The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

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*Re-assessing the reversibility of melengestrol acetate (MGA) implants in golden-headed lion tamarins (*Leontopithecus chrysomelas*): a comparison with golden lion tamarins (*L. rosalia*)*

*K De Vleeschouwer**†‡, *K Leus*† *and L Van Elsacker*†‡

† Centre for Research and Conservation, Royal Zoological Society of Antwerp, K Astridplein 26, B-2018 Antwerp, Belgium
‡ Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium
* Contact for correspondence and requests for reprints: kristel.de.vleeschouwer@zooantwerpen.be

Abstract

*The reversibility and flexibility of contraceptive methods generally allow for improved genetic and demographic management of captive populations. Earlier studies have produced conflicting results regarding the restoration of reproduction after using melengestrol acetate (MGA) implants in golden-headed (*Leontopithecus chrysomelas*, GHLT) and golden lion tamarins (*L. rosalia*, GLT): two closely related species that are physiologically and genetically very similar. The present study investigates the nature of this inter-species difference, presents new data on GHLTs and compares this with published data on GLTs. Analyses showed that around 34% of the GHLTs resumed breeding after their MGA implants were removed or had expired. Non-implanted GHLTs (control group) were significantly more likely to reproduce than females previously treated with an MGA implant, regardless of whether the implant was removed or left to expire. Younger and parous female GHLTs in the control group were more likely to start reproducing. In implanted females, only parity had an impact, with parous females being more likely to resume breeding than non-parous females. In contrast, data published on GLTs indicate that 75% of GLT females resume breeding, and that removing the implant increases the probability of reproduction occurring. Available data suggest that the observed inter-specific differences are related to differences in the weights of the implants used for the two species. For GHLTs, adjusting MGA doses and/or the sizes of the implants currently administered may be required in order to preserve the reproductive potential of individuals. Apart from potentially negative medical and welfare consequences for individual GHLTs, the reduced reversibility of MGA implants also impacts on management practices used to achieve the objectives of conservation breeding programmes. Finally, this study stresses the importance of evaluating the suitability of contraceptive methods at a species-specific level.*

Keywords: *animal welfare, callitrichids, contraception, melengestrol acetate, golden-headed lion tamarin, population control management*

Introduction

Controlling the growth of captive populations through the use of contraception has become an inevitable aspect of captive population management. Unequal representation of founder animals needs to be addressed, surplus animals are a common problem, and every successful conservation breeding programme at some point will have to control the number of animals that are involved in it (Leus 1999). Evidently therefore, the suitability and safety of contraceptive methods used in captive management and conservation breeding programmes are major concerns for animal managers and veterinarians (eg DeMatteo 1997; Sainsbury 1997; Glatston 1998). Frequently, the reversibility and flexibility of action of contraceptive methods are considered important features (Kirkpatrick & Turner 1991), which allow for improved genetic and demographic management (Ballou 1996; Wood *et al* 2001).

Several studies have raised questions regarding the medical effects and potential for restoring reproduction after treatment with melengestrol acetate (MGA) implants — the most frequently used contraceptive method in callitrichids and other mammals (DeMatteo 1997; De Vleeschouwer *et al* 2000a). Murnane *et al* (1996) reported changes in the reproductive organs of female Goeldi's monkeys (*Callimico goeldii*) and squirrel monkeys (*Saimiri sciureus*) following MGA implantation, which might have impaired their return to fertility. Adverse effects and/or delays or failures in the restoration of reproduction have been observed in several species of primate, cattle and felid (Zimbelman *et al* 1970; Seal *et al* 1975, 1976; Harrenstein *et al* 1996; DeMatteo 1997; Möhle *et al* 1999; De Vleeschouwer *et al* 2000a).

More specifically, a low degree of reversibility after MGA implantation has been observed in golden-headed lion tamarins (*Leontopithecus chrysomelas*, GHLT [De Vleeschouwer *et al* 2000a]). Only 19.2 % of GHLTs with implants that either were removed or had expired resumed breeding (28.4% if only removed implants are considered). In contrast, restoration of reproductive potential does not seem to be impaired in the golden lion tamarin (*Leontopithecus rosalia*, GLT [Wood *et al* 2001]). 75% of

