Review Article

In vitro fertilization embryo transfer processes and pathway: A review from practice perspective

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ABSTRACT

Infertility affects approximately 10%–15% of reproductive-aged couples. In Nigeria, it is an important reproductive health issue and a common reason for gynecological clinic consultations. Significant improvements in fertility treatment have made it possible for many patients to conceive with medical assistance. For example, women with fallopian tube blockage can conceive with in vitro fertilization (IVF). Modern IVF generally involves controlled ovarian stimulation with exogenous gonadotropins, oocytes collection through transvaginal ultrasonographic-guided aspiration, coculture of eggs and sperm in culture (or intracytoplasmic injection of sperm into the oocyte), and placement of the resultant embryos (2–5 d later) directly into the uterus. Some of the major drawbacks to IVF are its high cost of treatment and paucity of availability in our sub-Saharan region.

Key words: Infertility; *in vitro* fertilization; outcome; pathway; procedure; processes.

Introduction

Infertility which is defined as the failure to conceive (irrespective of cause) after 1 year of unprotected intercourse is a major challenge worldwide and it affects about 10%–15% of reproductive-aged couples. Although its overall prevalence has been stable for some years, a shift in the etiology mainly due to patient's age has occurred. As a woman's age increases, the risk of infertility also increases.

Despite the definition of infertility, the chances of achieving pregnancy per menstrual cycle (fecundability) are lower in older women. Withholding treatment for 1 year in a 40-year-old woman seeking fertility services may be inappropriate. In women older than 35 years, a complete evaluation after 4–6 months of trying to conceive is prudent because their response to treatment may be reduced due to diminished ovarian reserve.

To achieve pregnancy, the integrity of the female and male reproductive tracts must be intact and these involve the release of a normal oocyte, the production of adequate spermatozoa, the normal transport of the gametes to the fallopian tube where fertilization occurs, and finally, the subsequent transport of the cleaving embryo into a normal endometrial cavity for implantation and development. Male and female factors contribute about 35% each to infertility. Combined male and female factors contribute to 20% of infertility, and in the remaining 10% of cases, the cause is unknown. Other factors associated with an increased risk of infertility are lifestyle, environmental, and occupational factors. These include toxic effects of tobacco/marijuana or other drugs use, excessive exercise, inadequate diet associated with extreme weight loss or gain, and very importantly, advanced maternal age.

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Significant improvements in fertility treatment have made it possible for many patients to conceive with medical assistance. For example, women with fallopian tube blockage can conceive with *in vitro* fertilization (IVF). Men with severe oligospermia or obstructive azoospermia but have sperm on a testicular biopsy or epididymal sperm aspiration can have a family using IVF/intracytoplasmic sperm injection (ICSI).

Since the first successful human IVF treatment in 1978 with the delivery of Louise Brown in England,[1] there has been greater understanding of the ovulatory process. This pioneering work of Edwards and Steptoe has been replicated worldwide, and IVF is now recognized as an established treatment for infertility. Subsequently, helpless infertile couples are now able to achieve pregnancy and patients who were considered sterile in the past are now capable of having children.

The indications for IVF treatment have shifted from the narrow scope of tubal infertility to other indications that were almost impossible to overcome before now, including oligospermia and obstructive azoospermia. Patients with a history of endometriosis unsuccessfully treated medically or surgically and patients with failed conservative fertility treatment or those with unexplained infertility may benefit from IVF. Finally, adults and children with cancer can have fertility preservation done early in their treatment process thus making it possible for them to achieve their dreams of having their own babies.

