

## Research Article

# Restoration of Reproductive Potential After Expiration or Removal of Melengestrol Acetate Contraceptive Implants in Tigers (*Panthera tigris*)

Jason Y. Chuei,<sup>1</sup> Cheryl S. Asa,<sup>2\*</sup> Monica Hall-Woods,<sup>2</sup> Jonathon Ballou,<sup>3</sup> and Kathy Traylor-Holzer<sup>4</sup>

<sup>1</sup>Zoological Society of London, Regent's Park, London, England

<sup>2</sup>AZA Wildlife Contraception Center, St. Louis Zoo, St. Louis, Missouri

<sup>3</sup>Department of Conservation Biology, Conservation and Research Center, Smithsonian's National Zoological Park, Washington, DC

<sup>4</sup>IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota

The need for contraception in the successful management of captive wild animals is becoming increasingly apparent. Because concerns exist regarding the reversibility of the contraceptive implant melengestrol acetate (MGA), reproductive data for 94 female Amur (*Panthera tigris altaica*) and Sumatran tigers (*Panthera tigris sumatrae*) were analyzed using survival analyses to evaluate return to reproductive status after implant removal or assumed expiration. Females placed in potential breeding situations after MGA implants were surgically removed showed a 62% return to reproduction by 5.25 years, whereas females with implants that were assumed to have expired showed only a 30% return to reproduction by 6 years. Implanted females did not reproduce as successfully as non-implanted control females, which showed an 85% probability of reproducing after placement in a new breeding situation by 2.66 years. Parturition increased the probability of reproducing in non-implanted females, but not in implanted females. Litter size, stillbirths, and offspring survival were not significantly different between non-implanted, implant-removed and implant-expired female tigers. Ten female tigers reproduced both before and after implant placement, and the differences in litter size, stillbirths, and offspring survival were not significant, nor were they significantly different from non-implanted females. Prior parturition, age when implant was removed, and duration of implantation

\*Correspondence to: Cheryl Asa, PhD, AZA Wildlife Contraception Center, St. Louis Zoo, 1 Government Dr., St. Louis, MO 63110. E-mail: asa@stlzoo.org

Received 6 March 2006; Accepted 23 September 2006

DOI 10.1002/zoo.20137

Published online 8 June 2007 in Wiley InterScience (www.interscience.wiley.com).

did not affect the probability of reproducing for females after implant removal. These results show substantial reversibility of MGA implants, leading to 62% successful reproduction after implant removal. The reasons for lower successful reproduction in animals previously treated with the contraceptive compared to non-implanted females are not known, but a greater delay in reversibility was seen when implants were left in place and only presumed expired. *Zoo Biol* 26:275–288, 2007. © 2007 Wiley-Liss, Inc.

**Keywords:** contraception; captive breeding; MGA; felids

## INTRODUCTION

Although many species face declining numbers in the wild, contraception is important for the demographic and genetic management of captive wild animal populations [Cohn et al., 1996]. Population growth may be controlled via management, surgical, pharmacological and immunological methods [Asa and Porton, 2005b], but these may have consequences on social hierarchy and development and may have physiologically and pathologically adverse effects [Munson, 1995].

Melengestrol acetate, a synthetic progestin with greater biological activity than progesterone, in a slow-release implant containing 20% MGA by weight in a silastic matrix, is the most commonly used contraceptive in zoos. It has been effective in virtually all mammalian taxa in which it has been tested, and its use in tigers (*Panthera tigris*) began 30 years ago [Seal et al., 1976]. However, prolonged use in felids is associated with complications of health including uterine and mammary pathology [Munson et al., 2002], and possible effects on subsequent hormone function and reproduction, such as reversibility [Porton et al., 1990]. Reproductive delays and failures have been reported in several species, including lions (*Panthera leo*) [Seal et al., 1976]. Hence, it is recommended to only use MGA for two consecutive years, with a subsequent intervening pregnancy, and for no more than four years over the lifespan of the animal [Asa and Porton, 2005a].

The potential for successful reproduction after expiration or removal of MGA implants has previously been studied in golden lion tamarins (*Leontopithecus rosalia*) and golden-headed lion tamarins (*Leontopithecus chrysomelas*) [De Vleeschouwer et al., 2000], where concerns were raised regarding the viability of offspring, especially in females with presumed expired implants left in place. This study carried out similar analyses to investigate these potential effects on reproduction in tigers.

## MATERIALS AND METHODS

Female reproductive histories were obtained from the American Zoo and Aquarium Association (AZA) Amur tiger (*Panthera tigris altaica*) and Sumatran tiger (*Panthera tigris sumatrae*) regional studbooks [Traylor-Holzer, 2005a,b]. Breeding opportunities were assessed using studbook records and breeding recommendations made by the AZA Tiger Species Survival Plan (Tiger SSP) between 1989 and 2005 (Traylor-Holzer, unpublished data), and contraceptive histories were taken from the AZA Contraception Advisory Group (CAG) Database (Hall-Woods, unpublished data). Data for these two subspecies were combined to

maximize the power of statistical analyses, and as it was deemed that, separately, there were insufficient data. Other tiger species were eliminated from the study, as either no reproductive history was available or there had been no breeding recommendations in place for those that had previously been MGA implanted. The tigers involved in this study were housed at multiple facilities primarily in North America, with a few in Europe and Asia.

