Animal care and use in Reproductive Biology in a developing country

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Abstract

The field of Reproductive Biology is concerned about basic aspects of reproduction and includes factors affecting animal and human clinical reproductive health, which are studied by examining physiological, cellular, molecular and genetic mechanisms regulating reproductive function, yet reproductive biology and medicine has not been a focus of research to draw attention to problems of reproduction in tropical and under-developed countries. Fundamental mechanisms of development and reproductive toxicity, including endocrine studies have been carried out using different animal species as well as large domestic animals. When animals are use for studies, animal welfare is important because of the link between healthy, well-cared-for animals and sound science; hence research on animals should only take place within a framework that allows for continuous and challenging consideration of the ethical and welfare issues relating to this use of animals. Most National oversight mechanisms emphasize basic principles of humane science, which is none existent in many developing countries, in particular, Nigeria. This paper discusses the care and use of animals in the progress and advances of assisted reproductive Technologies as an approach to managing the burden of poor reproductive health particularly in sub-Saharan Africa where infertility constitutes one of the leading public health problems. Inspite of the cultural and ethical challenges arising from the use of Assisted Reproductive Technologies in developing societies, these technologies are rapidly becoming available and accessible to developing societies. This necessitates a continuation of refinement and evaluation of such technologies, which may also involve use of animals. Legislation on a National Animal welfare Act and Council is overdue in Nigeria and is suggested for other developing countries to accentuate the scientific progress in this and other biomedical research fields.

Keywords

Animal welfare; 3Rs; Federal regulations; Assisted Reproductive Technologies; Sub-Saharan Africa; Reproductive Health; Institutional Animal Care and Use Committee.

Introduction

Reproductive health is a state of complete physical, mental and social well-being, and not merely the absence of reproductive disease or infirmity[1]. It deals with the reproductive processes, functions and system at all stages of life[1]. Reproductive health is a crucial part of general health and a central feature of human development. It is a reflection of health during childhood, and crucial during adolescence and adulthood, sets the stage for health beyond the reproductive years for both women and men, and affects the health of the next generation. The health of the newborn is largely a function of the mother's health and nutrition status and of her access to health care[2].

Reproductive health is a universal concern, but is of special importance for women particularly during the reproductive years[2]. Although most reproductive health problems arise during the reproductive years, in old age general health continues to reflect earlier reproductive life events. Men too have reproductive health concerns and needs though their general health is affected by reproductive health to a lesser extent than is the case for women. Because reproductive health is such an important component of general health it is a prerequisite for social, economic and human development[2].

Poor reproductive health constitutes one of the leading public health problems in the world, particularly in sub-Saharan Africa (SSA) [3]. Not being able to procreate has severe social and economic repercussions in resource-poor countries[4] and causes severe suffering such as domestic violence, union dissolutions and sexual dysfunction in addition to the psycho-social consequences suffered by infertile couples. Although women carry the largest burden of suffering, the negative repercussions of infertility for men, especially at the level of the community, are considerable. Whether the infertility was caused by a female factor or male factor was an important determinant for the type of psycho-social consequences suffered[4].

Infertility is a problem of global proportions, affecting on average 8-12 percent of couples worldwide[5]. In some societies, however-particularly those in the "infertility belt" of sub-Saharan Africa-as many as one-third of all couples are unable to conceive. Factors causing high rates of tubal infertility in parts of the developing world include sexually transmitted, postpartum, and post-abortion infections; however, male infertility, which is rarely acknowledged, contributes to more than half of all cases[5].

Inspite of the cultural and ethical challenges arising from the use of Assisted Reproductive Technologies (ARTs) in developing societies, these technologies are rapidly becoming available and accessible to developing societies[6] where children are highly desired, parenthood is culturally mandatory, and childlessness socially unacceptable. Legitimacy of children born through ARTs, religious obligation, patriarchy, polygamy and value of children are cultural issues surrounding ARTs while decision making about it, discrimination against children born through ARTs, psychological problems and loss of self esteem, side effects of the technologies and the cost of accessing them are the ethical challenges[7].

