FACULTEIT DIERGENEESKUNDE
accredited by EAEVE

CONTRACEPTION TECHNIQUES IN NON-HUMAN PRIMATES IN THE CONTEXT OF SHELTER AND REHABILITATION

Word count: <8.656>

Celine Lemmens
Student number: 01201642

Supervisor: Prof. Dr. Ann Van Soom
Supervisor: Drs Cyrillus Ververs
Supervisor: Drs Hester van Bolhuis

A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Veterinary Medicine

Academic year: 2017 - 2018

UNIVERSITEIT
GENT

Preface

Wildlife medicine and conservation are my dearest passions. I believe it is extremely important to gain more knowledge in those fields. Not only to help animals in need and save them from extinction, but moreover to help humanity. The fading of borders between animals and humans, creating 'one world, one health', gives a new multidisciplinary approach towards health issues. By looking at animals, especially non-human primates, human health issues can be solved. Therefore, I am very grateful Stichting AAP gave me the opportunity to collaborate in writing my thesis.

I would like to give a special thanks to my supervisor, Cyriel Ververs, for his excellent guidance. He was a committed and inspiring coach with lots of experience in the field of wildlife. Moreover, I would like to thank prof. Ann van Soom and Jella Wauters from Ghent University for their cooperation.

Special thanks go to my supervisor at Stichting AAP, Hester van Bolhuis, who provided me with plenty of data, experiences and advice to accomplish my thesis. Her stories and the visits to Stichting AAP in Almere, encouraged my future dream to contribute in wildlife medicine and conservation. Additionally, I would like to thank all of the people from Stichting AAP who were involved in my study. Furthermore, I would like to thank Martine Van Zijll Langhout and Marno Wolters for their rides to Almere and the exchange of knowledge. Moreover, I wish to thank all of the respondents to my survey, without whose cooperation I would not have been able to conduct this dissertation.

Last but not least my parents, family and friends deserve a particular note of thanks. Their unconditional support and love kept me motivated.

I hope you enjoy your reading.

Table of Contents

ABSTRACT

Contraception is crucial in the management of zoo species. Implanon® (etonogestrel) is a widely used progestin based contraceptive in non-human primates. However, little is known about reversibility, side effects and adequacy creating reluctance to use the product. Moreover, the permanent surgical contraception techniques, castration and vasectomy, do not have widespread fixed protocols per primate species or family.

Therefore, we conducted a retrospective survey on 206 non-human female primates contracepted with Implanon® at Stichting AAP over the past 10 years. We questioned the zoos and reserves, where to primates were outplaced, and monitored implanted primates that stayed at AAP. Moreover, we mapped complications during and after vasectomy and castration performed at AAP in 88 male non-human primates.

Twenty out of 65 outplaced primates received prolonged Implanon® treatment. None of them showed signs of oestrus or pregnancy during therapy, which is indicative for the efficacy of Implanon®. In 27% of the outplaced primates oestrous signs were detected and only three out of 48 primates became pregnant after treatment. In total, only two out of six pregnancies resulted in healthy life young. Further research is indicated to investigate the link between the low results in fertility and the use of Implanon®. In 17 out of 30 post-mortem examinations, abnormalities of the genital tract could be found, i.e. endometritis, leiomyoma and sarcoma. Therefore a link between deviations of the genital tract and the use of Implanon® is suspected.

This retrospective survey indicated that castration is a safe permanent contraception technique, especially when monofilament resorbable suture material (Monocryl) is used. Furthermore, high (± 40%) complication rate after vasectomy with yet unknown origin was noticed. This is the first record of chronic fistulae after vasectomy in male macaques which seemed to be related to the use of polyfilament resorbable suture material (Vicryl) and could be prevented by using monofilament resorbable suture material. Moreover, small technical procedures are mentioned, which can be added to the vasectomy protocol of the addressed primate species to reduce complications.

In short, this survey is a contribution to the evaluation and optimization of effective contraception techniques in primates.

Samenvatting

Contraceptie is belangrijk in het beheer van species in de dierentuin. Implanon® (etonogestrel) is een wereldwijd gebruikt progestageen contraceptiemiddel in niet-humane primaten. Er is echter weinig gekend over reversibiliteit, bijwerkingen en vruchtbaarheid. Hierdoor is men bang om het product te gebruiken. Vasectomie en castratie, permanente chirurgische contraceptietechnieken, hebben daarnaast geen wijdverspreid, standaard protocol per primatensoort of -familie.

Om deze reden hebben we een retrospectieve studie uitgevoerd op 206 vrouwelijke niet-humane primaten die Implanon® hebben ontvangen bij Stichting AAP over de voorbije 10 jaar. We hebben dierentuinen en reservaten ondervraagd die de primaten hebben opgevangen en de primaten met een implantaat bij AAP gemonitord. Verder hebben we complicaties tijdens en na vasectomie en castratie opgevolgd in 88 mannelijke niet-humane primaten.

Twintig van de 65 uitgeplaatste primaten ontvingen een aanhoudende Implanon® behandeling. Geen enkele primaat toonde tekenen van oestrus of zwangerschap gedurende de behandeling. Dit is indicatief voor de werkzaamheid van Implanon®. Bij 27% van de uitgeplaatste primaten werden er tekenen van oestrus gezien en maar drie van de 48 primaten werden zwanger na behandeling. Van de zes zwangerschappen resulteerden er slechts twee in gezonde levende nakomelingen. Verder onderzoek is nodig om de link tussen deze lage resultaten in vruchtbaarheid en het gebruik van Implanon® te bestuderen. In 17 van 30 autopsieën werden abnormaliteiten aan het genitaal stelsel gevonden, o.a. endometritis, leiomyoma en sarcoma. Om deze reden wordt er een link verwacht tussen afwijkingen aan het genitaal stelsel en het gebruik van Implanon®.

Deze retrospectieve studie over castratie duidde aan dat castratie een veilige permanente contraceptietechniek is, vooral wanneer monofilament, resorbeerbaar hechtmateriaal (Monocryl) wordt gebruikt. Verder zien we een hoge graad (±40%) van complicaties na vasectomie met een vooralsnog ongekende oorzaak. Dit is de eerste vermelding van chronische fistels na vasectomie bij mannelijke makaken. Dit leek gerelateerd te zijn aan het gebruik van polyfilament, resorbeerbaar hechtmateriaal (Vicryl) en kon vermeden worden door monofilament, resorbeerbaar hechtmateriaal. Verder worden er technische handigheden vermeld die kunnen worden toegevoegd aan het vasectomie protocol om het aantal complicaties te reduceren.

Kortom, deze studie is een bijdrage aan de evaluatie en optimalisatie van effectieve contraceptietechnieken in primaten.

1 LITERATURE REVIEW

As EGZAC (EAZA Group on Zoo Animal Contraception) stated: ‘‘Wildlife contraception is an important tool in the genetic and demographic management of zoo species’’. Effective contraception is employed to prevent uncontrolled population growth in captivity and reduces inbreeding. Contraception methods also help to diminish inappropriate sexual behaviours and aggression, and decrease the physiological demands due to pregnancy on preferential individuals.[1]

1.1 THE ORDER PRIMATES

This study will focus on contraception in non-human primate species. The taxonomy of the order Primates is depicted in table 1 (Burvenich, 2014).

Table 1. Taxonomy of the order Primates

Strepsirhini (wet-nosed primates)	Lemurs, lorises, pottos..
Haplorhini (dry-nosed primates)	Tarsiers
	New World monkeys (e.g. squirrel monkey)
	Old World monkeys (e.g. rhesus macaque)
	Lesser apes (i.e. gibbons and siamangs)
	Great apes (i.e. orangutans, gorillas, chimpanzees, and bonobos)
	Humans

The order primates includes an enormous range of species with a wide variability in reproductive characteristics (see table 2: Reproductive parameters for representative primate species). Nonetheless, primates as a group are characterized by long lifespans, delayed reproductive maturation, low fecundity, and high investment in each offspring (Zimmermann & Radespiel, 2007). Like humans, the *Haplorhini* primates except for the tarsiers, do not show a limited period of sexual receptivity (oestrus). Those primates are continuously sexually active with a slight periovulatory enhancement, the so called ‘flattening of sexual activity’ (Burvenich, 2014). An other special feature of the sexual cycle of primates is the menstrual cycle which *Haplorhini* primates (except for the tarsiers) undergo. Moreover females of many species display cyclic changing in coloration and tumescence of the external genitalia and so-called sexual skin, which peaks periovulatory. The changes aim to enhance their attractiveness to males and are thought to play a key role in sexual selection (Dixson, 1998).