Statistical analyses were carried out in Microsoft Excel 2000 [Microsoft Corporation, 1999] and SPSS 13.0 for Windows [SPSS Inc., 2004] and are outlined below.

### **Implanted Females**

Ninety-four female tigers that had been implanted, often with multiple, successive implants (51%), for at least 4 months (mean  $\pm$  SE =  $48.94 \pm 3.55$ ) and had subsequently been placed in potential breeding situations for at least 4 months after implant removal or expiration were included in the analysis. A minimum of 4 months for implantation was chosen to allow sufficient circulating concentrations of MGA and to maximize the number of females available for analyses, and at least 4 months of potential breeding opportunity was chosen to allow familiarization with potential mates and to maximize the number of females available for analyses. Contraception database analyses have indicated that the minimum length of efficacy of the implants is 2 years, so they typically are considered expired at that time. However, MGA release rates decline with time and metabolic clearance rates have not been determined for any species, so actual length of efficacy cannot be predicted for individual animals [Asa and Porton, 2005a]. Because most users assume expiration at 2 years, we compared reproductive rates in females with implants still in place after 2 years to those of females with implants removed. Implants were removed from 24 females and were assumed to have expired in 70 females. Ten of 94 tigers reproduced both before and after implant placement.

### **Control Females**

The control group consisted of 46 non-implanted female tigers that had been placed into new breeding situations between 1992–2005 for Amur tigers and 1998–2005 for Sumatran tigers. Several females received more than one breeding recommendation, resulting in a total of 72 breeding opportunities for the control group. This control group provided baseline data on the probability of reproducing after placement in a new breeding situation, litter size, rates of stillbirth (that is, any animal that was born dead, or died on the day of its birth), and offspring survival rates to 30 days. In 31 instances (42%), the female had reproduced previously.

### **Probability of Reproducing**

The records for each implanted and control female were examined to determine whether she had produced offspring previously, age while at risk of breeding, date of parturition, and the date and reason that data collection ceased. Data collection may have ended due to death or euthanasia of the female or her mate, removal from breeding opportunity, insertion of another implant, medical reasons, and miscellaneous or unrecorded reasons. Females between 2–14 years of age, inclusive, were considered to be of reproductive age.

Because the length of time that individual females could reproduce varied, a survival analysis was adapted to describe the change in rate at which females reproduced over time after implant removal or expiration, using parturition (rather than death) as the factor of interest. Hence, the survival curves indicated the probability of reproducing by time increments after implant removal or expiration ( $t = 0$ ). Time  $t = 0$  for implant expired females was 24 months after implant placement, because implants were assumed to have expired after 2 years. The dates at which females entered the study, reproduced or left the study were established relative to  $t = 0$ . Females that left the study without reproducing were removed from the study (right censored) on the date they were no longer at risk of reproducing. Survival analysis is ideal for these data as it takes into account the amount of time females are in the study when calculating reproductive probabilities and allows for females to enter and leave the study at different times without biasing the results [Petrie and Watson, 1999].

Survival analyses were also used to determine whether there were differences in reproductive probabilities between females with implants removed and those with implants that expired, between females that had previously produced a litter and nulliparous females, and between implanted and non-implanted females. The probability of reproducing within a specified interval after implant removal or expiration was calculated using 1 minus the Kaplan-Meier survival estimator. Cox proportional hazard models and partial log-likelihood tests were used to compare reproductive probability curves for different groups (e.g., implant removed vs. expired) and to simultaneously test for the effect of independent variables (e.g., age and duration of implantation). Differences in reproductive probabilities were defined in terms of their proportional hazard ratios [Parmar and Machin, 1995].

### Litter Size, Rates of Stillbirth, and Survival Rates

Litter size, number of stillbirths per litter, and number of offspring surviving to 30 days per litter were recorded for each female implanted previously and each control female. Zoo staff often do not observe the act of parturition. However, for the purposes of this study, stillbirth represents any animal that was born dead as well as those who died on the day of their birth. Prior parturition and age of female at time of parturition were also recorded for each litter. For implanted females, the duration of implantation was recorded and only the data on litters produced after implant removal or assumed expiration were collected.

A  $t$ -test was used to compare average litter sizes between implant-removed and control females. Chi-square tests were used to compare stillbirth and offspring survival rates. The effects of female age and implant duration on survival rates were tested using log-likelihood ratio tests. A paired  $t$ -test was used to compare litter sizes in the 10 females that reproduced both before and after implantation, and a  $\chi^2$  test was used to compare offspring survival rates before and after implantation.

