The New Techniques of Assisted Reproduction

Olufemi A.O. Oloyede [1] ***and Osato F. Giwa-Osagie*** [2]
Department of Obstetrics and Gynaecology, [1]Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State, and [2]Lagos University Teaching Hospital, Idi-Araba, Lagos, Nigeria.

Abstract

Background: Infertility remains one of the most pressing gynaecological problems worldwide. In Nigeria, as in other countries in sub-Saharan Africa, the incidence has continued to increase due to the rising incidence of severe oligozoospermia and azoospermia as well as tubal damage. All these are secondary to previous infection. The success rates of conventional methods of treatment are not encouraging, thus necessitating the introduction of assisted methods of treatment. Assisted reproductive techniques are not widely available in Nigeria. Details about the methodology and complications of these techniques are also scarce.

Objective: This review is aimed at providing a systematic analysis of the various methods of assisted reproduction and their complications. Controversial aspects of the techniques are also discussed

Source: This review is based on articles selected from a *Medline* search and a systematic search of locally published journals as well as standard texts on assisted reproductive techniques.

Conclusion: Despite the limitations of inadequate facilities and low success rates associated with these techniques, they are recommended for couples that fail to benefit from the conventional methods of managing infertility.

Key Words: Infertility, Assisted Reproductive Techniques, IVF. [Trop J Obstet Gynaecol, 2003, 20: 67-73]

Introduction

Infertility remains one of the most pressing gynaecological problems that have attracted considerable attention because of its impact on reproductive health [1]. Its occurrence worldwide has been reported to vary from region to region as a reflection of societal awareness of problem, literacy level, and socio-economic status of the society as well as the medical bias of the particular society. The prevalence is however increasing globally [2, 3]. On the average, about 8-15% of couples experience subfertility or infertility during their reproductive lives [2, 4, 5]. The prevalence is much higher in Africa, especially in Cameroon and Sudan, where the figure is as high as 20–30% of couples. In our environment therefore, infertility is a public health issue.

An understanding of the basic nomenclatures and definitions in the field of infertility is necessary for focused investigation and management [1] and the comparison of results from different centres. Infertility is the inability of a couple to achieve a clinically recognizable pregnancy after 12 months of regular, unprotected intercourse [2,3,4]. Infecundity is the inability of a couple to achieve a live birth after 12 months of regular unprotected intercourse. Primary infertility is when there has been no previous pregnancy and secondary infertility is when there was a preceding pregnancy irrespective of the out come [2,3,4]. Either may occur in the man or the woman.

Indication/Basis for Assisted Reproductive Techniques.

The chance of conception should be expressed in terms of fertility of the couple rather than individual partners. Despite this however, the age of the female followed by the duration of infertility are the most powerful indices of prognosis [2, 5]. Female factors are responsible in about 40-55% of couples, male factor in about 20-40%, combined factors in about 10-30% and unexplained in about 4-12% of couples [5, 6, 7].

Modern investigation of infertility includes the conduct of seminal fluid analysis, hormonal assays, abdominal/pelvic ultrasonography, hysterosalpingography and laparoscopic chromopertubation. After a detailed investigation, treatment could either be by conventional methods or with the newer Assisted Reproductive Techniques (ART)

The conventional methods are often associated with frustrations and failure [6]. This is largely attributed to the fact that the leading causes of infertility found in male are severe oligozoospermia and azoospermia and in the female, severe tubo-peritoneal damage. All these are largely attributable to previous episodes of reproductive tract infection from various causes [1,7,8,9]

Correspondence: *Dr. O.A. Oloyede, Department of Obstetrics & Gynaecology, Olabisi Onabanjo University Teaching Hospital, P.M.B. 2001, Sagamu, Ogun State, Nigeria.*
E-mail: oloyedeoao@yahoo.com .