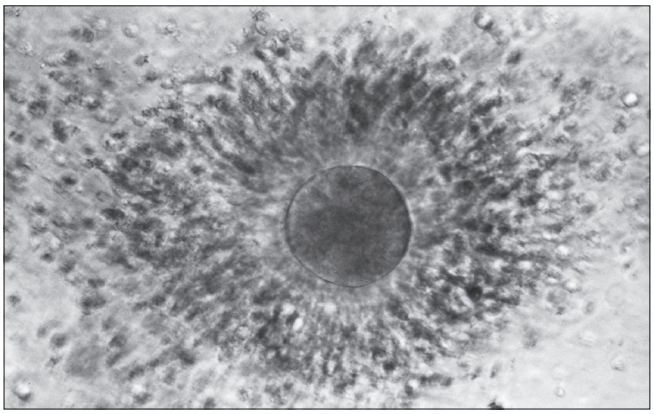

Figure 1: Metaphase II preovulatory oocyte

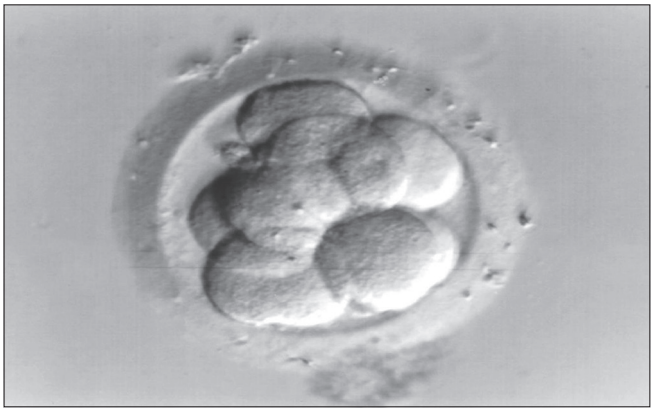

Figure 3: Eight cells stage embryo

In Vitro Fertilization Procedure

The procedure for IVF treatment comprises retrieving preovulatory oocytes from the ovary, subsequent fertilization with sperm in the laboratory and embryo transfer into the endometrial cavity. The following steps are required during an IVF cycle:

- Ovarian stimulation
- Follicular aspiration/egg collection
- Oocyte classification
- Sperm preparation
- Oocyte insemination
- Embryo culture
- Embryo transfer.

Ovarian Stimulation for *In Vitro* Fertilization

The success of an IVF treatment is dependent on the patient's age and the quality of embryos transferred into the endometrial cavity, among other factors.[2-4] The number

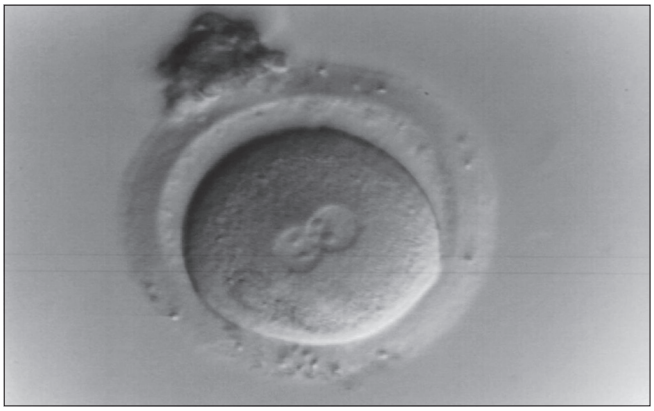

Figure 2: Two pronuclei stage embryo – 18 h postinsemination

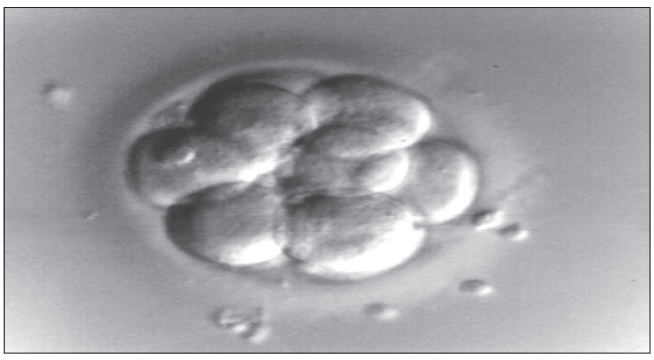

Figure 4: 10–12 cells embryo

of embryos obtained in any given IVF cycle depends on the number of oocytes retrieved after ovulation induction.