Table 1 shows the number of animals in the control and implanted group with implants removed and implants assumed expired and the number of females with and without previous offspring. Mean age when placed in a breeding situation and mean implantation duration are also shown.

**TABLE 1.** Number of female tigers in different categories used for the analysis, mean ( $\pm$ SE) age at implant removal, expiration or placement in new breeding situation ( $t = 0$ ), and mean time implanted (months)

Analysis group	Number implanted			
	Total	Implant removed	Implant expired	No. of controls
Total	94	24	70	72
Proven breeder	39	9	30	31
Non-proven breeder	55	15	40	41
Number producing a litter	28	12	16	57
Reproductive proven breeder	10	4	6	29
Reproductive non-proven breeder	18	8	10	28
Number not reproductive	66	12	54	15
Non-reproductive proven breeder	29	5	24	2
Non-reproductive non-proven breeder	37	7	30	13
Mean age at $t = 0 \pm$ SE (range) (months)	85.85 $\pm$ 4.64 (29.65–224.05)	84.54 $\pm$ 4.64 (29.65–188.48)	86.30 $\pm$ 4.67 (30.71–224.05)	88.85 $\pm$ 4.73 (26.20–206.30)
Mean time implanted $\pm$ SE (range) (months)	48.94 $\pm$ 3.55 (3.72–133.51)	11.46 $\pm$ 0.57 (3.72–19.86)	61.78 $\pm$ 3.15 (24.76–133.51)	–

## RESULTS

### Reversibility of Contraception

Twenty-eight (30%) of the 94 implanted females produced young after implants were removed or expired. However, as females were exposed to a breeding situation for varying lengths of time, it is more appropriate to present the results as the probability of reproducing relative to time after implant removal or expiration (Fig. 1). The probability of reproducing reached a plateau of 38% by 6 years after implant removal or expiration.

The probability of reproducing for females with implants that were removed ( $n = 24$ ) and for females with implants that were assumed to have expired ( $n = 70$ ) differed significantly (proportional hazard ratio = 3.654,  $df = 1$ ,  $P = 0.001$ ). The proportional hazard ratio indicates that the implant-removed females reproduced at almost four times the rate as implant-expired females. Females placed in breeding situations, and that reproduced, did so in a range from 6.44–44.09 months after implant removal. The probability of reproducing after implant removal was 62% by 5.25 years (Fig. 2). Females placed in breeding situations after assumed expiration of implant reproduced in a range from 24.76–103.23 months after placement, that is, 0.76–79.23 months after assumed expiration. The probability of reproducing by 5.25 years after assumed implant expiration was 24%, eventually reaching 30% by 6 years (Fig. 2).

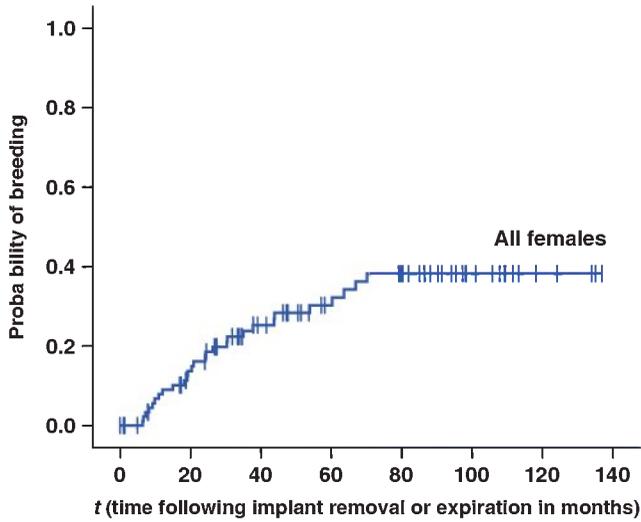


Fig. 1. Probability of reproducing of all implanted females after implant removal or expiration (vertical lines indicate censored data).

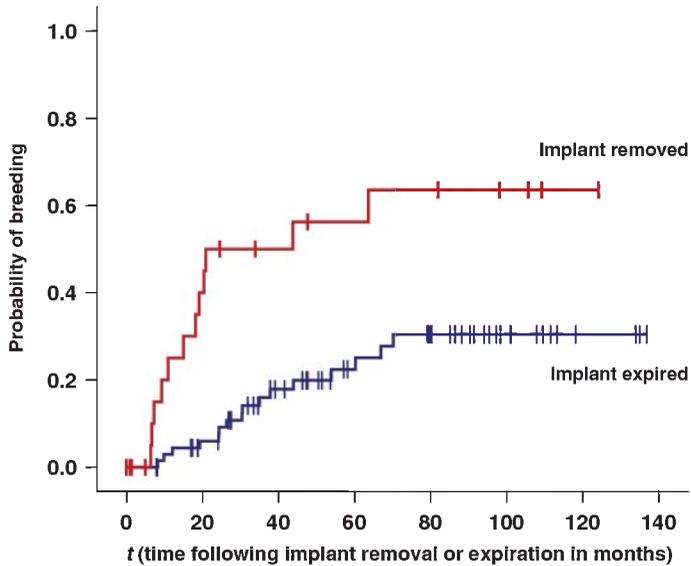


Fig. 2. Probability of reproducing of implant removed and expired females after implant removal or expiration (vertical lines indicate censored data).