The limitations of conventional treatment options such as antibiotics or reconstructive and anastomotic surgery are well documented. Post treatment prognosis is usually discouraging. It is therefore inevitable that alternative or advanced methods have to be offered to improve on the prognosis of infertility treatment. [10]

The Techniques of Assisted Reproduction

Assisted Reproductive Techniques (ART) are procedures done to enhance the chances of achieving pregnancy outside of the normal mechanism of conception from copulation between male and female. ART is a scientific and reliable method of treating both male and female infertility. The acceptance of ART as a method of assisting infertile couples to achieve conception came into being with the landmark delivery of Louise Brown on July 25th, 1978 in the United Kingdom through the process of *in vitro* fertilization and embryo transfer (IVF–ET) [3]. Since then, a number of related techniques have been developed, based on the various causes of infertility, to overcome barriers to spontaneous fertilisation. The commonly used techniques have fanciful acronyms and include:

A.I.	Artificial (Therapeutic) Insemination
I.U.I.	Intrauterine Insemination
I.V.F.	*In Vitro* Fertilization
G.I.F.T.	Gamete Intra Fallopian Transfer
Z.I.F.T.	Zygote Intra Fallopian Transfer
Z.D.	Zonal Dissection
P.Z.D	Partial Zonal Dissection
S.U.Z.I.	Sub Zonal Insemination
I.C.S.I	Intracytoplasmic Sperm Injection
T.E.S.A.	Testicular Sperm Aspiration
P.E.S.A.	Percutaneous Sperm Aspiration
M.E.S.A.	Micro-Epididymal Sperm Aspiration

Explaining the details of the procedure to the couple during their initial visit enhances patient compliance and cooperation, as well as a favourable outcome of the process. The session would include explanations on the possible reasons for infertility in the couple, the treatment options available to them, their side effects and complications, as well as success rates. A broad classification of the indications for ART is matched against the possible therapeutic options in Table 1.

The technique utilised for any particular couple depends on the identified aetiological factors. Occasionally more than one method may be employed. The application and relevance of the various methods is best understood if discussed in the light of the various steps that are mandatory for the success of ART.

Table 1

Aetiological Factors in Infertility and Appropriate ART

Aetiological Factor	*ART*
Male Factors	
Oligozoospermia	AI, IUI, ZD, PZD, SUZI, IVF, ICSI
Azoospermia	AID TESA, PESA, MESA
Female Factors	
Tubo-Peritoneal	IVF, ICSI
Endometriosis	IVF, ICSI
Cervical Hostility	AI, IUI
Ovarian Failure	Oocyte Donation
Idiopathic	
Unexplained Infertility	IVF, GIFT, ZIFT

Controlled Ovarian Stimulation (COS)

Appropriate ovarian response is required for a successful outcome of ART. Controlled Ovarian Stimulation (COS), otherwise referred to as super-ovulation, refers to the process whereby ovulation is deliberately programmed in a woman who may have been ovulating before, albeit irregularly, or a woman who is anovulatory. The aim is to stimulate the development of several mature follicles from the ovary in order to increase the chances of obtaining fertilizable eggs [5]. Clomiphene citrate is the first line agent employed in most patients. Usually between 50-150mg of clomiphene is given on Days 2-6 of the cycle. Other drugs that are used, either singly or in combination, include tamoxifen, human menopausal gonodotrophin (hMG) and recombinant follicle stimulating hormone (FSH) [5, 11, 12, 13, 14]. The introduction of gonadotropin releasing hormone analogues (GnRH analogues) such as buserelin and goserelin for the purpose of down-regulating the pituitary has made super-ovulation much easier to accomplish and their use prior to, or in conjunction with ovarian stimulation, is now an integral part of most IVF programmes. Administration of hMG or FSH is continued until human chorionic gonadotrophin (hCG) or Luteinising hormone (LH) is used to trigger the matured follicle to ovulate [5,13].

Successful maturation and ovulation of follicles is usually monitored with the ultrasound scan, even though other methods such as cervical mucus

assessment, serial oestradiol (E_2) and luteinising hormone (LH) measurement have also been used in the past [5,12,11,14,15]. COS is necessary for practically all the various types of ART.

Semen Collection, Preparation and Preservation.

Semen samples for ART are best collected by masturbation rather than by coitus interruptus [5, 14]. Where this is not achievable, special condoms may be used to assist the male partner. Sperm aspiration techniques have been found particularly useful in cases of obstructive azoospermia. Examples of these are the microsurgical epididymal sperm aspiration (MESA), percutaneous sperm aspiration (PESA) and the testicular sperm aspiration (TESA) [13,14,16,17]. The semen sample is allowed to liquefy, and then subjected to high centrifugation, "swim up" or percoll gradient technique in order to obtain motile healthy spermatozoa for the various types of ART [13,18]. "Sperm washing" removes the seminal plasma, while "swim up" or a percoll gradient ensures that the most motile fraction is obtained. Processed semen is kept in culture medium prior to its use for AI, IUI, IVF – ET, GIFT, ZIFT and ICSI.