In this study we focus on the primate species frequently sheltered at the rescue and rehabilitation centre, AAP (Animal Advocacy and Protection) (the Netherlands), in particular Barbary macaques *(Macaca sylvanus)*, long-tailed macaques *(Macaca fascicularis)*, rhesus macaques *(Macaca mulatta)*, chimpanzees *(Pan troglodytes)* and common marmosets *(Callithrix jacchus).*

[1] http://www.egzac.org/. Last consulted: 8-5-‘18

Table 2. Reproductive parameters for representative primate species (redrawn from: Norris and Lopez, 2010)							
Species	Age at sexual maturity-males (days)	Age at sexual maturity-females (days)	Ovarian cycle length (days)	Menstruation	Birth seasonality	Gestation Length (days)	Modal litter size
Macaca fascicularis **(long-tailed macaque)**	1544	1238	29,4	Overt	Weak	164	1
Macaca fuscata **(Japanese macaque)**	1369	1483	28	Overt	Strong	173	1
Macaca mulatta **(rhesus macaque)**	2,007	1,231	26,6	Overt	Strong	165	1
Pan troglodytes **(chimpanzee)**	2920	3376	37,3	Overt	Weak	238	1
Callithrix jacchus **(common marmoset)**	382	477	28,6	Absent/ covert	Weak/ bimodal	148	2
Lemur catta **(ring-tailed lemur)**	912	595	39,3	Absent/ covert	Strong	135	1
Saguinus oedipus **(cotton-top tamarin)**	550	548	22,7	Absent/ covert	Weak	168	2
Cebus paella **(brown capuchin)**		1703	20	Slight	Weak	154	1
Papio hamadryas **(hamadryas baboon)**	1762	1514	30	Overt	Weak	170	1

1.2 The endocrine system

The hypothalamic-pituitary-gonadal axis regulates the reproduction cycle in both sexes. Gonadotropin releasing hormone (GnRH) triggers the release of follicle-stimulating- and luteinizing hormone (FSH and LH) by the adenohypophysis. Follicle-stimulating hormone regulates folliculogenesis and the production of oestrogen in females and plays an important role in the development of gonads in males. Luteinizing hormone coordinates steroid hormone production and triggers ovulation. A notable exception to this mechanism can be seen in some New World monkeys (e.g. common marmoset) in which the hypophysis secretes chorionic gonadotropin (CG) instead of LH (Norris and Lopez, 2010). Oestrogen is produced in the ovary, placenta, testes and adrenal cortex. It is the primary female sex hormone. It causes development (e.g. secondary sexual characteristics) and regulation of the female reproductive system and peaks periovulatory. Progesterone is produced in the corpus luteum, placenta and adrenal cortex and is high during gestation. Steroid hormones with a progesterone-activity are called gestagens. Progesterone is involved in the initiation and maintenance of gestation and embryogenesis. Testosterone is the primary sex hormone in males. It is produced in the testis of males and, to a lower extent, in the female ovary. It plays a key role in the development of the male reproductive system (Davidson and Stabenfeldt, 2007).

1.3 Contraception techniques

Since the late 90s several contraception techniques have been developed for use in male and female primates. In females surgical techniques like ovariectomy, ovariohysterectomy and tubal ligation are effective for permanent contraception. Temporary or reversible contraception techniques are another option compromising a large amount of products as depicted in table 3.[1]

Table 3. Temporary or reversible contraception techniques in female primates[1]

Temporary contraception technique	Product	Active ingredient	Method of administration
Progestins	Birth control pill (possibly plus **oestrogens**) e.g. Norgeston®		Oral
	Microgynon® 30	Levonorgestrel Ethinylestradiol + levonorgestrel	
	Ovarid/Megace®	Megestrol acetate	Oral
	Implanon/Nexplanon®	Etonogestrel	Implant
	Norplant®	Levonorgestrel	Implant
	Mirena®	Levonorgestrel	Intra-uterine device
	Depo-Provera®	Medroxyprogesterone acetate	IM injection
	Delvosteron®	Proligestonum	SC injection
GnRH agonists	Suprelorin®	Deslorelin	Implant
	Lupron	Leuprolide acetate	Depot injection
GnRH antagonists	Acyline		Oral or SC injection
Anabolic steroids	Cheque drops	Mibolerone	Oral
Immunocontraceptive methods (anti-GnRH vaccines)	Native PZP vaccine	Derivative of porcine zona pellucida proteins	IM injection
	Improvac®	GnRH protein conjugate	SC or IM injection
Copper bearing devices	Flexi-T®	Copper	Intra-uterine device

In males castration and vasectomy are used in permanent contraception. As for females temporary contraception can be achieved with the use of hormone based products like progestins, GnRH agonists (e.g. deslorelin (Suprelorin®)), GnRH antagonists (acycline) and anti-GnRH vaccins (Improvac®, PZP vaccins). In addition, spermatogenesis inhibitors like bisdiamine (Fertilysin™), immunocontraceptives like sperm antigens (Eppin) and a recently discovered Vasalgel™ (Colagross-Schouten et al., 2017) seem to be effective[1].

1.4 Contraception policy

To meet the requirements mentioned in the introduction, Stichting AAP follows a strict contraception policy since 2013. All animals who are sheltered will be permanently contracepted. In order to preserve the sexual behaviour of primate species, vasectomy in males and tubal ligation in females is performed. Species selected to be managed under an EEP (European Endangered Species Programme) or ESB (European Studbooks) programme are excepted from this policy; males are left untreated and females

are treated with etonogestrel (Implanon®). Species which are considered too small for tubal ligation (e.g. common marmoset) are gonadectomised or receive no treatment. *Papio hamadryas* (hamadryas baboon) is ovariectomised in order to prevent hormone concentrations which result in huge swelling and cysts of the perineal area.

In this thesis we focus on **2 contraception techniques**, reversible as well as permanent. We carried out retrospective surveys on Implanon® in females and on permanent surgical contraception in males.

1.5 IMPLANON®

Etonogestrel (Implanon®, N.V. Organon) is a progestin regularly used as a temporary contraceptive in female primates. Etonogestrel inhibits ovulation, thus suppressing oestrus and prevents spermatozoa passage by influence on cervical mucosa. At AAP, etonogestrel 68mg is inserted subcutaneously in the (inner) upper arm around the age of 4 years. The dose is taxon specific, depending on the weight of the animal. In general, ovulation is inhibited 1 day after insertion preferably on day 1-5 of the ovarian cycle. As the right stage during ovarian cycle is often unknown, it is advised to use other contraceptive methods for at least 7 days after insertion of the implant. The implant is removed and replaced by a new one in the other arm after 2,5 to 3 years. In non-human primates progestins do not interfere with parturition in late pregnancy. However, it is contra-indicated to use etonogestrel during lactation as the hormone gets excreted in the milk. Some side effects are possible weight gain and increased or decreased frequency of bleeding during menstruation[1]. In the zoo community there is caution and reluctance to contracept endangered primate species with Implanon®. There is fear of non-reversibility after removal or elaboration of the operation and failure during contraceptive treatment. This concern is probably based on several case-reports of etonogestrel failure in humans and one report of failure in koalas (Bensouda-Grimaldi et al., 2005; Harrison-Woolrych and Hill, 2005; Hynes et al., 2010). Moreover, in humans there are reports of complications like intravascular migration of the subdermally placed implant (D'Journo et al., 2015; Kew et al., 2017), unscheduled vaginal bleeding (Zigler and McNicholas, 2017) and links between progestin-based anticonceptives and depression (Skovlund et al., 2016). However other studies argue etonogestrel has a great efficacy (Agrawal and Robinson, 2005; Funk et al., 2005; Adams and Beal, 2009; Darney et al., 2009; Brunson et al., 2017). A recent study shows that the etonogestrel 68mg implant in humans is effective for 4-5 years instead of 3 years which is assumed in primate species (McNicholas et al., 2017). There are only very few studies in primates due to reluctance to use etonogestrel. One study investigated the effect on the behaviour of Barbary macaques, showing etonogestrel can result in increased aggression, more self-scratching and more time self-grooming, demonstrating anxiety (Maijer and Semple, 2016). In 2002 on the scientific meeting of the European Association of Zoo and Wildlife Veterinarians (EAZWV) Goodman presented a retrospective survey on the use of Implanon® in three female chimpanzees at Edinburgh zoo[2]. No swelling or mating was observed during Implanon® treatment. However the duration of efficacy could not be determined accurately. To increase knowledge EGZAC started collecting information in a database concerning the use of contraceptives in captive wildlife within Europe. With the current study we try to help them in accomplishing this goal.