Ovarian stimulation is done mainly using gonadotropins (human menopausal gonadotropin such as Menogon Humog, pure follicle-stimulating hormone (FSH), and recombinant FSH/luteinizing hormone (LH) such as Menopur and Gonal-F). The doses of gonadotropins vary from 150 to 450 IU/day, depending on the patient's age, ovarian reserve, and previous history. The use of gonadotropins in ovarian stimulation has led to an overall improvement in IVF. This is due to increase in the number, quality, and synchronization of the retrieved oocytes per cycle leading to the improvement in fertilization rates, increase in the number of embryos, and the subsequent pregnancy rate.[5]

Furthermore, because more embryos are available, an opportunity exists for cryopreservation of the excessive number of embryos for the future transfer(s). In general, patients start treatment on the 2nd or 3rd day of the menstrual cycle. The response is basically monitored using transvaginal ultrasonography. Once at least four follicles reach 17–18 mm in diameter, the gonadotropins are discontinued, and 10,000 IU of human chorionic gonadotropins (HCGs) is administered. Oocyte retrieval is performed about 34–36 h later.

The two most common protocols available for ovarian stimulation are the gonadotropin-releasing hormone (GnRH) agonist and the GnRH antagonist protocols and both are based on the principles of follicular recruitment, selection, and dominance. Examples of the commonly used GnRH agonist in our environment are buserelin, luprodex, and zoladex, and for the antagonist, we have cetrotide.

In the GnRH agonist's protocol, the pituitary gland undergoes an initial downregulation leading to the prevention of spontaneous LH surge and premature luteinization. The GnRH agonists can be used in two protocols known as the flare-up protocol and the luteal-phase protocol.[5-8] The flare-up protocol has the advantage of using the transitory elevation of FSH (agonist effect) that occurs during the first 4 days of the follicular phase. This elevation helps in the follicular recruitment process, and the administration of gonadotropins must be initiated on the third menstrual cycle day.[5]

In the luteal-phase protocol, GnRHa is started in the mid-luteal phase, and by the onset of the subsequent menstrual cycle, the phenomenon of pituitary downregulation has been achieved. The administration of the gonadotropins commences on the 2nd day of bleeding.[7,8]

Potential risks and disadvantages with the use of GnRHa protocol include increased requirements of gonadotropins, increased costs due to additional days of therapy, and the risk of ovarian hyperstimulation syndrome.

