

Review

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EMERGING ROLES OF ANATOMISTS: DEVELOPMENT OF ASSISTED REPRODUCTIVE TECHNOLOGY IN WEST AFRICA

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SUMMARY

"Modern Anatomy" includes molecular biology which encompasses molecular embryology and genetics. Molecular biology is, indeed, rapidly gaining prominence in Human Anatomy departments. In the field of assisted reproduction, worldwide success rates culminating in live births from in – vitro fertilization are rapidly increasing. The role played by anatomists to this solution to the contemporary problem of infertility in Sub-Saharan Africa remains unclear. The article outlines the progress of in - vitro fertilization in Nigeria to illustrate the emerging roles of the 21st Century Anatomist. In Nigeria, during 1980s, there were only a few dedicated fertility centres, located in teaching hospitals. Most of them had no human sperm or gamete banks. Research work and full in vitro fertilization work started in Lagos University Teaching Hospital in 1983, culminating in the birth of the first in vitro fertilization baby in Nigeria and Sub Saharan Africa in 1984. Subsequently, the demand for in vitro fertilization services increased exponentially. The local sperm and embryo cryopreservation programme was hence initiated at Department of Anatomy, Lagos University. These revealed that freezing in ultra-low electrical freeze before storage in liquid nitrogen produces a significantly better post – thaw mortality after 4 weeks storage. In conclusion, the advent of assisted reproductive technology created a definite role for Anatomists and especially embryologists in the field of assisted conception. This implies that the 21st Century Anatomist has a huge potential role in applying molecular anatomy and other related fields hitherto not in the domain of morphology.

Key words: In Vitro Fertilization, Anatomist, Nigeria

INTRODUCTION

Infertility affects both the male and female patients equally (Cates et al., 1985; Gerais and Rushwan, 1992; Ashiru et al., 1993; Dyer, 2007; Maheshwari et al., 2012). The treatment, across the world is very expensive, and largely unaffordable by many patients especially in low-resourced areas of the world including parts Africa

continually on the increase. In Nigeria, prior to the birth of baby Olusola Ehosu Oni, preliminary scientific work in assisted reproductive technologies (ART) started at the Lagos University Teaching Hospital (LUTH) with the involvement of Professors Ashiru of Anatomy Department and Giwa-Osagie of Obstetrics and Gynaecology

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research by Professor Ashiru and Dr. Abisogun, laid the foundation for IVF success. Further research by Profs Ashiru, Giwa Osagie, Dr Abisogun, Mr Sanyaolu and Mr Aro gave rise to the establishment of full human reproductive medicine research and assisted reproductive technology at LUTH. The research work took about five years and culminated in the successful pregnancy and birth of Olushina Eghosa Oluwaremilekun to the family of Mr and Mgrs. Pius Oni (Ashiru et. al., 1986; Giwa-Osagie et. al., 1988). This pioneering work by Ashiru led to the active involvement of Anatomists and Embryologist in clinical medicine with regards to infertility.

1980-1984

Robert Edwards and Patrick Steptoe pioneering research work in IVF led to the delivery of Louise Brown in July 25, 1978 (Steptoe and Edwards, 1978). The IVF was

repeated successfully by Drs Howard and Georgeanna Jones in the United States and Elizabeth Carr was delivered in December 28, 1981 by Caesarean section (Jones et al., 1984). The first success in Africa was reported in 1984 by the Nigerian team of Professors Giwa-Osagie and Ashiru, Dr Abisogun, Mr. Sanyaolu and Mr. Aro. The birth of baby Olusola Eghosa Oni in 1989 followed diligent research work in IVF which had commenced in 1983, by the team at the LUTH. Their research and success was investigated and corroborated by a governmental investigative panel of 2 eminent professors at that time, Professors Adeleye and Grillo. The case was titled "IVF in Lagos, Nigeria - by Ashiru & Giwa-Osagie" and presented at Annual meeting of The Anatomical Society of West Africa (ASWA).



