

Improving the success of ART and reducing multiple pregnancies through a microdroplet-egg and embryo staging-extended culture/transfer protocol

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There has been overwhelming interest recently in using blastocyst culture for the transfer of embryos in assisted reproductive technology programs. However even though pregnancy rates have been proven to be high with blastocyst culture there has been concern that not all patients can be offered a blastocyst for transfer and many may go home disappointed given the fact that the optimum blastulation rates in vitro have been 60% to 70%. We have modified our ovarian stimulation and laboratory protocols and offer a microdroplet extended culture-egg/embryo staging program with day -3, -4 or -5 transfers that provide high pregnancy rates in each category to overcome some of the pitfalls of blastocyst culture.

Denuded oocytes are graded for quality according to the morphology of the first polar body, microinjected or inseminated and then grown in microculture droplets (5 to 10 ul) of sequential culture medium under oil in singles and not groups. Growth of embryos in singles allows accurate scoring of individual embryos throughout the extended culture period of 6 days.

Pronuclear embryos are scored using polarized parameters at 20 hours post insemination/post-injection and the first cleavage checked at 25 hours. The embryos are then grown on days-1 and -2 in sequential G1.2 medium (Vitrolife, Sweden) followed by growth in G2.2 medium (Vitrolife) on days -3 to -5/6. Daily scoring of embryos is carried out on each day of 1 to 5/6 using morphological characteristics and polarized parameters. Based on a collective score for all days prior to transfer, two to 3 embryos (based on maternal age) are replaced on either day-3, -4 or -5/6 depending on the quantity and quality of embryos available on each day. For older patients and those with several previous failures the zona pellucida is enzymatically softened or totally removed on day -5/6 with pronase prior to transfer to encourage implantation.

In a large clinical trial of 582 patients using this novel microdroplet extended culture/egg and embryo staging protocol, clinical pregnancy (36.1%, 38.8%, 47.0%), implantation (20.5%, 21.3% 29.1%) and multiple pregnancy rates (39.7%, 36.2%, 47.0%) were high for day-3, -4 and -5/6 transfers respectively. All patients received transfers with no patient disappointed. Pregnancy outcome and multiple pregnancy rates were greater for zona-free versus zona-intact blastocyst transfers suggesting that multiple pregnancies can be reduced by transferring one to two zona-free blastocysts without compromising on pregnancy outcome. This novel approach necessitates the keen eyes of a skilled embryologist to score and monitor embryos daily, select those embryos with optimum pregnancy potential for transfer and advise the clinician as to which day is the most suitable for embryo transfer.