General and Comparative Endocrinology 176 (2012) 182–191

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

The reproductive cycle of the Tasmanian devil (*Sarcophilus harrisii*) and factors associated with reproductive success in captivity

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ARTICLE INFO

Article history:
Received 14 March 2011
Revised 13 January 2012
Accepted 13 January 2012
Available online 28 January 2012

Keywords:
Non-invasive monitoring
Pregnancy
Captive breeding
Dasyurid

ABSTRACT

Numbers of wild Tasmanian devils are declining as a result of the fatal, transmissible Devil Facial Tumor Disease. A captive insurance population program has been initiated but current captive breeding rates are sub-optimal and therefore the goal of this project was to increase our understanding of the estrous cycle of the devil and elucidate potential causes of failed male–female pairings. Temporal patterns of fecal progestagen and corticosterone metabolite concentrations were examined for females ($n = 41$) in three categories of reproductive status (successful: viable young, $n = 20$ estrous cycles; unsuccessful: paired with a male but no young confirmed, $n = 44$ estrous cycles; non-mated: no access to a male during estrus, $n = 8$ estrous cycles) but substantial differences were not found. Females were more likely to produce pouch young if pairing with the male extended into late proestrus ($P < 0.05$), thereby decreasing the time between pairing and presumed ovulation. The interval between the end of proestrous elevation in progestagen metabolite concentrations and the beginning of the luteal phase was 7.6 ± 2.3 days in successful females. The length of the luteal phase in successful females was 12.5 ± 1.4 days which was not different from unsuccessful or non-mated females ($P > 0.05$). Unsuccessful females had 1–3 estrous cycles within a single year. Successful females were predominantly wild-caught (17/19, 90%) and most produced young following the first estrous cycle of the season (18/20, 90%). Unsuccessful females were predominantly captive born (20/27, 74%) in this study. It is possible that a proportion of females that do not produce pouch young achieve conception but the timing of reproductive failure continues to be elusive in this species.

1. Introduction

The Zoo and Aquarium Association (ZAA) of Australia has initiated a partnership with the Tasmanian Department of Primary Industries, Parks, Water and the Environment (DPIPWE) in conjunction with the Save the Tasmanian Devil (STTD) program, to develop an insurance population of captive Tasmanian devils as a buffer against the rapid decline of the wild populations [2]. The wild population of devils has declined by more than 70% since the first sighting of the fatal, contagious cancer Devil Facial Tumor Disease (DFTD) [13]. Wild devils have been captured from the west coast of Tasmania, the only disease-free area left, and transferred to mainland institutions over several years (2006–2008) to become founders for the current captive breeding population [2]. Population models developed during a recent strategic planning workshop [2] indicate that to achieve long-term goals of gene diversity retention and demographic stability, the DPIPWE–ZAA insurance population needs a minimum base of 150 founders and a minimum effective population size of 500. To retain 95% of genetic diversity for 50 years (minimum recommended for the Tasmanian devil), the captive breeding program must achieve a female reproductive rate of at least 57% in the 2 and 3 year-old age-classes and at least 35% in the 4 year-old age-class with an additional 4–8 founders added every 3 years [2]. Further, increasing reproductive rates to around 67% and 40% for the 2–3 year-old and 4 year-old age class, respectively, would significantly increase captive numbers for future reintroduction to wild habitats [2].

Our understanding of female devil reproduction has increased slowly through research spanning the last 110 years. Early studies into dasyurid reproduction used histological examination to evaluate the reproductive anatomy and cycle of the female [6,18,32,33]. Much like other marsupials, the female devil reproductive tract is characterized by bilateral uteri, attached to the vaginal complex by extended uterine necks [6,34]. The uterine necks empty into the anterior vaginal canal, which in the devil are separated by a fully formed septum [6,34]. The urethra and bilateral vaginas both

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doi:10.1016/j.ygcen.2012.01.011

empty into an extended urogenital system (UGS) which ends at a cloacal opening [6,34]. Devils may produce and ovulate an excess of oocytes (observed range 11–114) compared to the maximum of four young that can be supported by the dam's teats [22]. Although the fertilization rate is quite high, development of individual embryos is slightly asynchronous and less than half of the ovulated oocytes reach the expanded blastocyst stage [22]. In dasyurids, changes to the reproductive tract associated with pregnancy have been reported to occur in the absence of embryos in distended uteri [11,20,31].

Originally thought to be mono-estrous within a short breeding season peaking around March [10], recent research has confirmed that devils can undergo a second estrus if there is a failure of conception or early pouch young loss [15]. Female reproductive cycles have been documented to extend from January until June [15] but there have been accounts of successful reproduction as late as August which was originally thought to represent "out-of-phase" breeding [10].

Although estrus, courtship, mate selection and copulation have been examined in the devil (M. Jones; T. Eastley, personal communication), a detailed and systematic account of reproductive behavior has not yet been published. Brief descriptions of the estrus and mating behavior of captive devils have been made by several zoological institutions [23,40,41], and have recently been included in the development of a husbandry manual for the captive breeding program [24]. In captivity, estrus has been observed to occur in conjunction with one or more of the following signs; decreased appetite, development of a loose, fluid-filled neck roll, increased nesting behavior, lethargy and changes in pouch condition [14,23,24,40,41]. Once paired, a male may grip the loose neck roll of the receptive and submissive female with his teeth and drag or escort her around the enclosure until he finds an appropriate den in which to mate [24,40]. The male will then mate-guard and may continue to copulate with her for up to 10 days [24].