GLT females reproduce within 2.5 years of having their implant removed, which is similar to the level of reproduction observed in a control group of females that had never been implanted (Wood *et al* 2001). GHLTs and GLTs are closely related species that are physiologically and genetically very similar (Forman *et al* 1986; French *et al* 2002). The observed difference between the species in terms of their response to the same contraceptive method is therefore unexpected and requires further investigation. However, the nature of the data published, and in particular the difference in the methodologies used, renders this difficult. De Vleeschouwer *et al* (2000a) used an 'all or none' approach, by looking at the percentage of GHLTs that resumed breeding regardless of how long females were observed after implant expiration or removal. The influence of potentially confounding variables that may have affected the females' capability of conceiving (eg age, mate familiarity, parity, implant removal versus expiration) was investigated and discussed, although not statistically. In contrast, Wood *et al* (2001) analysed data on GLTs using survival analyses, which take into account the time that females are included in the study. They also investigated the impact of confounding variables and compared the reproduction of implanted females with a control group of non-implanted females.

In the present paper we elaborate on these findings using data presented in De Vleeschouwer *et al* (2000a) and new data collected since this previous paper was published. These are analysed using survival analyses similar to those used for golden lion tamarins (Wood *et al* 2001). This allows for 1) the control of potentially confounding variables that might affect the restoration of reproductive potential, and 2) a reliable comparison between GHLTs and GLTs of the restoration of reproductive potential following MGA implantation.

Material and methods

Implanted females

Our data set included two types of data: 1) data on 24 contraceptive bouts from GHLT females treated with an MGA implant, which were also used in an earlier publication (for collection methods, see De Vleeschouwer *et al* 2000a), and 2) new data collected since December 1997 (the date when collection of data presented in De Vleeschouwer *et al* [2000a] was completed). These new data include follow-up data on females included in the earlier study and new data on females that received MGA implants since December 1997. They were obtained through personal correspondence with KL or KDV through the annual GHLT questionnaires (information complete up to 31 December 2000), and from the International Species Information System (ISIS) database (information complete up to 19 July 2001 at the latest; exact date known and dependent on the institution). A total of 11 new contraceptive bouts were available.

Special care was taken to ensure that the methods of data collection, accuracy, criteria for inclusion in the analyses and criteria for assessing reversibility, were similar regardless of which data were used. Specifically, reversibility was assessed only for females that had been at risk of reproduction for at least the duration of a normal pregnancy (ie 125 days or four months [De Vleeschouwer *et al* 2000b]) after the implant was removed or potentially had expired. Implants were considered potentially expired when they had been in place for two years. This criterion was based upon the recommendation of the Contraceptive Advisory Group (CAG) to replace implants after two years (DeMatteo 1997). We considered two years as an approximation of the duration of implant efficacy, and a minimum 'lower margin' time point beyond which implants might have expired. All females were housed in male/female groups with at least one adult male being present at the time when the restoration of reproductive potential was being investigated.

Control females

As a control group, we used studbook information on the reproduction of 87 females that had never been implanted, and were in potential breeding situations. We did not select females based on specific characteristics, but rather included all those for which sufficient and reliable information was available. In addition, we used data only from the period 1988–2000, and only from females housed in European and North American institutions, in order to be consistent with the study period and geographic location of the implanted females. Control females either came directly from their natal group, from single-sex groups or from former breeding situations (either because their previous mate had died or because the pair had been split up). The date that pairs were formed was usually known accurately to the day, either through the annual studbook questionnaires, through personal correspondence with KDV or through the respective International Studbook Keepers for the golden-headed lion tamarin over the years. We did not have accurate pair formation dates for 18 pairs. In these cases, we took the pair formation date to be the earliest possible day that the animals may have been put together, ie the day that either the male or the female arrived at the respective institution (whichever arrived latest). Since animals frequently have to go through an isolated quarantine period before being introduced to their respective mates, we thus might have overestimated the time that these particular pairs took to produce their first litter. In this way, any possible error would have obscured any differences between the control and implanted groups, rather than exaggerating them.

Survival analysis

In order to control for variation in the time that females were included in the study (ie the time that they were at risk of reproduction after implants were removed or potentially had expired), we used survival analyses similar to those presented by Wood *et al* (2001) for GLTs. Giving birth, rather than infant survival, was the event of interest, and analyses presented the probability of breeding by a time 't' (measured in months) after pair formation, implant removal or implant expiration. No distinction was made between first litters that were stillborn or live born. The time ($t = 0$) when females entered the study was determined by the date on which control females were placed in breeding situations, or, for implanted females, the date of implant removal or of

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Table 1 Overview of the number and mean age (± standard error) of females in the different categories used for analyses, and of the mean time to first reproduction and duration of follow-up for 87 control and 35 MGA-implanted female golden-headed lion tamarins.