Assisted reproduction technologies (ARTs)

An estimated 3.5 million children have been born to date using ARTs[8]. In view of the global burden of subfertility, efforts are required to make assisted reproduction more effective, less burdensome and more equally accessible. New reproductive technologies are

frequently introduced in clinical practice without a sound evaluation of their efficacy, effectiveness and/or safety. Safety issues in this context refer both to patients (mostly women) undergoing the relevant medical procedures, and to the health of children born as a result[9].

With the advancement and introduction of newer techniques in ARTs there is an increase in the micromanipulation of gametes and embryos in vitro and extended exposure to the in vitro environment[10]. These include the use of gonadotropins for superovulation, intracytoplasmic sperm injection (ICSI), blastocyst culture and transfer, vitrification, assisted hatching, and preimplantation genetic diagnosis. Although these approaches aim to enhance pregnancy rates and its outcome, the risk of associated long-term health hazards in children born by these practices cannot be disregarded[11]. There is a theoretical potential risk of developing an excess of childhood malignancies and imprinting disorders in children born after ARTs[10]. Pregnancies achieved by IVF with or without ICSI are at higher risk for obstetrical and perinatal complications than spontaneous pregnancies, and close surveillance during pregnancy should be considered[12]. It remains unclear if these increased risks are attributable to the underlying infertility, characteristics of the infertile couple, or use of assisted reproductive techniques[12].

More recently, novel equipment that needs to be validated before it enters routine clinical use is being developed for IVF. With technologies such as producing gametes from stem cells around the corner, it is vital to ensure that the necessary research and development is conducted before bringing new techniques into clinical practice. Ideally, this should include preliminary work on animal models, such as mice/rats/rabbits/larger mammals, followed by studies on human embryos donated for research and finally well-designed randomized clinical trials (RCTs) with a follow up of all children born from the procedure[11].

Animal models of reproductive Biology studies

Animal studies are unique in that assessment of developmental and reproductive toxicity (DART) studies are not performed in controlled clinical trials; therefore, pre-clinical safety assessment forms the basis for risk assessment for marketed drug products. The regulatory agencies around the world including Food and Drug Administration (FDA) generally requires DART testing of all new drugs to be used by women of childbearing age or men of reproductive potential[13]. The nonhuman primates (NHPs) are most frequently used for DART testing when commonly used rodents and/or rabbits are not pharmacologically relevant species. NHPs are used in many areas of biomedical research where their similarities to humans make them exclusively valuable animal models. The use of NHPs in pre-clinical testing is expected to increase due to the increase in the development of biological compounds for therapeutic uses[13].

Understanding the long-term effects of environmental chemicals (EC) exposure on reproductive health in humans requires animal models and exposure to 'real life', environmentally relevant, mixtures during development, a life stage of particular sensitivity to ECs[14, 15]. Developmental exposure of male sheep to real-world mixtures of ECs has been shown to result in major reduction in germ cell numbers, indicative of impaired sperm production, in a proportion of exposed males[14]. In addition, mutations in mismatch repair and tumor suppressor genes, which could potentially lead to genomic instability, have been identified in some infertile men and animal models[15].

Endocrine studies in rat, rabbit and bovine models has raised our understanding on the paracrine and autocrine control of ovarian antral follicle development and ovulation by ovarian tissue rennin-Angiotensin system (RAS), using different in vivo and in vitro experimental approaches to study the role of RAS in the ovary[16]. It is widely accepted that there are marked species differences in RAS function in follicle development. Examples of species-specific functions of the RAS in the ovary include the involvement of Ang II in the regulation of follicle atresia in rats and the requirement of this peptide for the dominant follicle development and ovulation in rabbits and cattle. More recently, Ang-(1-7), its receptor, and enzymes for its synthesis; Neutral endopeptidase (NEP), Prolyl endopeptidase (PEP) and Angiotensin converting enzyme II (ACE2) were identified in bovine follicles, implying that Ang-(1-7) has an ovarian function[16].

Fertility depends mainly on the success of processes involving ovulation, fertilization, implantation, placentation and embryo development; processes that seem to be affected in obese females. Translational animal research and clinical studies have been used to evaluate the factors, mechanisms and pathways involved in the reproductive failures of individuals with metabolic disorders during the critical period from ovulation to completion of placentation and early-embryo development[17]. Evaluation of the survival rate and hatching rate of bovine blastocysts following vitrification using a method designed for oocytes has been developed, with a view to introducing this methodology into human assisted reproduction technology and reproductive medicine[18]. Hentemann and Bertheussen (2009)[19] develop sequential media for extended culture of embryos in human IVF, with use of a mouse embryo assay. The BlastAssist system was developed. The combination of blastocyst culture and single ET should be an effective means of treatment in patients and could help to eliminate multiple gestations. The BlastAssist system is commercially available[19].

