AN EVALUATION OF THE SUITABILITY OF CONTRACEPTIVE METHODS IN GOLDEN-HEADED LION TAMARINS (*LEONTOPITHECUS CHRYSOMELAS*), WITH EMPHASIS ON MELENGESTROL ACETATE (MGA) IMPLANTS: (II) ENDOCRINOLOGICAL AND BEHAVIOURAL EFFECTS

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Abstract

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In order to be suitable, a contraceptive method should have little or no effects on social organization or behaviour. In callitrichids, changes in socio-sexual interactions between group members, due to hormonal changes induced by contraception, may have consequences for the reproductive inhibition of offspring in their natal group. This may lead to an increased rate of inbreeding. In this paper we report on the endocrinological and behavioural effects of contraception in golden-headed lion tamarins, using data obtained through a world survey and an observational study. Hormonal analysis of urinary oestrone conjugate levels in melengestrol acetate (MGA)-implanted females confirmed earlier preliminary findings (Van Elsacker et al *1994): MGA implants inhibited reproduction through the suppression of ovulation and regular ovarian cycles in the implanted female, while the occurrence of ovarian cycles in the oldest female offspring of each group was not affected. Sexual interactions between the dominant adults still occurred but underwent temporal changes. Reproductive inhibition in female offspring was maintained. Social interactions between group members altered in a non-consistent way but did not have an impact on the stability of the study groups during the study. In principle, MGA implants do not have a detrimental impact on the behaviour of group members. The suitability of MGA implants from a behavioural point of view depends on the extent to which those involved wish to preserve the entire range of natural behaviours for this species. The behavioural effects of other contraceptive methods are still largely unknown.*

Keywords: *animal welfare, callitrichids, contraception, golden-headed lion tamarins, population control management, reproductive inhibition*

Introduction

The use of various contraceptive devices has become an important and essential means of controlling the number of animals maintained in captivity (DeMatteo 1997; De Vleeschouwer *et al* 2000a). In general, an appropriate contraceptive device should comply with the following standards (Kirkpatrick & Turner 1991): i) a high degree of effectiveness; ii) no toxic or harmful side-effects; iii) reversibility and a flexible duration of action, to preserve the reproductive and genetic integrity of the animal; iv) a low cost; v) minimal or no effects on social organization or behaviour; and vi) remote delivery, preferably in a single administration, which makes it easy to use in terms of daily animal management and animal welfare. In practice, the most suitable method will probably depend on the species and/or the individual to which it has to be applied, as well as the objectives of the zoological institution involved and those of the conservation breeding programme (if the species is a part of this).

Changes in behaviour following contraception may be most profound if hormonal contraception is being used, or in cases of ovariectomy or castration. These procedures will seriously alter the levels of reproductive hormones such as oestrogen and/or progesterone in females and testosterone in males. This in turn will have an effect on all behaviours that are under the control of these hormones. In callitrichids, these changes may have a larger impact on socio-sexual interactions within the group than in any other family of primates. Reproductive inhibition mechanisms in callitrichids allow offspring to reside in their natal group for several years after sexual maturity, without reproducing. This reproductive inhibition is sometimes physiological in nature, ie with female offspring lacking regular ovarian cycles (for a review see Abbott *et al* [1993]). In golden lion tamarins, *Leontopithecus rosalia*, and golden-headed lion tamarins, *Leontopithecus chrysomelas*, however, female offspring do show regular ovarian cycles while still in their natal group (French *et al* 1989; Van Elsacker *et al* 1994; Chaoui & Häsler-Gallusser 1999). Therefore, inhibition of reproduction in the genus *Leontopithecus* is probably mediated by behavioural cues, such as aggression by the mother (French *et al* 1989; Abbott *et al* 1993). Reproductive function in subordinate callitrichid males seems not to be impaired by their lower social status (Abbott & Hearn 1978; French *et al* 1989).

Administering contraceptives to the dominant female may cause changes in her behaviour or dominance status that might affect further inhibition of reproduction in the offspring. Her oldest female offspring may start inbreeding with their father or brother(s). In this way, not only will contraception fail to prevent the production of more infants, but these would be inbred infants of reduced value for the conservation breeding programme. Inbred infants also have a lower probability of survival (Ralls & Ballou 1982). In golden-headed lion tamarins, 25 per cent of inbred infants are stillborn, as opposed to 16 per cent of non-inbred infants (based on stud-book data from 154 inbred and 948 non-inbred infants: De Vleeschouwer unpublished data). A similar effect has been described for Goeldi's monkey, *Callimico goeldii* (Lacy *et al* 1993). Stillborn infants have, on average, a higher body weight (Hiddleston 1978; Rothe *et al* 1992). This may cause birth complications such as dystocia that lead to the death of the female (Poole & Evans 1982; Rothe *et al* 1992; Leus 1998).

Hormonal contraception, mostly in the form of MGA implants, has been widely used in golden-headed lion tamarins as well as in several other species (DeMatteo 1997; De Vleeschouwer *et al* 2000a). Given that female offspring are capable of regular ovarian cycles and thus of conceiving, the chances of incestuous breeding due to behavioural changes following contraception of the dominant breeding female may be higher in this species than in other callitrichids. Indeed, this was suggested by the preliminary study of Price (1998).

As part of a study on the effects of contraception in golden-headed lion tamarins, we investigated the occurrence of sexual behaviour in animals that had received contraception and the effect of this contraception on reproductive inhibition in their offspring. The data presented here were collected in two ways: i) through distributing surveys to all official institutions holding golden-headed lion tamarins; and ii) through detailed observations conducted on four groups of golden-headed lion tamarins in which the breeding female received an MGA implant. Data on the effectiveness, reversibility and medical side-effects of contraceptive methods, as well as data on the stability of non-breeding groups collected as part of this study are presented in companion papers (De Vleeschouwer 2000; De Vleeschouwer *et al* 2000a). The endocrinological data collected as part of this study have been partially published elsewhere (Van Elsacker *et al* 1994).

