# EFFECTS OF NORPLANT IMPLANTS ON BEHAVIOR AND PHYSIOLOGY OF CAPTIVE CHIMPANZEES

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TABLE OF CONTENTS


| Chapter | | Page |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | OVARIAN HORMONE LEVELS IN FEMALE CHIMPANZEES IMPLANTED WITH NORPLANT IMPLANTS | 3 |
| | Abstract | 4 |
| | Introduction | 5 |
| | Materials and methods | 12 |
| | Results | 21 |
| | Discussion | 24 |
| | Conclusions | 31 |
| | Acknowledgements | 31 |
| | References | 33 |
| III. | EFFECTS OF NORPLANT IMPLANTS ON GENITAL SWELLING PATTERNS IN FEMALE CHIMPANZEES | 50 |
| | Abstract | 51 |
| | Introduction | 52 |
| | Materials and methods | 61 |
| | Results | 66 |
| | Discussion | 70 |
| | Conclusions | 76 |
| | Acknowledgements | 76 |
| | References | 78 |

IV. EFFECTS OF NORPLANT IMPLANTS ON THE SOCIOSEXUAL BEHAVIOR OF FEMALE CHIMPANZEES ..... 98

Abstract ..... 99
Introduction .....100
Materials and methods .....112
Results .....119
Discussion .....125
Conclusions .....130
Acknowledgements .....130
References .....132

V. CONCLUSIONS .....160

APPENDIX - ETHOGRAM .....162

LIST OF TABLES

Chapter II.

Table Page

1. Life History Data on the Seven Female Chimpanzees in this Study ..... 42

2. Genital Swelling and Menses Data in Relation to Time of Insertion of Norplant for Seven Female Chimpanzees ..... 43

3. Mean Duration of Menstrual Cycle Components Before and During Norplant Treatment ..... 44

4. Mean Level of Levonorgestrel (LNG), Occurrence of Genital Swelling, Menses and Ovulation ..... 45

5. Mean Levels of Estrone-Glucuronide ($E_1G$) and Pregnanediol-Glucuronide (PdG) in Chimpanzees During Phases I and II of the Study ..... 46

Chapter III.

Table Page

1. Life History Data on the Seven Female Chimpanzees in this Study..... 90

2. Genital Swelling and Menses Data in Relation to Time of Insertion of Norplant for Seven Female Chimpanzees ..... 91

3. Genital Swelling Cycle Variables on Five Cycles Before and Seven Cycles Following Insertion of Norplant Implants..... 92

4. Results of Pearson's Correlation Tests Comparing Cycle Length and Swelling Phase Duration with Age, Weight and Time Since Implant ..... 93

Chapter IV.

Table Page

1. History of the 12 Cycling Females in this Study .....142

2. Composition of the Four Social Groups in this Study .....143

3. Total Number of 15-Minute Observations on Each Female During Phases I and II of the Study .....144

4. Percent of Observations on Each Subject During Different Levels of Genital Swelling .....145

5. Breakdown of the Behaviors Included in Each of the Behavioral Categories and Subcategories Analyzed for this Study .....146

6. Results of Student's t-Tests and Rates of Behavior of Five Female Chimpanzees (Experimental Group) for All Days Before and After Receiving Norplant Implants .....147

7. Results of Student's t-Tests and Rates of Behavior of Five Female Chimpanzees (Experimental Group) for Days With Full Swelling, Before and After Receiving Norplant Implants .....148

8. Results of Student's t-Tests and Rates of Selected Behaviors for Five Female Chimpanzees (Experimental Group) on All Days Before and After Receiving Norplant Implants .....149

9. Results of F-Tests on Variances of Behaviors Exhibited by Five Female Chimpanzees (Experimental Group) Before and After Receiving Norplant Implants .....150

10. Results of Student's t-Tests and Rate of Interaction by Partner Category for Five Female Chimpanzees (Experimental Group) Before and After Receiving Norplant Implants .....151

11. Results of Student's t-Tests and Rates of Behavior of Four Female Chimpanzees (Control Group) for All Days During Phases I and II of the Study .....152

12. Results of Student's t-Tests and Rates of Behavior of Four Female Chimpanzees (Control Group) for Days with Full Swelling During Phases I and II of the Study .....153

13. Results of F-tests of Variances of Behaviors Exhibited by Four Female Chimpanzees (Control Group) During Phases I and II of the Study .....154

14. Results of Student's t-Tests and F-tests Comparing Rates of Behaviors and Variances Between Experimental (n=5) and Control (n=4) Groups During Phase I of the Study .....155

15. Results of Student's t-Tests and F-tests Comparing Rates of Behaviors and Variances Between Experimental (n=5) and Control (n=4) Groups During Phase II of the Study .....156

16. Results of Student's t-Tests and Rates of Behavior for Experimental (n=6) and Control (n=6) Groups for Observations on All Days.....157

17. Results of Student's t-Tests, F-Tests and Rates of Behavior for Experimental (n=6) and Control (n=6) Groups for Observations During Full Swelling .....158

18. Results of Analysis of Variance and Rates of Behavior by Social Group (Corral) During Phase II of the Study (n=12 females).....159

LIST OF FIGURES

Chapter II.

Figure Page

1. Example of Patterns of Urinary Excretion of Luteinizing Hormone (LH), Estrone, Pregnanediol and Level of Swelling in an Adult Female Chimpanzee, as Published in McArthur et al., 1981.....47

2. Concentrations of Estrone Glucuronide ($E_1G$), Pregnanediol Glucuronide (PdG), and Level of Genital Swelling Exhibited by Three Female Chimpanzees Before and After Receiving Norplant Implants.....48

3. Concentrations of Estrone Glucuronide ($E_1G$), Pregnanediol Glucuronide (PdG), and Level of Genital Swelling Exhibited by Two Female Chimpanzees with Anovulatory Cycles During Norplant Treatment.....49

Chapter III.

Figure Page

1. Genital Swelling Cycles of Multiparous Female BD Before and After Insertion of Norplant Implants (0 = No Genital Swelling, 1 = Partial Genital Swelling, 2 = Maximum Genital Swelling).....94

2. Genital Swelling Cycles for Nulliparous Female MS Before and After Insertion of Norplant Implants (0 = No Genital Swelling, 1 = Partial Genital Swelling, 2 = Maximum Genital Swelling) ..... 95

3. Genital Swelling Cycles for Subject GG Before Insertion of Norplant Implants, and Genital Swelling During Two Cycles Following Implant Placement (0 = No Genital Swelling, 1 = Partial Genital Swelling, 2 = Maximum Genital Swelling) ..... 96

4. Genital Swelling Cycles of Female BT Before Receiving Norplant Implants, Immediately Following Implant Insertion, and Six Months Following Implant Insertion (0 = No Genital Swelling, 1 = Partial Genital Swelling, 2 = Maximum Genital Swelling) ..... 97

# CHAPTER I

# INTRODUCTION

This thesis is composed of three manuscripts formatted for submission to Zoo Biology. Each of the following three chapters is complete as written and needs no additional support material.

The objective of this study was to assess the effects of Norplant[1] implants on ovarian hormone secretion, genital swelling patterns and sociosexual behavior of female chimpanzees. Subsequent chapters address each of these three issues, respectively.

The study was divided into two phases. Phase I refers to the period before the subjects received the implants. Phase II refers to the period following the insertion of Norplant implants.

Urine, to be analyzed for ovarian hormone content, was collected on five females during Phase I and on the same

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[1]Norplant™ is the Population Council's trademark for subdermal implants containing levonorgestrel.

five females plus two additional females during Phase II. Genital swelling data recorded daily by facility staff was available for all seven females during both Phases of the study.

Experimental design for the behavioral portion of the study utilized a double control. During Phase I, behavioral data were collected on nine cycling females. The subjects were divided into two groups: the experimental group, designated to receive Norplant at the end of Phase I, and the control group which would not receive contraception. During Phase II, behavioral data were collected on the same nine females, plus three additional subjects. This resulted in data on six cycling females in the control group and six cycling females in the experimental group during Phase II.

The fifth and final chapter is a brief conclusion, pulling together the major findings reported in the previous chapters.

# CHAPTER II

# OVARIAN HORMONE CONCENTRATIONS IN FEMALE CHIMPANZEES WITH NORPLANT IMPLANTS

# OVARIAN HORMONE CONCENTRATIONS IN FEMALE CHIMPANZEES WITH NORPLANT IMPLANTS

**ABSTRACT**

The purpose of this study was to assess the effects of Norplant implants on ovarian hormone secretion in female chimpanzees during contraceptive treatment with Norplant. Urine samples were collected during one natural cycle (n=5 females) before and one cycle following (n=7 females) insertion of Norplant implants. Genital swelling data were recorded on all seven females. Two females exhibited minimal ovarian activity consistent with anovulation. Hormone data on five subjects indicate both follicular and luteal activity, suggesting that ovulation may have occurred during Norplant treatment. Among the cycles presumed to be ovulatory, concentration of estrone glucuronide did not differ from concentration of cycles during the control phase. However, mean ($p=.001$) and peak ($p=.003$) concentrations of pregnanediol glucuronide were significantly lower during Norplant treatment. The results of this study indicate that Norplant provided effective contraception for the chimpanzees. Additionally, in five of the subjects, the cyclic occurrence of genital swelling was not inhibited during the Norplant regimen.

**INTRODUCTION**

Documentation of the effects of contraceptives on chimpanzee (Pan troglodytes) physiology and behavior has become a critical concern. Until recently, researchers have strived to increase reproductive rates in captive chimpanzee groups. As a result of changing management practices, attention is now being focused on the safety and effectiveness of various methods of contraception developed for use in humans, but currently used with chimpanzees.

Although the chimpanzee is endangered in the wild, captive propagation programs have become quite successful. To ensure a self-sustaining population in captivity, zoos and laboratories have begun genetic management of their breeding populations (Schobert and Fulk, 1993; National Institutes of Health, 1994). However, the number of births which can occur each year is limited by the availability of space (Schobert and Fulk, 1993). As a result, breeding priority is given to chimpanzees which are inadequately represented in the gene pool.

Maximizing genetic diversity of the captive chimpanzee gene pool is not the sole concern of the breeding programs. The American Association of Zoos and Aquariums (AZA) and the National Institutes of Health (NIH) are committed to maintaining captive populations of chimpanzees that exhibit competent sociosexual and maternal behavior. To accomplish this goal, chimpanzees in their breeding colonies are

maintained in complex social groups containing members of several age classes and both sexes (Schobert, 1990; National Institutes of Health, 1994).

The dilemma facing managers of captive chimpanzees is determining which method of contraception will temporarily prevent reproduction in certain individuals while, at the same time not interfere with normal social behavior of the group. Nadler et al. (1992, 1993) studied the effects of combined oral contraceptives on chimpanzee behavior and physiology. Their study reported that the contraceptives suppressed endogenous ovarian hormone secretion, altered the expression of genital swelling patterns (Nadler et al., 1992) and reduced the rate of copulatory behavior exhibited by the treated females (Nadler et al., 1993, 1994). Therefore, oral contraceptives do not produce the desired behavioral results.

Chimpanzee behavior is closely linked to the presence or absence of the genital swelling of the female (Wallis, 1982; Wallis and Lemmon, 1986; Alford et al., 1990; Nishida, 1990; Bloomsmith et al., 1991; Shefferly and Fritz, 1992; Wallis, 1992; Bettinger et al., 1993). In turn, the genital swelling is linked to the endocrine environment provided by ovarian hormone secretion.

Chimpanzees exhibit a 36-day menstrual cycle which can be monitored by two external features: the genital swelling and menses (Yerkes and Elder, 1936; Elder, 1938; Young and

Yerkes, 1943; Graham, 1981; Nadler et al., 1985). Menses lasts about three days and often can be visually detected. The genital swelling, which encompasses the labia and perianal region, fluctuates in size and turgidity in response to changing levels of ovarian hormones (Yerkes and Elder, 1936; Allen et al. 1936; Fish et al., 1941; Clark and Birch, 1948; Graham et al., 1972; Nadler et al., 1985).

Variation in cycle length in chimpanzees has been attributed to differing durations of the follicular phase (Elders and Yerkes, 1936; Young and Yerkes, 1943; Graham, 1981; McArthur et al., 1981; Dahl et al., 1991). Graham (1981) suggests that the prolongation of the pre-swelling phase (a part of the follicular phase) may actually be a delay in the initiation of a new cycle rather than a prolongation of the cycle itself. Additionally, fertility is only slightly correlated with cycle variability (Young and Yerkes, 1943; Graham, 1981; Dahl et al., 1991).

During menstruation, estrogen and progesterone are at their lowest level (Allen et al., 1936; Graham et al., 1972; McArthur et al., 1981; Nadler et al., 1985). High levels of follicle stimulating hormone (FSH) are found early in the follicular phase (Reyes et al., 1975; Nadler et al., 1985). As follicular development proceeds, estrogen levels rise to a midcycle peak (Allen et al., 1936; Fish et al, 1941; Graham et al., 1972; McArthur et al., 1981; Nadler et al., 1985). The midcycle peak of estrogen is concurrent with or

followed closely by peaks in FSH and luteinizing hormone (LH) (Reyes et al., 1975; McArthur et al., 1981; Nadler et al., 1985). Figure 1, from McArthur et al. (1981), illustrates the fluctuating concentrations of estrone, pregnanediol, LH, and the genital swelling pattern of female chimpanzees during natural cycles.

Progesterone levels remain low throughout the follicular phase, rising rapidly after ovulation, to a concurrent peak with estrogen during the midluteal phase (Graham et al., 1972; Reyes et al., 1975; McArthur et al., 1981; Nadler et al., 1985; Dahl et al., 1991). The biphasic pattern of estrogen secretion is not found in monkeys and may be unique to humans and apes (Graham et al., 1972; Reyes et al., 1975; Graham, 1981; McArthur et al., 1981; Nadler et al., 1985). However, the midcycle peak in humans is larger than the luteal peak while in chimpanzees they occasionally may be equal in size.

Chimpanzee reproductive hormone secretion more closely resembles humans than it does monkeys (Graham et al., 1972; Reyes et al., 1975; McArthur et al., 1981; Nadler et al., 1985). Although not identical, this similarity is sufficient to allow cautious extrapolation between humans and chimpanzees. Therefore, studies on the effects of Norplant in humans may provide information regarding its possible utility as a chimpanzee contraceptive.

The Norplant system consists of six silastic tubules,

2.4 mm in diameter and 34 mm in length, each containing 36 mg of levonorgestrel (LNG). The tubules are inserted subcutaneously into the medial surface of the upper left arm. The initial dose provided by the system is about 85 μg/day decreasing to 50 μg/day by 9 months and 35 μg/day by 18 months (Wyeth-Ayerst Laboratories, 1990). The majority of circulating LNG is bound to sex hormone binding globulin (Olsson et al., 1987). A negative correlation has been found between plasma LNG concentration and body weight (Croxatto et al., 1981; Sivin et al., 1988; Brache et al., 1990) suggesting that the progestin may be held in adipose tissue.