1.6 SURGICAL CONTRACEPTION TECHNIQUES

Surgical contraception techniques like **vasectomy and castration** are permanent techniques in male individuals. Vasectomy (in human) is sometimes considered to be a reversible technique, however this is still controversial. Depending on the species after the vasectomy technique the individual will be infertile after 2 months or more[1]. In a survey of Harrison et al. in 1977 in rhesus monkeys, no complications were observed after vasectomy. However in bonnet monkeys and macaques complications were described. These included orchitis, epididymitis, surgical site infection, epididymal

[2] http://www.ivis.org/proceedings/eazwv/2002/Repro/Repro.pdf#nameddest=3. Last consulted: 13-5-'18

and ductal granulomas and even atherosclerosis due to constant sperm antigen leakage causing immune complex formation (Alexander, 1981; Clarkson and Alexander, 1980; Seppan and Krishnaswamy, 2014; Ekanayake-Alper, 2018). Little research about the best method and technique per primate species has been performed.

The SmithKline Beecham Pharmaceuticals Laboratory for Animal Science described a conventional surgical technique for vasectomy in common marmoset (Morris and David, 1993). In this technique one ventral midline incision cranial to the scrotum was made (figure 1). The testes were dissected, exteriorized and doubly ligated at the ductus deferens and the portion between the ducts was excised. The tunica vaginalis was not closed. Braided nylon 3/0 non-absorbable suture material was used to ligate the ducts. Polyglactin suture material should not be used as it gave rise to recanalization. The wound was closed with a simple subcutaneous mattress sutures using polyglactin suture 3/0. Additionally, the free edges of the incision were closed with surgical adhesive. No post-operative analgesia or antibiotics were required. In 2012 a new minimal invasive method of vasectomy in rhesus macaques was recorded, namely no-scalpel vasectomy by elektrocauterization (Kumar and Raj, 2012). A single prescrotal skin incision above the median raphae was made with a cautery pencil. The spermatic cord was exteriorized, vaginal tunic was opened and the ductus deferens was separated from the plexus pampiniformis. Two location 3-4 cm apart of the ductus deferens were cauterized at 60 watt current and resected. No sutures were applied to close the wound. Post-operative care was provided by injectable antibiotics and NSAIDS (enrofloxacin and meloxicam). No complications after surgery were noticed. The no-scalpel technique aims to reduce adverse events like hematomas, infection and pain and reduces the operation time compared to the conventional incision technique. A vasectomy technique in Drill Monkeys was described (Egbetade et al., 2014). A 2,5 – 3,0 cm cranio-caudal incision was made above the spermatic cord, which is externally palpable at the skin area cranial to the neck of the scrotum. The cord was elevated with the use of a curved forceps, exteriorized and the tunica vaginalis was incised. The ductus deferens was clamped at both ends and ligated. About 4 cm of the ductus in between the ligations was cut off. The tunica vaginalis was not closed. The skin incision was closed with simple interrupted intradermal buried stiches with 3/0 absorbable polyglactin suture material. Post-operative care was provided with long-acting amoxicillin, flunixin, aluminium spray and anti-tetanus toxoid. No post-op complications were encountered. Seppan and Krishnaswamy recorded another vasectomy technique in bonnet monkeys (Seppan and Krishnaswamy, 2014). A vertical incision of 1–2 cm over the scrotum was made. The spermatic cord was exposed and incised. The ductus deferens was gently squeezed to move the sperm from the incision site. The ductus deferens was ligated with the use of two ligatures at either end using 5/0 silk suture threads. The blood vessels were separated from the ductus deferens by using an operative microscope (22× magnifications). The intermittent segment of about 1 cm was excised. The wound was closed in layers. No antibiotic was administrated postoperatively. Analgesics were administered for a postoperative period of 2 days. No postoperative complications were experienced.

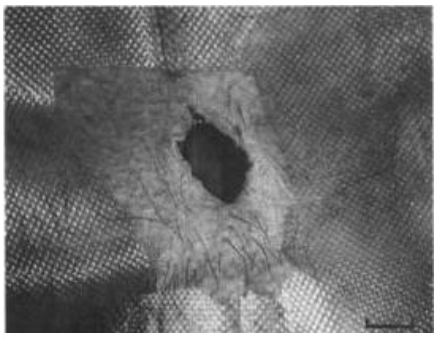

Figure 1. Ventral midline incision in the common marmoset.
From: Morris and David, 1993

Castration in non-human primates is successfully performed with the use of the open technique in macaques (Buyukmihci, 2017)[3]. The scrotum is incised and the testes exteriorized and excised after ligation of the spermatic cord. There is no scientific report of complications in the sex organs after castration in non-human primates.

[3] Buyukmihci, NC., 2017. Castration for Population Control of Macaques In a Sanctuary Setting. *UC Davis*. https://escholarship.org/uc/item/3cg050sp. Last consulted: 13-5-'18

In this study we try to map complications of surgical vasectomy and castration in various non-human primates performed at AAP. We link complications to technique and suture material.

In short, we try to make a contribution to the evaluation and optimization of effective contraception techniques in primates all over the world.

2 PROBLEM STATEMENT

Contraception in non-domesticated animals is an important method for population management. Implanon® (etonogestrel) is a widely used progestin based contraceptive in primates. Still, data on reversibility, side effects and efficacy are scarce. Therefore, in the zoo community there is caution to use the implant. Surgical techniques like vasectomy and castration are permanent contraception techniques. In some species complications like orchitis occur after vasectomy. However, in other species no complications were observed. There is no consensus in which method and technique is most applicable per species.

3 AIM

In this study we try to elucidate some of the previous contraception issues. By collecting data from female primates contracepted with etonogestrel by AAP and outplaced over the past 10 years, as well by monitoring primates with the implant who stayed at AAP, we hope to create more clarity about the effect and reversibility of Implanon®. Moreover, we will map from a retrospective follow up study, complications during and after vasectomy and castration performed at AAP in male non-human primates. We will try to determine the best technique and suture material and give recommendations to the used protocol in different primate families.

4 MATERIALS & METHODS

4.1 STICHTING AAP

Stichting AAP (Animal Advocacy and Protection) is a rescue and rehabilitation centre in the Netherlands, Almere. Stichting AAP tries to rescue primates and other exotic animals who are forced to live under poor conditions. Upon arrival, all animals go into quarantine and receive a thorough check-up by the vet. Unless solitary by nature, the animals are resocialised with conspecifics after the period of quarantine. As soon as possible the animals are outplaced to other zoos or reserves. At the outplacement address the animals are housed with fertile partners, nonfertile partners or species of the same sex. The above-mentioned contraception policy is used to preserve the sexual behaviour in the merged groups. This behaviour would not be preserved if gonadectomy was performed.

