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The Use of Artificial Insemination (AI) Technology in Improving Milk, Beef and Reproductive Efficiency in Tropical Africa: A Review

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Abstract

The objectives of the study are to review AI practices as rapid means of improving milk, beef production and reproductive efficiency in tropical Africa. It is also to showcase the place of AI in livestock industry with the aim of encouraging farmers to adopt the technology for better livestock production and food sufficiency. AI is one of the assisted reproduction technologies (ARTs) used in many domestic species including bees and human beings. The use of AI is on the increase in horses, beef cattle, sheep, goats, deer, buffalo and dogs. AI has, also, been successful in conservation breeding of endangered species such as primates, elephants and wild felids. AI allows for widespread use of genetically superior sires that would normally not be available to breeders because they are too expensive to purchase. AI allows for faster and increased genetic improvement in cattle allowing for improved herd performance and productivity. It is the most commonly used assisted reproduction technologies (ART) in livestock, revolutionizing the animal breeding industry during the 20th century. In contrast to medical use, where intra-uterine insemination (IUI) is used only occasionally in human fertility treatment, AI is by far the most common method of breeding in intensively kept domestic livestock, such as dairy cattle (approximately 80% in Europe and North America), pigs (more than 90% in Europe and North America) and turkeys (almost 100% in intensive production). The other assisted reproduction technologies (ARTs) in animals are generally confined to specialist applications or for research purposes, since the cost would be prohibitive for normal livestock breeding. It is recommended that, Government and well to do stake-holders in the industry should encourage farmers by supplying semen, reliable methods of estrus detection and training AI personnel to achieve higher conception rates.

Keywords: Powerful tool; Milk; Beef production; Reproductive efficiency; Africa

Abbreviations: HF: Holstein Friesian; NAPRI: National Animal Production Research Institute; AI: Artificial insemination; ART: Assisted reproduction technologies; ET: Embryo transfer; IVF: *In-vitro* fertilization; ICSI: Intra-cytoplasmic sperm injection; GIFT: Gamete intra-fallopian transfer

Introduction

Reports have shown that, the rapid means of improving milk, beef production and reproductive efficiency is to combine the adaptability and hardiness of the Bos indicus with the genetically high reproductive and milk yield potentials of the Bos taurus through cross-breeding .To utilize the genetic advantage of the cross-breeding, many decades ago, Nigeria imported several Holstein Friesian (HF) sires for cross-breeding with the local breeds, especially Bunaji (White Fulani) cows. This effort resulted in a considerable improvement in milk production .The results of studies conducted at the National Animal Production Research Institute (NAPRI), Shika, Nigeria, on the performance of Friesian-Bunaji crossbreds indicated an improvement of about 60% in milk yield of the first cross, and further increase in the level of Friesian blood resulted in an additional gain in yield, but with decreasing magnitude and marked reduction in calving interval and age at first calving. Subsequently, livestock owners and breeders begin to show high interest in the use of exotic breeds or their frozen semen to upgrade the local indigenous dairy cows. The objectives of the study are to review AI practices as a rapid means of improving milk, beef production and reproductive efficiency in tropical Africa. It is also to showcase the place of AI in livestock industry with the aim of encouraging farmers to adopt the technology for better livestock production and food sufficiency.

Artificial insemination (AI)

According to DeForest [1] and Blacksburg [2] artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female animal by a method other than natural mating. AI is one of the technologies usually referred to

as assisted reproduction technologies (ART), in which, offspring are produced by enabling the meeting of gametes (spermatozoa and oocytes). Other techniques encompassed by ART include the following: in-vitro fertilization (IVF) where fertilization takes place outside the body; intra-cytoplasmic sperm injection (ICSI) which is a single spermatozoon caught and injected into an oocyte; embryo transfer (ET) where embryos that have been derived either in-vivo or in-vitro are transferred to a recipient female to establish a pregnancy; gamete intra-fallopian transfer (GIFT) where spermatozoa are injected into the oviduct to be close to the site of fertilization in-vivo; and cryo-preservation where spermatozoa or embryos, or occasionally oocytes are cryo-preserved in liquid nitrogen for use at a later stage.