Assisted reproductive technologies have been successfully applied in several mammals, including humans, thanks to the ability of oocytes and embryos to face maturation, fertilization and first development in vitro[20]. However, efficiency and safety of these protocols has called for the use of large domestic animals been proposed for research in the reproductive field as models to improve human ARTs considering that mammalian oocytes and early embryos are transcriptionally inactive, and rely exclusively on maternal RNAs and proteins, deposited during oocyte growth, until embryonic genome activation (EGA). Such transcriptional quiescence needs complex post-transcriptional and post-translational mechanisms to coordinate meiotic maturation, fertilization, and reprogramming of the nascent genome. A deep knowledge of these early phases of development is crucial to understand the core mechanisms of life onset, and to optimize the safety and efficiency of in vitro reproductive technologies[20].

Additionally, research on animal models has demonstrated that stress, and the resultant adaptation to conditions during pre-implantation stages, can affect pregnancy loss and fetal growth. In addition to the ability of a culture system to produce a single top-quality embryo for transfer, it is also necessary to enhance the cryotolerance of sibling embryos so that they can survive freezing or vitrification[21]. It is therefore important to understand the role of each medium component and to identify possible sources of cellular stress to the embryo that will ultimately affect the function and viability of the conceptus[22]. The elucidation of the metabolic requirements of human embryos in vivo or in-vitro remains, despite being intensively investigated, a work in progress. The adoption of extended embryo culture to the blastocyst stage during the last decade has entailed new challenges[23]. With the increased

attention to culture media formulations, more evidence on the sensitivity of embryos to their early environmental conditions is accumulating which might affect phenotype and developmental potential[24].

Alternative method used in IVF – In-vitro methods

In Vitro maturation (IVM) of human oocytes is an emerging infertility treatment with great promise[25, 26]. To be successful, this future assisted reproductive technology must entail both nuclear and cytoplasmic maturation of the oocytes and give rise to human embryos that have the same developmental potential as embryos resulting from the golden standard of human IVF. The aspiration of immature oocytes from small to medium size antral follicles followed by their maturation In Vitro present an attractive alternative to the hormonal stimulation of patients in In Vitro fertilization (IVF) treatment, since administration of exogenous hormones is a costly treatment and may cause severe health problems[27]. At present, the In Vitro maturation techniques are highly successful in mice[25], variable successful in domestic species and still regarded experimental in the human clinic due to suboptimal fertilization rates and embryo quality. IVM has the promise of being tomorrow's gold standard in treatment of human infertility if most of the important components of oocyte maturation are understood and can be adequately addressed In Vitro[27,28].

Animal Welfare and Regulations.

The study of Reproductive Biology addresses basic aspects of reproduction as well as factors affecting animal and human clinical reproductive health, by examining physiological, cellular, molecular and genetic mechanisms regulating reproductive function, yet reproductive biology and medicine have not been a focus of research to draw attention to reproduction problems in tropical and under-developed countries. Research on animals should only take place within a framework that allows for continuous and challenging consideration of the ethical and welfare issues relating to this use of animals. Consideration of whether, and how, animals are used in experiments involves value judgements that may change with time and with the perspectives, priorities, interests and expertise of those making them (and with the context in which they are made) [29].

"…. the validity of your research outcome is dependent upon the satisfactory welfare of your research animals……" (Prof E. A. Caxton-Matins, 1995; Personal communications). Animal welfare is important because of the link between healthy, well-cared-for animals and sound science. Most national oversight mechanisms emphasize basic principles of humane science, in particular the "three R's" tenet of replacement, reduction, and refinement of animal use[30]. However, the oversight of animal care and use occurs through a wide variety of local, national, and international mechanisms, some based on legislation [the European Union (EU)][31]; others on peer review or other forms of non-legislated oversight (Canada) and yet others on a combination of legislated and non-legislated oversight United States, New Zealand[32].