4.2 IMPLANON®

The first contraception technique we focused on is a retrospective survey on the use of etonogestrel (Implanon®) in female primates. As mentioned in the literature review Stichting AAP makes use of a strict contraception policy. However, species who are selected to be managed under an European Endangered Species Programme (EEP) or European Studboods Programme (ESB) are treated differently. Males are left untreated and females are treated with Implanon®. The size of the implant (1/4, 1/3, ½, 1) is dependent on the weight of the animal, < 1kg: ¼, 1 kg- 5kg: 1/3, 5kg-30kg: ½, >30 kg: 1 implant. With the use of data from Stichting AAP, which is closely monitored in the programme ZIMS, we analysed 141 female non-human primates who received Implanon® and were not outplaced to another zoo or institution. After 2.5 yr - 3 yr (chimpanzees) Implanon® was replaced and the expired implant removed. Table 4 gives an overview of the concerned primate species with their scientific and common name. We focused on the appearance of pregnancy and deviations of the genital tract on post-mortem examination when animals had died.

Table 4. Number of female primates (not outplaced) incorporated in the study population of Implanon®

Callithrix jacchus (common marmoset, callitrichidae)	12
Callithrix penicillata (black-tufted-ear marmoset)	2
Cercocebus lunulatus (white-naped mangabey)	1
Chlorocebus aethiops (grivet)	3
Lemur catta (ring-tailed lemur)	7
Macaca fascicularis (long-tailed/crap-eating macaque)	8
Macaca leonina (northern pig-tailed macaque)	2
Macaca nemestrina (sunda pig-tailed macaque)	4
Macaca sylvanus (barbary macaque)	68
Miopithecus ogouensis (northern talapoin monkey)	3
Papio anubis (olive baboon, Old World)	3
Papio hamadryas (hamadryas baboon)	2
Pan troglodytes (chimpanzee)	24
Papio ursinus (chacma baboon)	1
Saimiri sciureus (common squirrel monkey)	1
TOTAL	**141**

Moreover, we followed female primates who were outplaced after insertion of Implanon® at AAP over the past 10 years. We made use of a survey containing 18 questions. The survey was distributed with use of the programme SurveyMonkey®. The questions concerned follow-up, side-effects, oestrus signs and pregnancies during and after the use of Implanon®, implant removal and post-mortem evaluation

when animals had died. In the addendum you can find the template of the survey (addendum 1: Implanon® survey). We addressed 33 zoos or institutions that had received one or more female primates contracepted with Implanon®. We received 21 suitable responses compromising 65 primates. Table 5 gives an overview of the concerned primate species with their scientific and common name. Our respondents were located in Belgium, Denmark, Finland, France, Great Britain, Poland, Portugal, Romania, Spain, Switzerland and the Netherlands.

Table 5. Number of female primates (outplaced to zoos) incorporated in the study population of Implanon®	
Callithrix jacchus (common marmoset, callitrichidae)	3
Callithrix penicillata (black-tufted-ear marmoset)	1
Cebuella pygmaea (pygmy marmoset)	2
Eulemur fulvus (brown lemur)	2
Eulemur rufifrons (red-fronted brown lemur)	1
Lemur catta (ring-tailed lemur)	8
Macaca fascicularis (long-tailed/crap-eating macaque)	13
Macaca leonina (northern pig-tailed macaque)	5
Macaca mulatta (rhesus macaque)	9
Macaca nemestrina (sunda pig-tailed macaque)	1
Macaca sylvanus (barbary macaque)	12
Pan troglodytes (chimpanzee)	2
Saimiri sciureus (common squirrel monkey)	1
Saguinus oedipus (cotton-top tamarin)	4
Varecia rubra (red ruffed lemur)	1
TOTAL	**65**

4.3 Permanent surgical contraception

The second contraception technique we focused on in this survey is surgical contraception in male non-human primates. The study population consisted of different primate species that were presented to AAP , Rescue Center for Exotic Animals over a 3 year period (2014-2017). The different species were categorised in groups according to the performed surgical technique and contained Old World monkeys, Cebidae (capuchin monkeys, squirrel monkeys), Callitrichidae (marmosets, tamarins) and Prosimiae (lemurs). Table 6 gives an overview of the concerned primate species with their scientific and common name including their family.

We looked at two different techniques in 88 male primates. 78 primates were vasectomised (ligation and removal of a part of the ductus deferens) and 15 primates were castrated (removal of the testis, including 5 primates who were castrated after vasectomy).

4.3.1 Vasectomy

Stichting AAP makes use of a standardised protocol to perform vasectomy:

Preparation
Preparation for surgery was performed by shaving and cleaning the skin surface with Betadine® scrub (povidone iodine 7,5%, Meda Pharma B.V.) and Sterilium® (Hartmann). In Callitrichidae a Betadine® solution (povidone iodine 10%, Meda Pharma B.V.) could be used instead.

Surgical technique
A skin incision over and parallel to each spermatic cord was made. The spermatic cords were palpable on the ventral side of the pelvic bone cranial to the scrotum. A closed forceps was placed underneath the spermatic cord to dissect and exteriorize the ducti. Thereafter the tunica vaginalis was opened and separated from the plexus pampiniformis by blunt dissection. The ductus deferens was ligated at both ends and a small portion of the ductus was excised. The tunica vaginalis was closed in some species (see family protocol specifications and table 7). The closure of the subcutis and skin was family dependent (see family protocol specifications and table 7).

Old World monkeys and Cebidae
The ductus deferens was ligated at both ends with Monocryl Plus (poliglecapone 25, Ethicon) 3/0 or 4/0. 1,5 cm of the ductus was excised. If possible, the tunica vaginalis was closed with 1 or 2 Monocryl stitches. However, it could be left open. The subcutis was closed with continuous sutures of Monocryl Plus 3/0 or 4/0. The skin was closed intradermally with the same suture material.

Callitrichidae
The ductus deferens was ligated at both ends with Monocryl 5/0. 0,5-1,0 cm of the ductus was excised. The tunica vaginalis was not closed. The skin was closed with 1 or 2 (cross) stitches of Monocryl 5/0. No subcutaneous stitches were applied.

Prosimiae
The ductus deferens was ligated at both ends with Monocryl 5/0. 0,5-1,0 cm of the ductus was excised. If possible, the tunica vaginalis was closed with 1 or 2 Monocryl stitches. However, it could be left open. The subcutis was closed continuously and the skin intradermally with Monocryl 5/0.

Post-operative care
Aftercare was provided by meloxicam (Novacam®, AST Farma) 0,1 mg/kg PO for 5 days in all animals. Antibiotics were only indicated if complications occurred.

Table 6. Number of primate species incorporated in the study population of permanent surgical contraception in males

Cercocebus torquatus **(red-capped mangabey, Old World)**	1 (castration)
Cercopithecus mitis **(blue monkey, Old World)**	1
Cercopithecus mona **(mona monkey, Old World)**	1
Chlorocebus aethiops **(grivet, Old World)**	1
Chlorocebus cabaeus **(green monkey, Old World)**	1
Macaca fascicularis **(long-tailed/crap-eating macaque, Old World)**	11 (3 castrations)
Macaca fuscata **(Japanese macaque, Old World)**	11 (5 castrations)
Macaca mulatta **(rhesus macaque, Old World)**	19 (5 castrations)
Macaca radiata **(bonnet macaque, Old World)**	2
Papio anubis **(olive baboon, Old World)**	3
Papio hamadryas **(hamadryas baboon, Old World)**	13 (1 castration)
Callithrix jacchus **(common marmoset, callitrichidae)**	12
Cebus **(capuchin monkey, cebidae)**	4
Cebus olivaceus **(weeper capuchin, cebidae)**	1
Sapajus paella **(black-capped capuchin, cebidae)**	2
Lemur catta **(ring-tailed lemur, prosimiae)**	5
TOTAL	**88**

Adjustments
The described fixed protocol is in use since 2016. Before that time, Vicryl (polyglactin 910, Ethicon) suture material was used. In January 2016 Vicryl suture material was changed to Monocryl in Old World monkeys, Cebidae and Prosimiae. In August 2015 Vicryl was changed to Monocryl in Callitrichidae. Aluminium spray (Aluspray, 4% aluminium, Vetoquinol) was applied on the wound up to 2016.