Methods

Definitions

Sexual maturity: females were considered capable of regular ovarian cycles at an age of 515 days (17 months) based on unpublished endocrinological data collected on 4 females in the colony of golden-headed lion tamarins housed at the Royal Zoological Society of Antwerp and findings reported for golden lion tamarins (French *et al* 1989).

Implantation bout: the period starting from the day on which the implant was put in place until the day it was either removed, expired (as determined from the assumed longevity of the implant), replaced by the same or another contraception method, lost (loss had to be confirmed), or when the animal died or was transferred to another institution and lost to follow-up (De Vleeschouwer *et al* 2000a). The longevity of MGA implants was assumed to be about 2 years.

Control period: the period of observation conducted when the female was not treated with any contraceptive method.

Implant period: the period of observation conducted when the female was implanted with a contraceptive (ie covering part of an *implantation bout*).

For simplicity, the dominant male will hereafter be referred to as 'father', the implanted female as 'mother', female offspring as 'daughters' and male offspring as 'sons'.

The survey

A survey was distributed among the 81 institutions listed as official holders of golden-headed lion tamarins (Leus 1998). A copy of the survey and details of the distribution, data collection and response rate have been published in De Vleeschouwer *et al* (2000a). The present paper addresses the occurrence of sexual behaviour in the male or female which received contraception and in any other males or females present (both before and after contraception); and the occurrence of reproduction in any males or females housed with a male or female that received contraception.

The observational study

Animals

The four family groups of golden-headed lion tamarins were housed at the Royal Zoological Society of Antwerp, Belgium. Table 1 summarizes data on the identity of the animals and group composition. All groups contained only one sexually mature daughter at the time of observation. Their housing conditions have been fully described elsewhere (Van Elsacker *et al* 1992; De Vleeschouwer *et al* 2000b). Preliminary data on the effects of MGA implants in groups 1 and 2 have been published in Van Elsacker *et al* (1994).

Table 1 Composition of the study groups. (Ages at the time of contraception were calculated by subtracting birth dates from the dates of contraceptive implantation unless otherwise noted. Numbers and names are as in the international stud-book for this species[Leus 1998].)

	Group 1	Group 2	Group 3	Group 4
Mother	150 Josepha	152 Fabiola	961 Ulrika	604 Roberta
Age	>11yrs[1]	>11yrs[1]	4yrs 8mths	6yrs 11mths
Father	153 Bonaventure	151 Boudewijn	652 Sam	930 Thierry
Age	>11yrs[1]	>11yrs[1]	6yrs 11mths	4yrs 8mths
Oldest daughter	916 Tina	712 Trees	1326 Xania	1276 Wiske
Age	17 mths	20mths	21mths	2yrs 1mth
No of offspring present[2]	9	7	7	3(2)[3]
Sex ratio of offspring (males:females:unknown)	5:4	2:7	3:3:1	2:1 (1:1)[3]

1 Animals are wild-born founders, with unknown birth dates. Their ages were calculated from the year when they first appeared in the captive population (Leus1998).
2 Including the oldest daughter.
3 The oldest son was evicted from the group 1week after the end of the control period.

Implantation procedure

Nursing mothers were implanted, 3–4 days after parturition. They were taken from the group during the morning, immediately following a suckling bout and the transfer of the infants to another group member. They were sedated with ketamine hydrochloride (Anesketin; Eurovet, Sint-Martens-Latem, Belgium) at a dose of 10mg kg^{-1} body weight, injected intramuscularly. A MGA implant (E Plotka, Marshfield Medical Research Foundation, Wisconsin, USA) was placed subcutaneously between the scapulae. The implants contain 20 per cent melengestrol acetate by weight in a silastic matrix and have an expected longevity of 2 years (see, the website of the Association of Zoos and Aquaria Contraception Advisory Group: http://www.worldzoo.org/cag). The incision was sutured with polyglactin sutures (Vicryl 2/0®; Ethicon®, Norderstedt, Germany). The females were returned to their groups after 3h. Capture time and procedure were similar for the female of group 4, who was not nursing at the time of implantation.

Observations

Behavioural observations were conducted at intervals from November 1993 until December 1997. Figure 1 explains the observation protocol for the four family groups. Due to difficulties in the availability of breeding pairs at comparable moments of the reproductive cycle, the timing of observation periods differed between groups. Groups 1 and 2 were followed throughout the end of a pregnancy (control period), continuing after parturition and implantation (implant period). The control period for group 3 included the period following a parturition after which the mother received no implant, while the implant period included the period following a parturition after which the mother was implanted. In group 4, the mother had stopped reproducing for some unknown reason. Endocrinological data collected at the time indicated that she was still experiencing regular ovarian cycles. The control period for this group started at an arbitrary chosen moment, and lasted for 40 days. Then, after a period of 2 months, the female was implanted, and observations recommenced.