Animal care and use in Nigeria as a case study

In Nigeria for instance a lot of data are published based on Animal experimentations from many Universities and Institutions (just a few have ethics committees). There are nine Universities Training Veterinarians in the Country while many Research Institutions and Pharmaceutical industries exist in the country (local & multinational corporations) with or without an R&D Unit, testing compounds and drugs on animals, YET there are no laws/acts to regulate these processes. Available literature showed that little attention has been given to

animal welfare in Nigeria, however, the right of animals under captivity or domesticated, has been defined by both the Criminal code and Penal code of Nigeria[33,34], which is summarized as follows: *"The right to be free from hunger; discomfort; fear and distress and Freedom to express normal behavior",* being laws relating to the responsibilities of owners and others in charge of animals. In the Nigerian Criminal/penal code there is provision for penalty for harming animals in Section 495 and Cap. 196 of the penal code. The list of offences of cruelty to animals include: beating, kicks, ill-treatment, over riding, over driving, overloading, torture, terrifying or cause or process or permit any animal to be so treated, cause or permit unnecessary suffering.

In USA, the Animal Welfare Act[35] was signed into law in 1966 and amended in 1970, 1976, 1985, 1990, 2002, 2007 and 2008 to regulate animal use activities, enforced by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). Violation of the Act is punishable under federal law and could lead to research program suspensions, fines and/or imprisonment. The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animal[36] is another guide that is applicable any time the Public Health Service funds research involving animals. The Office of Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH) administers the PHS Policy through the Research Extension Act. Deviation from the PHS Policy could result in loss of funding from Public Health Service organizations. These regulations and policies basically ensure that animals are used in research only when it is absolutely necessary and when animals are used in research, they are humanely treated. In this instance animals can be used when there are no other alternatives, when confirmation has been made that research activities are not unnecessarily duplicating previously conducted experiments and when experiments involving animals are relevant to human or animal health, will advance scientific knowledge, or will be for the good of society.

Federal and state agencies ensure that regulatory requirements are satisfied by establishment of Institutional Animal Care and Use Committees (IACUCs) with Regulatory Oversight[37]. Each Institution establishes an Animal Care and Use Program under an Institutional Official that appoints the IACUC membership. This committee will comprise of faculty and staff as well as a lay person non-affiliate with the Institution, empowered by law to ensure all research activities involving animals satisfy federal, state and local regulations & policies governing the use of animals in research. The IACUC is an "Institutional/University Governance" committee, legally required to oversee all animal care and use activities conducted in an institution[38] (Table 1). The IACUC reviews and approves all research, teaching or testing activities that involve animals before scientists begin their experiments[38, 39] to ensure: there are no alternatives to using animals, that research is not being unnecessarily duplicated, and that the experiment is relevant to human or animal health and will be for the good of society (Table 2).

The concept of the 3Rs has been internationally accepted as the basis of the care and use of animals for scientific purposes since it was first introduced by Russell and Burch (1959)[30]. These are: to Replace the need for animal use by alternative means, to Reduce the numbers of animals used to an unavoidable minimum, and to Refine any procedures necessarily used, so as to minimize the impact on animals, consistent with the achievement of a justifiable scientific purpose, and which is necessary because there is no other way of achieving that purpose. The incorporation of the 3Rs at the planning stages ensures that full consideration of the principle is exercised at every juncture of the process. However, it is unrealistic to expect

this to be possible in every area of scientific research in the immediate future. The main obstacle is still the difficulty of accurately mimicking the complex physiological systems of whole living organisms—a challenge that will be hard to meet[40].

Recommendations for the fashioning of Animal care and use for reproductive biology and medicine in developing countries

Developing countries need to be cognizant of world trends for the improvement of laboratory animal welfare. Animal ethical review in developing countries would benefit from experiences in Western countries in their evolution toward high standards of experimental animal ethics[41]. Biomedical research activities have been part of many Universities/Research Institutions in Nigeria in the absence of self regulatory mechanisms. These biomedical research activities, in most cases, require the use of animal models; in addition, nine Universities train veterinarians in the country using animals for teaching. It will therefore be apt at this point to suggest the establishment of a Nigerian National Animal Welfare Act and Council. Using animals for research activities is a privilege, not a right. It is a privilege that a scientist or an institution can lose if the ethical (e.g. legal and moral) responsibilities are not satisfied. The Animal Act should be comprehensive on the use of animals in research, education and testing, with particular emphasis for the Care and Use of Animals for Scientific Purposes. These regulations/laws should require any research facility or premises in Nigeria keeping and/or using animals for scientific purposes to:

(i) Be licensed by an appropriate state agency eg. the Ministry of Agriculture or Science & Technology and

(ii) Establish their respective Institution Animal Care and Use Committee (IACUC) [42].

