SAGE In Vitro Fertilization

Protocol for Single Embryo Culture

SAGE® In Vitro Fertilization
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Form # 81504 • Rev. 01/12

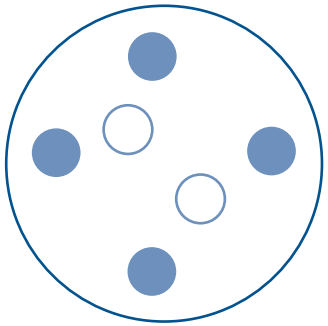
IVF Protocol For Single Embryo Culture

1. With cumulus-free oocytes and embryos up to Day 3 (D3), use 275-300 µm diameter pipette tips to minimize medium transfer between drops; transfer volume should be < 1 µL.

Day 1

2. At 4:00 in the afternoon before the day of oocyte retrieval, label 60 mm diameter BD Primaria® cell culture dishes (Falcon # 353802; or alternatively #353002). When making drops of medium, use a single-wrapped pipette tip, rinsing the tip twice with culture medium before making the drops.

3. ***For In Vitro Fertilization (IVF):*** Place 6 x 30 µL drops of ***Quinn's Advantage® (QA) Protein Plus Fertilization Medium*** (ART-1520) into the dish. Four drops should be at the 3, 6, 9 and 12 o'clock positions (used for culture); the 5th and 6th drops should be in the center of the dish (used for washing) — see diagram below.

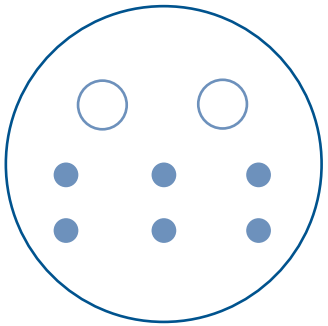


● = *30 µL drops used for insemination*

○ = *30 µL drops used for washing oocyte-cumulus complexes*

4. Immediately cover the drops with 9 mL of ***Oil for Tissue Culture,*** (ART-4008) and place the dish in the CO_2 incubator.

5. ***For Intracytoplasmic Sperm Injection (ICSI):*** Place 6 x 10 µL drops of ***QA Protein Plus Cleavage Medium*** (ART-1526) in the dish and 2 x 30 µL drops for washing in the dish as indicated below.



○ = *30 µL drops for washing oocytes*

● = *10 µL drops for culture of individual oocytes after ICSI*

6. Immediately cover the drops with 9 mL of oil and place the dish in the CO_2 incubator.

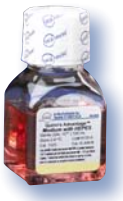
7. Prepare no more than 2 dishes at a time to minimize out-gassing of CO_2 and drift in the pH of the medium.

8. When placing the dishes in the incubator, gently remove the lid of the dish and set it at an angle on the side of the dish to allow for complete gas exchange. Dishes must gas for a minimum of 4 hours before use (or overnight).

Day 0 - Day of Oocyte Retrieval or Ovum Pickup (OPU)

9. ***For IVF on D0 at 4:00 pm:*** Prepare 60 mm diameter BD Primaria dishes as described in point 5 above for culture of fertilized oocytes in ***QA Protein Plus Cleavage Medium*** (ART-1526).

10. ***For both IVF and ICSI on D0 at 4:00 pm or D1 at 8:00 am before fertilization check:*** Prepare 60 mm diameter dishes with 9 x 10 µL drops of ***QA Medium with HEPES + 5 mg/mL HSA*** (HEPES+HSA: ART-1023 and ART-3001, respectively).



These can be prepared at ~ 4:00 pm on D0 and left at room temperature overnight, or prepared early on the morning of D1 warmed in air to 37˚C on a heating plate. In either case, warm the dishes to 37˚C on the morning of D1 before use.

11. ***For IVF late on D0 or early on D1:*** Prepare a wash dish using a Falcon organ culture dish. Use HEPES+HSA and place 1 mL of this medium in the center well and 2 mL in the moat.

Day 1 - Day of Fertilization Check

12. Gently remove the cumulus cells by "stripping" in the insemination dish. Gently wash the stripped oocytes in the well of the organ culture wash dish. Washing entails picking up the oocyte 2-3 times and moving the cells around within the well. Then place fertilized oocytes in individual 10 µL drops in the HEPES+HSA dish. For ICSI'ed oocytes, transfer fertilized oocytes to individual 10 µL drops of medium in the HEPES+HSA dish. Keep the dish containing the 10 µL drops of QA Protein Plus Cleavage Medium, in which the ICSI'ed oocytes were cultured overnight, in the CO_2 incubator for their return after pronuclei scoring described in point 13 below and their continued culture up until D3.

13. Score the inseminated/ICSI'ed fertilized oocytes under an inverted microscope for pronuclei and their alignment.

14. Place the fertilized oocytes individually into drops of ***QA Protein Plus Cleavage Medium,*** as described in point 5. Place only 6 embryos in each dish and handle one dish at a time to minimize increases in pH due to overexposure to air. Quickly return the culture dish to the incubator.

15. Follow the embryo scoring regime at the the times listed on accompanying procedure #F008 wherever possible.

Day 3 to the Blastocyst Stage

16. On D3 before 8:30 am, label 60 mm BD Primaria dishes with the patient's name.

17. Prepare culture drops of ***QA Protein Plus Blastocyst Medium*** (ART-1529) using the format described in point 5. These culture dishes must gas in the incubator for a minimum of 4 hours before use.

