in the outer moat.	HSA: 3.0 mL SPS: 5.0 mL

PREPARATION AND USE OF SAGE MEDIA IN A TYPICAL ART CYCLE: PREPARATION OF DISHES

NOTE:

The media series Protein Plus (ART-1520, ART-1526, and ART-1529) are equivalent to the respective QA series (ART-1020, ART-1026, and ART-1029) but already have the protein added so no additional protein is required.

NOTE 1:

All bicarbonate buffered media (QA or Protein Plus Fertilization, Cleavage and Blastocyst media (cat # ART-1020/1521, -1026/1526 and -1029/1529) require a minimum of 4 h incubation under a CO_2 atmosphere to fully equilibrate or incubation overnight.

If the color of the phenol red pH indicator in the bottle of medium appears excessively red, the medium can be gassed within the bottle with a sterile plugged 1 mL pipette attached to a cylinder of gas mixture containing 5% CO_2.

Use aseptic precautions and avoid excessive bubbling if the medium contains protein. The gas mixture should be blown over the surface of medium containing protein, not bubbled through it.

HEPES-buffered medium does *NOT* require CO_2 equilibration.

NOTE 2:

There are anecdotal data that human oocytes can be stored in QA Cleavage Medium (with 5 mg/mL SPS) or Protein Plus Cleavage Medium (which is manufactured to contain SPS), inseminated in this medium in regular IVF and cultured until Day 4 of beyond with acceptable results. It has also been suggested that embryos can be cultured to the blastocyst stage on Days 5/6 in Cleavage Medium, again, with acceptable results.

It is the responsibility of the end-user to validate this methodology in their own laboratory before using it as a routine procedure. It is also recommended that if this strategy is invoked, that the embryos be transferred to fresh Cleavage Medium on Day 3 or 4.

Aeulestrasse 23 – 72074 Tübingen – Tel: (+49) 07071 83958 - Fax: (+49) 07071 83995 – kb@supra-net.net - www.kbbiosystem.com