	Control	Implanted females		
	Total	Total	Implant left in place to expire	Implant removed
Total number of bouts*	87	35	23	12
Number of parous females	7	22	15	7
Number of parous males	20	19	12	7
Number of pairs with related individuals	8	11	10	1
Mean age of females at t = 0 (years)	3.19 ± 0.21 (range: 0.94–11.18)	7.39 ± 0.40 (range: 3.56–11.34)	7.34 ± 0.55 (range: 3.56–11.34)	7.48 ± 0.54 (range: 5.77–11.05)
Number of females that reproduced	58 (66.7%)	10 (28.6%)	6 (26.1%)	4 (33.3%)
Mean time to first reproduction (months)	8.62 ± 0.65 (range: 2.33–27.97)	11.87 ± 2.43 (range: 3.83–25.47)	15.20 ± 3.42 (range: 5.63–25.47)	6.88 ± 1.16 (range 3.83–9.47)
Mean duration of follow-up for females that did not reproduce (months)	24.12 ± 3.98 (range: 6.20–107.23)	32.58 ± 4.62 (range: 4.33–91.27)	32.65 ± 5.00 (range 4.33–71.33)	32.44 ± 10.37 (range 10.5–91.27)

* Contraceptive bout: the period starting from the day that the implant is put in place and ending on the day that the implant is either removed, potentially expires (ie in place for two years), replaced by the same or another contraception method, lost (loss must be confirmed), or when the animal dies or is transferred to another institution and is lost to follow-up (De Vleeschouwer *et al* 2003). For control females, a 'bout' refers to the period of time for which they were at risk of reproduction.

the implant being in place for two years. Females left the analyses without reproducing (ie were right-censored) at varying points of time, either because of death (self or mate), removal from a breeding situation, replacement of the contraceptive, reproductive senescence (14 years), or loss to follow-up (ie the date beyond which complete/accurate information was no longer available).

Cox Proportional Hazard Regression Models (proc PHREG in SAS® [Collett 1994]) were used to assess the impact of the following variables on the probability of reproduction: 1) relatedness between male and female (ie brother–sister or parent–offspring combinations versus other combinations); 2) age of the female on the day of pair formation, implant removal or potential implant expiration; 3) previous reproductive experience of male and female (ie non-parous versus parous); and 4) removal versus potential expiration of the implant.

The probability of reproducing by a specific time t after pair formation, implant removal or potential expiration was calculated using one minus the Kaplan-Meier survival estimator, which was generated using proc LIFETEST in SAS® (Collett 1994). Proportional hazard ratios or risk ratios were used to interpret the difference in reproductive performance between different groups of females (Collett 1994). Risk ratios larger than 1 indicate improved reproduction, whereas risk ratios less than 1 indicate that reproduction is worse.

Results

A total of 35 contraceptive bouts were available for analysis. Table 1 summarises data on the parity, age and relatedness of the animals included in the analyses. Reproduction occurred in 10 of the implanted females (28.6%), compared to 58 of the control females (66.7%). In 12 cases, implants were removed and four females resumed breeding (33.3%). Of the 23 females that had implants in place for at least two years, six reproduced (26.1%).

Reversibility of contraception

Survival analyses showed that the probability of reproduction after MGA implantation was not affected by whether the implant was removed or left in place to expire ($\chi^2 = 2.40$; $df = 1$; $P = 0.12$; see Figure 1). The probability of females resuming breeding was around 34%; both when implants were removed and when they were left in place to expire. Females that had their implants removed did seem to return to fertility faster than did those that had their implants left in place. Eight months after implant removal, 25% of the females had reproduced. In contrast, the same level of reproduction for females with implants still in place was only reached 25 months after the implant was expected to have expired (Figure 1). The average time to reproduction was approximately 7 months when implants were removed and 15 months when implants were left in place to expire (Table 1). Females that left the study without reproducing were monitored for an average of 32 months after implant removal or potential expiration. Given this lack of significant difference depending on whether implants were removed or not, all subsequent analyses were conducted by contrasting control females and implanted females (ie pooling data from females whose implants had been removed or had potentially expired; Figure 1).