Because a significant difference existed between implant-removed and implant-expired females, remaining analyses were carried out using only implant-removed animals to compare reproductive parameters with control animals. There was a significant difference in probability of reproducing between non-implanted control females and all implanted females ( $P=0.000$ ) and implant-removed females

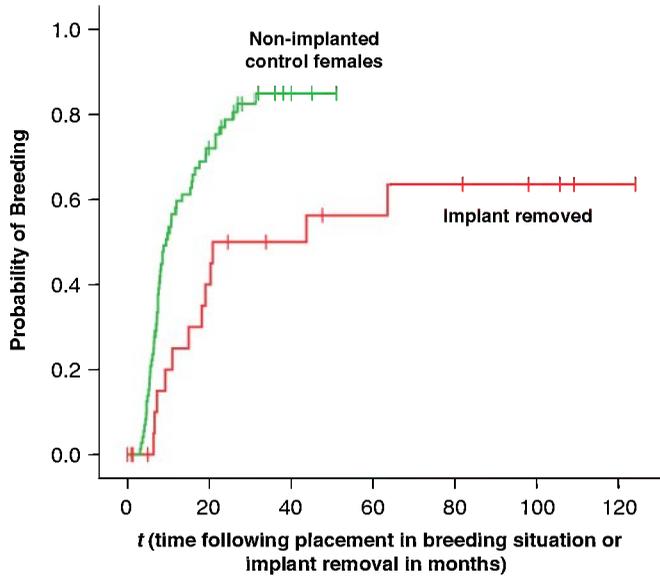


Fig. 3. Probability of reproducing of non-implanted control females compared to females that had implants surgically removed (vertical lines indicate censored data).

( $P = 0.019$ ; Fig. 3). Probability of implanted females producing a litter was only 5% by 1 year, 16% by 2 years, 24% by 3 years, and reached a plateau of 38.5% after 5.83 years. With implant-removed females only, the probability of reproducing was 25% by 1 year, 50% by 2 and 3 years, and reached a plateau of 62% by 5.25 years. Females in the control group reproduced successfully more quickly, with the probability of reproducing being 57% by 1 year, 77.5% by 2 years, and reaching a plateau of 85% after 2.66 years.

Within the implant-removed group, the probability of reproducing was unaffected by the female's age when the implant was removed ( $P = 0.519$ ) or by the length of time implanted ( $P = 0.130$ ). Similarly, there was no effect of age among control group females ( $P = 0.782$ ). There was no difference in probability of reproducing among implant-removed females with respect to reproductive experience before implantation ( $P = 0.460$ ). However, control females that had previously produced a litter had a significantly higher probability of reproducing than nulliparous females ( $P = 0.001$ ; Fig. 4).

### Litter Size

Twenty-four litters were born to females after implant removal, with a mean ( $\pm$ SE) litter size of  $2.33 \pm 0.20$  (range = 1–4; Table 2). There was no difference between this mean and that for the control tiger litters born in captivity to non-implanted females ( $2.68 \pm 0.14$ , range = 1–5,  $n = 57$  litters;  $t = -1.418$ ,  $df = 79$ ,  $P = 0.160$ ; Table 2). However, the average maternal age for the control tiger litters born in captivity was significantly lower than that for females with implants removed (87.33 months and 95.48 months, respectively). By disregarding litters born to females younger than 43 months from the control tiger litters, the mean age of the implanted and non-implanted control females could be equalized (95.78 months).

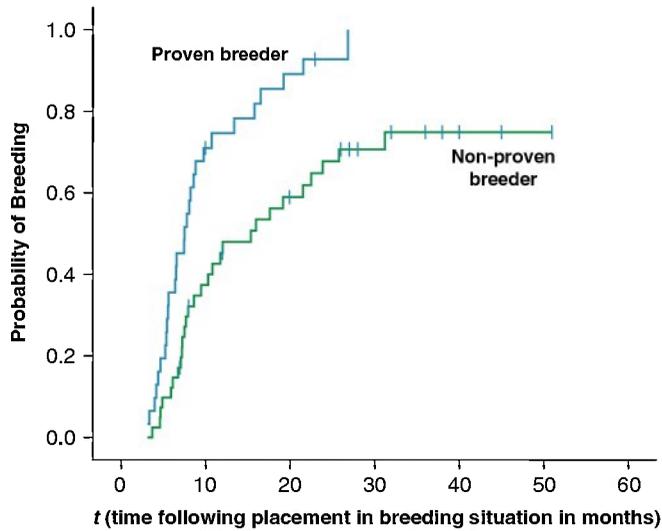


Fig. 4. Probability of reproducing of non-implanted proven and non-proven females after placement in a new breeding situation (vertical lines indicate censored data).