Ashiru and Blake in 1978 at the University of Nebraska started research in reproductive endocrinology on the effects of luteinizing hormone releasing hormone (LHRH) in phenobarbital-blocked rats (Ashiru and Blake, 1978; Ashiru and Blake, 1979; Blake et al., 1980). LHRH injection into the experimental animals restored their peri-ovulatory follicle-stimulating hormone surges. Other notable achievement by Ashiru and Blake in 1979 includes the stimulation of endogenous follicle-stimulating hormone release during oestrus by exogenous follicle-stimulating hormone or luteinizing hormone at proestrus in the phenobarbital-blocked rat (Ashiru and Blake in 1979).

Ashiru, Blake, Rush and others continued their research using experimental animals to elucidate follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol patterns after LHRH infusion in long-term, unanesthetized, ovariectomized and hypophysectomised rats (Blake et. al., 1980). The research shed further light on the interactions in hypothalamic-pituitary-ovarian axis in the periovulatory periods, showing that there is a significant increase in periovulatory plasma gonadotropins in the cyclic ovariectomised rat after exogenous estrogen and LHRH administration. Ashiru, Fagbohun, Dada, Blake and others also reported the effects of the blockade of the selective increase in serum FSH on the oestrous cycles of rats (Ashiru and Blake, 1980; Fagbohun, 1990).

At the college of medicine of the University of Lagos, with Okalawon and others the research continued with the study of the effects of food additives including maggi, local medications for example chloroquine phosphate (CQ, an antimalarial with amphiphilic properties), quinine and other environmental pollutants like 4-tert-octylphenol, on the reproductive functions of male and female experimental animals particularly in the Sprague-Dauley rat. Starting from 1992, Okanlawon, Noronha and Ashiru carried out series of experiments into the effects of CQ on reproductive and fertility potentials of male and female rats. CQ is known to induce generalized lipidosis. The studies suggested an adverse effect of administered CQ on the hypothalamo-pituitary-ovarian axis of the experimental animals (Okanlawon and Ashiru, 1992; Okanlawon et al., 1993). Using the optical dissector (with simple-point sampling of linear intercept lengths), the effects of CQ on rat testicular morphology were described. The rats were injected intraperitoneally with CQ and significant reductions in testicular weight, sperm production, total spermatid count per testis

and the star volume of the seminiferous tubules were noted. They noted, however, an increase in the total spermatocyte count per testis. These findings suggested that in CQ-treated rats there was a significant reduction in seminiferous tubular size and disruption of spermatogenesis (Okanlawon and Ashiru, 1998). Untreated female rats mated with treated male rats showed a dose-dependent decrease in the number of litter per female rat. In-vitro, more than 80% of spermatozoa were immotile in CQ-treated media suggesting an antifertility and sperm immobilising effects of CQ. It was also noted that in the female rat, administration of CQ disrupted ovulatory cycles; decreased serum oestrogen and luteinizing hormone with the exception of FSH which was unaltered (Okanlawon et al., 1993).

Nigeria is the second largest economy in Africa and represents the hub of the regional West African and sub-Saharan economic activity. In the early 1980s, there were few dedicated fertility centers in Nigeria. Most of the established fertility clinics then were located in the teaching hospitals; most had no human sperm or gamete bank. Research work and full IVF started at the LUTH in 1983 with part funding for the project from the Rockefeller foundation to Professor Ashiru. The research work centered on reproductive endocrinology and human fertility culminating in the delivery of the first IVF baby in Nigeria and Sub-Saharan Africa in 1984. Following the birth of the first IVF baby at LUTH, the demand for the service by patients increased beyond all expectations, the need thus arose to develop a local sperm and embryo cryopreservation programme at LUTH in the Anatomy Department of the CMUL to meet the demands. The research conducted by Akinola and Ashiru focused on the comparisons of semen cryopreservation protocols using different concentrations of

cryomedia including DMSO and Glycerol. The freezing protocols started at sub-zero temperatures (-20°C, ultralow-electrical refrigerator) to -196°C in liquid nitrogen. Fifteen semen samples were collected from consenting patients attending our fertility clinics for the study. The sperm count range was 50 – 200 million/ml and the motility ranges from 30 – 82%. The result demonstrated that freezing in ultralow-electrical freezer (-90°C) before storage in liquid nitrogen (-196°C) produced a significantly better post-thaw motility after 4 weeks of storage. Thus cryobanking of semen samples for assisted conception began in the Anatomy department of the College of Medicine, University of Lagos (Akinola and Ashiru, 1993).