One set of implants will effectively prevent conception in humans for five years. About 1% of five-year Norplant users become pregnant; the principal factor increasing failure rates is body weight over 70 kg (Darney, 1994). Norplant's effectiveness is achieved through several mechanisms: inhibition of ovulation in some cycles; luteal phase inadequacy through either reducing peak progesterone levels or shortening the duration of progesterone secretion; delayed maturation of the oocyte; or thickening of the cervical mucus (Croxatto et al., 1982; Shaaban et al., 1984; Brache et al., 1985; Alvarez et al., 1986; Croxatto et al., 1988; Brache et al., 1990; Faundes et al., 1991a; Faundes et al., 1991b; Segal et al., 1991; Shoupe et al., 1991a).

The most common side effect associated with Norplant is irregular bleeding patterns. By the end of the first year,

however, approximately 60% of the women have bleeding patterns similar to normal cycles (Fakeye and Balough, 1989; Shoupe et al., 1991b; Faundes et al., 1991a). One study reported that eight out of 10 Norplant pregnancies occurred among women expressing menses at normal intervals (Shoupe et al., 1991b). Shoupe et al. (1991b) found that although overall pregnancy rate was 4.4% for five-year users in their study, women with regular bleeding patterns experienced a 17.4% pregnancy rate over five years of use.

Anovulatory cycles are most frequent during the first year of implant use, and in some cases have been correlated with higher plasma levels of LNG (Croxatto et al., 1982; Brache et al., 1985; Faundes et al., 1991). Rate of cycles presumed to be ovulatory during the first year range from 11-36%, and by the fifth year may be as high as 60% (Croxatto et al., 1982; Shaaban et al., 1984; Croxatto et al., 1988; Brache et al., 1990). The highest incidence of presumed ovulation occurs in females with normal bleeding patterns. Ovulation is inferred to occur in 32-60% percent of cycles of these women (Brache et al., 1985; Faundes et al., 1991a; Faundes et al., 1991b; Shoupe et al., 1991a).

Unlike oral and injectable contraceptives, Norplant does not always suppress endogenous estrogen secretion (Collins, 1994; Lobo and Stanczyk, 1994). In the majority of women, whether the cycle was presumed ovulatory or not, plasma estrogen concentrations in Norplant users did not

differ significantly from levels expressed by naturally cycling controls (Shaaban et al., 1984; Alvarez et al., 1986; Croxatto et al., 1988; Brache et al., 1990; Shoupe et al., 1991a). However, cycles without luteal activity seldom show the biphasic estrogen pattern and are typically longer in duration (Faundes et al., 1991a). The absence of a luteal phase peak in estrogen in anovulatory cycles is consistent with the absence of a functional corpus luteum. In primates, the corpus luteum produces not only progesterone, but estrogen and testosterone as well (Speroff et al., 1989).

During cycles in which ovulation is presumed to have occurred, LH and FSH peaks are significantly lower than in control cycles (Shaaban et al., 1984; Alvarez et al., 1986; Croxatto et al., 1988; Faundes et al., 1991b; Shoupe et al., 1991a). These studies also found lower peak and mean concentrations of progesterone during the luteal phase. The findings to date all provide evidence of luteal phase inadequacy during presumed ovulatory cycles.

Findings indicate that Norplant implants may be an ideal contraceptive for use in chimpanzees. The lack of estrogenic inhibition should allow genital swelling to occur, thereby promoting species-typical behavior. Additionally, the ability to implant group-living, non-tractable female chimpanzees eliminates the problems associated with daily administration of oral contraceptives. Although Norplant has received U.S. Food and Drug

Administration approval for use in humans (Darney, 1994), its efficacy and safety has not been documented in chimpanzees.

The results presented in this study are a subset of data collected on seven female chimpanzees to address the effects of Norplant on ovarian hormone secretion, genital swelling patterns and sociosexual behavior. This paper presents results of urinary assays for levonorgestrel (LNG), estrone-3-glucuronide ($E_1G$) and pregnanediol-3-glucuronide (PdG) in female chimpanzees before and during treatment with Norplant implants. The data suggest that Norplant is an effective contraceptive in chimpanzees which works through mechanisms of action similar to that reported for humans.

## MATERIALS AND METHODS

### STUDY DESIGN

The study was divided into two phases. During Phase I, the control phase, urine samples were collected across one cycle from five naturally cycling females. At the end of Phase I, these five females, plus two additional females, received Norplant implants. During Phase II, six months following the insertion of the implants, urine samples were collected from all seven females.

### FACILITY AND HOUSING

The female chimpanzees in this study were maintained at

The University of Texas, M.D. Anderson Cancer Center's veterinary resource facility in Bastrop, Texas. The subjects lived in multimale/multifemale social groups housed in enriched, 22-meter diameter outdoor corrals with access to indoor dens (see Riddle et al., 1982 for a detailed description of the facility). Each social group contained 2-3 adult males, 1-2 immature males, 3-6 adult females (in various reproductive states), and 0-1 immature females.

Throughout the study, the seven subjects were housed with one to three adult males of proven fertility. Births occurred in two of the social groups one month prior to the onset of the study and one group during Phase I of the study. Two nulliparous females (from two social groups) became pregnant during Phase II of the study. Therefore, pregnancies and/or births occurred in three of the four social groups during the study period.

STUDY SUBJECTS

Table 1 provides life history data on the seven female chimpanzees included in this study. Management decisions required that these females be temporarily prevented from reproducing, therefore, subjects were not chosen at random. Reasons for placing the females on a contraceptive regimen included adequate or over-representation in the gene pool (n=5) and delaying first conception (n=2). Additionally, the two nulliparous females were housed in social groups

incompatible with breeding recommendations: one was housed in a social group containing her father and half brother; one female had blood type incompatible with the fertile male in her group.

Nulliparous female MS had begun cycling two and a half years prior to being placed on the Norplant regimen, however, she had been housed with a fertile male for only 10 months before receiving implants. The male, although a proven breeder, may have had reduced fertility as a result of Hansen's disease (leprosy).

Females BT and GR were placed on Norplant soon after their resumption of postpartum cycles. These two females had a history of conceiving within one to three postpartum cycles. Although the remaining multiparous females had been cycling for almost two years, their cycles were often irregular, possibly as a result of continued nursing of offspring. All multiparous females except BT were lactating and housed with offspring when placed on Norplant.

## NORPLANT INSERTION

Seven females received Norplant implants between July 29, 1993 and August 25, 1993 (see Table 1). Ten to 15 days following detumescence, each female was tested for pregnancy by evaluation of chorionic gonadotrophin in urine. Norplant implants were inserted during the early follicular phase of the female's cycle (Table 2).

Subjects were immobilized by an intramuscular injection of Telazol at a dosage of 3 mg/kg body weight (Manufactured by Elkins Sinn Inc., Cherry Hill, NJ for Aveco Co., Inc. Fort Dodge, Iowa). Confirmation of the non-pregnant status was obtained by ultrasonography of the uterus using a Ultramark 4 Plus ultrasound machine (manufactured by Advanced Technology Laboratories) equipped with a 3.5 megahertz linear probe. The uterus was examined at the ventral midline cranial to the pubic bone. Implant placement followed the protocol established for humans and provided in the Norplant packet (Wyeth-Ayerst, 1990). The six implants were inserted subcutaneously into the medial surface of the upper left arm using a 10 gauge trocar. A continuous intradermal suture pattern was used to close the skin incision. This type of buried suture pattern was used to deter the chimpanzees from removing the implants. The females received the implants in the morning and were returned to their social group the afternoon of the same day.

## VERIFICATION OF IMPLANTS AND NON-PREGNANT STATUS

Seven months following insertion of implants the subjects were immobilized to verify that the implants had remained in place (i.e. had not been removed). Manual palpation and ultrasonography, utilizing a 10 megahertz sector probe, were used to locate the silastic capsules. All

six implants were verified as being in place in six of the seven females. One female, BR, had only four implants. Confirmation that the other two implants had been lost externally and had not migrated from the original site of insertion was provided through assays on urine samples from other females in the same social group. On the day following BR's implant placement, urine samples from two of her cagemates, not on a contraceptive regimen, showed high concentrations (up to 8.4 ng/mg Cr) of LNG, thus indicating that these females had ingested the implant capsules.

Pregnancy tests were conducted in February and again in June of 1994 after insertion of implants. Additional confirmation of non-pregnant status was obtained by ultrasonic examination of each females' uterus during implant verification.

GENITAL SWELLING RECORDS

The genital swelling of each female was scored daily using a five-point scale (0 = no swelling; 1/4, 1/2, 3/4 = partial swelling; 4 = full swelling). For this study, only the 3 categories, 0 = no swelling, 1 = partial swelling and 2 = maximal swelling, were analyzed. Maximal tumescence was defined as maximum swelling of the labia minora and perianal tissues resulting in labial occlusion, a deeply recessed anus, and a taut and shiny appearance (following Dahl et al., 1991). Three facility employees were responsible for

the daily recording of genital swelling data, however, one person recorded 75% of these data. Kendall's coefficient of concordance found agreement among scorers to be 79.6% when comparing data from 8 test days.

URINE COLLECTION

Between June and August 1993, urine was collected from five subjects across one menstrual cycle prior to the insertion of Norplant (Phase I). Approximately six months following implant placement, urine was again collected across one cycle (Phase II). Samples were obtained two to three days weekly, with more frequent sampling during the periovulatory period of the control phase cycles.

Sampling began 10 days following detumescence of the genital swelling in one cycle and continued until the detection of menses or for 12 days following detumescence of the sample cycle. In the case of two females during Phase II, one exhibiting no genital swelling and one exhibiting erratic genital swelling, urine was collected across a six-week period.

Urine collections were made between 0800 and 1200 hours. Females were trained to enter a cage equipped with a metal pan for urine collection. Fifteen to 30 ml of urine were obtained from each female during each day of sampling. Urine was collected in a 50 ml syringe, aliquoted into two 15 ml polypropylene conical tubes and frozen immediately.

Samples were transported on dry ice and remained frozen until assayed.

URINARY ASSAYS

Urinary assays were conducted by Debbie Cougar under the direction of Dr. Bill Lasley at the University of California at Davis. Measurement of levonorgestrel concentrations was conducted using a levonorgestrel-specific enzyme immunoassay developed for use in humans (Munro et al., in preparation). Fifty microliters (ml) of rabbit anti-levonorgestrel antibody in coating buffer (.05M bicarbonate, pH 9.6), at a dilution of 1:15,000, were placed in each well of a 96-well microtiter plate and incubated overnight at 4° C.

Urine was extracted by adding two ml of petroleum ether and placing the mixture in a methanol and dry ice bath. The unfrozen supernatant was decanted into clean tubes and dried under a stream of air. Each tube then received 150 ml of phosphate buffered saline (PBS; .1M, pH 7.0, with .1% BSA) and was placed in a water bath at 60° C for 20 minutes. Standards were prepared using known concentrations of LNG diluted in ethanol and dried.

Fifty ml of sample, 50 ml of PBS and 50 ml of conjugate (horseradish peroxidase coupled to LNG) were placed in each well and allowed to incubate for two hours at room temperature. Unbound LNG was removed by washing each plate

five times in washing solution (.15M NaCl with .05% Tween 20). Conjugate binding was determined by adding 100 µls of 2,2'-azino-di-(3-ethylbenzthiazoline sulphonic acid) diammonium salt (ABTS) substrate solution (pH 4.0) and incubating for one hour at room temperature. The substrate reaction was terminated with 100 µls of .15M hydrogen fluoride stop solution. Optical density was measured at 405 nm in a Dynatec MR580 automatic microelisa plate reader. Standard curves and sample LNG concentrations were generated using an in-house computer program (Munro and Stabenfeldt, 1984). Parallelism was demonstrated using samples of human and chimpanzee urine during natural cycles. Ninety percent efficiency in the extraction technique was determined using radioimmunoassay comparisons.

Measurement of estrone-3-glucuronide ($E_1G$) and pregnanediol-3-glucuronide (PdG) was conducted using the method validated and described by Munro et al., 1991. Antibody dilution for $E_1G$ was 1:5000 and for PdG was 1:15,000. Unextracted urine samples were diluted 1:50 with distilled water. Horseradish peroxidase conjugated to $E_1G$ was used at a dilution of 1:5000; horseradish peroxidase conjugated to PdG was diluted 1:15,000. After two hours incubation at room temperature, measurement was conducted as described for LNG.

Sensitivity of the assay for LNG was .019 ng/ml urine. Munro et al. (1991) reported the sensitivity of the $E_1G$

assay to be <1.0 pg/well (10.6 nmol/L of urine) and sensitivity of the PdG assay to be <10.0 pg/well (<.05 μmol/L urine). Antiserum for $E_1G$ shows cross reactivity with free estrone (238%) and estrone-3-sulfate (66.6%), therefore the assay actually measures estrone conjugates (Munro et al., 1991). PdG antiserum showed a 60.7% crossreactivity with 20 α-hydroxyprogesterone (Munro et al., 1991). Average intra-assay coefficients of variation (CVs) for urine pools of low, medium and high concentration were: for $E_1G$ - 11.2%, 6.6% and 4.9%: for PdG - 11.0%, 6.9% and 5.2%, respectively. Interassay CVs on these same samples were: for $E_1G$ - 13.9%, 10.1% and 8.5%; for PdG - 13.6%, 7.8% and 5.6%.

To adjust for varying urine concentrations, all samples were indexed for creatinine (Cr) using the standard method described in Taussky (1954).

DATA ANALYSIS

Ovulation was inferred to have occurred in cycles showing luteal phase concentrations of PdG higher than 1.0 μg/mg Cr. For analysis, presumed ovulation was estimated to have occurred 24-48 hours prior to the first recorded PdG concentrations of .3 μg/mg Cr or higher. Urine samples were not collected daily, therefore, peak concentrations of $E_1G$ and PdG refer to the highest concentration measured in the available samples. To adjust for the greater number of samples obtained during the periovulatory period of cycles

during Phase I, consecutive day samples were averaged. Approximately one sample per three day interval per subject was used in this analysis (12-18 samples/phase/female). Values obtained from samples with creatinine levels below .20 mg/ml were discarded.

Student's t-tests were used to assess differences in menstrual cycle and genital swelling durations between Phases I and II. Differences in hormone concentration between Phase I and II were determined by analysis of variance, while differences in peak values were assessed by Student's t-tests.

## RESULTS

Pregnancy was not detected in any of the females during Phase I or Phase II of the study.

### MENSTRUAL CYCLE DURATION AND GENITAL SWELLING WITH NORPLANT

During the period of urine collection, five of the females exhibited genital swelling cycles. One female (SR) exhibited no genital swelling and had not done so since receiving the implants. The other female (BT) exhibited three genital swelling cycles following resumption of cycling, however, during the period of urine collection her genital swelling was erratic with no discernable pattern.

Table 3 provides means and results of t-tests on cycle variables. Among females exhibiting genital swelling cycles,

cycle length did not differ between Phase I and Phase II. Neither the follicular phase, measured from the onset of menses to the first day of genital swelling detumescence, nor the luteal phase, measured from detumescence of the genital swelling to the onset of menses, differed between control and implant cycles. There was a significant increase in the number of days with partial swelling ($p=.039$), however, number of days with full swelling, although fewer, was not statistically different (Figure 2).

LEVONORGESTREL LEVELS

Mean LNG concentration was between .01-.02 ng/mg Cr for six of the females. The remaining female had a mean concentration of .08 ng/mg Cr. Daily levels fluctuated from below assay sensitivity to .30 ng/mg Cr among the females (Table 4).

