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ScienceDirect
Theriogenology 71 (2009) 113–122
Theriogenology
www.theriojournal.com

The importance and potential of artificial insemination in CANDES (companion animals, non-domestic, endangered species)

B.S. Durrant *

Zoological Society of San Diego, Conservation and Research for Endangered Species, 15600 San Pasqual Valley Road, Escondido, CA 92027, USA

Abstract

Artificial insemination (AI) is the least invasive assisted reproductive technology, and is therefore of great interest to breeders of companion animals, non-domestic, and endangered species (CANDES). This most fundamental artificial breeding technique circumvents physical or behavioral impediments to natural mating and provides the means for genetic exchange between populations without transfer of live animals. In addition, because oocytes grow, mature and are fertilized in vivo and embryos are not subjected to in vitro culture conditions, AI eliminates the epigenetic effects on the female gamete that are inherent in more invasive assisted reproductive technologies. Although the management of CANDES differs significantly from current livestock husbandry practices, the cattle industry is a powerful example of the potential for AI to enhance the genetic health and sustainability of animal populations. Ultimately, successful AI requires sperm of adequate quality and quantity, oocytes that have attained nuclear maturation and cytoplasmic competence, operational gamete transport systems, accurate timing, and proper placement of sperm in the female reproductive tract. Increased understanding of semen collection, evaluation and preservation techniques, estrus synchronization and superovulation, estrus and ovulation detection, and insemination instrumentation is needed for each CANDES before AI success rates will approach those of the livestock industry. Concentrated, collaborative research in these areas must be encouraged among private breeders, universities and zoological institutions to realize the full potential of AI in the management of CANDES.

Keywords: Assisted reproduction; Companion animals; Endangered species

1. Introduction

Stimulation of natural breeding in captivity is a goal to which all zoological institutions and managers of CANDES aspire. Productive mating represents the combined efforts of animal care personnel, nutritionists, veterinarians, behaviorists, physiologists, and enclosure designers, and is a reflection of the animals' overall health and well-being. Although assisted reproductive technology (ART) may be necessary to ensure the genetic contribution of valuable animals or to equalize founder effects, reliance on artificial reproduction is not desirable nor practical. Complex ART such as in vitro fertilization and embryo transfer often require extensive hormone treatments and handling of female animals, including anesthetic and surgical procedures. These manipulations incur some risk to the health and future fertility of the animal. In addition, the specialized equipment and personnel needed to perform complex ART procedures is beyond the budgetary means of

* Tel.: +1 760 291 5436; fax: +1 760 291 5428.
E-mail address: bdurrant@sandiegozoo.org.

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doi:10.1016/j.theriogenology.2008.09.004

many CANDES institutions. Despite the potential of ART to enhance captive propagation efforts, in its current state of development, it is rarely, if ever, as successful as natural breeding.

However, when all reasonable attempts to stimulate natural breeding are unsuccessful, artificial reproduction becomes necessary. It is most appropriate that this IETS CANDES symposium addresses the first and most fundamental assisted reproductive technique of artificial insemination (AI). As the least complex, invasive and expensive of the ART techniques, AI has the smallest negative impact on the reproductive process and is therefore the logical first choice for CANDES. Offspring have been produced in virtually all companion animal species through AI, but the technique has been successful in a small number of non-domestic and endangered species. Thus, the considerable potential of AI for the genetic management of small populations has not yet been fully realized [1,2].

2. History of AI

Many authors cite accounts of sixteenth century Arab chieftains stealing semen from the recently mated mares of their enemies to enhance the performance of their own equine stock. However, the first verifiable AI is credited to Lazzaro Spallanzani in the early 1780s [3], who studied mating and fertilization in frogs before successfully inseminating a domestic bitch. By the late 1790s, the first human AI was successful [4]. Within the next century, AI had been performed in domestic farm animals, dogs, foxes, rabbits and poultry [5,6]. Artificial insemination in domestic animals began in 1912 in Japan [7] and a bovine AI cooperative was organized in Denmark in the 1930s, quickly spreading across Europe and to the United States [8]. Semen collection was facilitated by the development of the artificial vagina (AV) in 1914 (see [9]) and the electroejaculator in 1936 [10]. Semen evaluation and preservation soon followed, and superovulation was introduced in cattle in 1951 [11]. The history and current use of AI in CANDES is reviewed by Wildt and Wemmer [2] and will be discussed in detail for specific taxa in this issue.

3. Theory and practice of AI

By definition, AI comprises the collection of sperm and its introduction into the reproductive tract of a female. It is a powerful tool for increasing the number of offspring produced by a single male, and for the transfer of genes between populations. In its simplest form, insemination of a naturally cycling, spontaneously ovulating female, AI does not disturb or modify normal folliculogenesis, ovulation, luteinization, or the hormonal milieu surrounding these processes. Sperm must negotiate and interact with at least a portion of the female reproductive tract, promoting capacitation and normal acrosome responses to egg vestments. Oocytes must travel to the oviduct, which has evolved to produce the perfect culture medium for fertilization and the early cleavage stages of the developing embryo. Conception occurs only if sufficient numbers of viable sperm meet mature, competent ova. Movement into the uterus and eventual implantation is co-directed by the mother and offspring as each responds to the chemical signals of the other [12]. Pregnancy loss after AI can be attributed to genetic defects in the embryo and/or anatomical, nutritional or physiological inadequacies of the dam, rather than epigenetic factors caused by exposure of oocytes or embryos to inappropriate *in vitro* conditions.

Artificial insemination is the first choice for assisted reproduction in CANDES because it is relatively non-invasive, requiring as few as a single chemical or physical restraint of the male and female. Separation of the sexes for AI eliminates exposure to disease and traumatic injury, and is most useful for aged, infirm or behaviorally incompetent males. Paternity identification is assured if the semen of a single male is inseminated and accurate records are maintained. Despite the myriad advantages of AI compared to more complex ART, its utility is limited for increasing female fecundity, unless it is preceded by superovulation and followed by embryo transfer. In addition, oocyte quality cannot be assessed *in vivo*, eliminating one avenue of diagnosing fertilization failure. Because greater numbers of normal, motile sperm are needed for AI than for IVF or ICSI, males producing ejaculates of poor quality may not be viable AI donors.

4. Semen collection

Obtaining a sufficient quantity of viable sperm is the essential first step in the AI process. Although many companion and domestic animals may be readily trained for manual or AV collection, non-domestic and endangered species usually require more invasive methods. A notable exception is the cheetah (*Acinonyx jubatus*), the most naturally non-aggressive of the big cats. The trainers of a 2-y-old hand-reared male cheetah at the San Diego Wild Animal Park reported the animal's sexual interest in his enclosure enrichment items. Shaping this behavior for semen collection was accomplished in a number of approximation steps using positive food reward. When heated to 40 °C, a specially