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Artificial insemination in marsupials

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Abstract

Assisted breeding technology (ART), including artificial insemination (AI), has the potential to advance the conservation and welfare of marsupials. Many of the challenges facing AI and ART for marsupials are shared with other wild species. However, the marsupial mode of reproduction and development also poses unique challenges and opportunities. For the vast majority of marsupials, there is a dearth of knowledge regarding basic reproductive biology to guide an AI strategy. For threatened or endangered species, only the most basic reproductive information is available in most cases, if at all. Artificial insemination has been used to produce viable young in two marsupial species, the koala and tammar wallaby. However, in these species the timing of ovulation can be predicted with considerably more confidence than in any other marsupial. In a limited number of other marsupials, such precise timing of ovulation has only been achieved using hormonal treatment leading to conception but not live young. A unique marsupial ART strategy which has been shown to have promise is cross-fostering; the transfer of pouch young of a threatened species to the pouches of foster mothers of a common related species as a means to increase productivity. For the foreseeable future, except for a few highly iconic or well studied species, there is unlikely to be sufficient reproductive information on which to base AI. However, if more generic approaches can be developed; such as ICSI (to generate embryos) and female synchronization (to provide oocyte donors or embryo recipients), then the prospects for broader application of AI/ART to marsupials are promising.

Keywords: Marsupial, Assisted reproduction, Artificial insemination, Conservation, Estrous synchronization, Cryopreservation, Cross fostering, Pouch young

1. Introduction

Marsupials include some of the most highly threatened species in Australia and highly iconic species in great demand by zoos. The development of assisted breeding technology (ART) and artificial insemination (AI) has the potential to advance the conservation and welfare of marsupials. Marsupial semen collection, handling and basic properties were the subject of a paper [1] at the 1977 Symposium of the Zoological Society of London, which led to Paul Watson's landmark book 'Artificial Breeding of Non-Domestic Animals' [2]. However, despite the three decades that have passed AI, and ART generally, have been demonstrated to have the potential to make a significant impact on conservation

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and reproductive management of only one marsupial species, the koala (*Phascolarctos cinereus*; [3,4]). In the koala, 32 pouch young have been produced and birth rates following AI using extended and chilled semen are only slightly below those achieved by natural mating [3]. Furthermore, AI technology has now been incorporated in state government koala management policy [5]. In only one other marsupial species, the tammar wallaby, *Macropus eugenii*, have live births been achieved after AI [6]. Hormone-induced superovulation and AI have been successfully used as a basic research tool, and in development of fertility control vaccines for the tammar wallaby and common brushtail possum, *Trichosurus vulpecula* using fresh [7], and in the case of brushtail possum, frozen-thawed sperm [8]. However, in these studies, fertilisation or cleaving embryos, not full term development, has been the endpoint [9–12], and these embryos failed to develop beyond the unilaminar blastocyst stage in the hormonally manipulated females [13]. Other than these three species, the literature on AI or conventional ART for marsupials is very limited.

The koala and tammar wallaby studies have thus far only involved zoo-based or captive colony bred animals. The captive husbandry of the koala is well established [14], and the reproductive biology of the tammar wallaby is one of the best studied for any marsupial (for review see [15]). Arguably the koala and tammar wallaby are special cases, because the timing of ovulation can be predicted with considerably more confidence than in any other marsupial. The koala is an induced ovulator and techniques to artificially induce ovulation have been successfully developed [3,4,14,16–18] and the tammar has a highly predictable post-partum estrus, which can be tightly synchronized by removing pouch young, and a well-defined window of ovulation [19–21]. In a limited number of other marsupials, such precise timing of ovulation has only been achieved using hormonal treatment, which is likely to have undesirable effects.

Many of the challenges facing AI and ART for marsupial conservation or animal welfare outcomes are shared with other wild species; nevertheless, marsupials also offer unique challenges and opportunities. For the vast majority of marsupials, there is a dearth of knowledge regarding female reproductive anatomy, the endocrinology of estrus, and the dynamics of sperm transport and ovulation to guide an AI strategy. For threatened or endangered species, only the most basic reproductive information is available in most cases, if at all. This highlights the importance of developing new techniques on closely related non-endangered analogue species [22]. However, there is a unique marsupial ART strategy which has been shown to have considerable promise; this is the transfer of pouch young of threatened species to the pouches of foster mothers of a common species as a means to increase productivity [23]. Recently, cross fostering has been adopted by some wildlife agencies and zoos in Australia as tools for the conservation and management, of captive and wild macropodoid marsupials (e.g. [24]).

2. Current status of knowledge

2.1. Collection and handling of semen

Semen collection by electroejaculation (EEJ) has been established in several macropodids (tammar wallaby, yellow-footed rock wallaby, Matchie's tree kangaroo, eastern and western grey kangaroos), plus the common wombat, southern-hairy-nosed wombat, koala and common brushtail and ring-tailed possum [25–31]. Electroejaculation has allowed for the assessment of seasonal changes in semen quality observed in at least three marsupials: the tammar wallaby [32], southern hairy-nosed wombat [33], and koala (Allen et al., unpublished). Semen collection by electroejaculation has also recently become possible in the grey short-tailed opossum (*Monodelphis domestica*; Paris et al., unpublished) but is still sub-optimal.

The electroejaculation of dasyurid marsupials (Australian carnivores and insectivores) appears particularly problematic. For example, there have been a number of attempts to electroejaculate the largest of all dasyurids, the Tasmanian devil (*Sarcophilus harrisi*) [22]; although spermatozoa have been collected, the ejaculate was of limited volume or contaminated with urine and the sperm concentration low (Johnston and Blyde, unpublished observations). Semen collection by electroejaculation in the majority of dasyurids is likely to be even more difficult, given their small size (20–50 g) and as many have a highly acute seasonal production of spermatozoa. However, small diameter rectal probes have been constructed and used with partial success in other small marsupials (e.g. 100 g grey short-tailed opossum; Paris et al., unpublished) and 60 g phascogale (Taggart, unpublished observation) and may be applicable to small dasyurids. Conversely, natural breeding is well established for several small dasyurids in captivity (reviewed in [34]) and thus AI may not be a high priority for this group.

However, radiographic examination of the male tracts of animals infused with radiopaque dye into the vas deferens has been a useful approach to better understanding of electroejaculation in other marsupials, including the tammar wallaby [35] and such an approach