In 2015 when male macaques showed chronic fistulae after vasectomy, the method was evaluated and the suture material and surgery technique were adjusted. The fistulae occurred when Vicryl suture material was used to ligate the ductus deferens and close the subcutis. In 3 out of 7 cases the fistulae were so severe the animal needed to be castrated. Therefore the suture material was changed to Monocryl after 2016 which resulted in less irritation at the surgery site.

We stopped applying Aluminium spray on Monocryl skin sutures as it would result in loosening of the sutures with dehiscence of the wound. Moreover, in Callitrichidae the tunica vaginalis was not closed as the knots of suture material provoked wound irritation.

Table 7. Variations in surgical vasectomy technique according to family

Ligation ductus deferens	Old world monkey/Cebidae	Monocryl Plus 4/0
	Prosimiae / Callitrichidae	Monocryl 5/0
Closure tunica vaginalis	Old world monkey/Cebidae/ Prosimiae	Yes
	Callitrichidae	No
Closure subcutis	Old world monkey/Cebidae	Continuous Monocryl Plus 3/0 - 4/0
	Prosimiae	Continuous Monocryl 5/0
	Callitrichidae	No
Closure skin	Old world monkey/Cebidae	Intradermally Monocryl Plus 3/0 - 4/0
	Prosimiae	Intradermally Monocryl® 5/0
	Callitrichidae	1 or 2 (cross) stitches Monocryl® 5/0

4.3.2 Castration

Castration is performed according to the following protocol:

Preparation
Preparation for surgery was performed by shaving and cleaning the skin surface with Betadine® scrub and Sterilium®. In Callitrichidae a Betadine® solution could be used instead.

Surgical technique
One midline skin incision rostral of the scrotum was made. The tunica vaginalis was incised at 1 side. The spermatic cord was ligated with Monocryl 3/0. The cord was cut and the testis removed. Thereafter the tunica vaginalis was closed with continuous sutures of Monocryl 3/0. This procedure was repeated at the other side. The subcutis was closed continuously and the skin intradermally with Monocryl 3/0.

Adjustments
Vicryl suture material was used until 2016. Since 2016 Monocryl was used instead.

5 RESULTS

5.1 IMPLANON®

5.1.1 Survey on not-outplaced primates at Stichting AAP

Health and fertility data of 141 female non-human primates that received Implanon® and were not outplaced to another zoo or institution were analysed. Since the beginning of our study 35 primates had died on which 27 autopsies have been performed. In 13 animals no abnormalities of the genital tract could be found. In the remaining others the main deviations included:

- 8 animals had a thickened and/or hyperaemic wall of the uterus often filled with red/white/brown fluid (exudative endometritis)
- 3 animals had a leiomyoma (white-naped mangabey, sunda pig-tailed macaque and chimpanzee)
- 1 animal had uterus adenomyosis (chimpanzee)
- 1 animal showed a corpus luteum on one of the ovaries indicating implant failure (common marmoset)
- 1 animal showed a severe peri-anal swelling with ulcerations on both sides, which turned out to be a tumor of fatty cells (Barbary macaque)

2 primates, a Barbary macaque and a common marmoset, gave birth to healthy young. The barbary macaque was on Implanon® but had lost the implant. The common marmoset was not on Implanon® when she had conceived. However Implanon® was inserted 10 days before she gave birth. She gave natural birth to two young without problems. However the day after a section caesarea had to be performed to give birth to a third young with a bent spine. Afterbirths were expelled. Unfortunately the common marmoset could not recover from the event and died three days after. On post-mortem examination the uterus showed a big lumen with patchy, dark red mucosa. Cervix and vagina were diffuse dark red. The skin and underlying abdominal muscles were focally ruptured. Through this defect a segment of the small intestine was herniated. On histology multifocal necrosis with accumulation of bacteria causing bacterial sepsis was found.

Moreover, one still-birth occurred in a hamadryas baboon. Implanon® was inserted 5 years before (not replaced after 3 years). She was found lying down in dark, haemorrhagic and foul smelling vaginal discharge with a death young in her arm. She ate the young shortly after. The reason for still-birth was not found and obviously no post-mortem examination on the foetus could be performed. The hamadryas baboon herself died 12 days after parturition. No post-mortem examination was performed. The differential diagnose included endometritis, hypothermia and possibly sepsis.

Table 8 gives a short overview of the most important findings.

Table 8. Overview of the most important Implanon® findings of the primates that were not outplaced and stayed at Stichting AAP	
Number of female non-human primates	141
Number of pregnancies	3 (2 primates died shortly after)
Number of still-births	1
Number of deviations of the genital tract on PM examination	14/27

5.1.2 Survey on outplaced primates

Health and fertility data of 65 female non-human primates who received Implanon® at AAP over the past 10 years and were outplaced to another zoo or institution were analysed. The Implanon® contraception was still effective or followed by etonogestrel (Implanon®/Nexplanon®) at the outplacement address in 20 primates. Thirty-one primates did not receive follow-up reversible contraception at the outplacement address. In 14 primates the state of contraception was unknown. None of the 20 primates who received prolonged Implanon® therapy at the outplacement address showed signs of oestrus or pregnancy during contraceptive treatment. Eleven of these females showed no side-effects during treatment. In 9 primates this data was unknown (see figure 2).

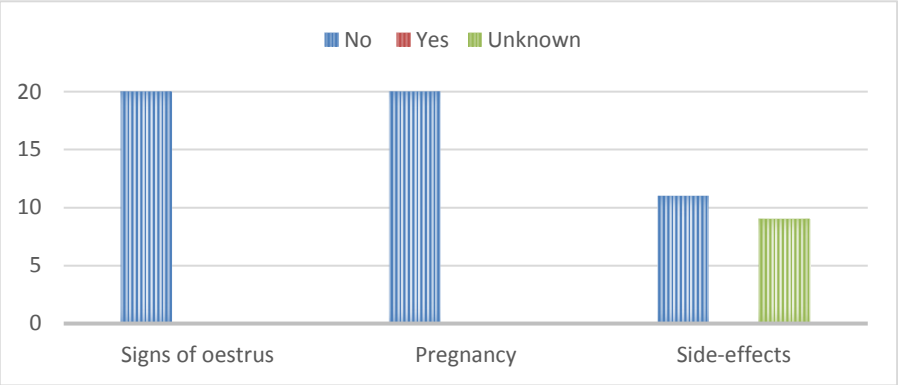

Figure 2. Fertility data during Implanon® treatment in 20 primates

Forty-eight primates were permanently or temporarily without contraception after Implanon® treatment. Table 9 shows an overview of the main fertility data after the Implanon® treatment.

Table 9. Fertility data after Implanon® treatment

Action		No of animals	Details
Implant removal		3	
Signs of oestrus	Absent	19	
	Unknown	16	
	Present	13	Very briefly (3x) Very randomly bleedings (5x) Only once than out of sight After 5y9m (2x) After 7m (implant was removed) After 1m (implant was removed)
Pregnancy	No, without fertile partner	26	
	No, with fertile partner	14	
	No, unknown if with fertile partner	1	
	Unknown	4	
	Yes, went well, live birth	1	Time between stop contraception and pregnancy = 3m (rhesus macaque)

	Yes, young died	2	Born alive, died later that day, implant was removed (time between stop contraception and pregnancy = 0-1m) (red ruffed lemur) Two sets of twins both to full term: first got stuck, died and was removed by vet. The second set was born alive but died shortly after (time between stop contraception and pregnancy = 1y3m/1y11m) (cotton top tamarin)

Since the beginning of our study 12 primates have died of which 3 autopsies have been performed. In all of the autopsies abnormalities of the genital tract could be found. The main deviations included:

- Endometrial hyperplasia, cystic mammary carcinoma (ring-tailed lemur)
- Endometrial stromal sarcoma with purulent, necrotic rearrangements (barbary macaque)
- Internal haemorrhages and bruises of the genital tract (cotton-top tamarin)

5.2 Surgical contraception techniques

In the group of male primates that were castrated or vasectomised 35 out of 88 primates showed a moderate to severe complication.