Semen collection

According to Nafarnda [3] in most domestic animals, semen is collected by means of an artificial vagina, for example, from a bull, ram or stallion, after allowing the male to mount either an estrous female or a phantom. The artificial vagina comprises of a lubricated liner which is inserted into an outer jacket, between the two spaces filled with warm water. The pressure inside the artificial vagina is increased by addition of air. The ejaculate is collected and deposited into an insulated vessel attached to the end of the liner. But boar and dog semen are usually collected by manual stimulation. In some species such as dogs and marmoset monkeys that are adapted and can be easily handled, it is possible to collect semen by palpation or through vaginal washing after natural mating. However, in this situation, the spermatozoa have been exposed which may be dangerous to sperm survival. Human males can usually supply a sample by masturbation, except in the case of spinal injury when electro-ejaculation may be necessary. Some other primates can also be trained to supply semen samples on request in the same manner like human beings. In non-domestic species, electro-ejaculation is the only possible means of obtaining semen samples. The problem with electro-ejaculation is that, the secretions of the accessory glands may not be present in the usual proportions, which may have a detrimental effect on sperm survival.

Semen constituents

Semen consists of spermatozoa contained in a watery fluid known as seminal plasma that represents the combined secretions of the different accessory glands, such as the seminal vesicles, bulbourethral gland and prostate. The contributions of these different glands vary between species and environmental conditions. In some species, such as most primates, the semen coagulates immediately after ejaculation and then liquefies over a period of approximately 30 minutes. In most other species, the ejaculate remains liquid, the exception being in camels where the seminal plasma is highly viscous and does not liquefy readily in vitro. The addition of enzymes has been suggested as a means of liquefying primate or camel semen. However, all the enzymes tested thus far (collagenase, fibrinolysin, hyaluronidase and trypsin) have been seen to cause acrosomal damage in spermatozoa and are contraindicated if the spermatozoa are to be used for AI. Recent advances have shown that, camel semen extended 1:1 volume to volume, will liquefy in 60-90min at 37 °C [4].

Seminal plasma contains an energy source (fructose), proteins and various ions such as calcium, magnesium, zinc and bicarbonate. Seminal plasma not only activates the spermatozoa, which have been maintained in a quiescent state in the epididymis, but also functions as a transport medium to convey the spermatozoa into the female reproductive tract and to stimulate her to allow spermatozoa to swim to the site of fertilization. It has been suggested that, seminal plasma, at least in horses, is also a modulator of sperm-induced inflammation, which is thought to play an important role in sperm elimination from the female reproductive tract [5]. Various types of protein in the seminal plasma, such as spermadhesins and the so-called CRISP (cysteine-rich secretory proteins) are thought to be associated with sperm viability. It may that, these protein types bind to spermatozoa immediately, setting in motion a series of intracellular events through a second-messenger pathway. In some species, small membrane-bound vesicles have, also, been identified in seminal plasma, seemingly beginning from distinct accessory glands in various species. These vesicles, variously named prostasomes, vesiculosomes, or epididysomes depending on their origin, fuse with the sperm outer membrane, increasing motility and possibly being involved in sperm capacitation and acquisition of fertilizing ability. However, their exact mechanism of action is yet to be elucidated.