Oocyte Retrieval and Preparation

Oocyte collection normally follows a successful superovulation regimen. Transvaginal ultrasonic scan-guided recovery is now the standard approach, although a laparoscopic approach may be used instead [5, 11, 12, 13]. The follicular content is aspirated and pipetted several times to aid the separation of the oocytes from the cumulus–oocyte complexes under microscope [12, 13]. The separated oocytes are placed in fresh culture medium and incubated at 37^0C. [12, 13].

Fertilisation

"Assisted fertilisation" can be achieved by any of the methods of ART, which are either macro- or micro-manipulation techniques. A brief description of the peculiarities of the various methods is highlighted in the discussion below.

Macro-Manipulation

Techniques involving macro-manipulation include AI, IUI, IVF-ET, GIFT and ZIFT.

Artificial Insemination

Artificial Insemination has been variously referred to as therapeutic insemination (TI) or intra uterine insemination (IUI) [5,14,19]. As a method of assisting couples with male infertility, its application in Nigerian is recent and in very few centres. The steps that are relevant in artificial insemination to ensure a successful fertilisation are controlled ovarian stimulation and sperm collection and preparation. Insemination is intra uterine on 1 or 2 occasions using specially designed catheters like Wallace's or Rocky Catheters. Pregnancy rate appears to be higher with intrauterine than with intra-cervical or intra-vaginal insemination [5]. Reports from centres both in Nigeria and other countries demonstrated pregnancy rates of between 54% and 75% for donor insemination and 20%-30% for insemination with husband's semen [14,19]. These rates tend to improve with the number of cycles of insemination attempts.

In Vitro Fertilisation (IVF)

IVF was originally designed for infertility due to damaged tubes [10]. Today however, the indications have widened to include male infertility, unexplained infertility as well as failed artificial insemination [5, 11]. The essential steps of the process include controlled ovarian stimulation, sperm collection, preparation and storage, oocyte retrieval and preparation, and fertilization [5, 13].

Oocytes are pre-incubated for between 3 to 5 hours before insemination with 1-2 x 10^5 spermatozoa [5,12]. Further incubation is continued, with changing of the culture media at between 12 and 24 hours, and later at between 36 and 48 hours after starting incubation. Identification of 2 pronuclei indicates fertilisation [5]. Various attempts have been made to improve fertilisation through sperm preparation and insemination techniques [13]. These include motility stimulation, increased concentration of sperm for insemination and insemination of small volume of semen [19, 20, 21].

Embryo transfer involves the return of a maximum of the best three embryos using a special non-toxic intrauterine catheter introduced into the uterine fundus [5]. The clinical pregnancy rate varies between 15-35% per cycle [3, 5]. IVF success rates are about equal for the first two cycles, decline modestly for the third cycle and drop significantly after the fourth cycle.

Gamete Intra-Fallopian Transfer (GIFT)

GIFT is an adaptation or variant of IVF. At least one functional fallopian tube is mandatory for the deposition of the egg-sperm mixture into the ampullary portion of the fallopian tube through the fimbrial end [3]. This is done immediately after mixing the gametes, using a special GIFT catheter. Clinical pregnancy rate is however slightly higher than for IVF, being about 33 - 37%.

Zygote Intrafallopian Transfer (ZIFT)

Zygote Intrafallopian Transfer shares some features with the IVF and GIFT in terms of the mandatory steps of controlled ovarian stimulation, oocyte retrieval and processing as well as sperm collection and processing. Fertilisation is confirmed after mixing the sperm with the oocyte after which the zygote is immediately transferred into the fallopian tube [23, 24]. A functional fallopian tube is mandatory as in GIFT. Clinical pregnancy rates hover between 30 and 40%.

Micro-Manipulation Techniques

The search for better ways of treating infertility, especially in severe male factor infertility and failed IVF, has led to the introduction of assisted fertilisation techniques involving gamete manipulation to facilitate fertilisation [5, 13]. A basic feature common to these techniques is the by-passing of the zona pellucida, a glycoprotein that surrounds the oocyte [13]. The techniques are also referred to as "assisted hatching".