5.2.1 Castration

Fifteen out of 88 primates were castrated (removal of the testis), including five primates who were castrated after vasectomy. Out of 15 castrated primates four individuals showed complications during and after castration. Table 10 shows the occurring complications including severity (severe, moderate, none), suture material and species.

Description of the used terminology of complications:

- Severe = tumour suspicion
- Moderate = very small testes, haemorrhage from inside tunica, severe inflammation of the wound
- None = slightly swollen and reddish wound, but closed and no discharge (approx. 1 week after surgery)

Table 10. Complications during and after castration

Severity	Complication	Suture material	Species
Severe	One very big (tumour suspicion) and one very small testis	Not noted	*Macaca mulatta* (rhesus macaque)
Moderate	Very small testes (two separate incisions lateral of the penis needed)	Vicryl 3/0	*Papio hamadryas* (hamadryas baboon)
	Haemorrhage from inside tunica vaginalis	Vicryl 3/0 + Monocryl 4/0	*Macaca fuscata* (Japanese macaque)
	Severe inflammation of the wound, slightly open wound (after surgery)	Vicryl 2/0	*Cercocebus torquatus* (red-capped mangebey)

Moreover, four castrations performed after a vasectomy in rhesus macaques (*Macaca mulatta)* were complicated due to the previous vasectomy. The complications included enlarged testes, attachment to the tunica vaginalis, abscesses on the vasectomy locations and swollen stumps leading to prolonged swelling.

Only four out of the 15 primates in our study population showed complications, and these all, except for one case, occurred before or during castration. Complications were due to deviations caused by a previous vasectomy, pathologies of the testes or a small bleeding during the procedure. Only one primate showed a complication after castration. The 7 primates who did not show any complications were sutured with Monocryl suture material (3x), Vicryl suture material (2x), a combination of both (1x), and one was not noted.

5.2.2 Vasectomy

Seventy-eight out of 88 primates were vasectomised (ligation and removal of a part of the ductus deferens). Out of the 78 vasectomised primates 31 individuals showed complications during or after vasectomy. Table 11 shows the occurring complications including severity (severe, moderate, none), suture material and species.

Description of the used terminology of complications:

- Severe = fistula of the vasectomy wound, severe swelling of the testis, apathy/dehydration after surgery with death
- Moderate = severe inflammation or infection of the wound, irritation of the skin, swelling of the scrotum, abscesses on the vasectomy location, small haemorrhage during the procedure
- None = slightly swollen and reddish wound, but closed and no discharge (approx. 1 week after surgery)

Table 11. Complications during and after vasectomy

Severity	Complication	Suture material	Species
Severe	Fistula of the vasectomy wound with enlarged testes and attachment to the tunica vaginalis -> castration needed + hernia inguinalis (amputation)	Not noted Vicryl 5/0 + 3/0	*Macaca mulatta* (rhesus macaque) (2x) *Macaca fascicularis* (long-tailed macaque)
	Fistula of the vasectomy wound -> no castration needed	Vicryl 3/0 Not noted Vicryl 5/0	*Macaca fascicularis* (long-tailed macaque) *Macaca fascicularis* (long-tailed macaque) (2x) *Macaca mulatta* (rhesus macaque)
	Severe swelling of the testis, blue and pockmarked	Not noted	*Callithrix jacchus* (common marmoset)
	Apathy/dehydration after surgery with death	Not noted	*Callithrix jacchus* (common marmoset)
Moderate	Severe inflammation of the wound	Not noted	*Macaca mulatta* (rhesus macaque)
	Infection of the wound	Vicryl 3/0	*Macaca fascicularis* (long-tailed macaque)
	Irritation of the skin	Monocryl 3/0	*Macaca mulatta* (rhesus macaque)

		Not noted	*Macaca mulatta* (rhesus macaque)
		Not noted	*Papio anubis* (olive baboon)
	Swelling of the scrotum	Not noted	*Macaca mulatta* (rhesus macaque)
		Monocryl 4/0	*Macaca mulatta* (rhesus macaque)
		Monocryl 4/0	*Callithrix jacchus* (common marmoset)
		Monocryl 4/0 (t.vaginalis open)	*Lemur catta* (ring-tailed lemur) (3x)
	+ small spontaneous bleeding after removal of a piece of the spermatic duct	Monocryl 4/0 + 5/0 (t.vaginalis closed)	*Lemur catta* (ring-tailed lemur) (2x)
	+ cyst of the spermatic cord	Monocryl 5/0	*Macaca fascicularis* (long-tailed macaque)
		Vicryl 3/0	*Callithrix jacchus* (common marmoset)
		Vicryl 3/0	*Chlorocebus aethiops* (grivet)
	+ hernia inguinalis (amputation)	Not noted	*Callithrix jacchus* (common marmoset) (2x)
		Monocryl 3/0	*Papio anubis* (olive baboon
	Abscess(es) on the vasectomy location	Vicryl 5/0	*Macaca mulatta* (rhesus macaque)
		Not noted	*Macaca mulatta* (rhesus macaque)
	Small haemorrhage in spermatic cord	Not noted	*Callithrix jacchus* (common marmoset)

About 40 % of the primates (31/78) in our study population showed complications. Most complications occurred after vasectomy. Complications after vasectomy included fistulae, scrotal swelling (see image 1), severe inflammation or infection of the wound, abscess of the wound, irritation of the skin and apathy/dehydration with death after surgery. During vasectomy small haemorrhages and cysts of the spermatic cord and hernia inguinalis were recorded.

In male ring-tailed lemurs swelling of the scrotum occurred frequently in 2017 using Monocryl suture material. In 2 lemurs the tunica vaginalis was closed after the procedure. However, in 3 lemurs the tunica was left open. This implicates closure of the tunica made no difference in occurrence of the swelling.

After 2016 when Monocryl suture material was persistently used, swelling of the scrotum and irritation of the skin, could still be seen, but moderate to severe complications did not occur anymore.

Image 1. Swelling of the scrotum in a common marmoset after vasectomy
Source: Stichting AAP

6 Discussion / Conclusion

6.1 IMPLANON®

Etonogestrel is a commonly used reversible contraceptive in captive female non-human primates. In literature, only little information on the effect of Implanon® in female non-human primates is available. In one retrospective survey on Implanon® treatment in chimpanzees (Goodman, 2002) no conclusions could be made about the duration of effect or reversibility of etonogestrel. We tried to create more clarity about the use of Implanon® by performing a large retrospective survey on 206 female non-human primates.

This study shows evidence of the efficacy of Implanon® during treatment. No signs of oestrus or pregnancies were recorded during the use of Implanon® in 20/20 implanted females. This is consistent with the previous retrospective survey on three chimpanzees of Goodman (EAZWV scientific meeting).

It is often feared that after removal or elaboration of the Implanon®, fertility will not be restored. We looked at signs of oestrus and pregnancies after the use of Implanon® to elucidate this question.
In only 27% of the primates oestrous signs were detected at various moments after treatment. The signs were mainly covert and brief. However, records of oestrous signs are dependent on the construction of the enclosure and the monitoring of the animals. Some subtle signs might have been missed if the animals were not monitored closely.
Only three out of 48 primates became pregnant after the use of Implanon®. In one out of three primates the implant was removed. Further research is needed to investigate the link between implant removal and resumption of fertility. Three out of 141 primates got pregnant during treatment. However, the implant was lost, elaborated or not yet replaced by a new one so we can conclude those primates became pregnant after the use of Implanon®. In short, this resulted in six pregnancies after the use of Implanon®. In only two out of six pregnancies healthy young were born. The remaining four pregnancies resulted in still-birth, neonatal death, or even death of the mother. In two out of six pregnancies the young got stuck due to deformities. In others, the stillbirth was of undetermined etiology. This death rate is high (67%) compared to the rate of still-birth in long-tailed macaques without contraception devices, namely 12% (Sesbuppha et al., 2008). However, our study population is too small to draw conclusions between the use of Implanon® and stillbirths or neonatal death.
14 out of 48 primates were housed with a fertile partner after the use of Implanon®. However, they did not get pregnant. We need a control group without the implant to see if this behaviour is normal in the particular conditions of a rehabilitation centre or the implant might have influenced behaviour.

