Patrick Quinn

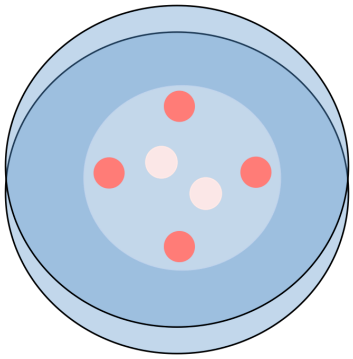
IVF PROTOKOL FOR SINGLE EMBRYO CULTURE

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

DAY -1

2. At ~ 4.00 pm on the day before ovum pickup (OPU), ie D-1, label 60 mm diameter Falcon Primaria dishes (Falcon # 353802; Fisher cat # 08-772-4C). An alternative dish is Falcon # 353002; Fisher cat # 08-772B. When making drops of medium, use a single-wrapped pipette tip, rinsing the tip twice with culture medium before making the drops.

3. **For IVF:** Place 6 x 30 uL drops of ***QA Protein Plus Fertilization Medium*** (Sage IVF Ref # 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.

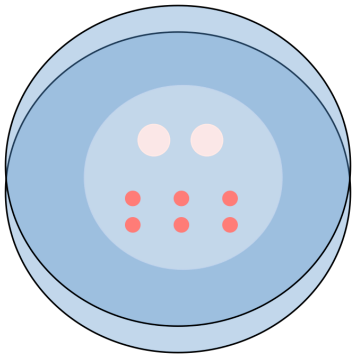


= 30 uL drops used for insemination.

= 30 ul drops used for washing oocyte-cumulus complexes

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

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



= 30 uL drops for washing oocytes

= 10 uL drops for culture of individual oocytes after ICSI

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

7. Prepare no more than two dishes at a time to minimize out-gassing of CO_2 and a 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 h before use (or overnight).

DAY 0 (Day of oocyte retrieval or ovum pickup – OPU)

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

10. ***For both IVF and ICSI on D0 at ~ 4 pm or D1 at ~ 8am before fertilization check:*** Prepare 60 mm diameter dishes with 9 x 10 uL drops of ***HEPES-HTF + 5 mg/mL HSA*** (HEPES+HSA: Sage Ref # ART-1023 and ART-3001, respectively). These can be prepared at ~ 4 pm on D0 and left at room temperature overnight, or prepared early on the morning of D1 and warmed in air to 37^{0}C on a heating plate. In either case, warm the dishes to 37^{o}C on the morning of D1 before use.

11. **For IVF on 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.

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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 it around within the well. Then place fertilized oocytes in individual 10 uL drops in the HEPES+HSA dish. For ICSIed oocytes, transfer fertilized oocytes to individual 10 uL drops of medium in the HEPES+HSA dish. Keep the dish containing the 10 uL drops of QA Protein Plus Cleavage Medium in which the ICSIed 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/ICSIed 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. Only place 6 embryos in each dish and handle one dish at a time to minimize increases in pH because of too long an exposure to air. Quickly return the culture dish to the incubator.

15. Follow the embryo scoring regime at the times listed on Form 020607-1 wherever possible.

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DAY 3 to the Blastocyst stage

16. On D3 befor 8.30 am, label 60 mm Falcon Primaria dishes with the patients name.

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

18. **On D3 between 10.00 am and 2.00 pm after Blastocyst Medium dishes have equilibrated for at least 4 h:** 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 uL drops of Blastocyst Medium in the Blastocyst Medium culture dish after washing the embryos through the 30 uL wash drops of Blastocyst Medium that are in the same dish. Only culture 6 embryos in a dish and handle one dish at a time

19. **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 extend, ie 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.

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DAY 5

20. **On morning of D5:** Score embryos for development to the blastocyst stage. For ET, select the best 2 embryos for ET. They should be at least grade 4AA (scoring by Gardner parameters – see Embryo Scoring Form 020607-1). Any blastocysts not transferred should be cryopreserved by vitrification.

21. Any embryo that has not formed a grade 3 or 4 blastocyst (ie 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 h before transferring embryos to it.

REFERENCE

Gardner DK Human embryonic development in vitro. In: In-vitro Maturation of Human Oocyte. Eds SL Tan, R-C Chian, WM Buckett. 2007 Taylor and Francis, Boca Raton, FL, Chapt 22.