CYCLES DISPLAYING FOLLICULAR AND LUTEAL ACTIVITY

Urinary assays of the five females that exhibited cyclic genital swelling patterns, showed fluctuating levels of ovarian hormones consistent with follicular and luteal development (Figure 2). Mean follicular and luteal phase concentrations of $E_1G$ did not differ between Phase I and Phase II of the study. Neither, peak $E_1G$ values at midcycle nor during the luteal phase, differed between control and treatment (Table 5).

No difference was detected in follicular concentrations of PdG between Phases I and II of the study. However, luteal concentrations were significantly lower after the subjects received the implants ($p=.001$), as were peak PdG ($p=.003$) values (Table 5). Maximum levels of PdG measured in cycles during the treatment phase were between 1.1 and 2.2 $\mu$g/mg Cr compared with 3.0 to 4.3 $\mu$g/mg Cr during the control phase. Additionally, PdG concentration remained elevated for 4-12 days during the control phase and only 1-5 days during treatment with Norplant.

One female, GG, exhibited genital swelling cycles which were occasionally shorter or longer than average cycles. Assays on urine collected across two short cycles revealed that one cycle exhibited the presumed ovulatory pattern of hormone secretion while one cycle was anovulatory. The cycle with presumed ovulation was included in the analysis described above. The anovulatory cycle showed two $E_1G$ spikes, five days apart, without a subsequent rise in PdG. Additionally, following the rapid drop in PdG concentration during the presumed ovulatory cycle, genital swelling resumed. The swelling reached maximal tumescence immediately, remained for three days then receded. In the anovulatory cycle, genital swelling did not accompany the rise in $E_1G$, even though values above 70 ng/mg Cr where measured. However, during this period, LNG values of .03-.11 ng/mg Cr - the highest recorded in this female, were

measured.

FEMALES EXPRESSING MINIMAL FOLLICULAR ACTIVITY

The nulliparous female (SR) which failed to resume genital swelling cycles after receiving Norplant showed minimal follicular activity. $E_1G$ concentration was below measurable amounts in 71% of the samples on this female. The remaining samples, which occurred within a seven-day period, ranged between 0.7-2.5 ng/mg Cr. PdG levels did not rise above 0.2 μg/mg Cr (Figure 3).

The multiparous female (BT) which exhibited erratic genital swelling during the urine collection period also showed minimal levels of $E_1G$ and PdG. $E_1G$ concentration was below measurable levels in 77% of the samples on this female. The remaining samples, which occurred within a five-day period, ranged from 0.4-0.6 ng/mg Cr. PdG typically measured 0.1 μg/mg Cr, with a maximum of 0.2 μg/mg Cr (Figure 3).

**DISCUSSION**

Levonorgestrel concentrations were low in the chimpanzees in this study after receiving the implants. Currently, data are not available on LNG concentrations in human urine with which to compare. However, the low concentration of LNG in chimpanzee urine does not indicate insufficient amounts of circulating LNG, as pregnancy was

not detected in any of the subjects. It does suggest that the metabolic pathway of LNG in chimpanzees may result in LNG being excreted through the feces rather than urine. A similar hypotheses has been proposed to explain the relatively low concentration of PdG in chimpanzee urine (Graham et al., 1972; Wright et al., 1981). Levonorgestrel is a synthetic progestin and therefore, may utilize a similar excretory route.

Reports of plasma LNG levels in humans indicate that plasma concentration is highest within the first 100 days, declining rapidly to concentrations of approximately .41 to.29 ng/ml for up to six years following insertion (Nash et al., 1978; Croxatto et al., 1981; Diaz et al., 1982; Robertson et al., 1983; Shaaban et al., 1984). Studies have produced conflicting results on the correlation between anovulation and LNG concentration. Originally, Croxatto et al. (1982) reported that presumed ovulatory rate was negatively correlated with concentration of LNG and similarly, Faundes et al. (1991b) reported that concentrations of LNG were lower, although not significantly, in subjects showing presumed ovulatory cycles. However, Brache et al. (1985) reported that some women with high levels of LNG did ovulate while some women with low concentrations were anovulatory.

The results of the present study indicate similar variation among the chimpanzees. Female SR had the highest

mean concentration of LNG and suppression of follicular development. However, subject BT also lacked evidence of follicular activity but exhibited a mean LNG concentration consistent with the females expressing both follicular and luteal activity. Additionally, concentrations up to 30 ng/mg Cr were found in presumed ovulatory and anovulatory cycles. These results indicate that individuals may vary in their response to chronic LNG stimulation.

The results on ovarian hormone secretion reported in this study suggest that Norplant may exhibit its contraceptive effect through several mechanisms. As is found in humans, Norplant worked by inhibiting ovulation in some cycles and depressing mean and peak levels of PdG in cycles with presumed ovulation (Shaaban et al., 1984; Faundes et al., 1991a; Faundes et al., 1991b; Shoupe et al., 1991a). The five females which exhibited cycles containing follicular and luteal development, also showed luteal phase inadequacy through lower peak and mean concentrations of PdG. Levonorgestrel works directly on the ovary, suppressing corpus luteum activity (Mukherjee et al., 1972; Brache et al., 1985). Additionally, the effect on the hypothalamus and pituitary axis results in lower levels of FSH and LH during Norplant treatment, which may interfere with normal oocyte maturation (Alvarez et al., 1986; Faundes et al., 1991; Segal et al., 1991)

Ovulation was inferred to have occurred in cycles with

PdG concentrations above 1.0 μg/mg Cr. However, the rise in PdG may have occurred as a result of luteinization of the follicle. This has also been suggested in studies on humans (Croxatto et al., 1982; Faundes et al., 1991b; Shoupe et al., 1991b). However, Alvarez et al. (1986) reported that during laparotomy, a corpus luteum was present on the ovary of two women with presumed ovulation during Norplant treatment. Additionally, PdG concentrations above 1 μg/mg Cr resulting from a luteinized follicle would be unusually high (B. Lasley, personal communication).

Data on one female across two cycles showed one cycle to contain luteal activity and the other cycle to be anovulatory, thus demonstrating the variability which can be found even within one female. Also consistent with data on humans (Shaaban et al., 1984; Faundes et al., 1991a; Faundes et al., 1991b; Shoupe et al., 1991a), estrogen levels in the anovulatory cycle were comparable to the presumed ovulatory cycles.

The suppression of ovarian and genital swelling activity in the adolescent female in this study may have resulted from her reproductive immaturity at the time of implant. Few studies have been conducted on human adolescents using Norplant (Berenson and Wiemann, 1993; Cullins et al., 1994). Studies to date have found no significant difference in side effects experienced by adults and adolescents (Berenson and Wiemann, 1993; Cullins et al.,

1994). However, most adolescents in these studies had one previous pregnancy, indicating they were reproductively mature at the time of implant placement. The adolescent chimpanzee in this study was still exhibiting irregular adolescent cycles (Tinklebough, 1933; Young and Yerkes, 1943; Clark and Birch, 1948) and may not have undergone menarche.

Ovarian hormone suppression was also found in one multiparous female in this study. However, during this period she was exhibiting fluctuating levels of genital swelling, including maximal tumescence. The reason for this disparity between chronically low levels of estrogen and erratic genital swelling during this period cannot be explained at this time.

Several studies have shown that chronic stimulation with exogenous hormones can induce daily fluctuations in level of genital swelling (Clark, 1947; Clark and Birch, 1948; Graham et al., 1972; Nadler et al., 1992; Bettinger, in press). Clark and Birch (1948) suggested that normal genital swelling is a result of the synergistic activities of progesterone and estrogen. However, the authors found that under constant progesterone stimulation, genital swelling would resume with minimum levels of estrogenic stimulation, a phenomenon they called "progesterone escape" (Clark and Birch, 1948). Modern studies have shown that this "escape" results from the down regulation of progesterone

receptors under chronic stimulation of a progestin (Speroff et al., 1989).

Graham et al. (1972) reported that genital swelling was not enhanced by progesterone and only occurred at certain ratios of estrogen to progesterone: 1:10 results in partial swelling, 1:20 in detumescence. However, in at least one case detumescence was transitory. Nadler et al. (1985) suggested that it is not the ratio but the absolute levels of progesterone which inhibit genital swelling. Additionally, some female chimpanzees exhibit genital swelling during pregnancy, a period when both, levels of estrogen and progesterone, are high. This occurrence of genital swelling during pregnancy has yet to be clearly explained (Faiman et al., 1981), however, genital swelling has been shown to occur more frequently in younger females than in older females (Wallis and Lemmon, 1986; Wallis and Goodall, 1993).

The variation in the genital swelling response suggests that individuals may differ in their response to ovarian, as well as hypothalamic and pituitary hormone stimulation. Additionally, chronic stimulation by estrogen and/or progesterone causes a down regulation in the concentrations of receptors which in turn diminishes the steroids ability to exert its effect (Speroff et al., 1989; Collins, 1994).

Five of the eight cycles (62.5%) analyzed, approximately six months following Norplant placement, were

presumed to be ovulatory. This rate is higher than rates of presumed ovulation reported among the general population of human females one year following implant placement (11-36% overall, Croxatto et al., 1982; Shaaban et al., 1984; Croxatto et al., 1988; Brache et al., 1990) and higher than that reported among women with normal bleeding patterns (30-60%, Brache et al., 1985; Faundes et al., 1991a; Faundes et al., 1991b; Shoupe et al., 1991a). This presumed rate of ovulation is more representative of fifth year Norplant users (Croxatto et al., 1982; Shaaban et al., 1984; Brache et al., 1990).

Additionally, if regular genital swelling cycle intervals of chimpanzees are comparable to regular bleeding intervals of humans, five of the chimpanzees may be at higher risk of contraceptive failure (Shoupe et al., 1991a). Level of genital swelling appears to be more sensitive to the contraceptive than does estrogen secretion. The increase in the number of days with partial swelling and the slight decrease in number of days with maximal swelling suggests that monitoring genital swelling pattern may be a beneficial tool in determining the continued effectiveness of the contraceptive. Although Norplant is effective for at least five years in humans, the longevity has yet to be determined in chimpanzees.

**CONCLUSIONS**

1. Norplant implants prevented pregnancy in seven chimpanzees by inhibiting ovulation (n=3 cycles) or inducing luteal phase inadequacy during cycles presumed to be ovulatory (n=5 cycles).

2. Six of the seven females continued to exhibit genital swelling during Norplant treatment. However, irregularities did occur.

3. Adolescent females which are not exhibiting cycles comparable to adult intervals at the time of Norplant insertion, may revert to an acyclic phase once they are on the Norplant regime.

4. Genital swelling patterns may be more sensitive to the effects of Norplant than is estrogen secretion.

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TABLE 1. Life history data on the seven female chimpanzees in this study

| Female | Date of birth | Parity | Birth of last infant | Date of implant | Weight at implant (kg) |
|---|---|---|---|---|---|
| SR | 02/12/85 | 0 | -- | 08/25/93 | 52.0 |
| MS | 06/17/84 | 0 | -- | 08/11/93 | 55.0 |
| BD | 1964 | >2 | 1988 | 08/18/93 | 64.5 |
| GG | 1963 | >2 | 1988 | 07/29/93 | 63.0 |
| BR | 1967 | >2 | 1990 | 07/29/93 | 46.0 |
| BT | 1965 | >2 | 1993 | 08/11/93 | 78.5 |
| GR | 1968 | >2 | 1990 | 07/29/93 | 52.5 |

TABLE 2. Genital swelling and menses data in relation to time of insertion of Norplant for seven female chimpanzees

| Female | Days from last menses | Days from last maximal swelling | Level of swelling at implant | Number of days until next cycle |
|---|---|---|---|---|
| BR | 10 | 20 | 3 (day 1) | 53/202* |
| BD | 8 | 22 | 4 (day 4) | 27 |
| BT | -- | 13 | 0 | 61 |
| GG | 6 | 18 | 0 | 48 |
| GR | 8 | 13 | 0 | 54 |
| MS | 4 | 16 | 0 | 14 |
| SR | -- | 22 | 0 | -- |

*Although this female began exhibiting genital swelling cycles 53 days after the insertion of Norplant, the first cycle with full swelling did not occur for 202 days following implant placement.

TABLE 3. Mean duration of menstrual cycle components during Phases I and II of the study

| | Cycle length | Menses to detumescence | Detumescence to menses | Days with partial swelling | Days with full swelling |
|---|---|---|---|---|---|
| Phase I | 37.0±6.3 (n=6) | 25.2±7.3 (n=5) | 11.8±0.8 (n=5) | 2.0±1.1 (n=6) | 14.2±3.5 (n=6) |
| Phase II | 39.2±11.0 (n=5) | 30.3±11.4 (n=4) | 10.5±1.3 (n=4) | 7.4±5.4 (n=5) | 11.2±2.3 (n=5) |
| t-test | 0.416 | 0.813 | -1.836 | 2.410 | -1.607 |
| p | .687 | .443 | .109 | .039* | .142 |

TABLE 3. Mean level of levonorgestrel (LNG), occurrence of genital swelling, menses and ovulation

| Female | LNG (ng/mg/cr) | Daily range | Genital swelling | Menses observed | Ovulation |
|---|---|---|---|---|---|
| SR | .08 | 0-.30 | No | No | No |
| MS | .02 | 0-.10 | Yes | Yes | Yes |
| BR | .01 | 0-.30 | Yes | Yes | Yes |
| BD | .01 | 0-.05 | Yes | Yes | Yes |
| GG | .01 | 0-.11 | Yes | Yes* | Yes |
| BT | .01 | 0-.05 | Yes | No | No |
| GR | .02 | 0-.04 | Yes | Yes | Yes |

* Observed during maximal tumescence.

TABLE 5. Mean levels of estrone-glucuronide ($E_1G$) and pregnanediol-glucuronide (PdG) in chimpanzees during Phases I and II of the study (mean ± sd)

| | Phase I | Phase II | Statistic |
|---|---|---|---|
| Follicular $E_1G$ (ng/mg/cr) | 19.68±16.01 | 16.32±18.25 | F= 0.274 |
| Luteal $E_1G$ (ng/mg/cr) | 22.13±17.07 | 16.90±15.09 | F= 1.318 |
| Pre-ovul. peak $E_1G$ (ng/mg/cr) | 51.37± 8.47 | 53.07±16.10 | t= 0.187 |
| Luteal peak $E_1G$ (ng/mg/cr) | 34.06±13.46 | 32.98± 4.00 | t= 0.172 |
| Follicular PdG (ug/mg/cr) | 0.07±0.06 | 0.08± 0.10 | F= 0.077 |
| Luteal PdG (ug/mg/cr) | 1.35±1.20 | 0.60± 0.47 | F=12.253** |
| Luteal peak PdG (ug/mg/cr) | 3.38±0.93 | 1.44± 0.50 | t= 4.127* |

*p=.003
**p=.001

Fig. 1. Example of patterns of urinary excretion of luteinizing hormone (LH), estrone, pregnanediol and level of genital swelling in an adult female chimpanzee, as published in McArthur et al., 1981.