**TABLE 2. Litter sizes, rates of stillbirth, and rate of offspring survival to 30 days in implanted and non-implanted female tigers**

Group	Mean litter size $\pm$ SE (n)	Total no. of offspring	No. of stillbirths (%)	No. of offspring surviving to 30 days (%)
All control females (mean age = 87.33 months)	2.68 $\pm$ 0.14 (57)	153	17 (11.11)	113 (73.86)
Control females matched for removed maternal age (mean age = 95.78 months)	2.63 $\pm$ 0.16 (49)	129	16 (12.40)	90 (69.77)
Control females matched for expired maternal age (mean age = 107.90 months)	2.71 $\pm$ 0.19 (36)	103	15 (14.56)	67 (65.05)
All previously implanted females (mean age = 101.74 months)	2.16 $\pm$ 0.13 (56)	110	19 (17.27)	76 (69.09)
Implanted females after implants removed (mean age = 95.48 months)	2.33 $\pm$ 0.20 (24)	56	11 (19.64)	41 (73.21)
Implanted females after implants expired (mean age = 107.30 months)	2.00 $\pm$ 0.19 (27)	54	8 (14.81)	35 (64.81)
Ten matched females:				
Before implantation	2.17 $\pm$ 0.18 (23)	50	9 (18.00)	34 (68.00)
After implant removed or expired	2.07 $\pm$ 0.28 (15)	31	3 (9.68)	24 (77.42)

Litter size for these age-controlled females was  $2.63 \pm 0.16$  (range = 1–5,  $n = 49$  litters; Table 2), which was still not significantly different than that for the implanted females ( $t = -1.141$ ,  $df = 71$ ,  $P = 0.258$ ). There was no effect of prior breeding experience ( $P = 0.603$ ), age of female ( $P = 0.310$ ), or duration of implant ( $P = 0.239$ ) on litter size.

Twenty-seven litters were born to females after the implant was assumed to have expired, with a mean litter size of  $2.00 \pm 0.19$  (range = 1–4). There was no difference between this mean and that for implant-removed females ( $t = -1.209$ ,  $df = 49$ ,  $P = 0.232$ ), nor was there a difference between implant-expired litter size (mean age = 107.30 months) and non-implanted control tiger litters matched for maternal age (mean age = 107.90 months;  $2.71 \pm 0.19$ , range = 1–5,  $n = 36$  litters;  $t = -1.367$ ,  $df = 60$ ,  $P = 0.177$ ; Table 2).

For the 10 MGA-implanted females that produced offspring both before implantation and after implant removal (Table 2), average litter sizes were  $2.17 \pm 0.18$  (range = 1–4) before implantation and  $2.07 \pm 0.28$  (range = 1–4) after implantation, a difference that was not significant (paired  $t = 1.146$ ,  $df = 14$ ,  $P = 0.271$ ).

### Stillbirths

No significant difference was found between the 11.11% rate of stillbirth in non-implanted females and the 17.27% in all implanted females ( $\chi^2 = 0.239$ ,  $df = 1$ ,  $P = 0.743$ ; Table 2), nor between the 12.40% rate in the subset of non-implanted females controlled for maternal age and the 17.27% rate in all implanted females ( $\chi^2 = 2.056$ ,  $df = 1$ ,  $P = 0.203$ ; Table 2). Similarly, no significant difference was found when implanted females whose implants were assumed to have expired were excluded from the analysis (rate of stillbirth of 12.40% for non-implanted age-controlled females vs. 19.64% for implant removed females;  $\chi^2 = 1.642$ ,  $df = 1$ ,  $P = 0.256$ ; Table 2). Rate of stillbirth was not affected by prior reproductive experience ( $P = 0.346$ ), age of female ( $P = 0.101$ ), or duration of implantation ( $P = 0.774$ ).

No significant difference was found between the rate of stillbirth of implant-expired females (14.81%) and the rate of implant-removed females (19.64%) ( $\chi^2 = 0.448$ ,  $df = 1$ ,  $P = 0.616$ ), nor was it different from the rate of age-controlled non-implanted females (14.56%;  $\chi^2 = 0.002$ ,  $df = 1$ ,  $P = 1.000$ ; Table 2).

In the 10 females reproducing both before implantation and after implant removal, no significant difference was found between the rates of stillbirth before or after implantation (18.00% vs. 9.68%;  $\chi^2 = 1.050$ ,  $df = 1$ ,  $P = 0.356$ ; Table 2).

### Offspring Survival

No significant difference was found between offspring born to all females that had been implanted and offspring born to non-implanted (age-controlled) females with respect to rate of survival to 30 days (69.09% vs. 69.77%;  $\chi^2 = 0.013$ ,  $P = 1.000$ ), nor between offspring born to females whose implants had been removed (73.21%) and those of the non-implanted age-controlled females (69.77%;  $\chi^2 = 0.061$ ,  $P = 0.860$ ). Rate of survival to 30 days was not affected by prior reproductive experience ( $P = 0.277$ ), age of female ( $P = 0.106$ ), or duration of implantation ( $P = 0.208$ ).