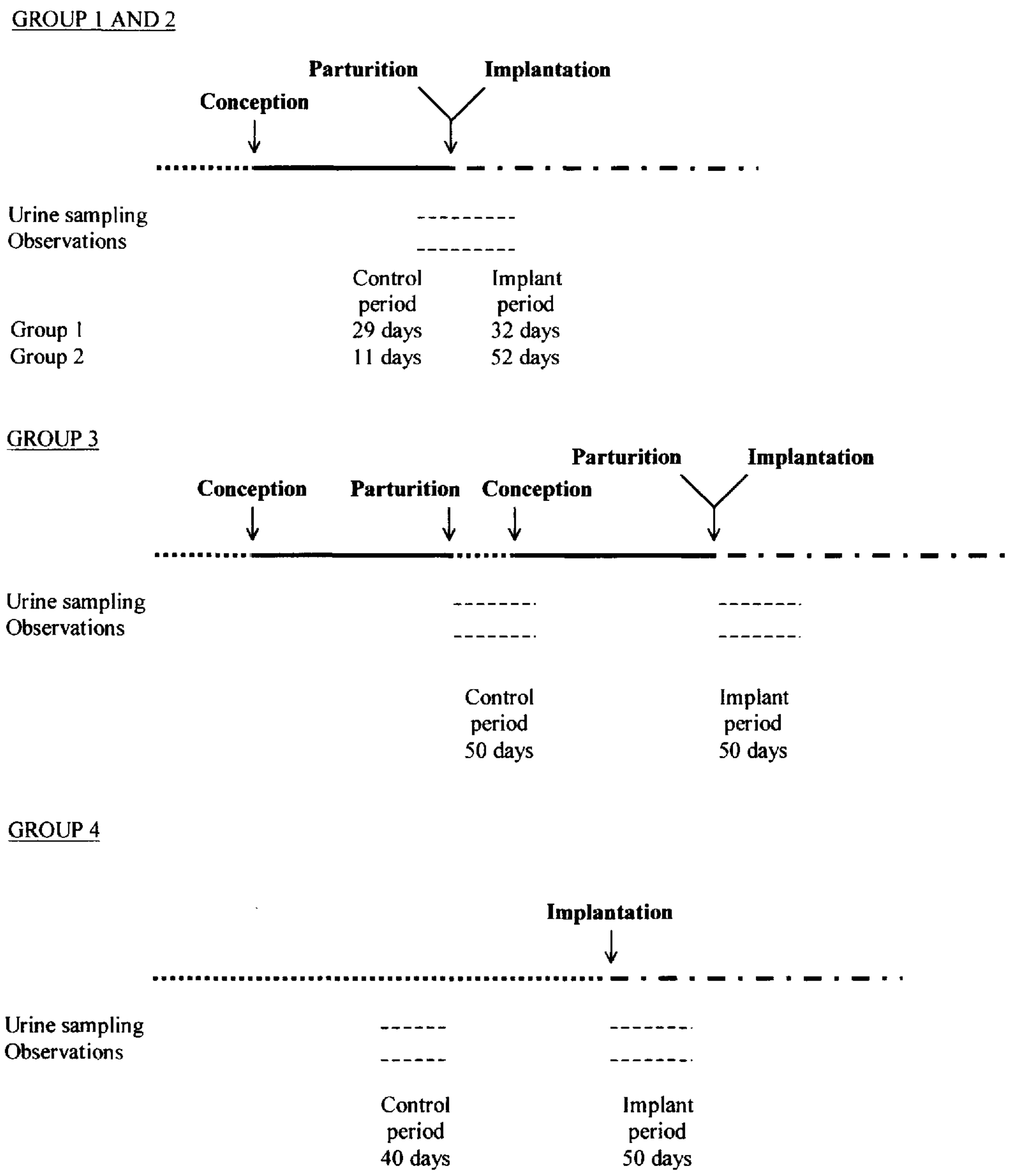

Figure 1 **Schematic representation of the timing and duration of the various observation and sampling periods among the four study groups. (Total line lengths represent the period over which groups were monitored to determine time of entry to the study [5mm = 25 days].•••••••• Breeding female shows regular ovarian cycles; ———— breeding female is pregnant;-·-·-·-·-·-·- breeding female is implanted;------------ period when urine samples were collected and observations made.)**

Behaviours were recorded using a combination of Focal animal sampling and continuous recording techniques (Altmann 1974). Focal animals were the father, mother and their oldest daughter. For groups 1 and 2, observations were conducted 6–7 days per week and lasted 30min per focal animal per day. Groups 3 and 4 were observed every other day and every focal animal was observed for 30min twice per day. The behaviours recorded were: sternal and circumgenital marking, anogenital sniffing, allogrooming, huddling, aggressive behaviour and male mounting. For groups 3 and 4, we also recorded all occurrences of female proceptive sexual presenting (for definitions of the behaviours see, Mack & Kleiman [1978], Hoage [1982] and Kleiman *et al* [1988]).

Urine sampling and hormone analysis

Urine samples from the mother and her oldest daughter were collected on a near daily basis during the control period and 3–4 times per week during the period of MGA implantation. The samples were analysed for concentrations of oestrone conjugates (E1C). Elevated levels of E1C are indicative of the luteal phase of an ovarian cycle in golden-headed lion tamarins (De Vleeschouwer *et al* 2000b). Thus, excretion of E1C represents a reliable indicator of female ovarian function in this, and other callitrichid, species. For details of the sample collection procedure, methodology of hormone measurements and some of the data obtained see: Van Elsacker *et al* (1994), Heistermann and Hodges (1995), and De Vleeschouwer *et al* (2000b).

Statistical analysis

The frequencies of behaviours were calculated per 30min of observation for every observation day. Given the heterogeneity of the control periods, data were analysed separately for each group, using Mann-Whitney U tests to compare behavioural frequencies between periods. Differences in E1C levels between control and implant periods were compared by a t-test for independent samples. Analyses were performed using Statistica®, release 5.0 (StatSoft Inc, Tulsa, USA). All reported frequencies are indicated below as mean (± SEM) frequencies per 30min of observations. The criterion for statistical significance was set at $P < 0.05$, while $P < 0.1$ was reported as a trend.