Seminal factors promote sperm survival in the female reproductive tract, modulate the female immune response, tolerate the concept us, and to condition the uterine environment for embryo development and the endometrium for implantation [6]. The action in the endometrium is through the activation of macrophages and granulocytes, and also dendritic re-modelling that improves endometrial receptivity to the implanting embryo. The cytokine release has embryotrophic traits and may also influence tissues outside the reproductive tract. Contact to semen induces cytokine activation into the uterine luminal fluid and epithelial glycocalyx lining the luminal space. These cytokines act together with the developing embryo as it traverses the oviduct and uterus preceding implantation. Several cytokines are thought to be involved, for example, granulocyte-macrophage colony stimulating factor (GM-CSF), a principle cytokine in the post-mating inflammatory response, which targets the pre-implantation embryo to promote blastocyst formation, thereby increasing the number of viable blastomeres by inhibiting apoptosis and facilitating glucose uptake [7]. According to Robertson, Mayerhofer, and Seamark [8]. Gutsche, Wolff, von Strowitzki, and Thaler [9] interleukin-6 (IL-6) and leukocyte inhibitory factor (LIF) are similarly induced after exposure to semen.

Clinical studies in humans showed acute and cumulative benefits of exposure to seminal fluid and, also, a partner-specific route of action. According to studies by Bellinge et

al. [10] and Tremellen et al. [11] live-birth rates in couples undergoing fertility treatments are improved if women engaged in intercourse close to embryo transfer. Research has also shown that, the use of seminal plasma pessaries by women suffering from recurrent spontaneous abortion is reported to improve pregnancy success. Partner-specificity of the response is suggested by increased rates of pre-eclampsia in pregnancies from donor oocytes or semen, when prior exposure to the donor sperm or concepts antigens has not occurred [12].

Processing of semen

Although seminal plasma plays an important role in the activation of spermatozoa and in the female reproductive tract, it is dangerous to long-term sperm survival outside the body. Under physiological conditions, sperm cells are activated by seminal plasma at ejaculation and then swim away from the site of semen deposition in the female reproductive tract. It is only during in-vitro storage that, sperm cells become in contact with seminal plasma long-term. Thus, it is customary to add a semen extender to the semen, to dilute toxic elements in seminal plasma, to provide nutrients for the spermatozoa during in-vitro storage and to buffer their metabolic by-products. The addition of extender, also, permits the semen to be divided into several semen doses, each containing a specific number of spermatozoa that has been determined to be optimal for good fertility in inseminated females [3].

Preservation of semen

Semen is used either immediately after collection (fresh) for example in turkeys, human beings; after storage at a reduced temperature (stored) for example in horses, pigs, dogs; or after freezing and thawing (cryo-preservation) for example in bulls [1-3].

Fresh semen: In contrast to animal species, human semen is not extended prior to processing and is not usually kept for more than a few hours before use. Poultry semen cannot be extended for too long as is done with other species since the sperm cells are adversely affected by increased dilution. Goat semen cannot be kept at 37 °C because an enzymatic component of the bulbo-urethral gland secretion hydrolyses milk triglycerides into free fatty acids, which adversely affects the motility and membrane integrity of buck spermatozoa [13]. For liquid preservation, goat semen can be stored at the temperature of 4 °C, although viability is retained for only 12-24 hours. The extension rate used for stallion varies among countries as 1:2, 1:3 or even 1:4 (v/v) semen extenders. The normal practice in some countries is to have 500 million or one billion progressively motile spermatozoa for fresh or cooled semen doses respectively. Boar semen doses contain three billion progressively motile sperm cells.

Stored semen: Storing of extended semen at reduced temperature helps to extend sperm life by slowing their metabolism as well as by inhibiting bacterial growth. Bacteria grows by utilizing the nutrients in semen extenders, thus competing with spermatozoa for these limited resources, and release metabolic by-products, thus creating an environment that is not conducive to maintaining viable spermatozoa. Furthermore, as bacteria die, they may release endo-toxins that are toxic to sperm cells. Nevertheless, cooled stored semen is the common method used for breeding horses and pigs, enabling the semen dose to be transported to different locations for insemination. Stallion semen is normally stored at the temperature approximately 6 °C while boar semen is stored between 16 and 18 °C. Most boar semen doses are sold and served as cooled doses. In contrast, some stallions produce sperm cells that do not tolerate cooling, rapidly losing progressive motility. In such cases, the only option currently is to use fresh semen doses for AI immediately after semen collection, although a new method of processing, centrifugation through a single layer of colloid, has been shown to solve the problem discussed [3].