No significant difference was found between the rate of survival of cubs to 30 days of implant-expired females (64.81%) and the rate of implant-removed females (73.21%) ( $\chi^2 = 0.908$ ,  $df = 1$ ,  $P = 0.411$ ), nor was it different from the rate of age-controlled non-implanted females (65.05%;  $\chi^2 = 0.001$ ,  $df = 1$ ,  $P = 1.000$ ; Table 2).

In the 10 females reproducing both before implantation and after implant removal, no significant difference was found between the survival rate for offspring born after implant removal (77.42%) and that for offspring born before implantation (68.00%;  $\chi^2 = 0.835$ ,  $P = 0.451$ ; Table 2). Also, there was no significant difference found between offspring survival for these 10 females before implantation and all non-implanted females (71.53%;  $\chi^2 = 0.220$ ,  $df = 1$ ,  $P = 0.717$ ), and no difference was found between offspring survival after implantation and non-implanted females ( $\chi^2 = 0.441$ ,  $df = 1$ ,  $P = 0.656$ ; Table 2).

## DISCUSSION

Reversibility with MGA implants was shown to be successful, based conservatively on birth of cubs, in most tigers if implants were removed surgically. This finding is consistent with previous studies in different species in golden lion tamarins (*Leontopithecus rosalia*) [Wood et al., 2001] and golden-headed lion tamarins (*Leontopithecus chrysomelas*) [De Vleeschouwer et al., 2000]. Concerns have been expressed regarding the reversibility of long-term MGA use with another *Panthera* species, the lion (*Panthera leo*), where high dose rates resulted in delayed parturition and subsequent death of the fetuses [Seal et al., 1976]. However, differences between lions and tigers may exist due to different dose rates or the method of analysis.

The use of survival analysis in this study has the advantage of taking into account the length of time females were in the study to calculate probability of reproducing. It allows females to enter and leave the study at different times for reasons such as transfer to other institutions, cancellation of breeding recommendation, death, ovariohysterectomy, and exceeding reproductive age, without biasing the results. Other studies [De Vleeschouwer, 2000] used total percentage of females; therefore, females with short periods of exposure and those with longer periods contributed equally, which can affect the outcome significantly.

If MGA implants were left in place, but were presumed to have expired, reversibility was significantly lower than when implants were surgically removed (24% vs. 62% respectively after 5.25 years). This indicates that implants were still effective after the recommended 2-year period. The most likely reason for this difference is that the implants typically continue releasing sufficient MGA after 2 years to remain effective. There are anecdotal reports to the Contraception Database on females with implants in place as long as 4 or 5 years, who begin to show signs of estrus soon after implant removal.

Several factors may have influenced the duration of efficacy of MGA implants in different females. These include the individual's physiological response to the implant; that is, some may have been more sensitive, especially with respect to implant location, because placement intramuscularly, as opposed to subcutaneously, results in better tissue uptake and placement stability [Asa and Porton, 2005b]. The effect of different doses, implant weight and implant size has yet to be investigated and may also have influenced effectiveness.

The mean age of implant-removed females (95.48 months = 7.96 years) was somewhat lower than that of implant-expired females (107.30 months = 8.94 years), which may have had an effect on probability of reproducing, as an increase in age is associated with a decrease in matings, conception rate, and birth, and therefore decreased probability of successfully producing offspring [Noakes et al., 2001]. However, no effect of age was observed in either the implanted or non-implanted females in this study, and it is unlikely that maternal age alone would account for the 38% difference.

Implanted females did not reproduce as successfully as non-implanted control females. Probability of reproducing of implanted females was only 5% by 1 year and reached a plateau of 38.5% after 5.83 years, compared to 85% by 2.66 years for non-implanted control females. Even using implant-removed females only, the probability of reproducing was still relatively low at 25% by 1 year and reached a plateau of 62% after 5.25 years. This suggests that although circulating MGA levels might have dropped below those needed to prevent conception, ovulation rate, fertilization, or implantation might have been affected. Because progesterone suppresses the final stage of follicle growth [Fukuda et al., 1980; Adams et al., 1992], fewer follicles might have ovulated or fewer embryos might have successfully implanted in an environment still influenced by MGA. Likewise, there might have been lasting negative effects on fertilization or implantation. In particular, changes in the uterine endometrium might hinder implantation [Munson, 2002]. However, mean ages of implanted females (85.85 months), implant-removed females (84.54 months) and non-implanted females (88.85 months) were similar and therefore, would not account for the differences observed.