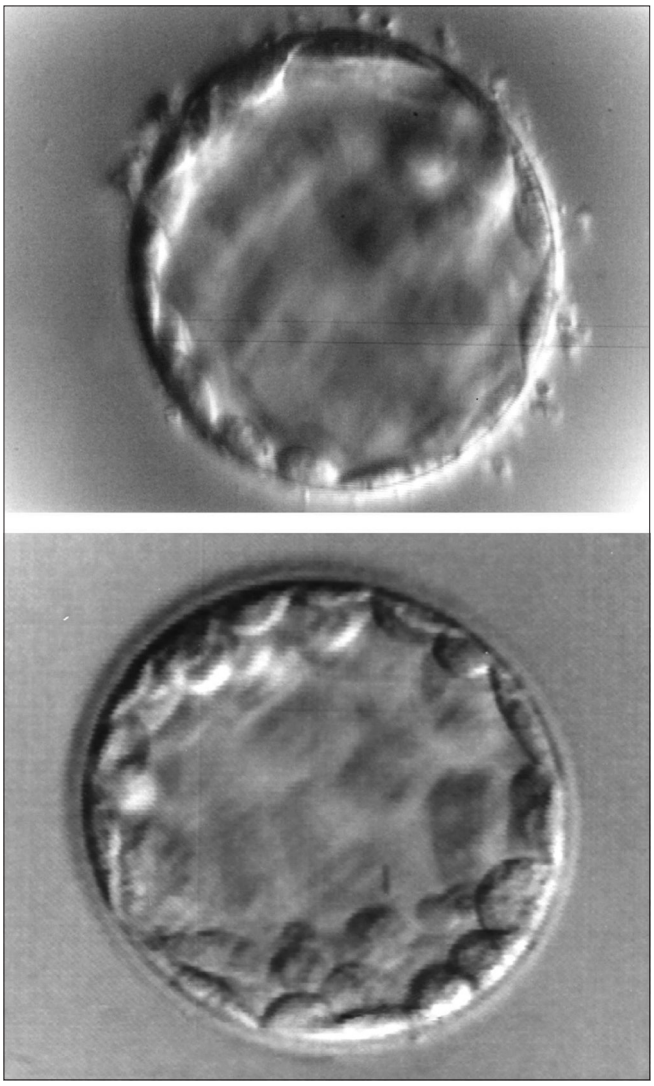

Figure 5: Blastocysts

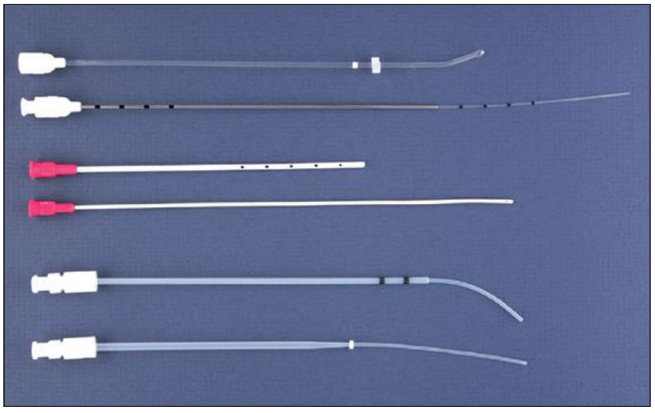

Figure 6: Catheters for embryo transfer

With the GnRH antagonist protocol, the GnRH antagonists are administered when the largest follicle reaches a diameter of 14 mm or when the LH levels in serum are >10 mIU/mL.[9] This is usually from day 6 or 7 of gonadotropins administration. GnRH antagonists have the advantage of blocking the LH surge at the periovulatory period; therefore, premature luteinization or spontaneous LH surge does not occur. Because the pituitary gland is not downregulated at the beginning of the menstrual cycle, smaller amounts of gonadotropins are required for ovarian stimulation. Another advantage with this protocol is that preovulatory LH surge can be achieved using GnRH agonist and therefore avoiding the long-term effects of the HCG injection that is responsible for triggering ovarian hyperstimulation syndrome.

Follicular Aspiration

Follicular aspiration is performed 34–36 h after the ovulation trigger. This is usually done under transvaginal ultrasound guidance. The patient is placed in the dorsal lithotomy position and sedation administered. The vaginal wall is cleaned with saline, and a transvaginal ultrasound probe with a sterile cover, attached to a needle guide is inserted in the vagina to localize the ovaries and the follicles. An oocyte retrieval needle is subsequently passed through the needle guide through the vaginal fornix into the ovaries, and the follicular fluid is aspirated. The fluid is then transferred to the IVF laboratory to confirm the presence of oocytes. The major risks of this procedure are infection and damage to the pelvic vessels and organs. These risks are, however, almost nonexistent when carefully performed and in trained hands.[10]

Oocyte Classification

The quality of the oocytes collected is an important determinant of IVF treatment success.[11] The follicular fluid is examined under a microscope for the oocytes which are graded according to the appearance of the corona-cumulus complex. The presence of a polar body (metaphase II stage) and/or germinal vesicle (prophase stage) is a determining factor for the preincubation time before the insemination.

Sperm Preparation and Oocyte Insemination

Semen sample is collected immediately before oocyte collection, usually after 3–5 days of sexual abstinence. The sample is then processed to retain only the motile and morphologically normal fraction of the sperm. This is done with centrifugation through a density gradient system or simple media wash for poor quality samples that may have poor recoveries with the gradient system. The sperm is incubated for 60 min in an atmosphere of 5% carbon dioxide in the air. Finally, the supernatant containing the motile fraction of sperm is removed, and oocyte insemination or ICSI performed.