FIG. 1

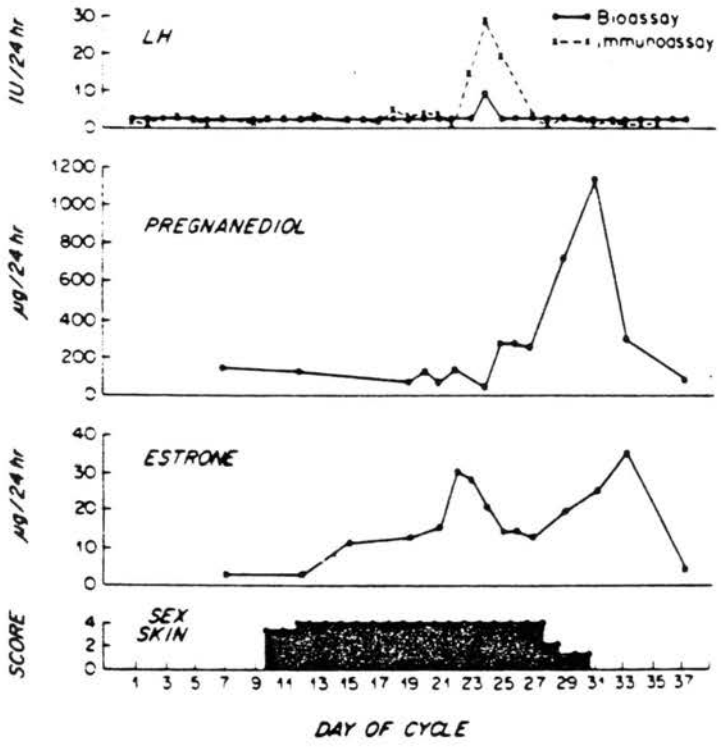

LH
Bioassay
Immunoassay
IU/24 hr
30
20
10
0
PREGNANEDIOL
µg/24 hr
1200
1000
800
600
400
200
0
ESTRONE
µg/24 hr
40
30
20
10
0
SEX SKIN
SCORE
4
2
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37
DAY OF CYCLE

Fig. 2. Concentrations of estrone glucuronide ($E_1G$), pregnanediol glucuronide (PdG) and level of genital swelling exhibited by three female chimpanzees before and after receiving Norplant implants.

FIG. 2

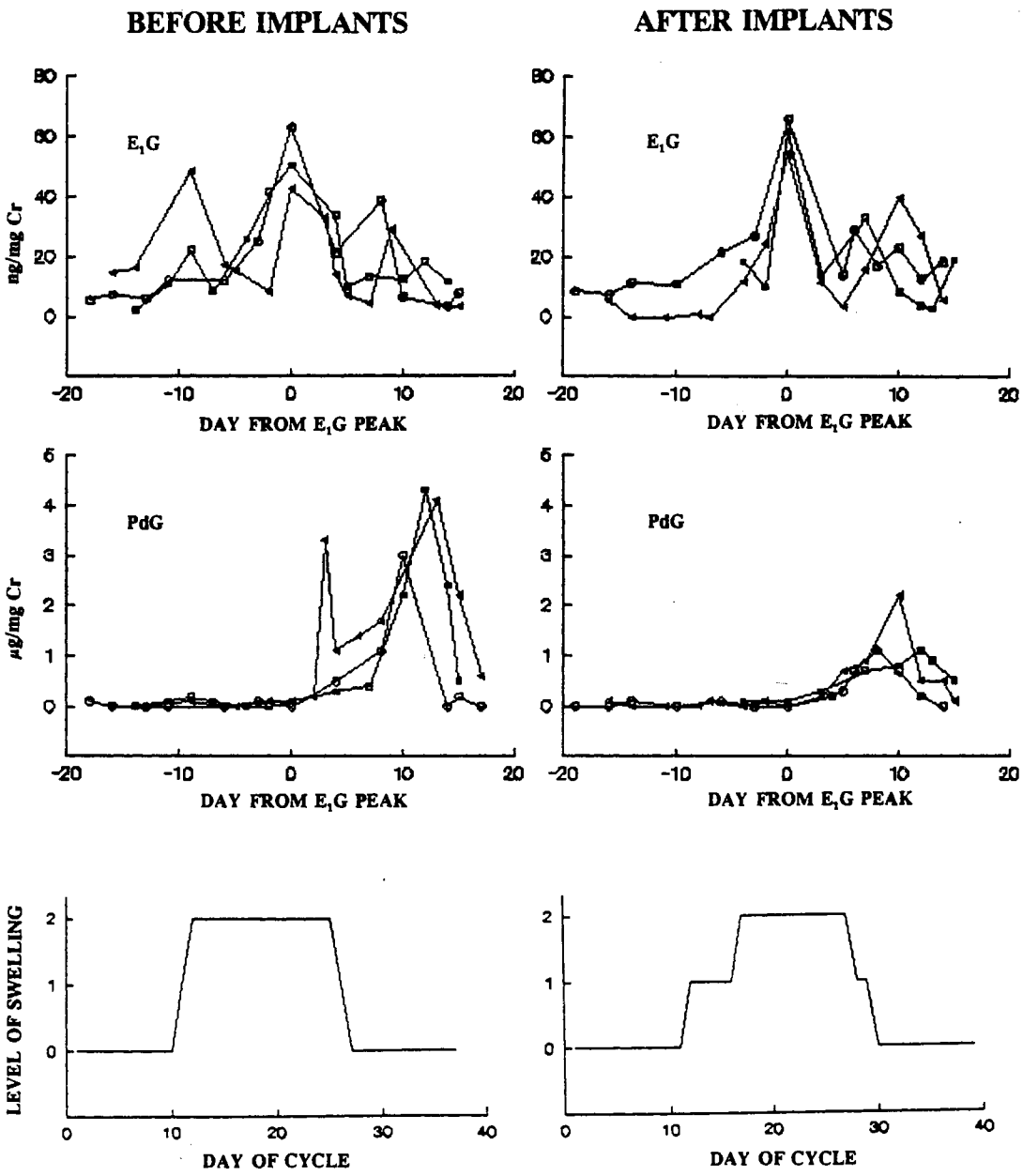
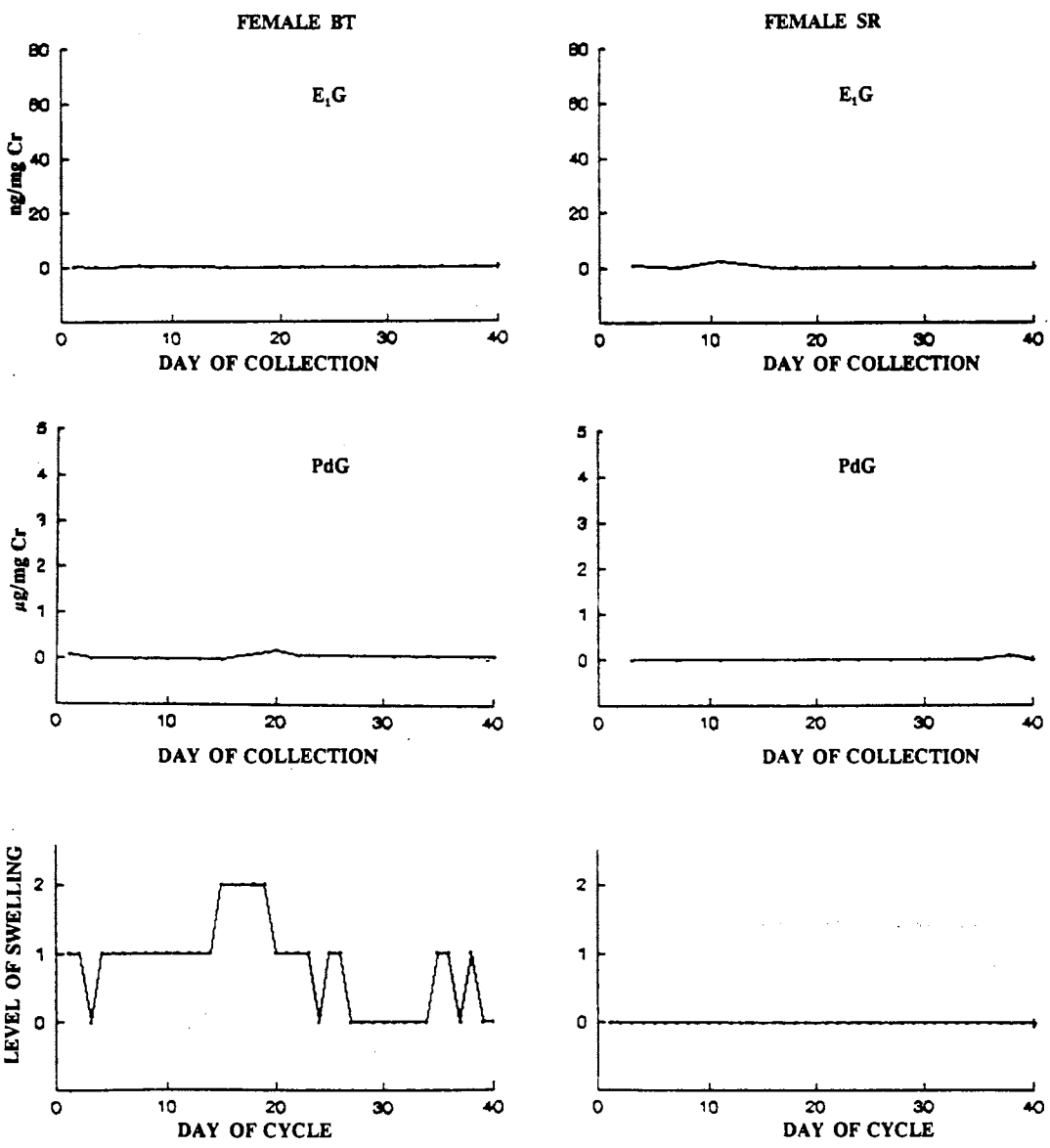

BEFORE IMPLANTS
AFTER IMPLANTS
E1G
ng/mg Cr
DAY FROM E1G PEAK
PdG
µg/mg Cr
LEVEL OF SWELLING
DAY OF CYCLE

Fig. 3. Concentrations of estrone glucuronide ($E_1G$), pregnanediol glucuronide (PdG) and level of genital swelling exhibited by two female chimpanzees with anovulatory cycles during Norplant treatment.

FIG. 3


AFTER IMPLANTS
FEMALE BT
FEMALE SR
$E_1G$
$E_1G$
ng/mg Cr
80
60
40
20
0
0
10
20
30
40
DAY OF COLLECTION
PdG
PdG
μg/mg Cr
5
4
3
2
1
0
DAY OF COLLECTION
LEVEL OF SWELLING
DAY OF CYCLE

# CHAPTER III

# EFFECTS OF NORPLANT IMPLANTS ON GENITAL SWELLING PATTERNS IN FEMALE CHIMPANZEES

# EFFECTS OF NORPLANT IMPLANTS ON GENITAL SWELLING PATTERNS IN FEMALE CHIMPANZEES

**ABSTRACT**

Genital swelling data on seven female chimpanzees was analyzed across five cycles before and seven cycles following insertion of Norplant implants. Repeated measures analysis of variance was used to assess differences in cycle variables before and after implant placement. Pearson's correlation coefficients were used to determine relationships between variables. Length of cycle did not differ after Norplant treatment ($F=1.415$, $p=.200$). Duration of the swelling phase ($F=3.311$, $p=.011$) and full swelling phase ($F=5.906$, $p=.011$) was shorter during Norplant treatment. However, number of days with partial swelling ($F=2.635$, $p=.011$) increased. No correlation was found between weight or age and the cycle variables. These data indicate that Norplant is an effective contraceptive for chimpanzees which does not inhibit the occurrence of cyclic genital swelling.

**INTRODUCTION**

There is an apparent contradiction in the use of contraceptives when managing endangered species. Why limit reproduction when the species is in danger of extinction? Although the chimpanzee is endangered in the wild, captive breeding programs have become quite successful (Schobert, 1990; National Institutes of Health, 1994). Therefore, contraceptives are currently used as a management tool (Porton and Asa, 1990). Birth control regimens are used both for genetic control of the captive gene pool and to limit the number of births each year ensuring that the captive population does not exceed its space allocation (Fulk, 1990; National Institutes of Health, 1994; also see Weise et al., 1993). Additionally, managers may wish to postpone first conceptions in young females, increase the interbirth intervals of parous females, or prevent reproduction in females exhibiting incompetent maternal skills.

Currently, there is not a contraceptive regimen that has been developed for use in chimpanzees. As a result, chimpanzees are often placed on a contraceptive regimen that has been approved for use in humans. An important consideration when choosing a contraceptive for chimpanzees is the effect it will have upon genital swelling. For many years researchers have recognized that the genital swelling can be used to monitor ovarian activity (Allen et al., 1936; Yerkes and Elders, 1936a, 1936b; Elder, 1938; Fish et al.,

1941; Graham et al., 1972; Nadler et al., 1985). More recently, researchers have found that the presence or absence of females with genital swelling strongly influences the frequency of many species typical behaviors within a social group (Merrick, 1977; Nishida, 1979; Wallis, 1982; Goodall, 1986; Wallis and Lemmon, 1986; Alford et al., 1990; Nishida, 1990; Bloomsmith et al., 1991; Lambeth et al., 1991; Bloomsmith et al., 1992; Wallis, 1992; Shefferly and Fritz, 1992; Bettinger et al., 1993; Wallis and Bettinger, 1993).

Studies have shown that the components of reproductive behavior in chimpanzees are learned during early development (Rogers and Davenport, 1969; Davenport and Rogers, 1970; Riesen, 1971; King and Mellen, 1994). These studies have found that the best method of ensuring future reproductive competency is to allow infants to be mother-reared within a social group. Therefore, a key behavioral concern is the ability to maintain multi-aged social groups which will produce competent adults for future breeding programs. To accomplish this goal, a contraceptive which allows the expression of genital swelling and normal sociosexual behavior is needed.

The anogenital swelling (also referred to as the genital swelling or sexual swelling), encompasses the labia and the perianal region and results from an accumulation of interstitial fluid (Elder & Yerkes, 1936; Clark & Birch,

1948) which can measure up to 1.4 liters (Elder & Yerkes, 1936). Duration of the menstrual cycle averages 35-38 days (Elders and Yerkes, 1936; Clark and Birch, 1948; Nadler et al., 1985; Dahl et al., 1991). However, individual variation is common (Graham, 1981). Early studies reported ranges from 22-187 days (Elders and Yerkes, 1936; Young and Yerkes, 1943), while more recent studies report ranges from 29-53 days (Nadler et al., 1985; Dahl et al., 1991).

Variation in cycle length in chimpanzees has been attributed to differing durations in the length of the follicular phase (Young and Yerkes, 1943; McArthur et al., 1981; Nadler et al., 1985; Dahl et al., 1991). Graham (1981) suggests that the prolongation of the pre-swelling phase (a part of the follicular phase) may actually be a delay in the initiation of a new cycle rather than an extension of the cycle itself. Additionally, infertility is only slightly correlated with cycle variability (Young and Yerkes, 1943; Graham, 1981; Dahl et al., 1991).

Duration of genital swelling phases also varies among individuals. Partial swelling phases range from 2-16 days, while maximal swelling duration ranges from 11-17 days (Nadler et al., 1985; Dahl et al., 1991). Dahl et al. (1991) reported that chimpanzees in their study exhibited genital swelling for an average of 41% of the menstrual cycle.