The probability of reproduction in control females differed significantly from that of implanted females ($\chi^2 = 5.02$; $df = 1$; $P < 0.05$; proportional hazard ratio = 0.336;

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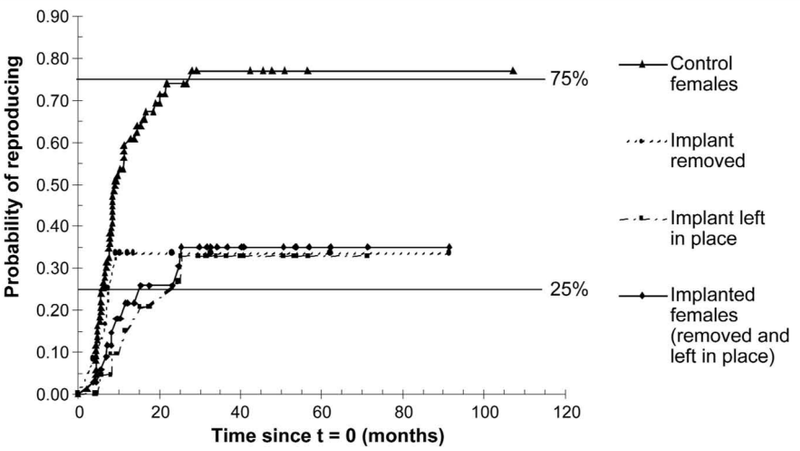
Figure 1



The probability of reproduction in control and MGA-implanted female GHLTs depending on whether the implants were removed or left in place to expire.

Figure 1). Control females had a probability of 77% of reproducing during the study, compared to 35% for the implanted females (Figure 1). 25% of the control females had reproduced by 6 months after pair formation, whereas 25% of the implanted females had reproduced by 15 months after implant removal/expiration. For control females, a probability of 75% of reproduction was reached at approximately 28 months after pair formation. The probability of reproduction for implanted females never reached this same level.

Reproduction in the control females

Female age influenced the probability of reproduction in the control group ($\chi^2 = 4.70$; $df = 1$; $P < 0.05$; proportional hazard ratio = 0.847), while the influence of parity was almost significant ($\chi^2 = 3.76$; $df = 1$; $P = 0.052$; proportional hazard ratio = 2.399). The parity of the male, and the relatedness between males and females did not have significant effects.

Compared to younger females, older females had less chance of reproducing after being placed in a potential breeding situation. On average, each year of age decreased the probability of reproduction by 10% (0.847/month). Given this effect of female age and the fact that the average age in the control group was considerably lower than in the implanted group (Table 1), we were concerned that the difference in reproduction between control and implanted females might be (partly) due to the implanted females being older. Therefore, we omitted females younger than 3.5 years at t = 0 from the control group, thus making the range of ages in both groups equal. Using this restricted control group, the difference in the probability of reproduction in control and implanted females was even larger than if the entire data set was used ($\chi^2 = 9.94$; $df = 1$; $P < 0.005$; proportional hazard ratio = 0.117), indicating that the difference in reproduction between control females and implanted females was not due to differences in female age. Within the restricted control group (n = 25), female age showed a non-significant trend towards a higher probability of reproduction in younger females ($\chi^2 = 3.05$; $df = 1$; $P = 0.08$; proportional hazard ratio = 0.721).

Parous control females were somewhat more likely to breed than non-parous females. Overall, 76% of the non-parous females and 86% of the parous females bred when placed in a potential breeding situation. Non-parous females took longer to start breeding. 25% of non-parous females had started breeding by 6.6 months after pair formation, whereas for parous females it took only 4.4 months to reach the same level. A 75% probability of breeding for non-parous females and parous females was reached at 28 and 11 months respectively (Figure 2).

Reproduction in the implanted females

Only the parity of the female affected the probability of reproduction after MGA implantation ($\chi^2 = 3.88$; $df = 1$; $P < 0.05$; proportional hazard ratio = 10.001; Figure 2). Parous females were about 10 times more likely to resume breeding after MGA implantation than non-parous females. Only about 15% of the non-parous implanted females resumed breeding, whereas 47% of the parous females did so.

Relatedness between the male and female, the parity of the male and the age of the female, did not affect the probability of reproduction after implantation.

Discussion

The results presented here support our earlier conclusion that there appears to be a problem with the restoration of reproductive potential in GHLT females that have been treated with MGA implants. Earlier data pointed to an overall reversibility of 19.2% regardless of whether the implants were removed or not, and a reversibility of 28.6% when considering removed implants alone (De Vleeschouwer *et al* 2000a). In the present study, in which the duration of follow-up and potentially confounding variables are controlled for, reversibility is still around 34%, and thus is much lower than the 77% probability of reproduction observed in control females that had never been implanted.