In order for an IACUC to be established, at least fifty percent of its memberships must undergo formal training on IACUC functions and duties.

A National Animal Welfare Council should be established that will also serve as the National Advisory Committee on Laboratory Animal Research (NACOMLAR). The Council will amongst other things develop and publish a set of comprehensive guidelines on the care and use of animals for scientific purposes. These guidelines should be developed in the context of the cultural, religious and socioeconomic values of the country and not a mere recapitulation of the guidelines in developed Western World, which however can be referenced.

A National Basic IACUC Training Course should be designed based on guidelines developed by NACOMLAR. The Course should offer participants the necessary formal training on the functions and duties of an IACUC. The course should provide a basic, yet comprehensive and practical, overview of the guidelines on the use and care of laboratory animals with the focus on equipping participants with knowledge and skills in IACUC management and administration. Participants should receive extensive resource materials including copies of relevant laws, regulations, policies, and guidelines. Speakers for the course should comprise of local and foreign experts, including government representation. There should be overseas representation from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International) until capacity is built for sustainability.

In Conclusion

Animal based studies are still very important, more today than before. We humans must have regard and respect for these surrogates in our care and use. Urgent legislation on a National Animal welfare Act and Council is overdue in Nigeria before the country becomes another "koko" waste dumping site for developed countries where the regulations are stricter. Ultimately, the efforts will bring about an attitudinal change from the users (scientists) and the public, who will eventually become interested in what researchers do with animal in their laboratories in the near future.

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Table 1. The Authority of IACUC

1	• **review** and approve or disapprove protocols and other proposed activities, or proposed significant changes in activities, related to agricultural animal care and use in research and teaching;
2	• **conduct**, at least twice a year, an inspection of agricultural animal facilities and study areas and review of the overall agricultural animal care and use program, and to provide a written report to the responsible institutional official regarding the institution's compliance with this guide;
3	• **investigate** concerns, complaints, or reports of noncompliance involving agricultural animals at the facility;
4	• **suspend** an activity involving agricultural animals when it is not in compliance with approved protocols or written operating procedures (see section on Written Operating Procedures);
5	• **make recommendations** regarding the development and implementation of institutional policies and procedures to facilitate, support, and monitor the humane and appropriate use of animals in agricultural research and teaching as well as any other aspect of the agricultural animal care program; and
6	• **perform** other functions as may be required by institutional need and by applicable laws, regulations, and policies. Other useful information about IACUC functions can be found in the

Table 2. IACUC considerations in the preparation and review of animal care protocols[35]

S/no	Item Check list
1	• Objectives and significance of the research or teaching activity;
2	• Unnecessary duplication of previous studies;
3	• Availability or appropriateness of alternative procedures or models (e.g., less invasive procedures, cell or tissue culture, or computer simulations) for the proposed research or teaching activity.
4	• Aspects of the proposed experiment or demonstration having to do directly with animal care and use, including justification for the species and (or) strain of animal used; justification for the number of animals used; and a description of procedures that may cause discomfort, distress, or pain and of methods of alleviation including anesthesia, analgesia, tranquilizers, and non-pharmacologic means, as well as justification for any procedures that involve unalleviated pain, discomfort, or distress;
5	• Appropriateness of procedures and post-procedural care;
6	• Criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful and stressful outcomes are anticipated;
7	• Unusual husbandry requirements (Note: describing a procedure as a "standard farm practice" may be acceptable if the institution's written operating procedure is being used or if the practice is needed to serve as an appropriate control);
8	• Aspects of animal husbandry not covered under written operating procedures
9	• Method of euthanasia or disposition of the animal; and
10	• Responsibilities, training, and qualifications of the researchers, teachers, students, and animal care personnel involved in the proposed activities.

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