Cryo-preservation: Semen is most useful for AI if it can be cryo-preserved, since this method of preservation ideally enables the semen to be stored for an unlimited period without loss of quality until needed for AI. Since the frozen semen does not deteriorate in viability, it can be examined until the male has been shown to be free from disease at the time of semen collection. However, the sperm cells of various species differ in their ability to survive cryo-preservation. Ruminant sperm cells survive well, whereas poultry sperm cells do not, with less than 2% retaining their viability on thawing [14]. For farm animal breeding, the cost of cryo-preservation and the likelihood of a successful outcome following AI must be considered when deciding whether to use fresh, cooled or frozen sperm doses.

The spermatozoa are mixed with a protective solution containing lipoproteins, sugars and a cryo-protectant, such as glycerol. These constituents assist to preserve membrane reliability during the processes of cooling and re-warming. However, sperm motility must also be maintained, so that the thawed sperm cells can reach the oocytes after insemination and fertilize them. In most species, the seminal plasma is removed by centrifugation before mixing with the cryo-extender, for example, stallion, boar, goat and human semen. The extended semen is packed in straws before plunging into liquid nitrogen for long-term storage. There is still considerable variation in the success of sperm cryo-preservation between different species, despite intensive research into the constituents of cryo-extenders and the rates of cooling and re-warming. Human spermatozoa can be frozen relatively successfully using commercially available cryo-extenders and programmable freezing machines. As previously mentioned, the ability of cryo-preserved spermatozoa to retain their fertilizing ability varies widely between species. New cryo-extenders and new protocols are being developed constantly in an effort to address this issue. One recent advance has been the introduction of dimethylsulphoxide and the amides formamide and dimethylformamide as cryo-protectants, in place of glycerol. These molecules seem to function better than glycerol for some individuals whose sperm cells do not freeze well, for example, some stallions. The clarification with this observation is that,

these molecules are smaller than glycerol and, therefore, may cause less damage when they penetrate the sperm membrane. However, no technique appears to be universally successful within one specie. As far as turkey spermatozoa are concerned, it seems that, the development of a successful freezing method will require more than new cryo-protectants and additives [15].

Semen evaluation

When choosing a male for breeding, especially for AI, it is imperative to assess its potential fertility by undertaking clinical and laboratory examinations. The in-vitro semen evaluation, complementary to the clinical examination, is of high diagnostic value for assessing testicular and epididymal function, and/or the genital tract of the male, allowing elimination of clear-cut cases of infertility or potential sub-fertility [16-18]. Likewise, the degree of normality of the semen before being processed for AI can be analyzed. The semen analysis routinely includes an immediate assessment of volume, appearance such as color, contamination, sperm concentration and motility, as well as later determination of sperm morphology and the presence of foreign cells. Once screened for normality, ejaculates preserved for AI are assessed for sperm concentration and sperm motility. These are the parameters most often used to determine sperm viability in post-thaw semen samples as well as to estimate breeding potential of a sire under field conditions [16,17,19]. Unfortunately, neither a simple semen analysis nor the routine evaluation post-thaw enables the determination a priori of the potential fertility level that the analyzed semen will reach, particularly after AI. The usefulness of these parameters to measure fertility of a semen sample accurately is controversial [20] and correlations between sperm motility and fertility have revealed large ranges of variation [21-24]. Correlations between sperm morphology and fertility have, also, been found to vary widely, and have most often been statistically non-significant when the semen of AI quality grade has been assessed [17]. Researchers have, also, used additional laboratory assays to predict accurately the fertilizing potential of a semen sample. Individual laboratory assays, which evaluate a single parameter, are not effective predictors of fertility. However, a combination of several assays may provide a better prediction of fertility [25,26]. The testing of a large number of parameters should lead to a higher accuracy because fertilization is a multi-factorial process [27]. However, most of these analyses are expensive and time-consuming and cannot be applied under field and/or commercial conditions. Sperm analysis conducted under commercial conditions leads to the detection of ejaculates of very poor quality associated with poor fertility. However, the pre-selection of the samples, the high number of sperm per dose and the high quality of the semen used in the AI programs reduces the variability, giving a low probability of detecting fertility differences associated with seminal parameters [28].