Zonal Drilling (ZD)

Zonal drilling is a micromanipulation method that requires the use of either chemical (acid tyrode or acid phosphate buffer saline solution) or mechanical (fine needle) means to drill a hole into the zona pellucida [13]. A capacitated and progressively motile sperm is required for the technique [13]. Oocyte injury as well as polyspermy are recognised complications of this procedure [23, 25, 26] Pregnancy rates are usually low [13].

Partial Zonal Dissection (PZD)

In PZD, a portion of the zona pellucida is removed using a fine needle, glass or metal micro-blade. The capacitated and acrosome-reacted sperm is now fused with the perivitelline membrane [13]. The fertilisation and pregnancy rates are low, just as in ZD. Polyspermy is also a recognised problem of this method.

Sub-Zonal Insemination (SUZI)

In SUZI, five to eight spermatozoa are injected into the perivitelline space after capacitation and acrosome reaction. The advantage of this is that biological selection of normal spermatozoa through the process of membrane fusion can take place [13]. Fertilisation and pregnancy rates are low as in ZD and PZD.

Intracytoplasmic Sperm Injection (ICSI)

Intracytoplasmic sperm injection (ICSI) is a highly sophisticated technique for injecting single live spermatozoa into the cytoplasm of the oocyte to achieve fertilisation [5, 13]. The method is regarded as a major break through in resolving cases of severe male factor infertility refractory to ZD, PZD, SUZI and IVF [13, 27] The mandatory steps of controlled ovarian stimulation, oocyte retrieval, sperm collection and preparation are the same as in IVF. Recent reports have shown that fertilization rate is independent of any method of sperm preparation such as swim-up, percoll gradient or wash [3]. Under an inverted microscope, fitted with a micro-manipulator, the intracytoplasmic injection of spermatozoa is performed with the aid of injection pipettes. A holding pipette is now used to transfer the injected oocyte into a culture medium for incubation for 16-18 hours [13]. Fertilisation is confirmed by the presence of 2 clearly distinct pronuclei containing nucleoli. Embryo transfer is done after 3 days of micro-injection using a special catheter such as an end-hole flexible catheter [13]. At present, pregnancy rates reported in various centres are between 30% and 40% [13, 26].

Complications of ART

ART like other medical or surgical procedures are associated with numerous complications. These range from the mild ones to severe and life-threatening ones. These complications may arise as a result of using ovulation induction drugs or from the procedure itself.

Multiple Pregnancy

This is perhaps the most frequent complication of ART especially, IVF and GIFT [29]. The chances of multiple pregnancy increase with the number of embryos transferred. In most centres, the maximum number of embryos transferred is three, in order to balance the risks of non-implantation with those of multiple pregnancy [5, 29]. Even with this practice, about 25% of IVF pregnancies are multiple, with a two to eight fold increase in the incidence of monozygotic twins [29, 30]. The risk of triplets is one in seven pregnancies.

Ovarian Hyperstimulation Syndrome (OHSS)

OHSS is widely regarded as the most dreadful complication of ovulation induction as a component of ART 31. An incidence of between 0.6% and 14%

has been reported from different IVF centres [31]. The condition commonly occurs with the use of GnRH analogues and gonadotropins, and occasionally with the use of clomiphene citrate [5, 31, 32]. Polycystic ovary and the administration of hCG appear to be major predisposing factors [29]. Measures such as non-use or reduction in the dosage of hCG as well as cryopreservation of embryos have been advocated to reduce the risk of occurrence of OHSS [5, 33, 34, 35]. However, the fact that its occurrence is associated with conception cycles makes it mandatory that a strategic approach to its management should be developed rather than reduce the chances of a woman getting pregnant by withholding the use of appropriate drugs.

Ectopic Pregnancy

Between 1 and 3% of all ectopic pregnancies occur after IVF [36]. These represent the embryos that migrate from the intra uterine environment into the fallopian tube. A high index of suspicion based on positive pregnancy test in the absence of intrauterine gestational sac after the 21st day of embryo transfer is required for early pick-up of cases.

Pelvic Abscess

Pelvic abscesses have been reported in about 0.6% of IVF treatment cycles [35]. It arises most commonly as a result of the process of oocyte retrieval, when pathogenic organisms are introduced into the pelvis. A high index of suspicion is necessary to pick up its early symptoms of abdominal pain, fever (with or without alteration in bowel habits), following egg retrieval. Ultrasound-guided transvaginal drainage is preferable to laparotomy [38].