Results

The survey

Through the survey, we obtained information on contraception in 44 females and 4 males. Contraception in the females consisted of 56 MGA implants, one Depo-Provera™ injection (Coopers-Pitman-Moore, Crewe, UK), two chlormadinone acetate implants (GS implant; Teikokuzouki Co Ltd, Tokyo, Japan), one hysterectomy and one sterilization. Contraception in the males included two vasectomies, one castration and one epididiectomy. More details are given in De Vleeschouwer *et al* (2000a).

Occurrence of sexual behaviour in MGA-implanted females

Information on sexual presenting was obtained for 14 females, 6 of which still showed this behaviour. Furthermore, copulations still occurred in 13 out of 19 females for which this information could be obtained. If sexual interactions occurred, this could be as early as within 3 months of implantation. More details of the occurrence and nature of sexual interactions will be presented in the section on the observational study (see below). All sexual interactions observed were with the fathers of the group.

Occurrence of incestuous breeding following MGA implantation

There were 10 social groups in which either at least one daughter was sexually mature when the mother was implanted (12 daughters in total), or in which at least one daughter reached sexual maturity during the implantation bout (13 daughters in total). Infants were born to two daughters in two social groups during the implantation bout. In one group, a subadult female reached sexual maturity and gave birth 23 months after contraception of her mother. In the other group, a daughter already sexually mature at the time of implantation gave birth 14.5 months after her mother was implanted. In this same group, a subadult female reached adulthood and gave birth 31 months after her mother was implanted. This indicates a conception date of 26.5 months after the start of the implantation bout. Given a presumed implant longevity of 2 years, conception thus occurred after the end of the implantation bout.

Occurrence of sexual behaviour and incestuous breeding in relation to other contraceptive methods used in females

Breeding of two subordinate females occurred in the group with the hysterectomized female, but one of these females had already conceived prior to her mother being hysterectomized. The sterilized female, the female receiving a Depo-Provera™ injection and the two females with chlormadinone acetate implants were housed in social groups without other sexually mature females.

Sexual behaviour was not reported in the sterilized female and in one of the females who received a chlormadinone acetate implant. The other female with the chlormadinone acetate implant was reported to copulate but not to present for mating. Sexual behaviour in the hysterectomized female and the female receiving a Depo-Provera™ injection was reported as unknown.

Effects reported for contraceptive methods in males

Sexual interactions between the epididiectomized father and the mother, including full copulations, occurred within 3 months of surgery and beyond that time. The only son in the group was 10 months old when his father was epididiectomized and 2 years old when the survey was conducted. Sexual interactions with his mother were not seen during this period.

The two vasectomized males were housed with one female: this female presented for mating but neither of the males initiated mating or copulated.

Sexual behaviour no longer occurred in the castrated male. His social group was put into free-ranging conditions 6 weeks after castration of the male, and the male subsequently disappeared along with five other group members. Prior to his disappearance, this male was never seen sexually interacting with any female group member. Infants sired by the oldest son were born to the breeding female of this group 10 months after the male was castrated. Calculation from the day of parturition indicated that conception had occurred after the castrated male had disappeared.

The observational study

Endocrinological data

Detailed hormonal profiles for the mothers and daughters of groups 1 and 2 have been published in Van Elsacker *et al* (1994). Table 2 summarizes the mean values of E1C for all females. The E1C levels measured after implantation and the occurrence of ovarian cycles are similar for all mothers. E1C levels decreased significantly after implantation (in all mothers for whom there was sufficient data) and hormonal profiles showed that ovulation was completely suppressed (Van Elsacker *et al* 1994). Data obtained for the daughters are less consistent. In groups 1 and 2, the daughters (Tina and Trees) showed regular ovarian cycles throughout the whole study (Van Elsacker *et al* 1994), with comparable average E1C levels both before and after implantation (Table 2). Xania, in group 3, showed low E1C levels before the implantation of her mother, but higher levels afterwards. The latter levels are within the range of those from the daughters of groups 1 and 2 and indicated regular ovarian cycles. Wiske, in group 4, showed low levels of E1C during the entire study period, without evidence of regular ovarian cycles.

Table 2 Comparison of mean (± SEM) levels of oestrone conjugate (E1C) and the occurrence of regular ovarian cycles pre- and post-contraceptive implantation in mothers and oldest daughters. (Hormone levels are expressed as µg E1C mg creatinin^{-1}; n – no of samples; ns – not significant.)

	Pre-implantation	Cycles?	Post-implantation	Cycles?	*t*-test result and *P* value
Mothers (and group)					
Josepha (group 1)	435.72 ± 30.46 (n = 32)[1]	pregnant	0.31 ± 0.04 (n = 28)	no	t_{58} = 13.36, P < 0.00005
Fabiola (group 2)	336.9 (n = 1)[1]	pregnant	0.39 ± 0.06 (n = 41)	no	–[3]
Ulrika (group 3)	19.2 ± 3.78 (n = 28)[2]	yes	0.6 ± 0.14 (n = 11)	no	t_{37} = 3.06, P < 0.005
Roberta (group 4)	21.0 ± 2.97 (n = 29)[2]	yes	1.71 ± 0.61 (n = 15)	no	t_{42} = 4.62, P < 0.00005
Oldest daughters (and group)					
Tina (group 1)	14.17 ± 2.1 (n = 32)	yes	19.31 ± 2.21 (n = 30)	yes	t_{60} = -1.69, P = 0.097
Trees (group 2)	10.05 ± 3.95 (n = 2)	yes	17.31 ± 2.22 (n = 28)	yes	t_{28} = -0.85, ns
Xania (group 3)	2.64 ± 0.58 (n = 30)	no	14.03 ± 4.24 (n = 18)	yes	t_{46} = -3.41, P < 0.005
Wiske (group 4)	2.89 ± 0.92 (n = 27)	no	1.41 ± 0.75 (n = 15)	no	t_{40} = 1.08, ns

1 Samples taken at the end of pregnancy (see, *Methods*).
2 Samples taken during regular ovarian cycles (see, *Methods*).
3 Statistics could not be computed as there was only one data point available for the pre-implant period.