Embryo Culture

The inseminated oocytes are incubated in an atmosphere of 5% carbon dioxide in the air with 98% humidity. Ideally, fertilization occurs approximately 18 h after insemination, and this is evidenced by the presence of two pronuclei and the extrusion of a second polar body. Fertilized oocytes (embryos) are transferred into growth media in the incubator and are subsequently monitored for divisions. A 4–8 cell stage embryo is seen about 36–48 h after insemination and then 10–16 cells after 48–72 h. The morula or blastocyst stage is observed after 96–120 h. Embryos are classified according to symmetry, presence of fragments, clarity, and number of blastomeres [Figures 1-5].[11]

Embryo Transfer

Transcervical transfer under transabdominal ultrasonography is the most common method used for embryo transfer. The procedure is usually performed on day 3 after oocyte insemination or when the embryo reaches the blastocyst stage on day 5.[12-15]

Several catheters have been designed for embryo transfer, and the choice of catheter is a matter of physician preference as it does not affect pregnancy rate [Figure 6]. The embryos are loaded with 15–20 μL of culture media, the catheter is advanced into the uterine cavity, and the embryos ejected into the mid-cavity, approximately 1–2 cm from the fundus. Elective single blastocyst transfer reduces multiple pregnancy rates.[16]

Management of the Luteal Phase

Recent publications support the benefits of supporting the luteal phase with exogenous progesterone which is usually commenced after oocyte retrieval.[2] The administration of exogenous progesterone stems from the possible induction of an abnormal endocrine balance from superovulation and aspiration of granulosa cells at the time of oocyte retrieval.[17] Several preparations are available including natural progesterone in oil base administered intramuscularly (e.g., gestone), vaginal progesterone suppositories (e.g., cyclogest), and capsules of micronized progesterone.[18] The administration of progesterone is continued until the day of the pregnancy test, and if the pregnancy test result is positive, the hormonal support may be continued until the 10th week of pregnancy.

Outcomes of Assisted Reproductive Technologies

It is estimated that more than 5 million children have been conceived worldwide since the first IVF pregnancy in 1978.[19,20] The ultimate goal of an IVF treatment is achieving a live birth; however, clinical pregnancy rate is the best indicator for evaluating IVF treatment. Globally, the average clinical pregnancy rate per embryo transfer ranges from 30% to 45%[21-23] although pregnancy and live birth rates vary significantly with the female partner's age. In one study, the overall pregnancy and live birth rates for all the IVF treatments in the United States of American were 35% and 28.6%, respectively.[23] The pregnancy rate by patient age was approximately 44.7%, 37.7%, 27.6%, 17.7%, and 9.2% for women younger than 35 years, 35–37 years, 38–40 years, 41–42 years, and 43–44 years, respectively. In that same study, the percentage of twins and other higher multiples were 28.8% and 2.1%, respectively. The incidence of miscarriages, stillbirths, congenital malformations, and chromosome abnormalities in IVF pregnancy is similar to that of general population.

Ectopic pregnancy has been reported after IVF probably due to the migration of the embryo through the corneal ostium. Ectopic pregnancy occurs in approximately 0.7% of cases. In some instances, ectopic pregnancy is associated with heterotopic pregnancy.

Conclusion

Since the delivery of the first IVF baby in 1978, millions of babies have been delivered worldwide through this process, and many infertile couples have been able to fulfill their dreams of having a family. However, the optimal strategy for treating infertile couples is not always clear, and IVF is not always the first treatment of choice for many infertile couples. Appropriate treatments for a couple with unexplained infertility may also include intrauterine insemination and ovulation induction, either alone or in combination as these treatments are less expensive and more cost-effective than IVF; however, the pregnancy rates associated with these methods (typically between 5% and 15% per cycle) are well below those achieved with IVF.

Some of the major drawbacks to IVF are its high cost and the paucity of available centers offering the treatment in the sub-Saharan region. There is poor government support, and in the majority of cases, the treatment is not supported by health management organizations or insurance companies, leaving the patients with a huge out of pocket expenditure for their treatment. It is, therefore, pertinent to ensure proper patient selection before commencement of IVF, as many patients will benefit from other cheaper forms of fertility treatment.

Its worthy to note that studies have not shown any significant difference in pregnancy outcomes between spontaneously conceived and IVF pregnancies.

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Conflicts of interest

There are no conflicts of interest.

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