It has long been recognized that the anogenital swelling of the normal adult chimpanzee is correlated with

ovarian activity (Yerkes & Elder, 1936a, 1936b; Allen et al., 1936; Fish et al., 1941; Clark & Birch, 1948). Subsequent studies using modern assay techniques have elaborated on these early studies and quantitatively demonstrated the relationship between hormone level and genital swelling (Graham et al., 1972; Reyes et al., 1975; McArthur et al., 1981; Graham, 1981; Nadler et al., 1985).

During menses and the postmenstrual period of genital skin quiescence, estrogen and progesterone are at their lowest level (Allen et al., 1936; Graham et al., 1972; Nadler et al., 1985). As follicular development precedes, level of estrogen rises and is accompanied by an increase in the size of the genital swelling. Approximately 10 days before the midcycle estrogen peak, the genital swelling reaches maximal size and turgidity.

The LH surge and ovulation occur on the last 1-2 days of maximum swelling (Elder, 1938; Graham et al., 1972; Graham, 1981; Nadler et al., 1985). Ovulation always precedes detumescence in the adult female (Graham, 1981; McArthur et al., 1981). Regression of the genital swelling is associated with the collapse of the Graafian follicle (Graham, 1981), decreasing levels of estrogen and increasing levels of progesterone (Graham et al., 1972; McArthur et al., 1981; Nadler et al., 1985). A functional corpus luteum is found on the day following detumescence (Graham 1981). A second estrogen peak occurs during the luteal phase of the

cycle, however, it is not associated with an increase in the size of the genital swelling (Fish et al., 1941; Clark & Birch, 1948; Graham et al., 1972; Nadler et al., 1985).

It was originally proposed that estrogen controlled genital swelling (Allen et al., 1936; Yerkes & Elder, 1936a; Elder & Yerkes, 1936). However, the discovery of the second estrogen peak during the luteal phase of the cycle (which can sometimes result in estrogen levels higher than in the follicular phase) casts doubt on this proposal (Fish et al., 1941). Researchers found that in castrated females, genital swelling could be induced with estrogen or with combinations of estrogen and progesterone (Clark & Birch, 1948; Graham et al., 1972).

Progesterone was shown to have both an inhibitory and synergistic effect on genital swelling: a 1:10 ratio of estrogen to progesterone resulted in genital swelling, while a 1:20 ratio resulted in no genital swelling (Graham et al., 1972). Graham et al. (1972) proposed that it is the ratio of estrogen to progesterone which regulates genital swelling. More recently, Nadler et al. (1985) suggested that genital swelling may be regulated by the absolute level of progesterone and that the ratio per se may not be wholly responsible.

Graham (1981) reported that there is no evidence that estrogen and progesterone act directly on the genital skin. The effects of estrogen and progesterone may be mediated

through the hormone system controlling water balance. Graham (1981) concluded that the genital swelling of baboons and presumably of chimpanzees may be a result of "an organ-specific estrogen-induced effect on hyaluronic acid and water accumulation."

Contraceptives work by interrupting the normal cycle of estrogen and progesterone production and are, themselves, synthetic forms of these hormones. Consequently, as would be expected, contraceptives have been shown to alter the normal pattern of genital swelling in chimpanzees (Nadler et.al, 1992; Bettinger, in press).

Studies on female chimpanzees administered combined oral contraceptives have shown that genital swelling does occur at several dose levels (Nadler et al., 1992; Bettinger, in press). However, Bettinger (1993) found variation among the females in the genital swelling response; some females seldom expressed swelling while others expressed constant swelling. In both studies, cyclicity of swelling was abolished and genital swelling continued throughout most of the placebo phase. Consistent with the findings of Clark (1947), Nadler et al. (1992) reported a day to day fluctuation in size of the genital swelling under constant hormone stimulation. Additionally, they found that increasing the dosage of ethinyl estradiol in relation to the dosage of norethindrone produced a larger genital swelling. However, maximum tumescence was not

reported by Nadler et al. (1992) and occurred only rarely in Bettinger's study (in press).

Nadler et al. (1992, 1994) found that the endogenous release of estrogen and progesterone was abolished in the female chimpanzees given oral contraceptives. This, in addition to the constant daily dosage of contraceptive, results in an artificially induced genital swelling cycle. This "artificial swelling" may be less attractive to male chimpanzees as well as have a negative influence on female sexual behavior. Indeed, Nadler et al. (1993; 1994) found a reduction in copulatory rate among these females. In contrast, Norplant implants may provide a contraceptive regimen which could permit the cyclic expression of genital swelling, thus allowing natural sexual behavior to occur.

Norplant implants, containing the synthetic progestin levonorgestrel (LNG), were developed for human use by the Population Council's International Committee for Contraceptive Research in the mid 1970s (Segal, 1983). Levonorgestrel acts upon the hypothalamus and pituitary to suppress the surge of luteinizing hormone responsible for triggering ovulation (Alvarez et al., 1986). However, ovulation has been reported to occur in 30-80% of the cycles studied and, is most frequent after the first year of implant use (Croxatto et al., 1982; Shaaban et al., 1984; Brache et al., 1985; Brache et al.1990, Shoupe et al., 1991a, 1991b; Faundes et al., 1991a, 1991b). In ovulatory

cycles, Norplant prevents pregnancy by thickening cervical mucous, depressing endometrial development and causing luteal phase inadequacy (Croxatto et al., 1982; Shaaban et al., 1984; Alvarez et al., 1986; Faundes et al., 1991a, 1991b; Segal et al., 1991).

The primary side effect associated with Norplant use is irregular bleeding patterns reported in some patients, especially during the initial months of implant use (Diaz et al., 1982; Shaaban et al., 1983; Sivin et al., 1983a, 1983b; Fakeye et al., 1989; Du et al., 1990; Shoupe et al., 1991a; Faundes et al., 1991a; Fakeye, 1991; Berenson et al., 1993; Cullins et al., 1994; Darney 1994). Irregular bleeding patterns include frequent menses, spotting and amenorrhea. By the end of the first six to 12 months, most women return to a bleeding pattern similar to pre-implant cycles.

Norplant is a popular contraceptive in humans due to its ease of administering, high efficacy rate, long duration of effectiveness, and the rapid return to fertility upon removal (Segal, 1983; Berenson et al., 1993; Darney, 1994). One set of Norplant implants will effectively prevent pregnancy for 5 years (Segal, 1983). Pregnancy rate among Norplant users is about 1% of 5-year users, with the principal factor related to failure being body weight above 70 kg (Darney, 1994). Ismail et al. (1987) found that after implant removal, ovulation was resumed in 80% of the patients by 3 weeks and in 100% by 7 weeks. Similarly,

cumulative pregnancy rates reported for ex-Norplant users was 76.5% (Affandi et al., 1987) and 86% (Diaz et al., 1987) by the end of the first post-implant year.

Many managers of captive chimpanzees view Norplant as a suitable solution to their colony contraceptive needs. The ability to implant group-living non-tractable female chimpanzees eliminates the problems associated with daily administration of oral contraceptives. Extrapolating from the data on humans, if ovulation is not inhibited in every cycle, then cyclic genital swelling may occur, thus promoting species typical sociosexual behavior. There are currently 15-20 chimpanzees in North American zoos and 13 chimpanzees in one laboratory on the Norplant regimen. However, until this study, the effects of Norplant on chimpanzee genital swelling, hormone level and behavior had not been documented.

Data on urine samples collected from seven female chimpanzees across one cycle six months following the insertion of implants indicated that ovulation may have occurred (PdG > 1.0 $\mu$g/mg Cr) in five of the seven females. Cyclic elevations in neither, estrone-glucuronide nor pregnanediol-glucuronide, were found in the cycle analyzed for the other two females. LNG concentrations were significantly higher in the nulliparous acyclic female than in the other six females (Chapter 2).

This report provides genital swelling data on seven

subjects before and after receiving Norplant implants. Daily genital swelling records were analyzed to determine the effects of the implants on genital swelling cycle duration, swelling phase durations and correlations between these variables with weight, age and time since implant. These data provide additional evidence that Norplant may provide an effective contraceptive that does not inhibit the cyclic occurrence of genital swelling in chimpanzees.

## MATERIALS AND METHODS

### FACILITY AND HOUSING

The female chimpanzees in this study were maintained at The University of Texas, M.D. Anderson Cancer Center's veterinary resource facility in Bastrop, Texas. The females lived in multimale/multifemale social groups housed in enriched, 22-meter diameter outdoor corrals with access to indoor dens (see Riddle et al., 1982 for a detailed description of the facility). Each social group contained 2-3 adult males, 1-2 immature males, 3-6 adult females (in various reproductive states), and 0-1 immature females. All females in this study were continuously housed with at least one male with proven fertility. Additionally, pregnancy or births occurred in three of the four social groups either immediately preceding or during the study period.

STUDY SUBJECTS

Table 1 provides life history data on the seven chimpanzees included in this study. All subjects were scheduled to be placed on contraceptives and were, therefore, not chosen at random. Reasons for placing the females on a contraceptive regimen included adequate or over-representation in the gene pool (n=5) and delaying first conception (n=2). Additionally, the two nulliparous females were housed in social groups incompatible with breeding recommendations; one was housed in a social group containing her father and half brother, one had blood incompatible with the fertile male in her group.

Nulliparous female MS began cycling two and a half years before she was placed on the Norplant regimen, however, she had been housed with a fertile male for only 10 months prior to receiving Norplant. The male, although a proven breeder, may have had reduced fertility as a result of Hansen's disease (leprosy).

Females BT and GR were implanted quickly upon resumption of post-partum cycling; these females had a history of conceiving within 1-3 postpartum cycles. Although the remaining multiparous females had been cycling for almost two years, their cycles were often irregular. This may have resulted from their continuation of lactation. All multiparous females except BT were lactating and housed with offspring when placed on Norplant.

NORPLANT INSERTION

The seven females received implants between July 29, 1993 and August 25, 1993 (see Table 1). Ten to fifteen days following detumescence, pregnancy tests utilizing the detection of chorionic gonadotrophin in urine, were conducted. Upon immobilization, the female's uterus was examined using ultrasonography for further evidence that she was not pregnant.

Norplant implants were inserted during the early follicular phase of the female's cycle (Table 2). Implant placement followed the protocol established for use in humans and provided in the Norplant packet (Wyeth-Ayerst, 1990). The six implants were inserted subcutaneously into the medial surface of the upper left arm. A continuous intradermal suture pattern was used to close the skin incision. This buried suture pattern was used to deter the chimpanzees from removing the implants. The females received the implants in the morning and were returned to their social group the afternoon of the same day.

VERIFICATION OF IMPLANTS AND NON-PREGNANT STATUS

Verification that the implants remained in place was conducted in March 1994 using manual palpation and ultrasonography. All six implants were located in six of the seven females. One female, BR, had only four implants. Confirmation that the other two implants had been lost

externally (i.e. had not migrated internally) was provided through urinary assay of other females in the same social group. On the day following BR's implant placement, urine samples from two of her cagemates, not on a contraceptive regimen, showed high levels of LNG (up to 8.4 ng/mg Cr). Thus, we assume these females ingested the two missing implants.

Pregnancy tests were conducted on all seven females in February and June of 1994 following insertion of implants. Additionally, each females' uterus was examined ultrasonically during implant verification.

GENITAL SWELLING RECORDS

The genital swelling of each female was scored daily using a five-point scale (0=no swelling, 1/4, 1/2, 3/4=partial swelling, 4=full swelling). For this study, only the 3 categories, no swelling, partial swelling and full swelling, were analyzed. A full swelling was defined as maximum swelling of the labia minora and perianal tissues resulting in labial occlusion, a deeply recessed anus and a shiny taut appearance (following Dahl et al., 1991). Three facility employees were responsible for the daily recording of genital swelling data, however, one person recorded 75% of these data. Kendall's coefficient of concordance found agreement among scorers to be 79.6% when comparing data from 8 test days.

DATA ANALYSIS

Data were obtained from the daily genital swelling charts maintained by the facility. Phase I refers to five genital swelling cycles before insertion of implants. During this period, the females were exhibiting genital swelling cycles without receiving contraception. Obtaining data on five cycles before implants for females BT and GR required utilizing data recorded between two to three previous pregnancies. Data for the other females were taken from five consecutive cycles preceding implant placement.

Phase II refers to the first seven consecutive genital swelling cycles exhibited following insertion of the Norplant implants.

Menstruation was not always detected in the females. Therefore, cycles were analyzed in relation to the occurrence of genital swelling. Calculations were made using the following definitions:

Genital swelling cycle length = number of days from the first day of sustained genital swelling in one cycle to the first day of sustained genital swelling in the next cycle.

Genital swelling phase = number of consecutive days with genital swelling within one swelling cycle.

Full swelling phase = number of days with maximal tumescence of the anogenital region within one swelling cycle.

Partial swelling phase = number of days within one genital

swelling cycle with genital swelling rated as 1/4, 1/2, or 3/4.

Only genital swelling cycles which conformed to Graham's (1981) description of typical durations of swelling phases were used in the Phase I cycle analysis (i.e. post-partum and adolescent cycles which exhibited fluctuating levels of genital swelling and/or extended periods of genital swelling or quiescence where not included).

Repeated measures analysis of variance and Student's t-tests using pairwise comparisons were used to assess differences in variables before and after implant placement. Pearson's correlation coefficients were used to test relationships among variables. Differences in variances were assessed using F-tests.

## RESULTS

### INITIAL RESPONSE FOLLOWING IMPLANT PLACEMENT

Five of seven females showed an extended period of genital swelling quiescence subsequent to insertion of the implants (Table 2). This prolonged interval of swelling quiescence lasted 48-61 days. Upon resumption of genital swelling, three of the subjects did exhibit maximal tumescence during the first Phase II swelling cycle. One subject, however, did not reach maximal swelling for 202 days following implant insertion. Two females showed no initial suppression of their genital swelling cycle.

The adolescent female SR did not resume genital swelling cycles after receiving the implants. This female's pre-implant cycles were characteristic of adolescent cycles; they were irregular in length and duration of swelling phases. Therefore, cycle data on this female, both before and after implants, were omitted from the analysis.

GENITAL SWELLING PATTERNS WITH NORPLANT IMPLANTS

Two months following implant placement, six of the seven females had resumed cyclic genital swelling. The subjects exhibited a pattern similar to that of the Phase I cycles: quiescence of the genital skin followed by partial swelling which lead to a phase of maximum swelling then detumescence and a return to swelling quiescence. Figures 1 and 2 illustrate the average genital swelling cycle for two of the subjects before and after receiving Norplant.

Variation among the subjects was common. Figure 3 shows genital swelling cycles of a third female which, although the pattern of swelling was consistent with Phase I cycles, exhibited periods of short and long cycles. Female BT's swelling cycles became more irregular as the time since implant placement increased. Her first three cycles showed a pattern similar to the other females (Figure 4). However, between February and July 1994, BT exhibited frequent genital swelling without a discernible pattern (Figure 4).

GENITAL SWELLING CYCLE LENGTH AND DURATION OF SWELLING PHASES

Repeated measures analysis of variance on five females (BT excluded) for 5 cycles during Phase I and 7 cycles during Phase II showed that cycle length did not differ between the two conditions ($F=1.415$, $p=.200$). However, during Phase II, the females exhibited significantly fewer days of genital swelling ($F=3.311$, $p=.002$) and days of maximal tumescence ($F=5.906$, $p<.001$), but a greater number of days with partial swelling ($F=2.635$, $p=.011$). Table 3 shows the means for each female, as well as overall means for cycle length and duration of swelling phases before and after implants.