Behavioural data

If an MGA implant was causing changes in a mother's behaviour that led to the her oldest daughter starting to reproduce, during the implant period one would expect to observe:

i) Decreased frequencies of affiliative (grooming and huddling) and sexual interactions between the father and the mother, indicative of a weakening of the socio-sexual bond between these two animals. (With regard to grooming, a decrease might only be expected in the father, while the mother might increase her frequencies of allogrooming, in an attempt to restore the relationship.)

ii) Increased affiliative and sexual interactions between the father and the oldest daughter, indicative of a stronger social bond and the formation of a sexual bond between these two animals.

iii) Decreased affiliative interactions between the mother and the oldest daughter, while aggressive interactions increased, both indicative of changes in their dominance relationship.

iv) A possible increase in sexual interactions between the oldest daughter and any sons present, indicative of the formation of sexual bonds between these animals.

Socio-sexual interactions between the father and the mother

Table 3 indicates that male anogenital sniffing of the female differed significantly between the control and the implant period in groups 1 and 3, with mean (± SEM) frequencies in both groups increasing during the implant period (group 1: control = 0.3 ± 0.09 vs implant = 1.1 ± 0.21, $P < 0.0005$; group 3: control = 1.73 ± 0.44 vs implant = 4.61 ± 0.47, $P < 0.00001$). Male mounting frequencies, however, only showed a trend to increase in group 2 (control = 0.0 ± 0.0 vs implant = 0.55 ± 0.13, $P = 0.06$).

Figure 2, however, shows that in all groups, changes occurred in the timing of sexual interactions after MGA implantation. The central graph of this figure shows the frequencies of female sexual presenting and male mounting during the control period in group 3, when the female was showing regular ovarian cycles. The temporary increase in sexual presenting by the female, coinciding with the increase in mounting by the male and recurring approximately every 20 days is typical of what is normally observed in pairs of golden-headed lion tamarins during the follicular and peri-ovulatory period of a regular ovarian cycle (De Vleeschouwer *et al* 1998; 2000b). The peripheral graphs in Figure 2 illustrate male mounting frequencies during the implant periods in groups 1, 2, 3 and 4, and female sexual presenting and male mounting during the implant periods in groups 3 and 4. The frequencies of female sexual presenting during the implant periods were generally low and no longer restricted to a limited period of consecutive days. The frequencies of male mounting also decreased during implant periods. For both female sexual presenting and male mounting, the normal interval of approximately 20 days was no longer apparent. In groups 1 and 4, the focal males only mounted sporadically, while in groups 2 and 3 male mounting occurred on many more days. The frequency of male mounting might have been influenced by the frequency of sexual presenting in the female: in group 4 the female only presented sporadically, while this was not the case in group 3.

Grooming interactions differed between groups 3 and 4 (Table 3). The fathers increased their mean (± SEM) rates of grooming the mothers (group 3: control = 0.07 ± 0.03 vs implant = 0.19 ± 0.06, $P = 0.055$; group 4: control = 0.43 ± 0.15 vs implant = 0.80 ± 0.17, $P < 0.05$). In group 4 only, the mother decreased her rates of grooming the father (control = 0.47 ± 0.13 vs implant = 0.17 ± 0.05, $P < 0.05$). Huddling significantly increased in groups 1 and 2 (group 1: control = 0.96 ± 0.27 vs implant = 2.44 ± 0.58, $P < 0.01$; group 2: control = 0.78 ± 0.53 vs implant =3.28 ± 0.61, $P < 0.05$); and significantly decreased in groups 3 and 4 (group 3: control = 2.59 ± 0.47 vs implant =1.17 ± 0.3, $P < 0.05$; group 4: control = 7.28 ± 0.89 vs implant = 4.17 ± 0.63, $P < 0.05$). The frequency of aggressive behaviour of the father towards the mother and vice versa did not alter significantly after implantation.

Socio-sexual interactions between the father and the oldest daughter

During the control period, male anogenital sniffing of the oldest daughter was observed once in group 1 and three times in group 2, but never during the implant period. In group 4, it

Table 3 Results of the statistical analysis, comparing the frequencies of socio-sexual behaviours between control and implant periods for the four study groups. The results are ordered according to the dyad of animals involved. (ns – not significant.)