The knowledge gained from several researches into the mechanism of ovarian sex steroid biosynthesis and research on pathophysiology of hypothalamo-pituitary-ovarian and testicular axis (Beall and DeCherney, 2012) has made treatment of infertility associated with ovulatory dysfunctions and other causes of infertility now feasible. The introduction of recombinant gonadotropins in the 1980s largely replacing urinary-derived human menopausal gonadotropin (HMG) made in the 1960s has further revolutionized ART treatments. Recent randomized controlled trials (RCTs) and metanalysis have demonstrated no significant difference in IVF outcomes when comparing the use of highly purified preparations of HMG for controlled ovarian hyperstimulation for IVF/ intracytoplasmic sperm injection (ICSI) and recombinant FSH (Youssef et al., 2011). Afnan (2009), however, suggested a significantly higher pregnancy and live birth rate with urinary HMG compared to rFSH treatment regimes. Furthermore, with the advent of ICSI, it is now also possible to treat infertility associated with a number of previously thought untreatable causes of male infertility like severe oligozoospermia.

Through the processes of IVF, about 5 million live births have been reported so far throughout the world, and in Nigeria it is estimated that altogether about 4,000 live births a year are delivered. Recent technology advances in IVF treatments including drug modifications, advent of new technologies, improvements in IVF equipments and expertise have led to better successes in outcomes and live births following fertility treatment. Improved patient selection processes to give patient-specific treatments, ICSI in selected cases (Tournaye et al., 2002; Fauser et al., 2009) and the advances in the in vitro culture equipments/environment and media (Thomas and Pool, 2004; Biggers and Summers, 2008) have further increased the treatment outcomes. World-wide pregnancy and live birth rates per treatment cycle have significantly increased from 10-15% in the mid to late 1970s to 50-60% lately. Interestingly, a number of ways that are hitherto social taboos are becoming socially acceptable including egg-donation, surrogacy, in vitro maturation (IVM) of immature oocytes, ovarian tissue/uterus transplant and other cutting edge treatments have further expanded the reach of fertility treatments (Siristatidis et al., 2011; Meseguer et. al., 2012). Other advances and modifications that have also occurred include individualisation of controlled ovarian hyperstimulation treatment processes, monitoring of treatment cycles, embryological processes and selections, mechanism of embryo implantation and cryopreservation (Beall and DeCherney, 2012). The live birth rate has increased significantly now up to 50% or more in well-established fertility clinics.

Suggestions for risk prevention in IVF include using, eligible patients, elective single embryo transfer, better management of controlled ovarian hyperstimulation, and close adherence to safe and quality

processes. This has resulted in further improvements in IVF outcome measures by recent optimization in culture techniques, media and embryo selection processes (Kirkegaard et al., 2012). Interests in the ovarian hyperstimulation techniques of mildly or minimally hyperstimulating the ovaries for IVF have gradually been explored in some centers around the world and are reported with at least equal success rates and in some centers better outcomes compared to the conventional IVF (Verberg et al., 2009 a,b). Conventional superovulation is reported to increase the incidence of chromosomal abnormalities of embryos (Verberg et al., 2009 a,b). Tarin et al. (1990) reported an increased incidence of diploid oocytes in patients with high response to gonadotrophins. High levels of FSH have also been related to the alteration of oocyte maturation, increased risk of aneuploidies in the MII oocyte (Roberts et al., 2005). In addition, high FSH concentrations used in the culture media for in vitro maturation of oocytes (IVM) experiments are reported to increase the proportion of aneuploidies in MII oocytes (Roberts et al., 2005). More improvements in outcome measures and results from mild/minimally stimulation of ovaries for IVF in centers actively using these techniques are awaited.