There was no difference in the probability of implanted females reproducing, depending on whether their implants were removed or had expired. The inclusion in our database

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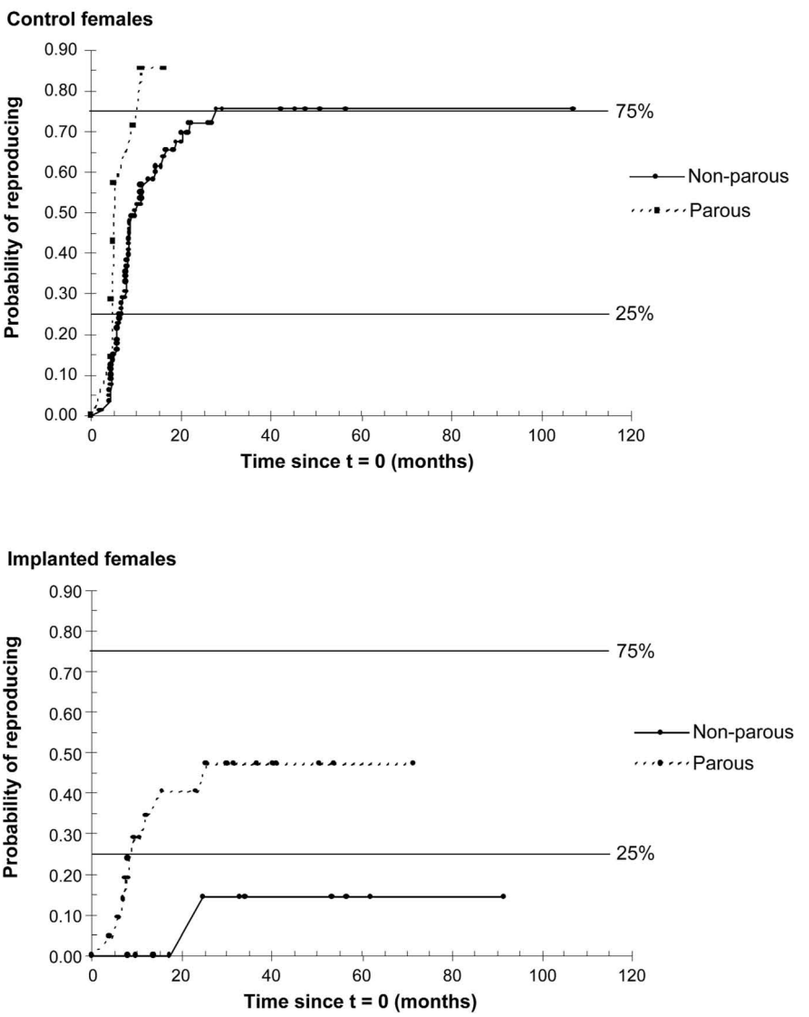
Figure 2

The probability of reproduction in control and MGA-implanted female GHLTs depending on whether the female was parous or non-parous.



of females with potentially expired implants that were still in place was offered as a possible explanation for the low level of reversibility observed in our earlier study (DeMatteo *et al* 2002). If implants are left in place to expire and no endocrinological evidence is available to assess whether normal ovarian cycles have been resumed, the absence of reproduction might indicate either that 1) implants are effective for a longer time than assumed, and/or 2) there is a reversibility problem. If females are fully capable of reproduction after being implanted, but expiration time is highly variable between implants, one would expect to see a gradually increasing number of females resume breeding as the time since implantation increases. Data on females with removed implants, however, reveal a high number of females resuming reproduction within a relatively short period after implant removal (De Vleeschouwer *et al* 2002). Recent analyses on the closely related GLT, in which the restoration of reproductive potential after MGA implantation is not impaired, confirm these expectations (Wood *et al* 2001). In order to assess the restoration of reproductive potential in GLTs, Wood *et al* (2001) therefore only consider implants that have been removed. Our analyses, however, show no such pattern. They do indicate that GHLT females with implants left in place take somewhat longer to resume reproduction (although not statistically) than females that have their implants removed. This is probably related to variation in the duration of efficacy of different implants. Thus, if females are followed for only a short time after their implants potentially expired, false conclusions regarding reversibility are possible. The implanted females in our study had been followed for an average of 32 months after implant removal/expiration — a considerably long period. The probability of breeding occurring in females whose implants had expired was very similar to that of females whose implants had been removed (around 34%). It is therefore highly unlikely that a higher percentage of breeding would have been observed if females with expired implants had been followed for longer.