F-tests showed that variance was significantly higher during Phase II cycles for all four parameters (cycle length $F=2.256$, $p<.05$; swelling duration $F=3.967$, $p<.01$; full swelling duration $F=2.398$, $p<.05$; partial swelling $F=2.851$, $p<.05$).

Menstruation was observed in at least one cycle on five of the females following insertion of implants. For cycles during which menstrual blood was detected, the intervals from detumescence to menses and menses to detumescence was analyzed. Paired t-tests found no difference in these intervals between Phase I and Phase II cycles (detumescence to menses $t=.783$, $p=.454$, $n=4$ females, 10 intervals; menses to detumescence $t=1.580$, $p=.158$, $n=4$ females, 8 intervals).

The mean interval between detumescence and menses before implants was 12.10 days (sd=2.132) and with implants was 13.80 days (sd=6.408). Mean interval between menses and detumescence was 20.63 days (sd=2.504) before implants compared to 26.63 days (sd=9.680) with implants. In both cases, the variance was greater during cycles exhibited after Norplant placement (detumescence-menses $F=9.03$, $p<.01$; menses-detumescence $F=14.948$, $p<.01$).

During Phase II, there were no correlations between age and swelling cycle length, swelling duration or full swelling duration. Nor was there a correlation between weight and these variables (Table 4). However, the subjects exhibited a greater number of days with genital swelling ($r=.557$, $p=.001$) and with maximal swelling ($r=.409$, $p=.022$) with successive cycles following implant placement.

There was a significant increase in body weight among the females when comparing weights obtained at the time of implant insertion with weights obtained approximately 6 months later ($t=2.925$, $p=.026$). Mean weight of the females at the time of implant was 58.36 (sd=11.452) compared to 61.79 (sd=11.814) six months later.

Pregnancy tests conducted at six and ten months following implant insertion were negative on all seven females.

**DISCUSSION**

The data presented in this study provide important supplemental information to the hormone data previously reported (Chapter 2). Although variability was greater among the females after receiving the implants, six of the seven did resume genital swelling cycles. However, four of the females exhibited an initial period of suppression of the genital swelling cycles.

Reports on LNG release rates in humans indicate that release is highest during the first 60-100 days following insertion (Croxatto et al., 1981; Croxatto et al., 1982; Robertson et al., 1983; Olsson et al., 1987). Additionally, the inflammatory response that follows insertion of the implants may contribute to the initially higher levels of LNG found in circulation (Shaaban et al., 1984). As the release rate decreases and fibrous tissue accumulates around the implants, circulating levels of LNG decline (Shaaban et al., 1984). Individual variation in LNG levels is reported to be common among human patients (Nash et al., 1978; Croxatto et al., 1981; Shaaban et al., 1984; Olsson et al., 1987) and may account for the variation among the chimpanzees in their response time from implant to first genital swelling cycle. Additionally, correlation data showed that the females exhibited progressively more days with genital swelling the longer they remained on the Norplant regime.

The lack of genital swelling in one of the nulliparous females is consistent with the assay results indicating minimal follicular activity (Chapter 2). The genital swelling cycles exhibited by this female before receiving the implants were irregular in length and duration of swelling phases. This irregular pattern is common among young female chimpanzees during the period of adolescent infertility (Tinklepaugh, 1933; Young and Yerkes, 1943). The Norplant data suggest that in adolescents that have not attained genital swelling cycles conforming to the adult pattern, ovarian activity may be suppressed. Little is known about the effects of Norplant in human adolescent females. Until its 1992 Food and Drug Administration approval (Darney, 1994), studies could not involve adolescents. Initial data show no significant differences between problems experienced by adults and adolescents (Berenson et al., 1993; Cullins et al., 1994). However, the majority of adolescents in studies thus far have had at least one previous pregnancy, indicating that the subjects were reproductively mature at the time of implant placement.

The six females that exhibited genital swelling did so at intervals comparable to cycles preceding Norplant treatment. However, the females exhibited genital swelling on fewer days than during natural cycles. The decrease in total number of days with genital swelling each cycle included significantly fewer days at maximal tumescence and

significantly more days with partial swelling when compared to normal cycles. These data are an important supplement to the hormone data (Chapter 2). Although estrogen concentration may not be significantly different between Phase I and Phase II cycles, the ability of estrogen to maintain genital swelling is decreased. This could be due in part to the depressive effect of LNG on the production of sex hormone binding proteins and competition between LNG and the ovarian hormones for the limited binding sites on the carrier proteins (Victor and Johansson, 1977; Shaaban et al., 1984).

Two of the females exhibiting genital swelling cycles showed some cycles with pronounced irregularities. The extended period of irregular genital swelling of one multiparous female (BT) cannot be explained given our limited information. Hormone data collected on this female during six weeks of this irregular genital swelling period indicated low, often below measurable concentrations, of estrone-glucuronide in the urine (Chapter 2).

Persistent follicles have been reported to occur in 50% of the human subjects in one Norplant study (Shoupe et al., 1991b). Studies on human females with cystic follicles report an increase in the circulating levels of testosterone (Laatikainen, et al., 1980). Additionally, the increase in total levels of estrogens associated with cystic follicles is due to peripheral conversion of androstenedione to

estrone (Wajchenberg, et al., 1988) which may not be secreted as estrone-glucuronide in the urine of chimpanzees.

An alternative explanation may be that this particular female is reacting to an interaction between the levonorgestrel and endogenous hormone secretion. Several studies have shown that chronic stimulation with exogenous hormones can induce daily fluctuations in the level of genital swelling (Clark, 1947; Clark and Birch, 1948; Graham et al., 1972; Nadler et al., 1992; Bettinger, in press).

Clark and Birch (1948) found that under constant progesterone stimulation, genital swelling would resume with minimum levels of estrogenic stimulation, a phenomenon called "progesterone escape" (Clark and Birch, 1948). Modern studies suggest that the "escape" occurs as a result of a down regulation in progestin receptors (Speroff et al., 1989). Graham et al. (1972) reported that detumescence could be induced by administering a progestin, however, in at least one case detumescence was transitory. Additionally, some female chimpanzees may exhibit genital swelling during pregnancy, a period when both, levels of estrogen and progesterone, are high, a phenomenon not clearly understood (Faiman et al., 1981). However, studies report that genital swelling occurs more frequently during pregnancy in young females than in older females (Wallis and Lemmon, 1986; Wallis and Goodall, 1993). These data indicate that many factors may influence the expression of genital swelling and

that the underlying mechanism has not been fully determined.

It has been suggested that among Norplant users showing presumed ovulatory cycles, one mechanism preventing pregnancy may be a luteal phase inadequacy (Shaaban et al., 1984; Faundes et al., 1991a, 1991b; Segal et al., 1991; Shoupe et al., 1991b). Four of the six females consistently showed genital swelling intervals comparable to normal cycles. Menstrual cycle data on these females were analyzed to determine if the luteal phase, calculated as the number of days from the last day of maximal swelling to menses, differed between cycles before and during implants. The data showed no difference in luteal phase length. However, variation was significantly higher among the cycles in Phase II indicating that, at least during some cycles, the luteal phase may be shortened or lengthened.

In humans, a negative correlation between body weight and circulating levels of LNG has been reported (Croxatto et al. 1981). Heavier females also experience higher pregnancy rates (Darney, 1994). No correlation was found in this study between body weight and mean number of days with genital swelling. This may be due to the small sample size of the study or the fairly even distribution of weight among the females. A better indicator may be a measure of body fat as opposed to body weight.

The females did exhibit a significant increase in body weight after receiving the implants. This has been reported

to be a common side effect among human patients as well (Shaaban et al., 1983; Sivin et al., 1983b; Berenson et al., 1993; Darney, 1994). Levonorgestrel is structurally related to testosterone (Lobo and Stanczyk, 1994) and has androgenic binding capacity (Collins, 1994). Weight increase is a side effect of synthetic progestins that are capable of binding with testosterone receptors (Collins, 1994).

The data in this study indicate that genital swelling may occur during contraceptive treatment with Norplant. Genital swelling duration appears to be more sensitive to the effects of levonorgestrel than does estrogen secretion. These data suggest that for most females, monitoring their genital swelling is a useful tool in assessing the continued effectiveness of the regimen.

It is important to note, however, that following implant insertion, the females exhibited a greater number of days with genital swelling with successive cycles. Comparison of the genital swelling cycle preceding insertion of implants to a cycle six to seven months after implant placement indicated that the number of days with full swelling, although lower, did not differ statistically (Chapter 2). Therefore in time, some females may exhibit genital swelling patterns consistent with cycles of females not on a contraceptive regimen. This rapid return to values of the control cycles may suggest a decreased effectiveness of the contraceptive. The longevity of Norplant in

chimpanzees must be addressed before we can assume that it will be effective for five years in chimpanzees as it is in humans.

**CONCLUSIONS**

1) The data presented in this study, in conjunction with the hormone data (Chapter 2), provide evidence that Norplant is an effective contraceptive in chimpanzees.

2) Although individual variation did occur, the females exhibited cyclic genital swelling, including maximal swelling, at intervals consistent with natural cycles.

3) None of the females became pregnant within the first year of treatment with Norplant. However, the longevity of the contraceptive effect must be addressed to ensure that Norplant will be effective for five years in chimpanzees, as it is in humans.

4) Genital swelling patterns appear to be more sensitive to the effects of the levonorgestrel than does estrogen secretion. This, coupled with the ease in monitoring, makes the genital swelling cycles an important tool for assessing the continued effectiveness of Norplant in chimpanzees.

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TABLE 1. Life history data on the seven female chimpanzees in this study

| Female | Date of birth | Parity | Birth of last infant | Date of implant | Weight at implant (kg) |
|---|---|---|---|---|---|
| SR | 02/12/85 | 0 | -- | 08/25/93 | 52.0 |
| MS | 06/17/84 | 0 | -- | 08/11/93 | 55.0 |
| BD | 1964 | >2 | 1988 | 08/18/93 | 64.5 |
| GG | 1963 | >2 | 1988 | 07/29/93 | 63.0 |
| BR | 1967 | >2 | 1990 | 07/29/93 | 46.0 |
| BT | 1965 | >2 | 1993 | 08/11/93 | 78.5 |
| GR | 1968 | >2 | 1990 | 07/29/93 | 52.5 |

TABLE 2. Genital swelling and menses data in relation to time of insertion of Norplant for seven female chimpanzees

| Female | Days from last menses | Days from last maximal swelling | Level of swelling at implant | Number of days until next cycle |
|---|---|---|---|---|
| BR | 10 | 20 | 3 (day 1) | 53/202* |
| BD | 8 | 22 | 4 (day 4) | 27 |
| BT | -- | 13 | 0 | 61 |
| GG | 6 | 18 | 0 | 48 |
| GR | 8 | 13 | 0 | 54 |
| MS | 4 | 16 | 0 | 14 |
| SR | -- | 22 | 0 | -- |

*Although this female began exhibiting genital swelling cycles 53 days after the insertion of Norplant, the first cycle with full swelling did not occur for 202 days following implant placement.

TABLE 3. Genital swelling cycle variables on five cycles before and seven cycles following insertion of Norplant implants

| | Phase I | | | | Phase II | | | |
|---|---|---|---|---|---|---|---|---|
| Female | Cycle length | All swelling | Partial swelling | Full swelling | Cycle length | All swelling | Partial swelling | Full swelling |
| BR | 49.8±9.9 | 21.4±0.5 | 2.0±1.0 | 19.4±1.1 | 38.5±18.2 | 14.7±9.7 | 11.1±6.5 | 3.2±4.4 |
| BD | 34.6±3.0 | 16.4±1.8 | 0.6±1.3 | 15.4±2.0 | 33.8±2.8 | 14.7±4.1 | 5.4±3.9 | 9.2±3.3 |
| MS | 32.6±3.9 | 16.2±1.9 | 3.2±2.2 | 13.0±0.7 | 36.7±4.0 | 16.0±3.6 | 4.6±2.5 | 11.4±2.9 |
| GR | 35.6±3.3 | 20.6±3.4 | 6.6±4.2 | 14.0±1.5 | 39.4±8.0 | 17.4±4.9 | 7.1±4.6 | 10.28±1.3 |
| GG | 41.0±5.4 | 16.6±3.2 | 1.8±1.8 | 14.8±2.2 | 36.0±20.4 | 7.2±3.2 | 1.7±2.4 | 5.7±31.8 |
| BT* | 37.0±5.8 | 18.2±1.3 | 6.8±3.1 | 11.4±3.7 | 45.7±12.9 | 16.7±5.8 | 9.3±4.6 | 7.3±4.6 |
| **Mean** | **38.4±7.8** | **18.3±3.0** | **3.1±2.9** | **14.7±3.2** | **37.6±12.4** | **14.2±6.3** | **6.3±8.5** | **7.9±4.2** |

* After implants n=3.

TABLE 4. Results of Pearson's Correlation tests comparing cycle length and swelling phase duration with age, weight and time since implant

| Comparison | n | r |
|---|---|---|
| Age by: | | |
| Cycle length | 6 | .146 |
| Swelling duration | 7 | .435 |
| Full swelling duration | 7 | .199 |
| Weight by: | | |
| Cycle length | 6 | .523 |
| Swelling duration | 7 | .313 |
| Full swelling duration | 7 | .417 |
| Number of cycles following implant by: | | |
| Cycle length | 6 | -.007 |
| Swelling duration | 6 | .557** |
| Partial swelling duration | 6 | .290* |
| Full swelling duration | 6 | .409 |

* p=.022
** p=.001

Fig. 1. Genital swelling cycles for multiparous female BD before and after insertion of Norplant implants (0 = no genital swelling, 1 = partial genital swelling, 2 = maximum genital swelling).