Sperm concentration

Accurate and precise determination of sperm concentration in an ejaculate is important for AI programs in order to produce uniform insemination doses containing an adequate number of sperm. A certain safety margin is often used by AI stations to ensure that, all insemination doses contain a minimal number of sperm. This, also, implies that, some insemination doses contain an excessive number of sperm and that males of high genetic value are not used efficiently. This safety margin, also, affects the average revenue per ejaculate for the AI station. The concentration of sperm in a straw is dictated by factors that affect semen quality, which are usually based on how the semen survives the freezing and thawing process. Factors include breed of bull, bull to bull variation, and the time of the year the semen is collected. Dairy semen usually freezes better than bull's semen. The average number of sperm cells/straw is 20-40 million. Proportion of sperm that endure the thawing process is between 30 and 80%, which is dependent on the factors listed above. The good AI sires will usually not release semen that has a post-thaw survival rate less than 30%. Average number of live sperm cells/insemination dose is 5-10 million. If the semen is gender selected, the straws will contain approximately 2 million sperm cells. Additionally, only 30% of the sperm survive the freezing and thawing process. Therefore, most companies that sell gender-selected sperm recommend that, it only be used on virgin estrous cycling yearling heifers [29-31].

The hemocytometer has often been referred to as the gold standard for assessing sperm numbers [29-31]. The equipment is slow, however, and multiple measurements of each sample are needed to obtain a precise result [31,32]. The use of a spectrophotometer is probably the most frequent method used by AI stations for assessment of sperm concentration [32,33]. For satisfactory results, periodic calibration of hemocytometers is necessary. The detection spectrum is inadequate for these instruments, and accurate quantification of sperm numbers in dilute or concentrated samples is challenging [29,34]. Spectrophotometers over-estimate sperm numbers in dilute semen samples and under-estimate sperm numbers in concentrated sperm samples. For individual raw ejaculates of boar semen, differences in the amount of gel particles or debris (cytoplasmic droplets, bacteria) can result in an inaccurate determination of the sperm concentration [33]. According to Evenson who reported that, electronic particle counters allow rapid determination of sperm concentration but tend to include any debris in the size range of sperm. Fluorometric measurements of the amount of DNA using DNA-specific fluorochromes have been studied by Fenton and Hansen [34,35] but this method requires stoichiometric staining of all DNA and minimal unspecific fluorescence from the extender.

Sperm motility

Most frequently, the semen quality of dairy bulls and boars in AI centers is evaluated using sperm concentration and motility in fresh and post-thaw semen for bulls. While studies by Correa et al. [23,36-38] have established a correlation between motility and field fertility, and others did not. Good progressive motility of spermatozoa is an indicator of both unimpaired metabolism

and intactness of membranes [39]. Estimation of motility has fundamental importance in daily quality control of semen. The percentage of motile sperm cells is used to calculate the required degree of dilution and to estimate the number of intact sperm cells per insemination dose. Regular motility checks of boar semen after dilution and during the holding period furnish information on the capacity for preservation of the semen of each boar and its individual peculiarities. Motility is usually assessed visually via a light microscope. It is inexpensive and quick, but accuracy depends on the subjective estimation by individuals even though, surprisingly, consistent results can be obtained [33]. Objective Computer Assisted Sperm Analysis (CASA) systems have become commercially available, but these systems are not frequently used in commercial AI-centers because of the high investment costs [40]. Encouragingly, small sampling errors and high correlations with fertility have been reported [41] but the reported procedures have to be applied to an independent data set to test their repeatability. The main problem in CASA systems is related to the standardization and optimization of the equipment and procedures [40,42]. A simple visual estimation of sperm motility remains a useful tool for routine semen assessment for research purposes and in the AI industry.