In total 30 autopsies were performed in females that had been treated with Implanon® during life. In more than 50%, namely 17 out of 30 cases, abnormalities of the genital tract were observed. The deviations included endometritis, leiomyoma and sarcoma. Leiomyomata (a benign fibroid tumor) have been reported in several non-human primate species, including chimpanzees (Videan et al., 2011). However, the use of hormonal contraception is linked to a decrease in leiomyomata. Due to lack of similar studies in non-human primates, our findings are discussed in relation to a post-mortem study of the genital tract in female wild boars without an implant. The study showed post-mortem abnormalities of the genital tract in only 10% of the cases (Malmsten, 2017). Compared to this study our post-mortem pathology rate in primates is high. Therefore a link between deviations of the genital tract and the use of Implanon® is suspected. This can only be suspected, not concluded as we don't have disposition of a control group without Implanon®. However, a 2-year comparative study of endometrial histology of human female Implanon® users shows no evidence of an increasing risk of endometrial hyperplasia, endometrial carcinoma, cervical intra-epithelial neoplasia or cervical carcinoma (Mascarenhas et al., 1998). The link between the use of Implanon® and deviations of the genital tract in female non-human primates can be an avenue for future research.

6.2 SURGICAL CONTRACEPTION TECHNIQUES

There is only one scientific record of a successful open castration technique in macaques. In this technique the scrotum is incised (Buyukmihci, 2017). No scientific report of complications in the sex organs after castration in non-human primates was published before. Stichting AAP made use of a closed castration technique via one midline skin incision rostral of the scrotum. During 15 castration procedures only one complication, a small haemorrhage, occurred and one complication occurred after castration. This suggests castration is a safe permanent contraception technique. The complication that was observed after castration seems to be related to the use of polyfilament resorbable suture material (Vicryl), suggesting Monocryl is safer. However, our study population consisted of only 15 non-human primates which is a small amount to conduct conclusions. A large study population consisting of Monocryl only vs Vicryl only suture material can be suggested for future research.

In literature several vasectomy techniques have been used in different non-human primate species using different surgical approaches (scalpel, elektrocauterization), incisions (prescrotal, scrotal, incision above the spermatic cord), suture material (braided nylon, vicryl, silk, no sutures) and post-operative management. There is scientific record of several complications during and/or after vasectomy. Stichting AAP made use of a scalpel surgical approach, an incision above the spermatic cord and Monocryl or Vicryl suture material.

Thirty-nine point seven percent of the primates in our study population showed moderate to severe complications of which most occurred after vasectomy. This complication rate is high compared to complications in humans, which show 2% haematomas, 2% infections and 1% epididymitis (anonymous, 1990). The reason for this discrepancy is not known. There seems to be a relation between the use of Vicryl suture material and complications but further research is needed.

This is the first record of chronic fistulae in male macaques after the use of Vicryl suture material during vasectomy. The suture material was used for ligation and closure of the tunica vaginalis and the skin. Veterinarians should be made aware of this risk in macaques. Monocryl suture material is advised to avoid reactivity in the body and possible infection. However, further research (and statistical analysis of data) is advised to determine if occurrence of fistulae after surgery is also occurring after the use of Monocryl suture material.

A prominent swelling of the scrotum in male ring-tailed lemurs occurred frequently in 2017 using Monocryl suture material. Closure of the tunica vaginalis played no role in the appearance of the swelling. The underlying cause needs to be further investigated and seems to be species-specific.

Moreover, in Callitrichidae the tunica vaginalis is not closed to prevent wound irritation. This can be an useful addition to the vasectomy protocol in other zoos and institutions. It was already recorded by Morris and David in 1993.

In conclusion, this retrospective study shows the contraceptive efficiency of Implanon® during use in female non-human primates. However, a high rate of stillbirth, neonatal death (4/6) and post-mortem deviations of the genital tract (17/30) were seen after the use of Implanon®. Additionally, in male non-human primates castration is represented as a safe permanent contraception technique. High complication rate after vasectomy with yet unknown origin is noticed. This seems to be related to the use of Vicryl suture material. It's a gathering of several technical procedures, e.g. opening of the tunica vaginalis in Callitrichidae, which can be applied during vasectomy to lower the rate of complications.

LIST OF REFERENCES

Adams, K., Beal, M.W., 2009. Implanon: a review of the literature with recommendations for clinical management. J Midwifery Womens Health 54, 142-149.

Agrawal, A., Robinson, C., 2005. An assessment of the first 3 years' use of Implanon in Luton. J Fam Plann Reprod Health Care 31, 310-312.

Alexander, N.J., 1981. Primates - Their Use in Research on Vasectomy. Am J Primatol 1, 167-173.
Anonymous, 1990. Serious complications after vasectomy rare, experts say. Contraceptive technology update 11, 185.

Bensouda-Grimaldi, L., Jonville-Bera, A.P., Beau-Salinas, F., Llabres, S., Autret-Leca, E., le reseau des centres regionaux de, p., 2005. [Insertion problems, removal problems, and contraception failures with Implanon]. Gynecol Obstet Fertil 33, 986-990.

Brunson, M.R., Klein, D.A., Olsen, C.H., Weir, L.F., Roberts, T.A., 2017. Postpartum contraception: initiation and effectiveness in a large universal healthcare system. Am J Obstet Gynecol 217, 55 e51-55 e59.

Burvenich, B., 2014. Fysiologie voortplanting & melkklier. In: Orgaanfysiologie 3. Course, Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Ghent University, Belgium.

Clarkson, T.B., Alexander, N.J., 1980. Long-term vasectomy: effects on the occurrence and extent of atherosclerosis in rhesus monkeys. J Clin Invest 65, 15-25.

Colagross-Schouten, A., Lemoy, M.J., Keesler, R.I., Lissner, E., VandeVoort, C.A., 2017. The contraceptive efficacy of intravas injection of Vasalgel for adult male rhesus monkeys. Basic Clin Androl 27, 4.

D'Journo, X.B., Vidal, V., Agostini, A., 2015. Intravascular pulmonary migration of a subdermal contraceptive implant. Ann Thorac Surg 99, 1828.

Darney, P., Patel, A., Rosen, K., Shapiro, L.S., Kaunitz, A.M., 2009. Safety and efficacy of a single-rod etonogestrel implant (Implanon): results from 11 international clinical trials. Fertil Steril 91, 1646-1653.

Davidson, A.P., Stabenfeldt, G.H., 2007. Reproduction and Lactation. In: Cunningham, J.G., Klein, B.G., Cunningham's Textbook of Veterinary Physiology, Fifth Edn. Elsevier, Hongkong, China, pp. 408-431.

Dixson, A.F., 1998. Sexual selection and sexually dimorphic traits. In: Primate Sexuality: Comparative Studies of the Prosimians, Monkeys, Apes and Human Beings, First Edn. Oxford University Press, New York City, New York, USA, pp. 201-214.

Egbetade, A.O., Gadsby, E.L., Idoiaga A., Jenkins P.D., 2014. Vasectomy in Drill Monkeys (Mandrillus leucophaeus) - A Population Management Tool. World Journal of Zoology 9 (2), 107-110.

Ekanayake-Alper, D.K., Wilson, S.R., Scholz, J.A., 2018. Retrospective Review of Surgical Outcomes and Pair-housing Success in Vasectomized Rhesus Macaques (*Macaca mulatta*). Comparative Medicine 68, 168-176.

Funk, S., Miller, M.M., Mishell, D.R., Jr., Archer, D.F., Poindexter, A., Schmidt, J., Zampaglione, E., Implanon, U.S.S.G., 2005. Safety and efficacy of Implanon, a single-rod implantable contraceptive containing etonogestrel. Contraception 71, 319-326.

Harrison-Woolrych, M., Hill, R., 2005. Unintended pregnancies with the etonogestrel implant (Implanon): a case series from postmarketing experience in Australia. Contraception 71, 306-308.

Harrison, R.M., Domingue, G.J., Heidger, P.M., Jr., Roberts, J.A., Schlegel, J.U., 1977. Vasectomy in rhesus monkeys I. Surgical techniques and gross observations. Urology 9, 639-644.