In most dasyurids, ovulation occurs several days after copulation [21,29] following a period of sperm storage [29,38,42]. For example, in the brown antechinus (*Antechinus stuartii*), optimal *in vivo* embryo production results from pairings which occur 5–13 days prior to ovulation [38]. Similarly, in the Eastern quoll (*Dasyurus viverrinus*), only developing follicles are observed 1–2 days post-mating and corpora hemorrhagica are not detected until 7–10 post-mating which coincides with initial increases in serum progesterone concentrations [21]. The marsupial luteal phase begins after ovulation and encompasses pregnancy or a non-pregnant luteal phase. In polyovulatory species such as the striped-face dunnart (*Sminthopsis macroura*) and Virginia opossum, (*Didelphis virginiana*), both ovarian (only examined in the former) and plasma progesterone increases by the first day after ovulation [12,28] most likely due to the production of this steroid by the large number of developing corpora lutea. In Tasmanian devils, implantation occurs during the final 2–2.5 days of the 13–14 day pregnancy (R. L. Hughes, personal communication). If fertilization does not occur, dasyurids will undergo a luteal phase in which changes to the ovary, uterus and mammary gland are indistinguishable from a pregnant animal [20,31,32].

The lack of basic knowledge of the reproductive endocrinology of the devil led to the recent examination of plasma and fecal steroid hormone concentrations in relation to reproductive events [15]. Fecal progesterone metabolites concentrations corresponded with changes in plasma hormone concentrations as well as reproductive events such as mating and parturition, confirming the application of non-invasive hormone monitoring as a useful tool for the devil [15]. Tasmanian devils have a bi-phasic progesterone estrous cycle with increased progesterone concentrations during proestrus and estrus (follicular phase) and the luteal phase, with low concentrations between [15]. These elevations in progesterone during estrus and pregnancy are similar to other dasyurids [1,5,21,29]. During proestrus, the oocytes mature and the female becomes receptive to males as she enters estrus. In most mammals, including the Tasmanian devil [15], the follicular or proestrous phase is characterized by elevated estrogen concentrations.

The proestrous, luteal, and intermediate (between the end of a luteal phase and the beginning of the next proestrous phase) phase lengths in non-mated captive female devils were 14.9 ± 1.5, 17.9 ± 0.3 and 33.7 ± 5.9 days respectively with an inter-estrous period of 80.3 ± 7.1 days (range 64–102 days) [15]. Frequency of mating varies between females with copulations occurring over 1–8 days (average of 3.4 days) before, during and after the proestrous rise in progesterone concentrations [15]. Luteal length, based on the interval between the rise in luteal progesterone concentrations after mating and the birth of young, was highly variable, ranging from 14 to 21 days with a mean of 16.7 ± 0.8 days [15]. Intervals from final mating to parturition also varied, ranging from 19 to 27 days [15]. That study concluded that the predominant increase in progesterone concentrations in a pregnant devil occurred during the latter half of the luteal phase and hypothesized that it was supplemented by the placenta during implantation [15]. It also inferred that the non-conceptive cycles featured an early increase in progesterone concentrations (during the first half of the luteal phase) while this early peak was absent in conceptive cycles [15]. The authors suggested that this difference could be used to confirm pregnancy in females which failed to produce viable pouch young in captivity [15].

The goal of the current study was to increase our understanding of the reproductive biology of the female Tasmanian devil to enhance captive management and breeding strategies and identify potential causes of unsuccessful pairings. To do this we examined the temporal relationship between reproductive and adrenal hormone concentrations and their correlation to signs of estrus and dates of pairing in captive devils. Changes in hormone concentrations were examined to identify differences between females of varying reproductive status. Specific objectives were to: (1) evaluate the temporal changes in fecal progesterone and corticosterone metabolite concentrations and compare endocrine profiles among successful (production of young), unsuccessful (provision of mating opportunities without production of young) and non-mated (no access to males) female devils; (2) examine the effect of the timing of mate introduction and pairing duration on reproductive success; and (3) refine our knowledge of the characteristics of the estrous cycle phases and their correlation to changes in female appetite and timing of pairings.

2. Methods

2.1. Study animals

Captive female devils (n = 41) between the ages of 2 and 4 years were used in this study over a three year time period (2008–2010). Of these, four females were examined over two breeding seasons and two females were examined over three breeding seasons. Wild caught devils (n = 20) were 1 year of age when brought into captivity as confirmed by size and teeth wear [26,35] and had been in captivity for 1–2 years at the time of study. Study animals were located at seven Australian mainland zoological institutions and individuals were studied over one to three breeding seasons (Table 1). All of the institutions included in this study have historically successfully bred Tasmanian devils. In some cases, not all of the females located at each institution were examined in each year for logistical reasons. All females were given breeding opportunities with the exception of five individuals in 2010 (non-mated females). A total of 36 individual males were provided for breeding