Chromosomal /Congenital Abnormalities

The micromanipulation techniques of ART have generated understandable concerns about the potential for the occurrence of chromosomal and congenital abnormalities. Such abnormalities are attributable to the fact that these methods bypass the natural sperm selection barrier offered by the zona pellucida and the oocytic cytoplasmic membrane [13]. Examples of such abnormalities are Down's syndrome, cheilopatoschisis, inguinal hernia and cystic fibrosis. ICSI and SUZI are the two methods commonly associated with these abnormalities [13].

Pre-eclampsia

A higher incidence of preeclampsia has been reported with the use of donor semen for A.I. The occurrence of multiple pregnancies has been adduced as a possible explanation for the higher incidence of preeclampsia in these patients.

Transmission of Infectious Agents

The risk of transmitting pathogenic organisms such as human immunodeficiency virus (HIV), hepatitis viruses and other sexually transmitted diseases (STD) through infected sperm is possible, though rare. This has led to the use of screened and stored semen rather than fresh semen for ART procedures.

Low Birth Weight and Prematurity

The risk of preterm delivery or low birth weight fetuses does not appear to be higher.

Ovarian Tumours and Early Menopause

These are possible long-term complications that need further evaluation.

Issues in ART

The introduction of ART has revolutionized the management of infertility. Expectedly, it is faced with a lot of problems ranging from acceptability to utilisation and standardization of practice. Some of these conflicting issues are worth discussing in an attempt to forge ahead.

Quality Control And Regulation Of Standard

ART, being a highly technical procedure, should have standard quality control measures to guide its operations. The Human Fertilization and Embryology Authority (HFEA) is a body legally established for the regulation of ART practice in the United Kingdom [5]. The minimum standards established by HFEA include the appropriate use of drugs, definition of indications for procedures, technical guidelines during the processes involved and consensus definition of how to assess the outcome of treatment [5]. The setting up of the HFEA dates back only to 1991. Such activities as outlined above that are to be guided are still not operational in most developing countries.

Efficacy of Methods and Success Rates

The success rates for the various methods of ART have been of concern to patient and gynaecologist alike. This is viewed against the background of the expense involved in the procedure. Another area of concern is that of chemical pregnancy rates being far above those of clinical pregnancy. Would it be satisfactory to the gynaecologist and/or the patient to regard chemical pregnancy as a measure of success of ART? There are several predictors of success such as the age of the patient, the status of the uterus and the specific aetiological factors [5].

Cost

The cost of setting up and maintaining ART programme is enormous, irrespective of the level of national development [3, 5, 13]. This has militated against wider utilisation of the services by prospective patients. Several innovations are being introduced to make the procedure more affordable. Some of these measures include:

(i) the invitation of voluntary organisations to subsidise ART programmes
(ii) re-organisation of government health policies to accommodate ART in health funding.
(iii) cryopreservation
(iv) gamete donation
(v) sperm storage / freezing
(vi) standardisation of equipment and bulk purchase of consumables
(vii) collaboration between centres

Religious and Socio-Cultural Factors

The influence of religion and cultural beliefs of the people is another issue to contend with in ART. To the deeply religious, ART contravenes divine injunctions as "man has literally taken over the work of God". In the traditional African setting, both donor and husband insemination seems to be acceptable to most couples after long period of waiting [19]. This is because when a woman is legally and traditionally married to a man, all the children belong to the man, no matter whom the biological father is [19]. Most couples would have little aversion to IVF but for the huge cost involved.

The Future of ART

The prevalence of infertility is no doubt on the increase worldwide [2, 3, 5]. Similarly, the need for and acceptance of assisted reproductive techniques is bound to increase. There is therefore the need to be able to rationalise the present with a view to improving on the future of the programme. At the moments all efforts should be made to ensure:

(i) universal standardisation of practice
(ii) reduction in cost
(iii) improvment in the standard of practice

Towards the future, there is need to critically examine the viability of the following:

(i) long-term effects on babies born through ART.
(ii) the use of immature spermatozoa such as round spermatids and intact DNA for fertilisation [13].
(iii) the introduction of pre-implantation diagnosis.
(iv) reductions in the risk of multiple pregnancy through the introduction of follicular aspiration sperm injection and assisted follicular rupture (FASIAR)
(v) the introduction of surrogacy and oocyte donations [39].