18. ***On D3 between 10:00 am and 2:00 pm after Blastocyst Medium dishes have equilibrated for at least 4 hours:*** For embryos that are to be cultured from D3 to D5/6, remove the embryos from the Cleavage Medium culture dishes and place in individual 10 µL drops of Blastocyst Medium in the Blastocyst Medium culture dish after washing the embryos through the 30 µL wash drops of Blastocyst Medium that are in the same dish. Culture only 6 embryos in a dish and handle 1 dish at a time.

PLEASE NOTE: There is anecdotal data that transferring cleaving embryos from Cleavage Medium to Blastocyst Medium on D2 or D4 may result in better results than the more traditional medium exchange on D3. It is the responsibility of each individual laboratory to determine their own protocol for when this embryo exchange from Cleavage Medium to Blastocyst Medium should be undertaken. To reach this decision, it should be kept in mind that the optimal day of exchange may be patient dependent to some extent, i.e., some patients may do better if the exchange is on D2, others, if the exchange is on D3, and others again, if the exchange is on D4. In addition, some embryologists leave the embryos for the whole culture period from D1 to D5/6 in cleavage medium.

Day 5

19. ***On morning of D5:*** Score embryos for development to the blastocyst stage. For Embryo Transfer (ET), select the best 2 embryos. They should be at least grade 4AA (scoring by Gardner parameters—see Embryo Scoring Form #F008). Any blastocysts not transferred should be cryopreserved by vitrification.

20. Any embryo that has not formed a grade 3 or 4 blastocyst (i.e., fully expanded) should be cultured in a fresh drop of QA Protein Plus Blastocyst Medium and assessed on D6; if suitable on D6, it should be cryopreserved by vitrification. For these embryos, make up a fresh dish of blastocyst medium on D5, as indicated in points 5 and 17 above, and allow it to equilibrate in the CO_2 incubator for a minimum of 4 hours before transferring embryos to it.

Related Products

SAGE In Vitro Fertilization has a full line of products for the Reproductive Medicine Specialist. SAGE Media™ products are designed to provide consistent results during every stage of fertilization and embryo development. Our Quinn's Advantage® Sequential Media series and Quinn's Advantage® Protein Plus Sequential Culture Media series are manufactured under tri-gas conditions to enable ease and accuracy in achieving optimal pH ranges during re-equilibration.

REF#	Description	Unit Size
ART-1020	Quinn's Advantage Fertilization (HTF) Medium	50 mL
ART-1021	Quinn's Advantage Fertilization (HTF) Medium	100 mL

REF#	Description	Unit Size
ART-1026	Quinn's Advantage Cleavage Medium	50 mL
ART-1027	Quinn's Advantage Cleavage Medium	100 mL

REF#	Description	Unit Size
ART-1029	Quinn's Advantage Blastocyst Medium	50 mL

REF#	Description	Unit Size
ART-1023	Quinn's Advantage Medium with HEPES	100 mL
ART-1024	Quinn's Advantage Medium with HEPES	500 mL

REF#	Description	Unit Size
ART-1520	Quinn's Advantage Protein Plus Fertilization (HTF) Medium	20 mL
ART-1526	Quinn's Advantage Protein Plus Cleavage Medium	20 mL
ART-1529	Quinn's Advantage Protein Plus Blastocyst Medium	20 mL
ART-1729	Quinn's Advantage Protein Plus Blastocyst Medium with insulin	20 mL

To find out more about **SAGE Media** go to **www.coopersurgical.com**.
To place an order call **800.243.2974.**

Reference

Gardner, DK (2006) Human embryonic development in vitro., In . SL Tan, R-C Chian, WM Buckett (Eds.), *In-vitro Maturation of Human Oocytes* (Ch. 22) Boca Raton, FL: Taylor and Francis.

Accompanying Procedure # F008 "Scoring of embryos during an ART cycle"

Patient's name:_____ **Patient's ID:**_____

Embryo # (Dish #_____)

Day	Date	Time	Event (points allocated)	1	2	3	4	5	6
0			Insemination						
1			**(16-18 h pi) 2PN check** nucleoli aligned = 20 not aligned = 0						
1			**(25 h pi)** Early cleavage to 2-cell and symmetric blastomeres = 30 Slightly asymmetric blastomeres = 25 no cleavage = 0						
1 or 2			**(25 h pi or 40-44 h D2)** 0% frag = 30 <20% frag = 25 >20% frag = 0						
2			**(40-44 h pi)** ≥ 1 cell multinucleated = deduct all previous points						
3			**(64-67h pi)** 7/8/9-cell 0% frag = 20 8-cell <20% frag = 20 for both groups score = 20 if full to some compaction, score =15 if no compaction 7/9/≥10 <20% frag = 10						
CUMULATIVE EMBRYO SCORE TO D3			**TOTAL POINTS (100 MAX)**						
5			1 = eB(<50% cavity) 2 = EB(>50% cavity) 3 = FEB(100% cavity) 4 = >100% cavity 5 = hatching 6 = fully hatched A = tightly packed, many cells B = loosely grouped, several cells C = few cells						
6			1 = eB(<50% cavity) 2 = EB(>50% cavity) 3 = FEB(100% cavity) 4 = >100% cavity 5 = hatching 6 = fully hatched A = tightly packed, many cells B = loosely grouped, several cells C = few cells						
			ETed = ET; Frozen = F; Discarded = D						

Comments

Form #F008