Oocyte and embryo: Assessment and scoring for IVF

Recent trend in IVF treatment processes have concluded that the outcome and goal of IVF should be for patients to safely/successfully deliver healthy singleton live births at the end of the processes (ASRM, 2012 a, b). Multiple and higher order gestations as a result of IVF treatment is a disadvantageous outcome. This is largely to avoid the associated obstetrics, maternal and fetal morbidity and

mortality of multiple and higher order gestations/ births; and to reduce the enormous physical, psychological and financial burden on the individual patients, their families and the society at large (ASRM, 2012a, b). Therefore, selection of quality embryos for elective single embryo transfer to curtail multiple/higher order pregnancy and associated complications, look promisingly as the norm in qualified patients. Furthermore, the significant improvement in the embryo culture systems including media, sequential- /co-culturing of embryos and equipments gave further boost to improvements in IVF treatment outcomes.

The use of proteomics, metabolomics, transcriptomics, and other newer non-invasive embryo assessment technologies including metabolic pathway identifications are still largely experimental with little practical applications (Katz-Jaffe et al., 2006). The non-invasive assessment of human embryos in vitro to select quality embryos prior to transfer in IVF treatment processes is therefore, currently based on their morphological features and development growth (Balaban et al., 2011).

Presently, there are many variations from one laboratory to the other in the scoring processes and no worldwide consensus yet. Based on published reports by Hardarson et al. (2001) and van Royen et al. (2003), the Association of Clinical Embryologists (ACE) and the British Fertility Society (BFS) (Cutting et al., 2008) recommended the following parameters for cleavage-stage embryo scoring: a combination of blastomere number, size symmetry and the degree of fragmentation (Cutting et al., 2008). For blastocysts based on the work of Gardner

and Schoolcraft (1999a, b) and Stephenson et al. (2007), the BFS and ACE suggested a three-part grading system looking at the expansion status, the inner cell mass and trophoctoderm morphologies. ESHRE and SIG Embryology Atlas project (Gianaroli et al., 2000) has made conscious efforts to forge a common ground and build a worldwide agreeable consensus on oocyte, zygote and embryo grading system to guide quality selection, thereby improve treatment successes, facilitates research studies and clinical trials comparability of new fertility drugs and technologies. Other goals include the need for durable international clinical and training quality assurance systems for oocyte and embryo morphology grading.

Oocyte: Assessment and scoring

Human oogenesis and embryogenesis is a rapidly evolving field. Optimal oocyte morphology consists of a spherical structure enclosed in a uniform zona pellucida. It normally has a translucent uniform cytoplasm without any inclusions and a polar body. Generally oocytes grading includes the assessment of the oocyte–corona–cumulus–complex and cytoplasmic / extracytoplasmic dysmorphisms (Balaban et al., 2011). Abnormalities of the oocyte will have grave consequences on its ability to fertilise and later preimplantation development. This can result in embryos that fail to implant; and if implanted are likely to be miscarried. Therefore oocyte grading and selection of normal oocytes prior to fertilisation in IVF will have positive consequences on treatment outcomes. Assessment of the human oocyte quality is based on the nuclear (genetic) and cytoplasmic

morphology. Any abnormalities in the meiotic process resulting in nuclear or cytoplasmic dysmorphism of the oocyte before fertilization with human sperm impact significantly on the implantation of the consequent embryos and therefore the success of IVF treatment (Van Blerkom & Henry 1992; Ebner et al., 2006). The intrinsic causes of oocyte dysmorphism and its consequent effects on the developmental biology of the oocyte and the embryo are largely unknown. However, embryos generated from a fertilised dysmorphic oocyte are reported to have significantly poor implantation rate and a higher risk of miscarriage (Balaban et al., 2011). Significant cytoplasmic anomalies also cause poor oocyte fertilising and developmental potential. These anomalies are sub-divided into intracytoplasmic and extracytoplasmic dysmorphisms.