Females whose implants were removed took approximately seven months to resume breeding, a period very comparable to the period taken by control females to start breeding after

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pair formation (8.5 months). This indicates that, provided that females are still capable of reproducing after having been treated with an MGA implant, they will do so equally as fast as females that have never been implanted. There appears to be no delay in restoring reproduction after implant removal. The fact that the probability of reproduction levels off sharply at 35% after 9.5 months, despite several females having been followed for much longer, further indicates that females that have not resumed breeding by that time are very unlikely to do so in the future.

All of the females in our study (this study; De Vleeschouwer *et al* 2002) were followed under conditions where conception was theoretically possible. In an earlier study (De Vleeschouwer *et al* 2000a) we suggested that social factors such as relatedness between mates, age and parity might have accounted to some extent for the low degree of reversibility that we observed. Our present analyses indicate that, while female parity indeed influences the restoration of reproductive potential in implanted females, female age, male parity and male/female relatedness do not. Parous females are more likely to resume breeding, but reversibility is still less than 50%. In the control group, both female age and to a lesser extent parity affected the probability of reproduction, with younger and parous females being more likely to reproduce.

Comparing the reversibility of MGA implants in GHLTs and GLTs

The results presented here are different from those obtained for GLTs (Wood *et al* 2001). In GLTs, neither female age nor parity affects the resumption of breeding in implanted females, while in control females, female age does have an influence, with younger females being more likely to breed. However, by far the most surprising result is the large difference between the two species in the percentage of females resuming reproduction after MGA implantation and the impact of removing implants. In contrast to our results for GHLTs, there is a significant difference in the percentage of GLT females that resume breeding depending on whether implants are removed or are left to expire, with only 30% of females in the latter group resuming reproduction (Wood *et al* 2001). Overall, 75% of the GLTs reproduced by 2.5 years after implant removal, compared to only 34% of the GHLTs. This raises questions about how two so closely related species can differ so much in their response to the same contraceptive.

Factors determining the duration of MGA implant efficacy and time to reversal

The results for implants that expired are similar for both species, with a reversibility of 30–35% (this study; Wood *et al* 2001). DeMatteo *et al* (2002) state that the reversibility of implants that have expired is determined by the duration of implant efficacy, which is said to be dependent on the MGA dose, implant size and physiology of the animal. GLTs and GHLTs are closely related and very alike in physiology: levels of circulating oestrogen, cycle length and other characteristics of reproduction are quantitatively and qualitatively similar in the two species (French *et al* 2002). Judging from the information that is requested when ordering implants, dose and size are determined by a female's age, parity and weight, and by whether she has been implanted previously (DeMatteo 1997). How each of these factors contributes individually to the final dose and size of the implant is unclear to us.

The major difference between the species lies in the results on implants that are removed versus those left to expire. According to DeMatteo *et al* (2002), the time to reversal after implant removal is determined by several factors, including female age, weight, parity prior to conception, mate access, reproductive quality of the mate and inter-individual differences. It seems reasonable to assume that similar factors will affect the time to reversal after implants have effectively expired. Our study and the study of Wood *et al* (2001) allowed for evaluating the role of these factors in GHLTs and GLTs. Female age did not influence the time to reversal in either species (this study; Wood *et al* 2001). Parity affected time to reversal in GHLTs only, but implanted parous females still had a considerably lower probability of reproducing (47%) than control parous females (86%). The impact of mate access and reproductive quality of the mate can be largely excluded since both GLT and GHLT females were followed for considerable lengths of time while in potential breeding situations — frequently with their former breeding partner (this study; Wood *et al* 2001; De Vleeschouwer *et al* 2002). Female weight is also very similar between GHLTs and GLTs (Leigh 1994), and thus unlikely to explain the differences observed between the species. Finally, potential inter-individual differences probably would not result in the kind of distinctly different pattern that is evident between the two species. Although certainly, no two individuals are alike, the use of this latter factor seems like a 'catch-all' term, which should be treated with caution. Inter-individual differences can be readily invoked to account for any case in which a female does not resume breeding where this cannot be attributed to any other parameter. In this way, the term tends to ignore the potential for irreversibility, because females that do have problems resuming breeding after being implanted might simply be considered to be individuals that for some reason need more time than others.