Alternatively, breeding management may have differed between these groups. Implanted females may have been more likely to be housed with a male rather than maintained separately, hence the need for contraception. The Tiger SSP recommends that females and males be maintained separately and only be introduced during estrus to maximize successful reproduction [Tilson et al., 1995]. Therefore, a potential bias in management may exist that might affect successful reproduction, but could not be evaluated in this study due to lack of sufficient management data.

Litter size was unaffected by MGA implant placement, with the mean litter size of implant-removed females (2.33 offspring/litter) being not significantly different from either all non-implanted control females (2.68 offspring/litter) nor age-controlled females (2.63 offspring/litter). In the 10 females reproducing both before (2.17 offspring/litter) and after (2.07 offspring/litter) implantation, there was no significant difference in litter sizes. Furthermore, the difference between mean litter size of implant-expired females (2.00 offspring/litter) and that of corresponding age-controlled females (2.71 offspring/litter), was not significant ( $P = 0.177$ ).

From the results, it can be seen that cub rate of stillbirth was unaffected by MGA implant use. These rates suggest that actual stillbirth rates are also not affected but is not a true indication, because it was not possible to determine the number of deaths on the day of birth that were stillbirths from the available data. Therefore, a true proportion could not be calculated.

Similarly, rates of cub survival to 30 days were unaffected by MGA implant use. The failure to reach significance with respect to litter size, cub rate of stillbirth, and rates of cub survival to 30 days may have been due to the limited sample size and

the conservative nature of the statistical analyses, but these results conclude that the use of MGA implants does not affect offspring survival to 30 days.

For the 10 females that reproduced both before and after implantation, one might expect a detrimental effect of MGA treatment, but this was not the case. These 10 animals were highly prolific and reproductively successful having produced 38 litters and 81 cubs among them, with 58 cubs surviving more than 30 days. Therefore, it may be construed that they were more likely to have required reproductive intervention, that is, contraception. Therefore, a bias may have ensued whereby reproductively successful females may have been used as test subjects, whereas relatively less successful females may have remained as non-implanted control animals. As breeding is primarily managed to diversify the genetic composition of populations, unsuccessful breeders, with low genetic representation within the population, may become reproductive targets and rise to the top of the mean kinship rankings. In this study, however, non-implanted females were found to reproduce more quickly than implanted females, which does not support such a bias.

Although prior reproductive experience, age at time of implant cessation, and duration of implantation did not have a significant effect on any of the parameters measured for implanted females, it is generally believed that prime age females have a higher probability of reproducing and are more reproductively successful than their older counterparts. As seen in the non-implanted females, prior reproduction is associated with increased successful reproduction. Of 101 breeding recommendations for Amur tigers between 1992–2001, females that had given birth previously showed a higher probability of producing a litter in a given year (58.5%) than nulliparous females (28.3%) [Traylor-Holzer, 2003]. These factors may not have had a significant effect in this study due to the limited sample size and the retrospective nature of the data collection. Hence, a prospective trial might render more precise and decisive results.

An extensive number of adverse effects have been reported with MGA use in wild felids, in particular, uterine and mammary pathology [Kazensky et al., 1998]. Therefore, the surgical alternatives of ovariectomy and ovariectomy should be considered for genetically unimportant females, as these methods are safe and have fewer adverse effects while allowing social interaction and pairs to be maintained. However, Amur tigers are critically endangered, with numbers estimated as low as 334–417 adult tigers in the wild and approximately 450 in captivity, and Sumatran tigers are also critically endangered with fewer than 400 estimated in the wild and around 250 in captivity [Mueller, 2005; Wildlife Conservation Society, 2005] (Tilson and Nyhus, personal communication). The AZA Tiger Species Survival Plan has allocated 450 spaces for tigers and is aiming for a target population size of 150 individuals for each of the three subspecies of Amur, Sumatran, and Malayan tigers [Tiger Species Survival Plan, 2005a,b]. Therefore, a decision to irreversibly sterilize females would eliminate the possibility for future breeding, which is especially unwise in genetically valuable and dissimilar individuals. Hence, reversibility is a critical consideration when using MGA contraceptive implants.

## CONCLUSIONS

In conclusion, this study has shown that MGA is an effective contraceptive in tigers that when removed surgically resulted in a subsequent 62% probability of

reproducing by 5.25 years. However, previously implanted females did not reproduce as successfully as non-implanted control females, which showed an 85% return to reproduction by 2.66 years. Prior parturition increased the probability of reproducing in non-implanted females, but not in implanted females. In this particular population, with the MGA doses the animals received, implants should not be assumed to have expired after two years of insertion, and up to 30% of females will reproduce if implants are not removed. Therefore, implants should be surgically removed from females when breeding is desired. Successful reproduction, in terms of probability of producing a litter within several years, may be lower if contraceptive implants are used.

## ACKNOWLEDGMENTS

This study was completed as part of the MSc in Wild Animal Health run by the Institute of Zoology and the Royal Veterinary College. The authors would like to thank T. Sainsbury of the Institute of Zoology, Dr. M. Fox of the Royal Veterinary College, and K. Bauman of the AZA Wildlife Contraception Center.