Intracytoplasmic dysmorphism includes aggregation of smooth endoplasmic reticulum, dense granulation vacuoles and refractile bodies; and extracytoplasmic dysmorphisms (first polar body morphology, perivitelline space size and granularity, discoloration, zona pellucida defects, shape anomalies). Abnormal aggregation of smooth-surfaced endoplasmic reticulum (sER) in the oocyte causes poor calcium signaling and mitochondrial functions (Otsuki et al., 2004; Ebner et al., 2008; Akarsu et al., 2009) and predictably have the worst prognosis. The zygotes and embryos generated from these oocytes have abnormal developmental process, early fetal demise and imprinting disorders e.g. Beckwith-Wiedemann Syndrome (Otsuki et al., 2004). Compared to oocytes derived from controlled ovarian hyperstimulation using minimal dose of gonadotropins; oocytes derived

from cycles stimulated with high dose of gonadotropins as in conventional IVF cycles are reportedly associated with significantly higher abnormalities of oocyte attributed to desynchronization of nuclear and cytoplasmic maturation of the oocyte (Miao et al., 2009). A normal fertilized oocyte should be spherical, have two polar bodies, and two centrally-juxtaposed symmetric pronuclei.

Pronuclear embryo

Grading of embryo to gauge their potential development and implantation capabilities is at the cleavage and blastocyst stages within a fixed time period (usually about 16 hours) post insemination. For zygote grading or scoring cellular polarization, the presence of a cytoplasmic halo, the number of pronuclei and pronuclear appearance are determined, while the expansion rate, trophoctoderm and inner cell mass morphometry are assessed for the blastocyst. At the pronuclear stage, the determinants of good quality embryos include the position and symmetry of the pronuclei as well as the number and the relative position of the nucleolar precursor bodies (NPB). Therefore, in a normally fertilized oocyte, the pronuclear embryo should possess two similar-sized and closely apposed pronuclei that are usually centrally located (Balaban et al., 2011) and about five to seven NPB. It is pertinent to note that pronuclear embryo scoring at days 1 and 2 provides a good measure of gamete quality and scoring embryos at days 3–5 reflects more about gene expression, differentiation and developmental controls.

Cleavage stage embryo

Zygote scoring and assessment using time-lapse technology is useful to determine the cleavage rates and identify abnormal morphological changes that are predictive of poor processes of embryogenesis, embryo quality and implantation potential (Lemmen et al., 2008). When assessing zygote or early cleavage stage embryo, the developmental time difference between those obtained from ICSI and standard IVF is paramount, as the ICSI process bypasses initial steps of oocyte fertilization including (Nagy et al., 1998).

Cleaving embryos reach up to 7 or 9 cells by day 3, usually with <15% fragmentation and without any cellular multinucleation. The cleavage rates, fragmentation, the presence of multinucleation and the relative size of the cleavage cells are considered for scoring. Optimally cleaving and developing embryos with less than 10% fragmentation are considered to be good with good implantation potential (Van Royen et al., 2001). Embryos with cleavage rate abnormalities (including accelerations, stagnations and/or slowing) and those with unequal blastomere size, significant number of multinucleated blastomere and excessive fragmentation (for example >35%) are associated with higher risk of chromosomal aberrations, poor implantation potential, miscarriages and pregnancy failures (Van Royen et al., 2003, Magli et al., 2007, Munné, 2007). Factors responsible for cellular multinucleation include culture media temperature (Winston et al., 1991), abnormal genetic composition and aberrations of the meiotic processes (Munné and Cohen, 1993).

Morula and Blastocyst stage

Embryos at Day 4 should have started compacting or are fully compacted. This process of morulation; must include all blastomeres; therefore cellular exclusions (especially involving >50% of the cells) portends abnormalities of morulation usually associated with poor development and implantation potential (Tao et al., 2002). Morular or Day 4 embryos are graded good, fair or poor (Balaban et. al.,

2011). An optimal or good morula will be in cleavage stage 4, with all cells compacting or are fully compacted. Fair grade includes those where almost all the cells are involved in compaction and those with less <50% cellular compactions are graded as poor. Parameters considered in blastocyst grading include expansion, inner cell mass and the trophectoderm morphologies.