Effects of the implants' characteristics on the time to reversal

Because none of the variables mentioned above seem likely to fully explain the inter-species differences observed here, we assume that additional factors are also involved in determining the time to reversal. Two variables that we suspect to be potentially important determinants for the time to reversal are the MGA dose and/or the size of the implant. Implants are supplied without information on the exact amount of MGA that they contain, although zoos are provided with the implant's weight. Implants contain 20% MGA by weight (http://www.stlzoo.org/downloads/CAGRecommendations2003.pdf). Thus, implant weights can be used to make a general assessment of the effect of implant dose on the resumption of breeding.

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For GHLTs, data on implant weights were available through our survey and through personal communication with institutions. We analysed data on only 21 females that had been implanted once and were followed for at least four months after implant removal/expiration. Also, we excluded three females for which the first litter after implant removal was stillborn (two of these females were not included in the study long enough to allow the production of another [live born] litter). We then performed a survival analysis similar to those presented above, to investigate the effect of implant weight on the probability of breeding in implanted females after implant removal/expiration, while simultaneously controlling for the variables: parity (male and female), relatedness between mates, and female age. Implant weight was the only significant factor affecting the probability of the resumption of breeding ($\chi^2 = 4.09$; $df = 1$; $P < 0.05$). The formerly significant effect of female parity had disappeared ($\chi^2 = 0.006$; $df = 1$; $P = 0.94$). Median implant weight for females that resumed breeding was significantly lower (median = 0.28 g; range = 0.21–0.35 g; n = 5) than for females that did not resume breeding (median = 0.39 g; range = 0.26–0.85 g; n = 17; Mann-Whitney *U*-test: $U = 14$; $P < 0.05$). Data on implants inserted in GLTs, as listed in DeMatteo (1997), indicate that the median weight of implants inserted in GLTs is 0.31 g (range = 0.15–0.70 g; n = 210), while implants inserted in GHLTs have a median weight of 0.37 g (range = 0.21–0.85 g; n = 52 [own data]). This difference is highly significant (Mann-Whitney *U*-test: $U = 3360.5$; $P < 0.00005$), indicating that GHLT females have received on average heavier implants.

An implant's weight is related to its dose but the actual dose of MGA to which implanted females are exposed will probably also depend on the permeability of the silastic matrix carrier that contains the MGA. Assuming that this carrier has remained the same over the years, the weight of the implant is also in relation to its size. Whichever of these three factors (dose, size or permeability) is most important, it is clear from these additional analyses that differences in implant weight have an impact on reversibility. Even more importantly, the higher median values of implant weight for GHLTs compared to GLTs, and the correspondingly lower reversibility observed for GHLTs, suggest that the types, doses and/or sizes of the implants used in female GHLTs have to date negatively affected their ability to resume breeding. Since the current manufacturer of the implants and/or the CAG probably have accurate information on the identity of the carrier and on how the dose and size of an implant are determined, and in view of the potential impact of these factors on the restoration of reproductive potential, we would encourage them to investigate this issue further.

Obesity may cause reproductive failure (Hutchins *et al* 1996) and is a common problem in captive animals (eg Taylor & Poole 1998), including primates (Schwitzer & Kaumanns 2003). In the present study, it was not possible to assess the influence of female (over)weight on the resumption of breeding. We had data on body weights for only a number of GHLT females at the time that the implant was ordered (sometimes dating from a few weeks to months before the actual order date). Data on female body weights at the time that the implant was inserted or at the time it was removed or presumed to have expired were not available. Females that were obese at the time of implant insertion may have lost weight by the time that the implant was removed or had expired. Conversely, weight gains during the contraceptive bout may have influenced the resumption of breeding. Given that there is no published information on the mechanism of action of the implant's active substance, it is difficult to predict whether and in what way obesity might have influenced the results and contributed to the inter-specific differences observed between GLTs and GHLTs. Data on the weights of the GLT females observed by Wood *et al* (2001) were not available. Leigh (1994) reports an average weight of 659 g for captive female GLTs and 550 g for wild GLTs. Mean body weight for female GHLTs housed at the Royal Zoological Society of Antwerp was 645 g (11 females). Data for wild GHLT females have not been published. Based on this information, the weights of captive GLTs and GHLTs seem comparable. Whether the pattern of higher weights in captive female GLTs versus wild GLT females is due to obesity and whether it also occurs in GHLTs cannot be determined at present. Given the similarity in the reproductive physiology of GLTs and GHLTs (French *et al* 2002), obesity might have a comparable effect in the two species. However, this point requires further investigation.