Behaviour	Group 1 N1 = 27; N2 = 25	Group 2 N1 = 10; N2 = 48	Group 3 N1 = 25; N2 = 25	Group 4 N1 = 20; N2 = 24
Father-mother				
Father sniff mother	$U = 143.5$; $P < 0.0005$	ns	$U = 77$; $P < 0.00001$	ns
Father mount mother	ns	U = 150; P = 0.06	ns	ns
Sexual presenting by mother	NI[1]	NI[1]	ns	$U = 167.5$; $P = 0.09$
Father groom mother	ns	ns	$U = 213.5$; $P = 0.055$	$U = 155.5$; $P < 0.05$
Mother groom father	ns	ns	ns	$U = 137$; $P < 0.05$
Huddling father-mother	$U = 195.5$; $P < 0.01$	$U = 123.5$; $P < 0.05$	$U = 191.5$; $P < 0.05$	$U = 131$; $P < 0.05$
Father aggresses mother	ns	ns	ns	ns
Mother aggresses father	ns	ns	ns	ns
Father-daughter				
Father sniff daughter	NO[2]	ns	$U = 191$; $P < 0.05$	ns
Father mount daughter	NO[2]	NO[2]	NO[2]	–[3]
Sexual presenting by daughter	NI[1]	NI[1]	NO[2]	NO[2]
Father groom daughter	$U = 170$; $P < 0.005$	ns	ns	ns
Daughter groom father	ns	ns	$U = 143$; $P < 0.001$	ns
Huddling father-daughter	ns	ns	ns	ns
Father aggresses daughter	ns	ns	ns	ns
Daughter aggresses father	ns	ns	ns	ns
Mother-daughter				
Mother groom daughter	ns	ns	ns	ns
Daughter groom mother	ns	ns	ns	ns
Huddling mother-daughter	$U = 177.5$; $P < 0.005$[4]	ns	$U = 215.5$; $P = 0.06$	ns
Mother aggresses daughter	ns	ns	$U = 225$; $P = 0.09$	$U = 150$; $P < 0.05$
Daughter aggresses mother	ns	ns	ns	$U = 144$; $P < 0.05$

[1] Behaviour was not investigated (see, *Methods*).
[2] Behaviour was never seen during observations.
[3] Father initiated mounts on daughter twice.
[4] N1 = 27; N2 = 26 (applies to all interactions between mother and daughter of group 1).

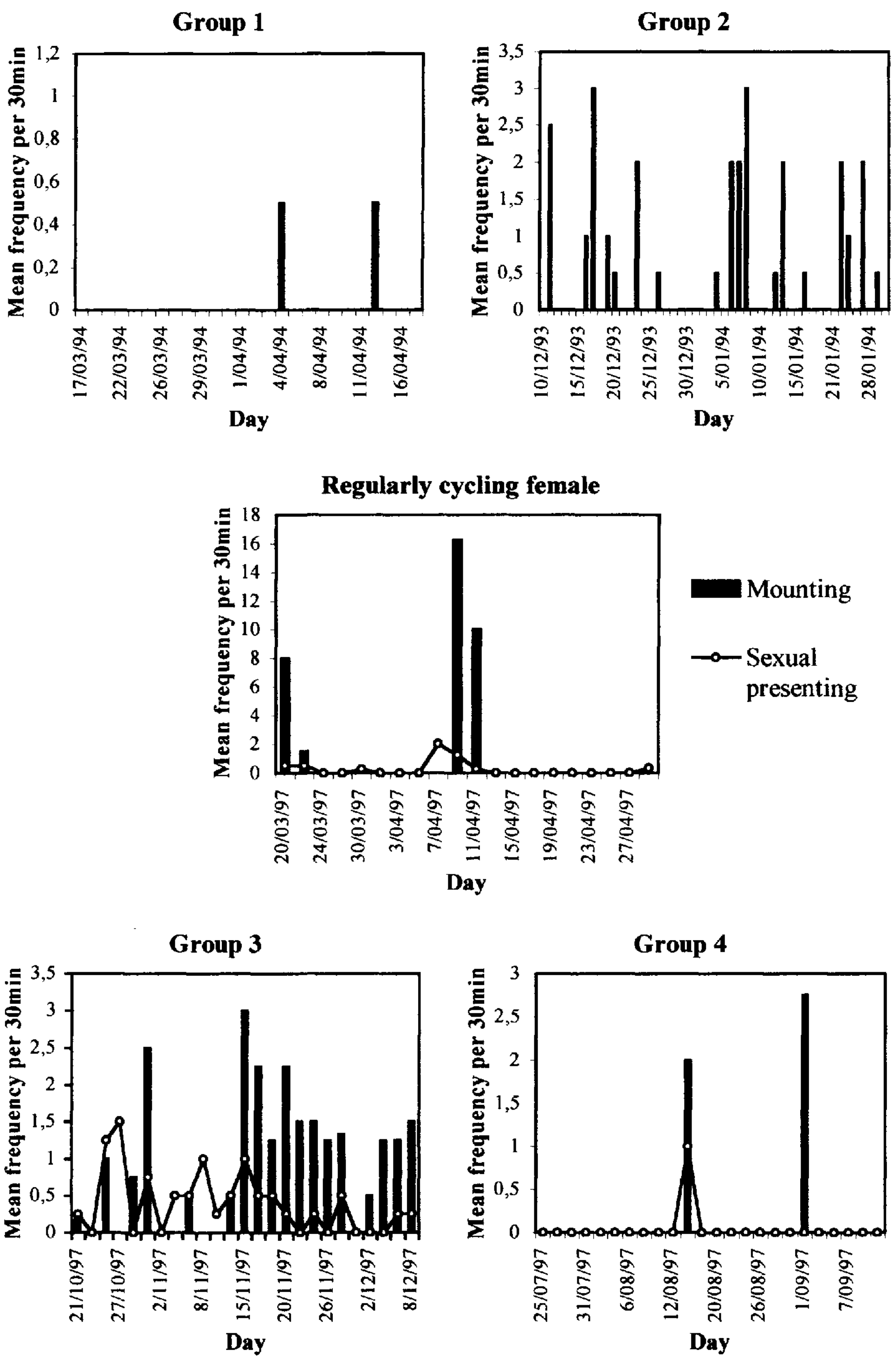

Figure 2 **Distribution of sexual interactions over time, before (central graph) and after (peripheral graphs) implantation of the mother. Note that the scales differ between graphs.**

occurred once during the control period and twice during the implant period. Only in group 3 did anogenital sniffing increase significantly during the implant period (control = 0.05 ± 0.03 vs implant = 0.21 ± 0.05, $P < 0.05$, see Table 3) – but these frequencies were still much lower than those observed for the father and the mother of this group. Mountings or copulations between the father and the oldest daughter were never observed in groups 1, 2 and 3, while the father of group 4 mounted his daughter on two occasions during the implant period. Sexual presenting by the daughter was never observed.

