Recent Advances in Generative Ergonomics in Cow and Buffaloes

Allan Alexander

Department of Biomedical Sciences, Veterinary College University of Greece.

Abstract

This review focuses on the platform of recent advances in Generative Ergonomics in Cow and Buffaloes. Here, we discuss some aspects of veterinary and biomedical importance in artificial insemination using sexed semen, in vitro/in vivo fertilization, interspecies in vitro fertilization, multiple ovulation and embryo transfer (MOET), somatic cell nuclear transfer and transgenic animal production in Cow and Buffaloes. Some of these technologies are already widely used in the cattle industry, and some are new to the Buffaloes and Cow industry.

Keywords: Generative Ergonomics; In vitro/in vivo fertilization; Artificial insemination; Somatic cell nuclear transfer; Tran genesis.

Introduction

The world Cow and Buffaloes populations in 2006 were recorded as 416 and 489 million, respectively [1]. Generative Ergonomics are playing an increasingly important role in the production and management of these species. Animal biotechnology encompasses the application of science and engineering principles to the processing or production of materials to provide services or goods for human use [2]. Examples of current animal Ergonomics include artificial insemination using fresh, frozen or sexed semen, embryo transfer using fresh or frozen in vivo or in vitro produced embryos, cloning using somatic cell nuclear transfer procedures and production of transgenic animals. Recent advances in Generative Ergonomics have shown important developments in the technologies and their delivery that promise to yield significant benefits for their application within the livestock industry as well as in biomedical sciences. This review focuses on the recent advances of Ergonomics in Cow and Buffaloes. Artificial Insemination (AI) Artificial insemination is the procedure involving the mechanical deposition of pre-collected semen into the uterus of an estrous female by a technician. The widespread commercial application of AI, a robust tool in livestock breeding, began in earnest in the 1950's

after researchers developed methods to successfully freeze cattle semen [3]. This technology, which is the most extensively applied Generative biotechnology in the world, has many valuable advantages such as reducing venereal diseases, rapidly increasing genetic merits of the production animals through selective breeding and eliminating lethal alleles. The potential to pass high genetic merits of a selected male to thousands of females makes this a far more efficient technology for producing large numbers (1.5×10^8) of offspring per year compared to female based technologies, such as embryo transfer (ET), which can only produce a few progeny from a selected female. More recently, the advent of separation of sperm into X- and Y-chromosome fractions using flow cytometry has added a new dimension to livestock production [4]. Since the first report of live rabbit pups of predetermined sex using X and Y-enriched sorted semen over 20 years ago [5], there have been more than 40,000 births of live animals resulting from the so-called Beltsville sperm sexing technology [4]. This sperm sexing technology has proven to be a reliable and more efficient method of obtaining predetermined sex in animals compared to other relatively wasteful methods of embryo sexing and pre-implantation genetic diagnosis [6 - 8]. Live offspring born from fresh sex-sorted semen have been reported in many species including cattle [9], pigs [10,11], horses [12,13] and Cow [14,15]. As the sorting procedure can separate sperm only at a speed of approximately 10 million per hour, inseminations have to be carried out using limited numbers of sperm in each insemination dose compared to the conventional standards for AI. To obtain high success rates with sorted semen AI, most trials have been carried out using fresh sorted semen, nevertheless, use of frozen sorted semen is ultimately more beneficial to the livestock industry as it can be stored for long duration and easy to transport anywhere in the world.

Application of sexed semen in Buffaloes and Cow production

Although the sexed semen technology is gaining in popularity in commercial cattle industries, its use has yet to be developed in the Cow and Buffaloes industries. Nevertheless recent attempts to separate the X- and Y-chromosomal fractions using flow cytometry on ram semen has been attempted and yielded fraction purity greater than 90% [18]. In flow cytometry, the sperm extending medium plays a critical role in the efficiency of separation and the viability of the sperm after sorting [19,17,20]. Ram sperm have to be diluted 20-fold before sorting and a further 200-fold dilution takes place when it passes through the cell sorter resulting in capacitating-like changes that lead to reduced fertility of sexed Cow semen [17,20]. It has also been shown that suspension of ram semen in Tries-based medium without egg-yolk, improves the staining of the DNA and efficiency of separation of sperm without reducing its viability