FIG. 1

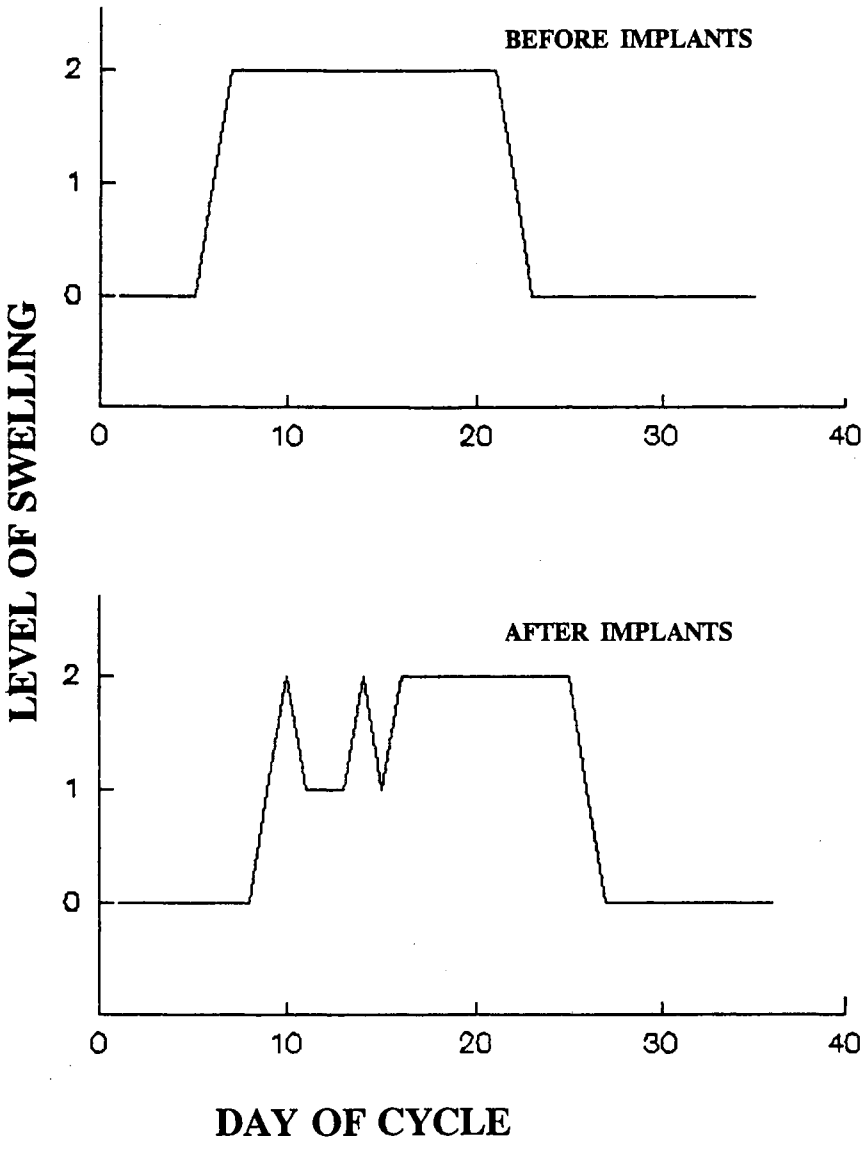

BEFORE IMPLANTS
AFTER IMPLANTS
LEVEL OF SWELLING
DAY OF CYCLE
0
1
2
0
10
20
30
40

Fig. 2. Genital swelling cycles for nulliparous female MS before and after insertion of Norplant implants (0 = no genital swelling, 1 = partial genital swelling, 2 = maximum genital swelling).

FIG. 2

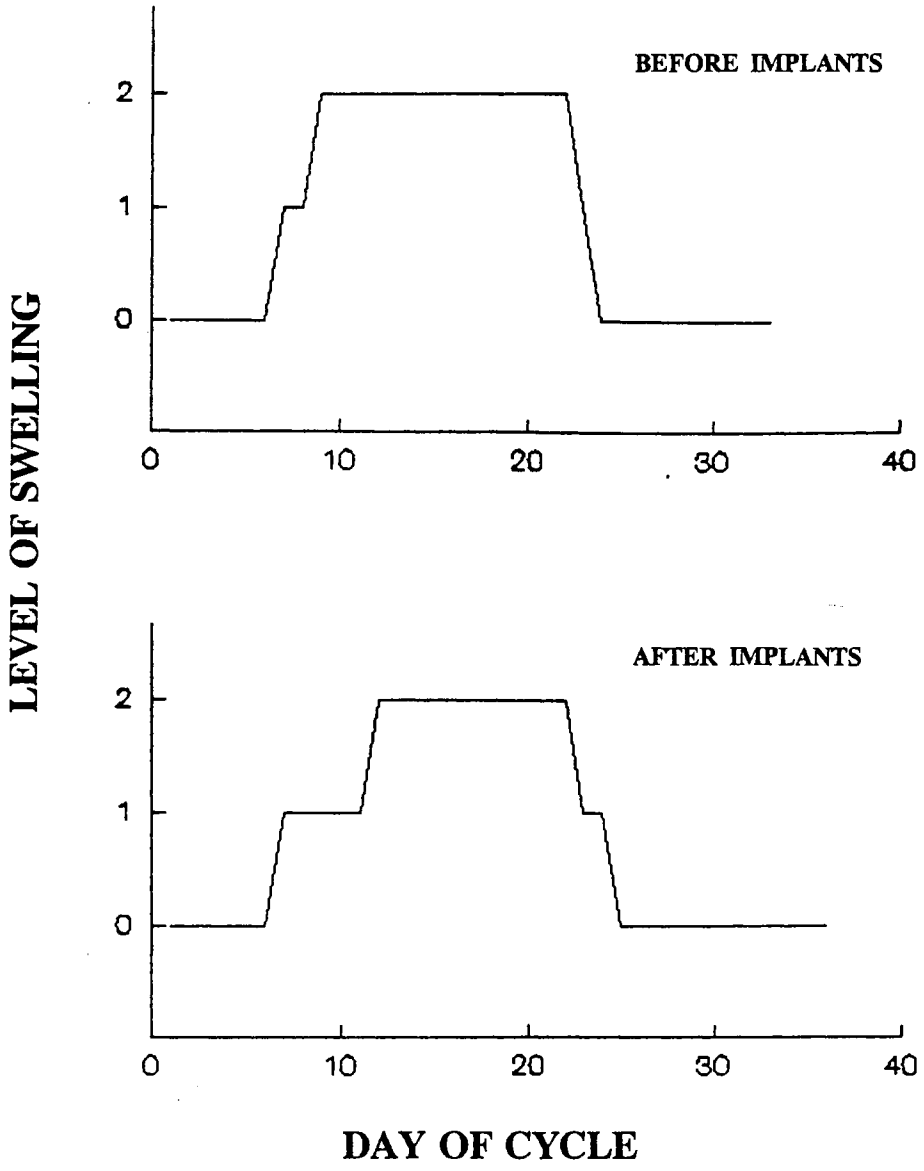

BEFORE IMPLANTS
AFTER IMPLANTS
LEVEL OF SWELLING
DAY OF CYCLE
0
1
2
0
10
20
30
40

Fig. 3. Genital swelling cycle for subject GG before insertion of Norplant implants and genital swelling during two cycles following implant placement (0 = no genital swelling, 1 = partial genital swelling, 2 = maximum genital swelling).

FIG. 3

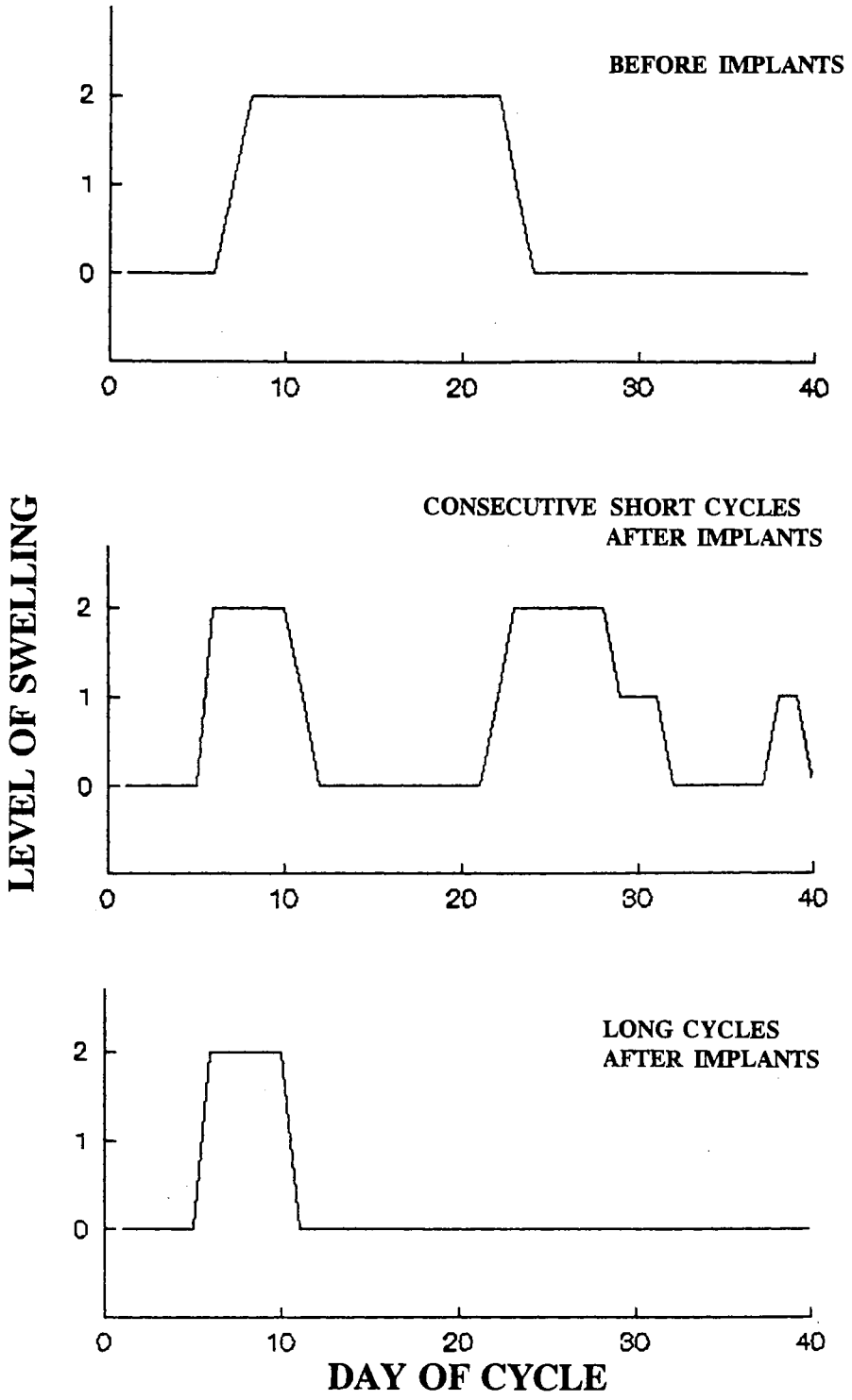
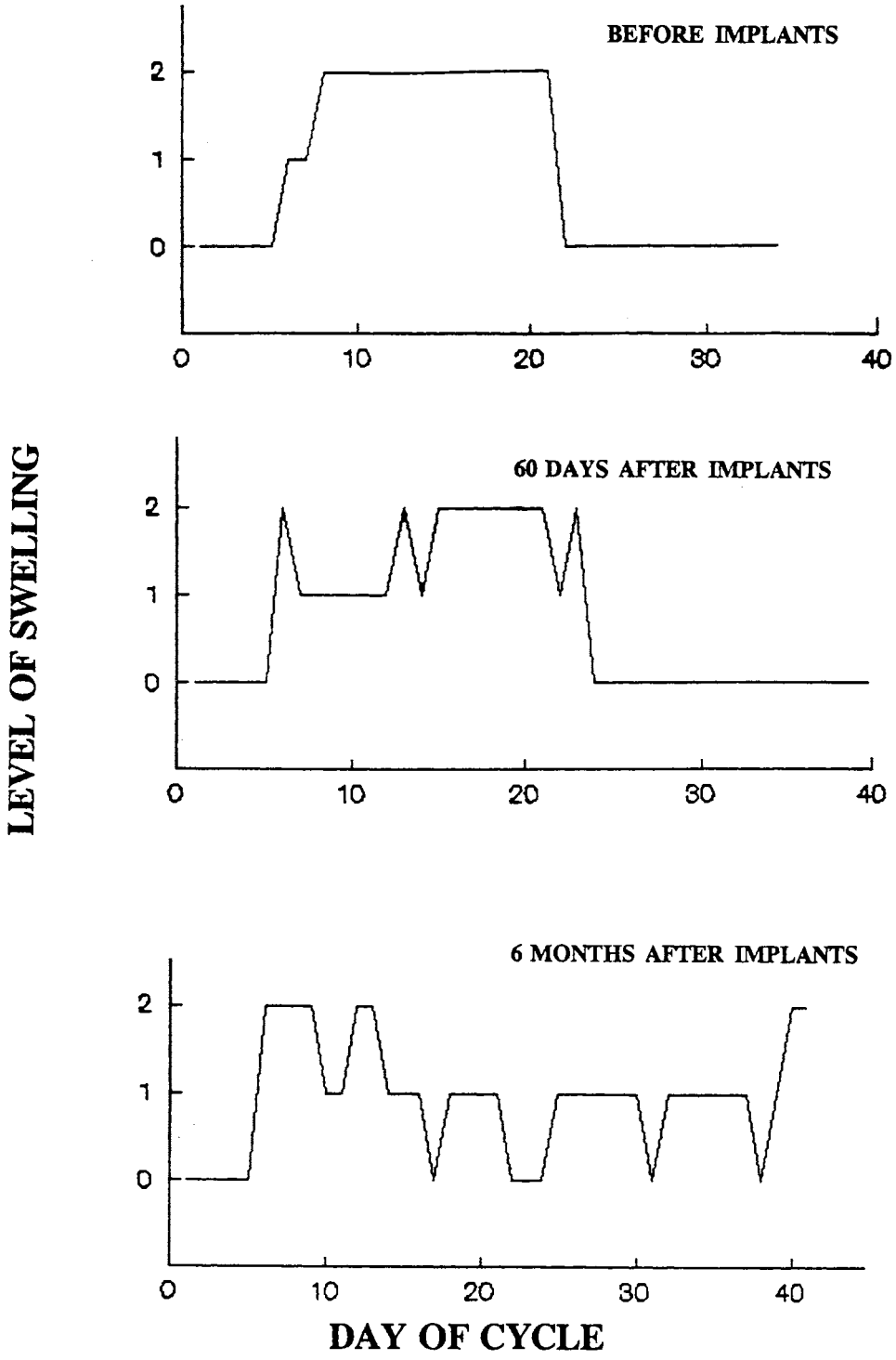

BEFORE IMPLANTS
CONSECUTIVE SHORT CYCLES
AFTER IMPLANTS
LONG CYCLES
AFTER IMPLANTS
LEVEL OF SWELLING
DAY OF CYCLE
0
1
2
0
10
20
30
40

Fig. 4. Genital swelling cycles of female BT before receiving Norplant implants, immediately following implant insertion, and six months following implant insertion (0 = no genital swelling, 1 = partial genital swelling, 2 = maximum genital swelling).

FIG. 4


BEFORE IMPLANTS
60 DAYS AFTER IMPLANTS
6 MONTHS AFTER IMPLANTS
LEVEL OF SWELLING
DAY OF CYCLE
0
1
2
0
10
20
30
40

# CHAPTER IV

# EFFECTS OF NORPLANT IMPLANTS ON THE SOCIOSEXUAL BEHAVIOR OF FEMALE CHIMPANZEES

# EFFECTS OF NORPLANT IMPLANTS ON THE SOCIOSEXUAL BEHAVIOR OF FEMALE CHIMPANZEES

**ABSTRACT**

The purpose of this study was to assess the effects of Norplant implants on the sociosexual behavior of female chimpanzees. Data were collected on five subjects before (Phase I) and six subjects following (Phase II) insertion of Norplant implants. Additional data were collected on six cycling females not designated to receive contraception. Neither rates of sexual ($t=-1.365$, $p=.244$) nor affiliative ($t=0.531$, $p=.624$) behavior differed between Phase I and Phase II of the study. There was a significant increase in the rate of agonistic behavior ($t=2.942$, $p=.042$), specifically submissive behavior ($t=3.28$, $p=.031$) after the females received implants. The control group did not differ in rates of behavior between Phases I and II. The results of this study indicate that Norplant can effectively prevent pregnancy without disruption to normal sociosexual behaviors of the social group.