Grooming interactions did not differ between control and implant periods in most groups, except for group 1 where the father groomed his daughter less (control = 0.62 ± 0.15 vs implant = 0.19 ± 0.08, $P < 0.005$); and in group 3 where the daughter groomed her father more (control = 0.02 ± 0.01 vs implant = 0.34 ± 0.09, $P < 0.001$) during the implant period (Table 3). Neither huddling frequencies nor aggressive interactions differed between control and implant periods in any of the groups.

Social interactions between the mother and the oldest daughter

These data are summarized in Table 3. Grooming frequencies did not differ in any of the groups. Huddling between the mother and her daughter significantly increased in group 1 (control = 1.34 ± 0.5 vs implant = 3.79 ± 0.85, $P < 0.005$), and showed a trend to decrease in group 3 (control = 0.88 ± 0.27 vs implant = 0.3 ± 0.17, $P = 0.06$). The frequencies of aggressive interactions between the mother and her oldest daughter did not differ after implantation in groups 1 and 2. Aggression of the mother towards her daughter showed a trend to decrease after implantation in group 3 (control = 0.15 ± 0.06 vs implant = 0.01 ± 0.01, $P = 0.09$). Aggressive interactions of the mother towards her daughter and vice versa decreased significantly after implantation in group 4 (mother aggresses daughter: control = 0.39 ± 0.09 vs implant = 0.15 ± 0.07, $P < 0.05$; daughter aggresses mother: control = 0.13 ± 0.04 vs implant = 0.02 ± 0.01, $P < 0.05$).

Sexual interactions between the oldest daughter and sons

Anogenital sniffing by sons was observed very rarely. During the control period, anogenital sniffing by the oldest son was seen four times on one day in group 1 and once in group 4, but was never observed during the implant period. In group 2 the daughter was sniffed twice by two different sons during the implant period, while the daughter of group 3 was sniffed once during the implant period. Only in group 2 did we observe copulation between the oldest daughter and the oldest son, prior to implantation of their mother. In the other groups, sexual interactions were never observed.

Marking behaviour of the father, mother and oldest daughter

Table 4 presents data on the changes in frequencies of marking behaviours. These did not show any consistency between groups and/or individuals. In group 1, the mother decreased her rates of circumgenital marking (control = 2.33 ± 0.42 vs implant = 0.8 ± 0.22, $P < 0.01$). In group 2, the father decreased his rates of both sternal and circumgenital marking (sternal marking: control = 4.44 ± 1.03 vs implant = 1.50 ± 0.31, $P < 0.005$; circumgenital marking: control = 3.89 ± 0.95 vs implant = 2.05 ± 0.36, $P = 0.051$). In group 3, the mother increased sternal marking (control = 0.58 ± 0.22 vs implant = 0.88 ± 0.2, $P = 0.054$). In group 4, the male increased his rates of sternal and circumgenital marking (sternal marking: control = 0.43 ± 0.28 vs implant = 0.92 ± 0.16, $P < 0.005$; circumgenital marking: control = 0.63 ± 0.21 vs implant = 1.06 ± 0.16, $P < 0.05$); the daughter increased her rates of sternal marking

Table 4 **Results of the statistical analysis, comparing the frequencies of marking behaviours between control and implant periods for the four study groups. (ns – not significant.)**

Behaviour	Group 1 N1 = 27; N2 = 25	Group 2 N1 = 9; N2 = 42	Group 3 N1 = 25; N2 = 25	Group 4 N1 = 20; N2 = 24
Sternal marking:				
Father	ns	$U = 77$; $P < 0.01$	ns	$U = 107.5$; $P < 0.005$
Mother	ns	ns	$U = 213$; $P = 0.054$	ns
Daughter	ns	ns	ns	$U = 101.5$; $P < 0.005$
Circumgenital marking:				
Father	ns	$U = 110$; $P = 0.051$	ns	$U = 132.5$; $P < 0.05$
Mother	$U = 191.5$; $P < 0.01$	ns	ns	$U = 170.5$; $P = 0.1$
Daughter	ns	ns	ns	ns

(control = 0.18 ± 0.07 vs implant = 0.83 ± 0.14, $P < 0.005$); and the mother showed a trend for increased circumgenital marking (control = 1.4 ± 0.29 vs implant = 2.17 ± 0.33, $P = 0.1$).

Discussion

Based on the results from a worldwide survey, combined with an endocrinological and behavioural study it can be concluded that:

i) MGA implants prevent pregnancy through the suppression of ovulation. This confirms the findings reported by Van Elsacker *et al* (1994).

ii) Sexual interactions between the breeding adults generally still occur after MGA implantation, but detailed data show that the distribution of these interactions over time is affected. Grooming interactions or aggressive interactions do not alter in a predictable way after implantation.

iii) The use of MGA implants in mothers does not seem to induce reproduction in their daughters. Endocrinological data did not reveal any changes in the ovarian cycles of daughters before and after implantation of the breeding females. Detailed observations show that implantation of the mother does not increase the frequency of sexual interaction between a father or any sons present and the oldest daughter. Affiliative or aggressive interactions between the father, the mother and the oldest daughter do not alter consistently for all groups after implantation.

iv) The data on other contraceptive methods used in females are very limited and do not provide a uniform picture.

v) The limited information available for males suggests that epididiectomy does not abolish the occurrence of sexual interactions, while vasectomy and castration do.