## REFERENCES

- Adams GP, Matteri RL, Ginther OJ. 1992. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone on heifers. *J Reprod Fertil* 96:627–40.
- Asa CS, Porton I. 2005a. AZA Contraception Advisory Group Recommendations 2005. AZA Wildlife Contraception Center and Contraception Advisory Group. Available at: <http://www.stlzoo.org/contraception>.
- Asa CS, Porton I. 2005b. Wildlife contraception: issues, methods and application. Baltimore, MD: Johns Hopkins University Press.
- Cohn PN, Plotka ED, Seal US. 1996. Contraception in wildlife-book 1. Lewiston, NY:Edwin Mellen Press Ltd.
- De Vleeschouwer K, Leus K, van Elsacker L. 2000. An evaluation of the suitability of contraceptive methods in golden-headed lion tamarins (*Leontopithecus chrysomelas*), with emphasis on melengestrol acetate (MGA) implants. I: effectiveness, reversibility and medical side-effects. *Anim Welf* 9:251–71.
- Fukuda M, Katayama K, Tojo S. 1980. Inhibitory effect of progesterone on follicular growth and induced superovulation in the rat. *Arch Gynecol* 230:77–87.
- International Union for Conservation of Nature and Natural Resource. 2004. Red list of threatened species. The IUCN Species Survival Commission. Available at: <http://www.redlist.org>
- Kazensky CA, Munson L, Seal US. 1998. The effects of melengestrol acetate on the ovaries of captive wild felids. *J Zoo Wildl Med* 29:1–5.
- Microsoft Corporation. 1999. Microsoft Excel 2000. Redmond, WA: Microsoft Corporation.
- Mueller P. 2005. 2004 International Tiger Studybook. Leipzig, Germany: Leipzig Zoo.
- Munson L. 1995. Health complications of contraception in zoo animals. American Zoo and Aquarium Association Annual Conference Proceedings. Sept. 15–19, Seattle, WA. p 389–92.
- Munson L, Gardner IA, Mason RJ, Chassy LM, Seal US. 2002. Endometrial hyperplasia and mineralization in zoo felids treated with melengestrol acetate contraceptives. *Vet Pathol* 39:419–27.
- Noakes DE, Parkinson TJ, England GCW. 2001. *Arthur's veterinary reproduction and obstetrics*. 8th Ed. Philadelphia, PA:Saunders.
- Parmar MKB, Machin D. 1995. *Survival analysis: a practical approach*. New York: John Wiley and Sons.
- Petrie A, Watson P. 1999. *Statistics for veterinary and animal science*. Oxford, UK:Blackwell Science. p 178–9.
- Porton I, Asa CS, Baker A. 1990. Survey results on the use of birth control methods in primates and carnivores in North American zoos. American Association of Zoological Parks and Aquariums Proceedings. Sept. 23–27, Indianapolis, IN. 489–97.
- Seal US, Barton R, Mather L, Olberding K, Plotka ED, Gray CW. 1976. Hormonal contraception in captive female lions (*Panthera leo*). *J Zoo Anim Med* 7:12–20.
- SPSS Inc. 2004. SPSS 13.0 for Windows. Chicago, IL: SPSS Inc.
- Tiger Species Survival Plan. 2005a. 2005 complete analysis and breeding plan for Amur tiger (*Panthera tigris altaica*). Tiger Species Survival Plan prepared by K. Traylor-Holzer. Silver Spring, MD: American Zoo and Aquarium Association.

- Tiger Species Survival Plan. 2005b. 2005 complete analysis and breeding plan for Sumatran tiger (*Panthera tigris sumatrae*). Tiger Species Survival Plan prepared by K. Traylor-Holzer. Silver Spring, MD: American Zoo and Aquarium Association.
- Tilson R, Brady G, Traylor-Holzer K, Armstrong D. 1995. Management and conservation of captive tigers. Apple Valley, MN: Minnesota Zoo.
- Traylor-Holzer K. 2003. Using computer simulation to assess management strategies for retention of genetic variation in captive tiger populations. [PhD dissertation]. St. Paul, MN: University of Minnesota.
- Traylor-Holzer K. 2005a. 2005 AZA Amur tiger regional studbook. IUCN Conservation Breeding Specialist Group. Apple Valley, MN: Minnesota Zoo.
- Traylor-Holzer K. 2005b. 2005 AZA Sumatran tiger regional studbook. IUCN Conservation Breeding Specialist Group. Apple Valley, MN: Minnesota Zoo.
- Wildlife Conservation Society. 2005. The 2005 Amur tiger survey. New York: The Wildlife Conservation Society.
- Wood C, Ballou JD, Houle CS. 2001. Restoration of reproductive potential following expiration or removal of melengestrol acetate contraceptive implants in golden lion tamarins (*Leontopithecus rosalia*). *J Zoo Wildl Med* 32: 417–25.