Is Test – Tube Baby is Really Test-tube

ANJANA RAY CHAUDHURI *

Test-tube baby- the particular term makes common people curious enough to find out whether the baby or its precursor formed in this process remains in a test-tube. Hence, the Infertility practitioners are frequently asked this question by their patients. Actually, it is the popular term used to designate a baby born out of the process of ‘in vitro fertilization’ (IVF), ie, fertilization of male and female gametes ‘outside’ the body,- in the incubator instead of being in the fallopian tube that occurs in vivo fertilization. In Latin ‘vitro’ means glass and in the beginning the laboratory part of the IVF procedure used to be carried out in a test-tube. This is how the name was originated. The procedure which was confined mostly to the laboratory and known to the scientists and clinicians associated with this field became popular world-wide and overnight with the birth of the world’s first IVF or test-tube baby Lewis Brown in England in July 1978. Immediately after that, India’s first test-tube baby Durga was born in Kolkata as the result of constant endeavour and research work of the great endocrinologist cut scientist late Dr. Subhas Mukherjee. It was him to whom the world owes the idea of cryopreservation ie, storage of embryo at a very low temperature.

IVF is an assisted reproductive technique (ART) in which the egg (female gamete) of the female partner of the couple under treatment are taken out of her ovaries and fertilized in the laboratory with her husband’s sperm (male gamete). The fertilized zygote (s) /embryo (s) are transferred into her uterus within 2-6 days. In the reproductive age group, a normally ovulating woman brings out one mature egg per menstrual cycle. However, availability of more number of eggs which is mandatory in IVF increases the chance of pregnancy, Hence, in IVF for multi-follicular development the female partners receive some hormone preparations in the form of recombinant FSH (Recagon, Gonal f) and / or hMG (GMH, IVF-M, Nugon, Pergonal, Menogon, etc.) which help recruitment and growth of a number of follicles at a time. Growth pattern of this follicular cohort is monitored by transvaginal ultrasonography (TV-USG). As the follicles reach certain size (eg, 18-20mm in diameter) ovulation or release of the eggs from the follicles is initiated by HCG hormone (Profasi, Pregnyl) injection . After 34-35 hours of HCG injection the eggs are taken out of the ovaries (ovum pick up or OPU) under ultrasound guidance.

Ovarian stimulation might require 12-22 days depending on individual response/recruitment. Development of multiple follicles and avoidance of unscheduled LH surge (leading to cycle cancellation) need pituitary down regulation for which the patient has to take either GnRH agonist or antagonist injections. GnRH agonist eg, suprefact/leuprolide/decapryl etc. is usually given as daily subcutaneous injection either from day 2l of the previous cycle (long down regulation protocol) or day 2 of the same cycle (short down regulation). In both the cases ovarian stimulation starts from day 2/3 of the cycle. For patients under GnRH antagonist protocol stimulation with p/r FSH followed by hMG starts from day 3 of the cycle in which IVF is
planned and antagonist Cetrotide, Orgalutran is given around day 7-8 when follicle size is 14 mm. Depending on the response of individual patient injection doses for stimulation are fixed.

Patients with severe seminopathy (very low sperm count or motility) or those with azoospermia, previous fertilization failure or less number of eggs or with advanced age of the female partner do not benefit from conventional IVF. They require a special technique called micromanipulation or intra cytoplasmic sperm injection (ICSI) in which a single spermatozoon (from a processed sample) is injected directly into an egg thereby increasing the chance of fertilization with minimum number of sperm available. For azoospermic patients some advanced microsurgical techniques called PESA/MESA/TESE are performed for sperm retrieval from either the epididymes or the testes.

After OPU the eggs are retrieved from the follicular fluid and kept in a Petri-dish (centre well or four well) containing pre equilibrated culture medium inside the incubator for pre insemination incubation in an atmosphere of 5% CO\textsubscript{2} at 37° C. After incubation period that depends on the maturity of the eggs retrieved is over these are inseminated with calculated volume of sperm suspension prepared from semen sample of the patient’s husband. For ICSI the egg are treated with enzyme form removal of the surrounding cells as a pre-requisite of injection. The inseminated/injected eggs are kept undisturbed for culture overnight in the CO\textsubscript{2} incubator. Fertilization is assessed the following morning when the fertilized eggs should either show two pronuclei (2PN stage) or be a zygote. Cleavage starts thereafter and the next day embryos with 2-6 blastomeres (known as 2-6 cell embryos) are ready to be picked up for transfer. Usually best quality (decided after scoring) embryos (2-3 in numbers) are put back to the uterus of the lady usually on day 2-day 3 (for cleavage stage embryo) and latest by day 5/day 6 (for blastocyst transfer) of OPU.

Estimation of serum beta-HCG level 14 days after embryo transfer (ET) confirms presence/absence of biochemical pregnancy whereas clinical pregnancy is established only when foetal cardiac activity is observed on ultrasound around 6 weeks. To help prepare the endometrium to accept the embryo(s) on implantation the patients after OPU receive some progesterone preparation (tablet/pessary/cream/injection) and/or oestrogen in combination as luteal support that continues till pregnancy test and is withdrawn if the test is negative. Any conception that occurs following IVF-ET continues like spontaneous ones. The fact that the patient after an IVF pregnancy has to be extra careful is due to the expensive and invasive nature of the procedure. Chances of miscarriage also do not increase significantly in such a case compared to spontaneous pregnancy.

As the caption of the article suggests there is lot of misconception about the IVF procedure in layman’s mind. One of them being that in IVF always stored heterologous sperm sample instead of the husband’s sperm is used. This idea is totally wrong since nowadays even in case of azoospermia sperm (or precursor cells) can be retrieved from the testes which enable the so far “unfortunate” man father a child. The hormone injections used for ovarian stimulation have never been proved to cause cancer of genital organs which is another myth popular among the commoners. Though the whole procedure possesses certain risks, these are the calculated ones and can be avoided with proper guidance and careful management in well equipped centers. **Days are not very far away, when IVF will become a procedure like appendicectomy or diagnostic curettage, because, its indications as well as applications are increasing rapidly even in developing countries.**