Conclusion

Infertility and its management are extremely stressful experiences for all couples [5]. The advent of ART has brought a radical departure from the past state of hopelessness. With the increasing rate of success, and wider acceptance by affected couples all over the world, there is no doubt that the future of ART is bright.

References

1. Adetoro OO, Komolafe F. Hysterosalpingography and laparoscopy in infertility management. *Trop J Obstet Gynaecol,* 1990; 8: 26-29.
2. World Health Organisation. *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple.* Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ (eds). Geneva, WHO; 1993.
3. Okonofua FE. The management of infertility in Africa. Proceedings of the 1997 Annual Symposium of the DOKITA Editorial Board, Ibadan. 1997:10-20.
4. Akande EO. Problems of infertility in Sub-Saharan Africa. *Dokita.* 1987,16: 23-27.
5. Cooke ID. Infertility. In Keith Edmonds (ed), *Dewhurst's Textbook of Obstetrics and Gynaecology for Postgraduates.* 6th edition. Oxford, London; Blackwell Scientific Ltd. 1999: 432-440
6. Hull MGR, Glazener CMA, Kelly NJ. Population study of causes, treatment and outcome of infertility. *Br.Med. J.*1985, 291: 1693-1697.
7. Giwa-Osagie OF, Ogunyemi D, Emuveyan, EE, Akinla OA. Etiologic classification and sociomedical characteristics of infertility in 250 couples. *Int. J. Fertil.* 1984, 29: 104-108.
8. Ogedengbe OK, Giwa-Osagie OF, Emuveyan EE. Implications of pattern of tubal disease for micro-surgery or in vitro fertilization in Lagos. *J. Nat. Med. Assoc.* 1987, 79: 510-513.
9. Idrisa A, Ojiyi E. Epidemiology of infertility at a teaching hospital in the North Easthern Nigeria. Society of Gynaecology And Obstetrics (SOGON) Annual Scientific Conference.1999. Abstract 7.
10. Wada I, Adamu, YM. The magnitude of need for assisted conception technologies in a Nigerian tertiary hospital. Society of Gynaecology and Obstetrics of Nigeria (SOGON) Annual Scientific Conference. 1999. Abstract 14.
11. Wada, I and Odoma, G. The success rates of intra-uterine insemination (IUI) and timed intercourse (TI) after superovulation with exogenous gonadotropins.

Society of Gynaecology and Obstetrics of Nigeria (SOGON) Annual Scientific Conference. 1999 Abstract 10.