As boar spermatozoa show a higher percentage of circular movement than those from other species, except stallions, it is recommended to estimate the different forms of motility, including proportions of progressive spermatozoa [39]. Estimates undertaken using phase contrast microscopy within 20-30 min of dilution cannot be integrated easily into the production processes. Stored semen should be examined regularly and motility values above 60% should be considered satisfactory [39].

Sperm morphology

Morphological abnormalities of sperm can have a detrimental impact upon fertilization and embryonic development [18,43]. Bulls and boars used for commercial AI are selected to a certain degree on the basis of a low incidence of morphologically abnormal spermatozoa, so that, statistical calculations concerning their correlation with fertility are not very informative [39,42], although some evidence for a relationship between sperm morphology and fertility in bulls has been presented [44,45]. A complete morphological examination is recommended when bulls and boars are introduced into the AI station and during subsequent regular routine examinations [39,45]. Principles for determining sample size for morphological assessment of spermatozoa were extensively discussed by Kuster, Singer and Althouse [46]. The percentage of cytoplasmic droplets in boar ejaculates used for AI should not exceed 15%, especially when stored semen is used. In addition to the incidence of cytoplasmic droplets, the percentage of other morphological alterations should not exceed 20% [39].

A number of classification systems exist for morphological abnormalities of sperm, including primary and secondary defects, which classify sperm abnormalities on the basis of their presumptive origin [47] Major and minor defects-a revised system where sperm defects are classified in terms of their perceived adverse effects upon male fertility [48]; Compensable and uncompensable semen traits according to a theoretical increase in numbers of functionally competent sperm that will or will not solve the problem [18,49,50]. A compensable defect is one where the defective spermatozoa either do not reach the site of fertilization or fails to initiate the fertilization process. Defects that lead to failed fertilization or early pregnancy loss are termed uncompensable.

The insemination dose

The number of sperm in the insemination dose is an important factor affecting the probability that a female will become pregnant after AI, and in litter-bearing animals, also, the litter size [51]. To maximize pregnancy rate, the number of sperm in a dose is intentionally set high, but this management approach tends to obscure differences among males that might impact outcome of breeding when fewer sperm are used [33,52-56]. Certain males achieve maximum fertility after AI with very few motile sperm (1 million for cattle), whereas for other males 20-30x more motile sperm are required to maximize fertility [51,57,58]. At high sperm numbers per AI dose, individual bulls differ in their maximal NR%. That is unrelated to the rate at which they approach this maximum [49,58]. Vice versa, sub-fertile bulls could not be restored to normal fertility by increasing numbers of sperm per insemination. Data for cattle are most comprehensive, but it would be erroneous to assume that this principle, which results from so called "compensable defects" of sperm [50], is not operational in other species. Actually, it has been stated by several authors that, insemination trials with reduced sperm numbers are needed to reveal sub-fertile males and/or to detect differences between males [33,33,52,56,59,60].

From the perspective of validating a diagnostic assay, the use of an excessive number of sperm when measuring fertility increases the probability that, the compensable defects in sperm will be masked [33,54]. A compensable defect is one in which low fertility can be overcome, at least in part, by increasing the number of sperm in the AI dose [12,58,50]. Low fertility caused by an uncompensable defect persists regardless of the number of sperm per insemination. Hence, with a compensable defect of sperm, the "problem" causing low fertility results from the failure of sperm characteristics being expressed before sperm enters the oocyte. An uncompensable defect involves an attribute (s) being expressed only after a spermatozoon enters an ovum [54]. When a spermatozoon with an uncompensable defect fertilizes an oocyte, it is unable to complete the fertilization process or sustain embryonal development, so pregnancy may not be detected.

There is an increasing interest among AI/breeding organizations to decrease the number of spermatozoa per straw to be used for AI, which may be related to economic revenues and the expected increased use of sex-sorted semen in bulls. It is generally accepted that, a total of 15 x 106 spermatozoa

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