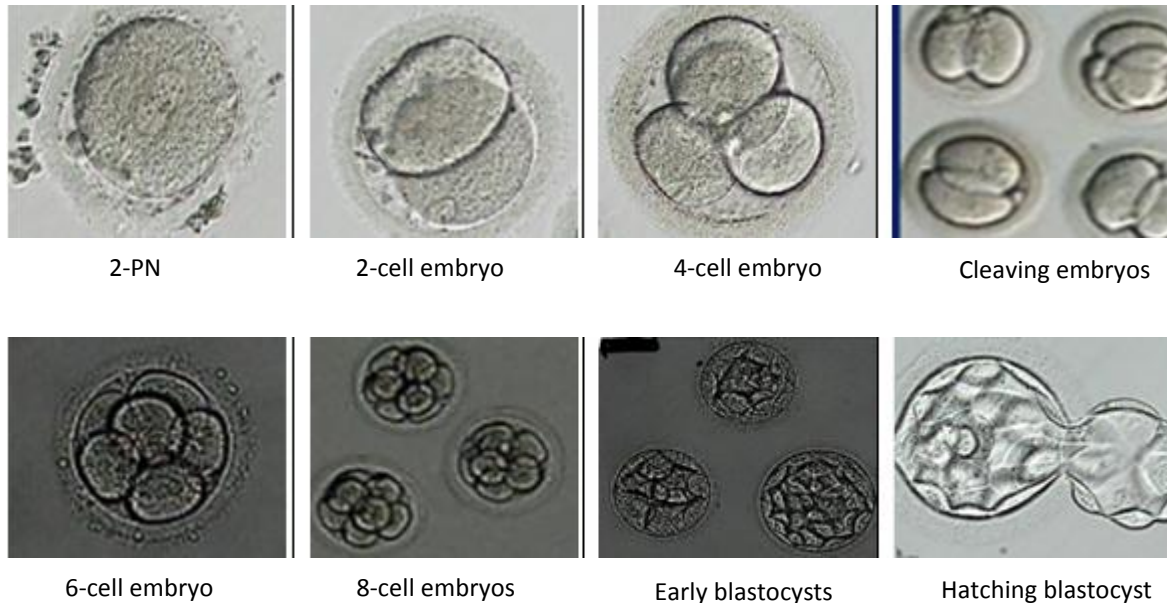
Grading / scoring of Blastocyst – 1AA score for an optimally developed blastocyst

Blastocyst expansion	
1	Blastocoele <50% of the embryo volume
2	Blastocoele >50% of the embryo volume
3	Blastocoele completely fills embryo volume
4	Fully expanded blastocyst with zona thinning
5	Hatching blastocyst
6	Hatched blastocyst

Inner Cell Mass (ICM)	
A	tightly packed ICM with many cells
B	loosely grouped ICM with many cells
C	ICM with very few cells

Trophectoderm (TE)	
A	many cells forming cohesive epithelium

B	few cells forming loose epithelium
C	TE with very few cells



Embryos and blastocysts at different stages of development

Research reports on embryogenesis have suggested that only few embryos with chromosomal abnormalities progress to the blastocyst stage (Munné 2007, Sandalinas et al., 2001; Magli et al., 2000). With extended in vitro culture processes it is feasible to monitor the proportion of Day 2-3 embryos that will progress to the morula and blastocyst stages. Extended or sequential embryo culturing is often considered favourably as surrogate to select viable/normal embryos as it eliminates embryos with post-meiotic abnormalities, but not those with aneuploidies, more commonly found in older women beyond 35 years of age. Chromosomal analysis of surplus blastocyst suggested little correlation between blastocyst morphology and

chromosomal abnormalities, therefore, extended culturing compared to PGD might not be the ultimate screening method to avoid selection of chromosomally abnormal embryos (especially aneuploid embryos) (Schoolcraft et al., 2010; Fragouli et al., 2010). Compared to embryo morphological scoring methods, newer embryo scoring using logistic regression modeling to calculate cleavage scores significantly predict embryo developmental potential, blastulation and implantation (Holte et al., 2007).

CONCLUSION

Further support from by the government, practicing reproductive medicine specialists, organizations and the society in most developing countries is required to optimize benefits from recent advancements in IVF.

Exploitation of infertile individuals or couples also presents as a major concern that needs to be addressed. A government regulatory strategy, akin to the HFEA in UK, will protect the society from abuse and exploitation of the ART process. In tandem with this, recent efforts by the Association of Fertility and Reproductive Health (AFRH)

in Nigeria are directed towards guidance on the code of practice of ART treatments for local practitioners, provision of a robust acceptable code of practice, periodic public consultations, periodic revision of the practice code in line with cutting-edge researches and societal beliefs.

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