The results from the detailed behavioural study indicate that MGA implantation does induce changes in the behaviour of individual group members, but these changes did not occur in a consistent, predictable way in the four study groups. Unfortunately, the heterogeneity of the control periods and the small sample size precluded a thorough comparison of the results and the formulation of any firm conclusions. However, this study does provide the first detailed information on combined behavioural and endocrinological effects in MGA-implanted golden-headed lion tamarins. Behavioural effects have been investigated in only a few primate and non-primate species (eg Rodrigues fruit bats, *Pteropus rodricensis* [Hayes *et al* 1996]; hamadryas baboons, *Papio hamadryas* [Portugal & Asa

1995]). Similarly, apart from the preliminary results on golden-headed lion tamarins (Van Elsacker *et al* 1994), endocrinological effects of MGA implantation have only been reported for common marmosets (Möhle *et al* 1999).

The most profound changes seemed to occur in the distribution of sexual interactions over time. Two patterns were apparent, either: i) absolute mounting frequencies increased and mounting occurred on many more days than during a regular ovarian cycle; or ii) the reverse pattern emerged, in which mounting occurred at a lower frequency but without the normal interval of approximately 20 days. It is interesting that in the two groups in which female sexual presenting was scored, the frequencies of male mounting seemed to depend on the frequencies of sexual presenting by the females. De Vleeschouwer *et al* (1998; 2000b) have shown that female sexual presenting increases during the follicular phase of a regular ovarian cycle and that this also coincides with an increase in male mounting. Although oestrogen levels in the females remained consistently low, apparently the interdependence of female sexual presenting and male mounting still exists after implantation of the breeding female – at least in our groups 3 and 4. The data obtained from the world survey do not seem to contradict this. Sexual presenting and/or copulation still occurred in several females. (Obviously, there are some problems with the accuracy of the data the institutions can provide: whether sexual interactions are reported is likely to depend on the time available to those involved in the management of the animals to observe and record the occurrence of such interactions.)

The changes in social-sexual behaviours following MGA implantation did not seem to affect the reproductive inhibition of female offspring. Similarly, oestrogen levels and the occurrence of regular ovarian cycles in the oldest daughters did not differ before and after implantation of their mothers. The survey's results showed that the number of female offspring reproducing in their natal group was extremely low. Only 2 out of 25 female offspring theoretically capable of reproducing during their mothers' implantation bouts actually commenced reproduction. Reproduction by subordinate female golden-headed lion tamarins in regularly breeding groups in captivity is rare, but it does occur sometimes (Price 1998; Chaoui & Häsler-Gallusser 1999; De Vleeschouwer 2000). The factors that determine whether subordinate females escape from the mechanism of reproductive inhibition are as yet unknown. Given that breeding by subordinates occurs only rarely – both in regularly breeding and non-breeding groups – it is unlikely that this is solely caused by contraception of the breeding female. Thus, although breeding in subordinate females will probably create management difficulties (De Vleeschouwer 2000), this is a general problem, unrelated to the use of contraception in itself.

In principle therefore, MGA implants can be used in golden-headed lion tamarins without the danger of producing inbred infants, since there seems to be no breakdown in the mechanism of reproductive inhibition. However, changes in behaviour do occur. It is as yet unknown whether these may have an impact on group structure in the long term. Group stability may be lower in non-breeding groups of golden-headed lion tamarins, as opposed to other callitrichids (Price 1997). This may, however, be influenced by factors such as group size, age composition and sex ratio. Given the close interdependence of social organization and infant care in callitrichids, the simple fact that infants are no longer produced may also have an impact on group stability in the long term. Offspring residing in their natal group, and thereby foregoing their own reproduction, no longer reap the benefits usually associated with this strategy (ie sibling rearing experience and a gain in inclusive fitness). The consequences of this may only be apparent after a prolonged period of contraception. The possible impacts of contraception on long-term group stability, including the impact of group size and other factors, is assessed in De Vleeschouwer (2000).

Due to the small sample size, little can be said about the impact of other contraceptive methods on the occurrence of sexual interactions and reproduction in subordinate animals. Normally one would not expect the disappearance of sexual behaviour following vasectomy or tubular ligation. Nevertheless, the two males and one female which underwent these operations were no longer seen to copulate or present for mating. Occurrence of sexual behaviour in the hysterectomized female was reported to be unknown. As stated earlier, the comprehensiveness/accuracy of the data obtained from the different institutions may be a problem. Clearly, more data are needed before any firm conclusions can be formulated. Therefore, it is difficult to assess the suitability of these methods for golden-headed lion tamarins at present. In principle, however, the permanent infertility associated with these methods makes them suitable only if one is absolutely certain that the animal will never be required to breed again. In such cases, sterilization is likely to be preferable to castration or ovariohysterectomy, because of the potentially high impact of the latter contraceptive procedures on the behaviour of the animals involved.

Animal welfare implications

Whether using MGA implants as a population control method is acceptable from a behavioural point of view, will ultimately depend on the desires of the institution involved and the objectives of their conservation breeding programme. The changes in behaviour demonstrated in this study may be acceptable to some institutions but not to others. One also has to keep in mind that this study did not investigate the entire behavioural repertoire of golden-headed lion tamarins. One class of important behaviour patterns to disappear completely is that related to infant care. The potential to participate in infant care is likely to contribute to the well-being of all group members since alloparental care is a prominent feature of callitrichid social systems. It may be more beneficial, from a welfare point of view, to allow reproduction while controlling population growth through other means (for more details see De Vleeschouwer *et al* [2000a]). Effectiveness, reversibility, medical effects and effects on group stability also need to be taken into account. Finding the most suitable contraceptive method for this, and other, species will involve finding an appropriate balance between all of these factors.

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