Species-specific assessment of reversibility

The current findings point to the importance of interpreting results at the level of the species. Historically, the CAG has assumed this task through annual surveys and an extensive database that contains more than 12,000 entries on over 300 species (DeMatteo 1997). Given this vast amount of data and the number of species encompassed, performing the kind of detailed analyses we present here on a species-specific level may not be feasible. Regional or international species co-ordinators might be capable of doing so, but would need more information. Published data on issues such as factors that influence the time to reversal, the duration of efficacy and how the dose of an implant is being determined, are very important but also very scarce (DeMatteo *et al* 2002). In our experience, animal managers find this lack of information frustrating. Increased and easy access to information on the mechanism of action of MGA, determination of the doses and sizes used, duration of efficacy, etc, would be much welcomed. Such information should be made readily available to those dealing with animals and making decisions about using contraceptives on an almost daily basis. Not only might this alleviate the concerns of the people actually using the MGA implants, but also it would allow them to perform the kind of detailed analyses required to monitor the impact of these implants at a species-specific level.

Animal welfare implications

The analyses presented above confirm that there are problems with the reversibility of MGA implants, as described for GHLTs in De Vleeschouwer *et al* (2000a), and provide ideas for further investigating implant reversibility in this and other species. Doing so is of the utmost importance both in terms of animal welfare and in terms of the objectives of conservation breeding programmes. The medical effects of MGA implants are still largely unknown and require further investigation (De Vleeschouwer *et al* 2000a; Wood *et al* 2001). Furthermore, analyses have demonstrated group stability to be impaired in groups of GHLTs that are prevented from breeding (Price 1997; De Vleeschouwer *et al* 2003). An increased rate of aggression results in more animals being at risk of injury and potential death. Evicted GHLTs may end up being housed alone, which should be avoided in a social species such as this. Thus, in addition to their possible medical effects, implants may have negative welfare consequences not only because they interfere with the reproductive integrity of the females, but also because their use (and the use of contraception in general) may affect group stability and the well-being of individual tamarins. Normal group stability would probably be maintained by allowing a certain level of reproduction, for example, at the rate of one litter per year (De Vleeschouwer *et al* 2003). However, this requires the reliable reversibility of any contraceptive methods used. In their current form, MGA implants do not meet this goal in GHLTs.

From the perspective of conservation breeding programmes, the fact that non-parous female GHLTs treated with a contraceptive are unlikely to breed after MGA implantation is very important. Contraception of such females should be avoided if they may be required to breed later. More far-reaching though, the presence of females rendered infertile by the use of contraceptives alters the effective size of the captive population (ie the number of males and females potentially contributing to the gene pool). Effective population size is a parameter used in analyses based on which the genetic and demographic management of captive populations is being determined. Thus, changes in effective population size as a result of the use of irreversible contraceptive methods are likely to influence the management of the entire captive population of a species. Management practices may need to be altered in order to meet the objectives of the programme. Before sound recommendations can be formulated, the ways in which the use of contraception affects effective population size need to be investigated in more detail, while controlling for potentially confounding variables.

Finally, this study emphasises the importance of taking into account inter-specific differences and implant dose/size effects when evaluating contraceptive methods. High dose MGA implants are recommended as the most appropriate contraceptive method for all callitrichids except Goeldi's monkeys (in which negative medical side effects have been described [Murnane *et al* 1996; DeMatteo 1997]). Several studies have investigated the effects of contraception in callitrichids (eg Murnane *et al* 1996; Möhle *et al* 1999) and other species (eg Seal *et al* 1975, 1976; Portugal & Asa 1995; Harrenstein *et al* 1996; Hayes *et al* 1996; Price 1997; Kazensky *et al* 1998). However, few studies have investigated reversibility on a long-term basis and of these, the use of different methodologies that are difficult to compare has prevented a thorough comparison such as the one presented here between GHLTs and GLTs. Nevertheless, the fact that, despite the validity of this comparison, very different findings are reported for these two closely related species, supports the argument that species-specific differences should be taken into account when trying to extrapolate findings between species. The reversibility, efficacy, safety and suitability of contraceptives should be determined for all species and analysed on a species-specific basis, and administered doses should be determined based on such analyses. Only in this way will it be possible to ensure the adequate management of captive populations so that neither the welfare of the animals involved nor the goals of conservation breeding programmes are impaired.

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