Hynes, E.F., Handasyde, K.A., Shaw, G., Renfree, M.B., 2010. Levonorgestrel, not etonogestrel, provides contraception in free-ranging koalas. Reprod Fertil Dev 22, 913-919.

Kew, E.P., Senanayake, E., Djearaman, M., Bishay, E., 2017. Migration of contraceptive implant into the left pulmonary arterial system. Asian Cardiovasc Thorac Ann 25, 537-539.

Kumar, V., Raj, A., 2012. No-scalpel vasectomy by electrocauterization in free range rhesus macaques (Macaca mulatta). Open Vet J 2, 6-9.

Maijer, A.M., Semple, S., 2016. Investigating Potential Effects of the Contraceptive Implanon on the Behavior of Free-Ranging Adult Female Barbary Macaques. J Appl Anim Welf Sci 19, 16-23.

Malmsten, A.,Jansson, G., Dalin, A. M., 2017. Post-mortem examination of the reproductive organs of female wild boars (Sus scrofa) in Sweden. Reprod Domest Anim 52, 570-578.

Mascarenhas, L., van Beek, A., Bennink, H. C., Newton, J. 1998. A 2-year comparative study of endometrial histology and cervical cytology of contraceptive implant users in Birmingham. UKHum Reprod 13, 3057-60.

McNicholas, C., Swor, E., Wan, L., Peipert, J.F., 2017. Prolonged use of the etonogestrel implant and levonorgestrel intrauterine device: 2 years beyond Food and Drug Administration-approved duration. Am J Obstet Gynecol 216, 586 e581-586 e586.

Morris, T.H., David, C.L., 1993. Illustrated guide to surgical technique for vasectomy of the common marmoset. Lab Anim 27, 381-384.

Saltzman, W., Tardif, S.D., Rutherford, J.N., 2010. Hormones and Reproductive Cycles in Primates. In: Norris, D.O., Lopez, K.H, Hormones and Reproduction of Vertebrates, Volume 5: Mammals, First Edn.Academic Press, Cambridge, Massachusetts, USA, pp.1-29.

Seppan, P., Krishnaswamy, K., 2014. Long-term study of vasectomy in Macaca radiata--histological and ultrasonographic analysis of testis and duct system. Syst Biol Reprod Med 60, 151-160.

Sesbuppha, W., Chantip, S., Dick, E. J., Jr., Schlabritz-Loutsevitch, N. E., Guardado-Mendoza, R., Butler, S. D., Frost, P. A., Hubbard, G. B., 2008. Stillbirths in Macaca fascicularis. J Med Primatol 37, 169-72.

Skovlund, C.W., Morch, L.S., Kessing, L.V., Lidegaard, O., 2016. Association of Hormonal Contraception With Depression. JAMA Psychiatry 73, 1154-1162.

Videan, E. N., Satterfield, W. C., Buchl, S., Lammey, M. L., 2011. Diagnosis and prevalence of uterine leiomyomata in female chimpanzees (Pan troglodytes). Am J Primatol 73, 665-70.

Zigler, R.E., McNicholas, C., 2017. Unscheduled vaginal bleeding with progestin-only contraceptive use. Am J Obstet Gynecol 216, 443-450.

Zimmermann, E., Radespiel, U., 2007. Primate Life Histories. In: Henke, W., Tattersall, I., Handbook of Paleoanthropology, Second Edn. Springer, Berlin, Germany, pp.1527-1592.

List of websites consulted:

http://www.egzac.org/. Last consulted: 8-5-'18

Buyukmihci, NC., 2017. Castration for Population Control of Macaques In a Sanctuary Setting. *UC Davis*. Retrieved from https://escholarship.org/uc/item/3cg050sp. Last consulted: 13-5-'18

The use of implanon® contraceptive in apes. European Association of Zoo and Wildlife Veterinarians (EAZWV) 4th scientific meeting, joint with the annual meeting of the European Wildlife Disease Association (EWDA) [Internet]. 2002 [cited 2015 March 15]. Available from http://www.ivis.org/proceedings/eazwv/2002/Repro/Repro.pdf#nameddest=3. Last consulted: 13-5-'18

ADDENDUM

Addendum 1: Implanon® survey

Introduction Implanon survey AAP

Contraception in non-domesticated animals is an important method for population management. Implanon (etonogestrel) is a widely used progestin based contraceptive in primates. Still, data on reversibility, side effects and fertility are not available. Together with EGZAC (EAZA Group on Zoo Animal Contraception) we hope to acquire more knowledge by following up female primates contracepted with Implanon at AAP, Rescue centre for Exotic animals (Almere, The Netherlands), over the past 10 years and trace them for fertility information.

We approach you because one or more primates that previously got Implanon by AAP as reversible contraception were outplaced to your zoo/institution. We would really appreciate if we could take a little of your time to fill in this survey. Please fill in all the questions given below. Based upon your answer inappropriate questions are skipped.

Thank you in advance.

1. Please enter the name of your zoo/institution:

2. Please enter the name of the animal:

3. Please enter the species of the animal (English name)

4. Was the contraception by Implanon followed up by reversible contraception at your zoo/institution?

- ◯ Yes
- ◯ No
- ◯ Unknown

* 5. Was the animal surgically ovariectomised / sterilised?

- ◯ Yes
- ◯ No
- ◯ Unknown

6. You mentioned the animal was surgically ovariectomised / sterilised. Were there any complications during or after surgery? If yes, please mention which complications occurred.

- ☐ Yes
- ☐ No

If yes, please explain which complications

7. Which products were used as reversible contraceptive(s) at your zoo/institution?

- ◯ Etonogestrel (Implanon/Nexplanon)
- ◯ Deslorelin (Suprelorin)
- ◯ Birth control pill
- ◯ Injectable progestagen (Depo-Provera)
- ◯ Other (please specify)

8. Could you give the begin and (possible) end date of the contraception (bout)? If Implanon was used repeatedly please fill in the different bouts in the provided spaces below.
Begin = moment the contraceptive was placed
End = date the contraceptive is removed or worn off (no action expected anymore)

Begin - end

Begin - end

Begin - end

9. Have you seen signs of oestrus during contraceptive treatment (sexual swelling, menstruation...)?

- ◯ Yes
- ◯ No
- ◯ Unknown

If yes, pleae specify

10. Has there been a pregnancy during contraceptive treatment?

- ◯ Yes
- ◯ No
- ◯ Unknown

11. Have you seen any side-effects during contraceptive treatment?

- ◯ No
- ◯ Yes, weight gain
- ◯ Yes, ↑/↓ frequency of bleeding during menstruation
- ◯ Yes, other:
- ◯ Yes, more aggression
- ◯ Yes, changes in hierarchy/dominance

12. Has there been a period without contraception?

- ◯ Yes
- ◯ No

13. Has the implant been removed?

- ◯ Yes
- ◯ No

Date of removal

14. Time span between stopping contraception & signs of oestrus cycle?

Time in months

15. Did the animal become pregnant after removal/stopping contraceptive method?

- ◯ Yes, but the pregnancy resulted in stillbirth (fetal death)
- ◯ Yes, but the pregnancy resulted in miscarriage
- ◯ Yes, the pregnancy went well, live young (please fill in the date below)
- ◯ Date
- ◯ Yes, the pregnancy went well, dead young (please fill in the date below)
- ◯ No, but not housed with fertile partner
- ◯ No, although housed with fertile partner

16. Time between stop contraception and pregnancy?

Time in months

17. Is the animal still alive?

- ◯ Yes
- ◯ No

Date of death

18. Did you take any post-mortem samples of the genital tract?

- ◯ No
- ◯ Yes, please specify the results

End of the survey

Thank you for sharing your information! Your efforts will be greatly appreciated and will contribute to the search and optimization of effective and safe reversible contraception in primates.

The final outcome of this survey will be shared with you at a later stage.
Thank you for your cooperation!

Best wishes,
Celine Lemmens (3th master veterinary medicine UGent, Belgium)
Hester Van Bolhuis (Stichting AAP, EGZAC)