12. Giwa-Osagie OF, Ashiru OA, Sanyaolu A, Fagbohun C. Clinical profiles, assessment for IVF and the outcome of IVF in Lagos. *Advances in Fertility and Sterility Series (IFFS).*1988, 2: 45-48.
13. Imade GE, Baker HWG, Otubu JAM. Intracytoplasmic sperm injection: an advance in *in vitro* fertilisation procedure for male infertility. *Trop J Obstet Gynaecol.* 1997, 14: 15-27.
14. Giwa-Osagie O F, N wokoro C, Ogunyemi D. Donor insemination in Lagos. *Clin Reprod Fert.* 1985, 3: 305-310.
15. Giwa-Osagie OF, Disu RF, Kayode OB, Aro T. Succesful outcome following *in vitro* fertilization and zygote intrafallopian tube transfer in a private clinic. Society of Gynaecology and Obsterics of Nigeria (SOGON) Annual Scientific Conference . (1999) Absract 9.
16. Tournaye H, Derroey P, Liu J, Nagy Z, Lissen W, Van Steritegham A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil Steril,* 1994, 61: 1045-1051.
17. Silber S, Nagy Liu J, Godoy H, Deveroey PJ, V an Steirteghem A. Conventional in *vitro* fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum Reprod.* 1995, 9: 1705-1709.
18. Palermo G, Joris H, Derde, M, Camus M, Devroey P, Van Steirteghem AC. Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil Steril,* 1993, 59: 826-835.
19. Megafu U, Okoye IJ, Ofodile A, Afifam G. Therapeutic insemination of semen: ultrasonic monitoring of ovarian follicular growth. *Orient J Med,* 1995, 7: 32-37.
20. Cohen J, Edward R, Fehilly C, Fishel S, Hewitt J, Pondy J.*et al.* In vitro fertilization: a treatment for male infertility. 1985.
21. Oehinger S, Acosta. AA, Morsbedi M, Veeck L, Swanson JR , S immans K , R osenwaks Z. Corrective measures and pregnancy outcome in *in vitro* fertilization patients with severe sperm morphology abnormalities. *Fertil Steril,* 1988, 50:283-287.
22. Yovich JM, Edirisinghe WR, Cummins JM, Yovich JC. Influence of pentoxyfulline in severe male factor infertility. *Fertil Steril,* 1990, 53: 715-722
23. Iritani A. Micro-manipulation of gametes for *in vitro* assisted fertilization. *Mol Reprod Dev,* 1991, 28: 199-207
24. Giwa-Osagie OF, Ashiru O, Sanyaolu A, Abisogun O. Human oocyte recovery for *in vitro* fertilization: the use of cervical mucus and plasma oestradiol as indices of follicular development . W Afr J Med, 1998, 7: 71-76
25. Fishel S, Dowell K, Tomline J, Green S, Hall J, Klentzerris I. Micro-assisted fertilization with human gametes. *Hum Reprod,* 1993, 8: 1780-1784.
26. Gordan. J.W, Grunfield I, Garrisi G.J, Talausky B.E, Richer C, Laufer N. Fertilization of h uman o ocytes by sperm from infertile males after zona pellucida drilling. *Fertil Steril,* 1988; 50: 68-73
27. Tarin, J.J. Subzonal insemination, Partial zona dissection or intracytoplasmic sperm injection. an easy decision? *Hum Reprod,* 1995, 10: 165-170.
28. Baker HWG, Liu DV, Bourne H, Lopata A. Diagnosis of sperm defects in selecting patient for assisted fertilization. *Hum Reprod,* 1993, 8: 1779-1780.
29. Fisk NM. Multiple Pregnancy. In Edmonds KD (ed), *Dewhust's Textbook of Obstetrics and Gynaecology for Postgraduates,* 6th edition. Oxford, London; Blackwell Scientific Ltd; 1999: 298-307.
30. Derom C , D erom R , V lietinck R , Maes H, Van De Berghe H. Iatrogenic multiple pregnancies in East Flanders, Belgium. *Fertil Steril,* 1993, 60: 493-496.
31. Rizk. B, Smitz. J. Ovarian hyperstimulation syndrome after superovulation for IVF and related procedures. *Hum Reprod,* 1992, 7: 320-327.
32. Giwa Osagie OF,Oyewunmi AF, Elesha F, Sanyaolu, A, Osaghae, J. An unusual reaction to GnRH analogue therapy in a 9-year old female child. Society of Gynaecology and Obstetrics of Nigeria (SOGON) Annual Scientific Conference.1999 Abstract 16
33. Rizk B. Ovarian hyperstimulation syndrome. In Studd J (ed), *Progress in Obstetrics & Gynaecology,* 1994, 11: 311-349.
34. Al-Ramahi M. Decreasing the risk of severe ovarian hyperstimulation syndrome. *Hum Reprod,* 1999; 14: 2421-2422.
35. Titinen A, Husa LM, Tuppala M. The effect of cryopreservation in prevention of ovarian hyperstimulation syndrome. *Br J Obstet Gynaecol.*1995; 102: 326-329.
36. Rizk B, Tan SL, Morcos S *et al.* Heterotopic pregnancies after IVF and ET. *Am J Obstet Gynecol,* 1991; 64: 161-164.
37. Bennett SJ, Waterstone JJ, Cheng WC, Parsons J. Complications of transvaginal ultrasound directed follicle aspiration: a review of 2670 procedures. *J Assist Reprod Genet.* 1993; 10: 71-77.
38. Dicker D, Ashkenaz J, Felberg D. Severe abdominal complications following transvaginal ultrosonographically guided retrieval of oocytes for *in vitro* fertilization and embryo transfer. *Fertil Steril,* 1993; 59: 1313-1315.
39. Esangbedo J, Braimo O. Live birth following transfer of *in vitro* fertilized donated oocytes. Society of Gynaecology and Obstetrics of Nigeria (SOGON) Annual Scientific Conference. 1999. Abstract.