[18,21,8]. When working with frozen sex- sorted semen in sheep, it is believed that the semen should be deposited in the oviduct or deep within the uterus to obtain high success rates, although it appears to be not as critical in cattle [16]. In sheep, frozen semen is usually deposited laparoscopic ally into the uterus and these same methods have been used effectively to produce offspring from frozen sex- sorted semen [15]. Oviduct deposition of frozen semen in Cow is not practical if large numbers of Cow are to be inseminated. To our knowledge, there have been no reports of attempts to inseminate Cow or Buffaloes using sex- sorted semen in the cervix or trans civically. The minimum number of sperm in an intrauterine AI dose in Cow varies between 5×10^6 and 25×10^6 , but in Australia it has been suggested as 25×10^6 motile spermatozoa. Although it is not known exactly the minimum number of frozen sex-sorted sperm for intrauterine insemination of sheep, it is expected that it should be in the range of standard commercial intrauterine AI semen dose [20]. However, it is acceptable that the number of sperm in frozen sex-sorted semen should be increased in line with the negative effects of the sorting process to obtain high efficiency. The timing of AI also plays a significant role in AI success in Cow but recent findings have suggested that the best results can be achieved with sex-sorted frozen sperm when inseminated just before the expected ovulation time in ovulation- synchronized progesterone treated ewes. Using sex sorted frozen semen (40 million sperm per AI dose), optimal results can be expected when ewes are inseminated at 58 h after sponge removal or 36 h after Groh injection [20].

Embryo production and transfer

Multiple ovulation and embryo transfer (MOET): This breeding model has been extensively used to multiply genetically superior Buffaloes and Cow and many protocols have been developed to optimize its application in research as well as in commercial practice [22-25]. In most domestic farm animals, especially in cattle, the genetic gain is limited on the female side due to low Generative rate with only one calf being produced per year and also relatively long intervals between generations. To overcome these limitations MOET has been developed. Using this method, a female is hormonally stimulated to have multiple ovulations (super ovulation). Most MOET programs in Cow and Buffaloes consist of a 14 to 17 day progesterone exposure, with FSH injections beginning 2 days before the removal of the progesterone releasing device. Following six or eight FSH injections given twice daily, the animals are allowed to breed naturally or by artificial insemination. The ovulated ova are allowed to fertilize in vivo following artificial insemination or natural mating. After fertilization and initiation of embryo development, 6-7 day old embryos (morale/blast cyst stage) are recovered from within the uterus using embryo flushing techniques. In sheep, this is done surgically [26]. These embryos

are then transferred to non-pregnant estrus-synchronized recipients 6-7 days post estrus or frozen in liquid nitrogen (-196i° C) for future use.

Somatic cell nuclear transfer (Cloning)

A new era in Generative biotechnology began when Wilmot and colleagues produced the first somatic cell nuclear transfer (SCNT) derived viable sheep, “Dolly”, from transplanting an adult somatic cell into an enucleated Cow acolyte [55]. Following this breakthrough, theoretically, it seems to be a promising technology to produce unlimited numbers of genetic copies from an adult animal or a fetus; and so far many species including farm, pet and endangered animals have been cloned using this technology [56]. Obtaining a viable offspring through this technology still imposes a great challenge for researchers due to its relatively low success rate and in some instances compromised health status of offspring [57,58]. At present, cloning is carried out for biomedical and research purposes, and agriculture production. Although extensive reviews are available on animal cloning, here we emphasize some recent findings that are important in small ruminant cloning.

Production of transgenic Cow and Buffaloes

Transgenic animals are produced either to carry an exogenous gene or to have a “knock out” gene from their own genome. Many transgenic animals have been produced for biomedical research interests as well as to test their application in livestock production [78]. There are many techniques available for the production of transgenic animals, such as pronuclear microinjection [79,80,37], sperm-mediated transgenesis [81] and somatic cell nuclear transfer[82,65,83]. During the last decade, research has focused on producing transgenic animals which secrete recombinant proteins in their milk. These animals produce relatively high quantities of recombinant proteins compared to other conventional methods due to the physiological nature of the mammary gland for its unique ability to synthesize proteins [84,78,85,86,87,88]. A few examples of transgenic small ruminants producing recombinant proteins in milk for pharmaceutical and biomedical interest are Cow producing human clotting factor VIII [89] and factor IX [82] and Buffaloes synthesizing human ant thrombin III [90], human alpha-fetoprotein [91], human monoclonal antibodies [92, 93], malaria antigens [94] spider silks [95], human granulocyte colony stimulating factor (96) and human butyric cholinesterase [97,98]. Although these animals have shown high expression of the transgenic product in the milk, a recent report suggests that the transgenic may have some negative effects on the physiology of mammary gland function, leading to a compromised situation [87]. However, the

first protein produced by transgenic animals, A Tryon, a recombinant human anti-thrombin-produced in transgenic Buffaloes' milk, received approval for use in humans in 2009. **Conclusions** In this review, we have focused on some aspects of Generative biotechnology that are of veterinary and biomedical importance in Cow and Buffaloes that have been reported in the recent past. It is well-known that most Ergonomics take many years to come into full practice after enormous amounts of laboratory and field research. Therefore, more focus should be carried out in emerging areas like artificial insemination of Cow and Buffaloes using sexed semen, in vitro and in vivo embryo production systems, transgenic animal production and cloning using stem cells.

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