**INTRODUCTION**

Contraception has become an important component of many captive breeding programs (Porton and Asa, 1990; also see Weise et al., 1993). However, documentation of the effects contraceptives have upon sociosexual behavior has received little attention (Nadler et al. 1993, 1994). In chimpanzees, understanding the behavioral effects of a contraceptive regimen may be a critical component to the ultimate success or failure of breeding programs. The study reported in this paper compares the sociosexual behavior of socially-housed female chimpanzees before and after receiving Norplant implants, and compares the behavior of these females to other cycling females.

There are currently two managed populations of chimpanzees in the United States: the North American zoo population and the National Institutes of Health (NIH) breeding population. In 1986, NIH enacted a breeding program for the approximately 350 chimpanzees held at its five breeding facilities (National Institutes of Health, 1994). In 1989, the American Association of Zoological Parks and Aquariums (AAZPA) developed a Species Survival Plan (SSP) for the 270 chimpanzees maintained in accredited North American Zoos (Schobert, 1990). Both programs were developed with the goal of meeting genetic and demographic requirements of a self-sustaining population (Fulk, 1990; National Institutes of Health, 1994). The importance of

these plans was emphasized in 1991 with the CITES (Convention on International Trade of Endangered Species) listing of wild chimpanzees being upgraded from threatened to endangered. Chimpanzees can no longer legally be imported from the wild, therefore, American zoos and laboratories must actively manage the reproduction of their captive populations to maximize genetic diversity within the limits of available space and the current gene pool.

Management of chimpanzees in captivity is constrained by space limitations. In North American zoos, there are 261 spaces allocated for chimpanzees; 55 males, 136 females and 65-70 dependent juveniles (Schobert and Fulk, 1993). NIH breeding facilities maintain approximately 350 chimpanzees dedicated to be used in their breeding program (National Institutes of Health, 1994). Both populations contain a sufficient founder base to establish a self-sustaining population (Fulk, 1990; National Institutes of Health, 1994). However, the major problem faced by managers of captive chimpanzees is space limitation (Schobert and Fulk, 1993; National Institutes of Health, 1994). Managing the gene pool within the constraints imposed by the current available space requires that matings be selective and limited to produce only the number of offspring that can be accommodated each year. Delaying first parturition of young females and/or increasing the interbirth intervals of parous females are two techniques which can be used to limit the

number of births each year.

Currently, there is no birth control drug designed specifically for chimpanzees. In the past, chimpanzees have been used as a model for the study of human reproduction (Elders and Yerkes, 1936; Elder, 1938; Yerkes, 1939b; Graham and Hodgen 1979; Winter et al., 1980; Graham, 1981; Nadler et al., 1985). Ironically, the tables are now turned and we extrapolate what is known about contraceptive use in humans to chimpanzees.

A major difference between the reproductive physiology of chimpanzees and humans concerns the cyclic occurrence of genital swelling exhibited by female chimpanzees. Female chimpanzees exhibit swelling of the anogenital region in response to the increased ratio of estrogen to progestin during the follicular phase of their reproductive cycle (Graham et al., 1972; McArthur et al., 1981; Nadler et al., 1985; Dahl et al., 1991). As ovulation approaches, the genital swelling is fully tumescent with a shiny pink appearance. Ovulation occurs during the last few days of maximal swelling or early on the first day of detumescence (Elder and Yerkes, 1936; Elder, 1938; Graham et al.; 1972; McArthur et al., 1981; Nadler et al., 1985; Dahl et al., 1991). After ovulation, the genital swelling rapidly decreases in size and turgidity (Elder and Yerkes, 1936; elder, 1938) in response to the rise in progesterone secreted by the corpus luteum (Graham et al., 1972; McArthur

et al. 1981; Nadler et al., 1985; Dahl et al., 1991). Thus, the genital swelling of the chimpanzee serves as an external monitor of ovarian activity.

The genital swelling is a critical component of chimpanzee sociosexual behavior (Yerkes, 1939a, 1939b; van Lawick-Goodall, 1968, 1969; Nishida, 1968, 1979; Tutin, 1979; Wallis, 1982; Wallis and Lemmon, 1986; Nishida, 1990; Hasegawa and Hiraiwa-Hasegawa, 1990; Wallis, 1992). The genital swelling of the female is a visual cue to male chimpanzees (Dixson, 1983; Goodall, 1986), signalling the approach of ovulation. Indeed, wild male chimpanzees have been reported to detect a female with a genital swelling from across a valley, almost a kilometer away (Goodall, 1986).

Not all female chimpanzees exhibit reproductive behavior as they approach ovulation (Rogers and Davenport, 1969; Riesen, 1971; Fritz and Fritz, 1979; Fritz, 1986). Within the captive population are a number of animals that exhibit what appears to be normal reproductive cycles but fail to copulate (Yerkes and Elder, 1936b; Fish et al. 1941; Fritz and Fritz, 1979; Fritz, 1986; Bettinger and Carter, 1992; King and Mellen, 1994). These individuals pose a problem for captive breeding programs. The reproductive incompetence of these chimpanzees precludes them from making a genetic contribution to the captive gene pool, thus decreasing genetic variability among the captive population.

Bingham (1928), in a study on the ontogeny of sexual behavior in chimpanzees, speculated that the lack of sexual behavior of one male chimpanzee in his study may have resulted from early social deprivation. Bingham (1928) suggested that the emergence of adequate sexual behavior results from a recombination of previously exhibited behaviors. However, this speculation received little attention until zoos and laboratories began experiencing problems with increasing numbers of non-copulating adult chimpanzees.

Subsequently, historical management regimes and early experiments on the effects of restricted rearing of chimpanzees have indicated that, indeed, sexual behavior in chimpanzees is, to a large extent, learned during early development (Rogers and Davenport, 1969; Davenport and Rogers, 1970; Reisen, 1971). However, as suggested by Bingham (1928), sexual behavior itself may not be what is learned. It is rather the components of sexual behavior, which are derived from behaviors learned during social interactions with the mother and other group members, that may be critical to the ultimate expression of sexual behavior (Rogers and Davenport, 1969; Davenport and Rogers, 1970). King and Mellen (1994) found evidence to support this hypothesis: in their study, 93% of the infants which were mother-reared for at least one year, copulated as adults.

Therefore, to enhance the chances of reproductive

competence among future breeders, infants should be mother-reared in a social environment (Rogers and Davenport, 1969; Maple, 1979; Riddle et al., 1982; King and Mellen, 1994). However, King and Mellen (1991) also found that infants exposed to females with genital swelling and that had observed reproductive behavior were more likely to copulate as adults than were infants who had not had these experiences. Therefore, the need to maintain the expression of sexual behavior within a group, while at the same time preventing conception in certain individuals, places constraints upon the types of contraception that can be used. The ideal contraceptive should not prevent genital swelling nor diminish the expression of sexual and affiliative behavior among members of the group.

Studies on both captive and wild chimpanzees are finding that several factors may influence the frequency of sexual interactions in chimpanzees. Repeated exposure to the same partner may decrease the rate of sexual interactions between individuals. For years, managers of captive chimpanzees have observed that individuals which were raised together often did not breed with one another as adults (Fritz et al., 1992). Coe et al. (1979) found that within groups, copulation most frequently occurred between chimpanzees which had been housed together for the shortest period of time. Additionally, Bloomsmith et al. (Bloomsmith et al., 1991, 1992) found that frequency of copulation in

their groups of chimpanzees that had been housed together for long periods was lower than that reported for groups housed together for shorter intervals.

Both male and female chimpanzees have been reported to show a "preference" for certain mates (Yerkes and Elder, 1936a, 1936b; Allen, 1981). Allen (1981) reported that the male chimpanzee in his study showed a "preference" for newly introduced females in the group, copulating more frequently with these females than females already residing within the social group. Allen (1981) called this the "strange female effect". Data on socially-housed chimpanzees which had visual access to other social groups, showed that the chimpanzees, both male and female, repeatedly solicited individuals housed in other social groups (Bettinger, unpublished data).

Similar data are reported on wild chimpanzees. Females that transfer between communities typically do so when they are exhibiting maximal swelling (Pusey, 1979; Goodall, 1986; Nishida, 1990). Nishida (1979) found that males mated with females which had recently immigrated into the group more frequently than they did with females already residing in the group's territory. Additionally, immigrant females more actively solicited sexual interactions with males than did the resident females (Hasegawa, 1989). Nishida (1990) reported that when new females entered the social group, they mated with almost all resident males within a few days.

Other data suggest that males may be able to detect swellings resulting from fertile cycles from those of infertile cycles. Physiologically infertile chimpanzees that continue to exhibit genital swelling have been reported to copulate less frequently than their fertile counterparts (Gigi at Gombe, personal communication, J. Wallis; female chimpanzees at San Francisco Zoo, personal communication, zookeepers). Among wild chimpanzees, the dominant male copulated almost exclusively with females exhibiting fertile swellings, and this most frequently occurred during the portion of the cycle immediately preceding detumescence (Wallis and Bettinger, 1993). Similarly, males in their prime have been found to mate more frequently with females exhibiting fertile swellings than did younger or older males (Hasegawa and Hiraiwa-Hasegawa, 1990).

Wallis (1982, 1992) found that nearly 74% of the copulatory attempts made by males occurred during periods of maximum genital swelling, and that most sexual interactions were confined to the follicular phase of the cycle. Thus, males may be cuing on certain characteristics of the genital swelling. Several studies have indicated that females exhibit full swelling for approximately 11-12 days prior to ovulation (Elder, 1938; Young and Yerkes, 1943; Graham et al., 1972; Nadler et al., 1985). However, the majority of copulations occurs during the periovulatory period (Yerkes, 1939a; Elder, 1938; Tutin, 1979, 1980; Wallis, 1982;

Hasegawa and Hasegawa, 1990; Wallis, 1992; Wallis and Bettinger, 1993).

Wallis and Lemmon (1986) reported that males not only inspected the genital area of pregnant females less frequently, but also groomed these females less than they did cycling females. These data suggest that not only are males differentiating between genital swelling of pregnant and non-pregnant females, but they are also altering both their sexual and social responses accordingly. Other studies have found that many social behaviors may be affected by the presence or absence of females with genital swelling. Females have been reported to receive more grooming from males when they have genital swelling (Merrick, 1977; Wallis, 1992), as well as more grooming from their offspring during periods of genital swelling (Nishida, 1988). Females may exhibit more assertive behaviors (Yerkes, 1939b; Nishida, 1979; Goodall, 1986) and show less submissive behaviors while fully tumescent (Bloomsmith et al., 1991). In newly established groups, aggression among males resulting in wounding has been associated with the presence of a female in estrus (Alford, 1990). Additionally, a positive correlation has been found between aggressive interactions among males and the number of females exhibiting genital swelling in captive (Lambeth et al., 1991; Shefferly and Fritz, 1992) and wild groups (Bettinger et al., 1993).

The literature reviewed thus far reveals two facts: 1) the cyclic pattern of the genital swelling results from changing ratios of estrogen and progesterone; and 2) sexual, affiliative and agonistic behaviors can all be affected by the presence of a female with genital swelling.

Contraceptives that inhibit the endogenous release of estrogen and are themselves high in synthetic progestin, have been shown to alter the genital swelling patterns normally exhibited by female chimpanzees (Nadler et al., 1993, 1994; Bettinger, in press). Thus, contraceptives may inadvertently influence the expression of the sociosexual behavior of both males and females.

Although there have been many studies addressing the effect of contraceptives on physiology in humans, few studies have addressed the effect they have upon behavior (see Bancroft and Sartorius, 1990 for review). Sexual behavior studies in humans are fraught with difficulties. Issues frequently addressed in such studies are difficult to quantify: sexuality, well-being and depression (Bancroft and Sartorius, 1990). Additionally, cultural, religious and personal beliefs may influence the study subjects' assessment of their response.

Contraceptive use in nonhuman primates is a relatively recent development (Porton and Asa, 1990). Consistent with data on humans, there have been more studies on the physiological effects (Porton and Asa, 1990; Nadler et al.,

1992; Goodrowe et al., 1992; Bettinger, in press) than on behavioral effects (Nadler et al., 1993, 1994). Nadler et al. (1993) addressed the behavioral and physiological (1992) effects of combined oral contraceptives containing varying levels of ethinyl estradiol and norethindrone on chimpanzees. The study reported that ovulation was suppressed in these females. Genital swelling did occur, however, level was affected by hormone dose; high doses of ethinyl estradiol resulted in a larger genital swelling than did low doses. Maximum swelling did not occur in the females (Nadler et al., 1992).

Nadler et al. (1993, 1994) used free access and restricted access pair-tests to evaluate the effect the contraceptives had on copulatory frequency. Copulation was reduced in females given contraceptives. Males and females that exhibited the most affiliative behavior during normal cycles continued to copulate, although at a lower rate, after the female was placed on oral contraceptives. However, copulatory behavior in pairs that exhibited less affiliative behavior during normal cycles (and copulated at lower rates) ceased to copulate once the female was placed on the oral contraceptive regimen.

Beach (1976) proposed that sexual behavior is composed of three components: attractivity, proceptivity and receptivity. Attractivity refers to the female's ability to evoke a sexual response from the male. In the chimpanzee,

examples include the male behaviors of inspecting, soliciting and following the female (van Lawick-Goodall, 1968; Nishida, 1970; King et al., 1980; Wallis and Lemmon, 1986; Bloomsmith et al., 1991). Proceptivity is defined as the female's initiation of sexual interactions. In chimpanzees, females may initiate sexual behavior by soliciting the male or presenting her hind quarters to him for inspection or copulation (Goodall, 1968; Nishida, 1970; King et al., 1980; Wallis and Lemmon, 1986; Bloomsmith et al., 1991). Receptivity is defined by the female's cooperation with the male which ultimately results in copulation.

All social behaviors contain the three components described above. Behavior can be initiated by a focal animal, it can be directed to the focal animal, or it can require cooperation between the animals involved in the interaction. This study addresses the behavior of group-living female chimpanzees from this perspective. Three categories of behavior were analyzed: affiliative, sexual and agonistic. These categories were then further defined by the direction of the behavior as described above.

The data reported in this study are a subset of data collected to evaluate the effects of Norplant implants on physiology and sociosexual behavior in chimpanzees. Data on ovarian hormone secretion indicated follicular and luteal activity occurred in cycles of five females. These data

suggest that ovulation may have occurred (Chapter 2). Additionally, the subjects continued to exhibit genital swelling cycles and reach maximal tumescence after receiving the Norplant implants (Chapter 3). This paper reports the effects of Norplant implants on sociosexual behavior. The results of this study suggest that Norplant implants may provide a form of contraception which shows minimal interference in sociosexual behavior of female chimpanzees.

## MATERIALS AND METHODS

### FACILITY

The study was conducted on chimpanzees housed at The University of Texas M.D. Anderson Cancer Center, Science Park, in Bastrop, Texas between June - August 1993 and February - June 1994. The subjects for this study were maintained in multimale/multifemale social groups housed in enriched, 22-meter diameter outdoor corrals with access to indoor dens (see Riddle et al., 1982, for a detailed description of the facility). Each social group had visual access to other social groups through windows at ground level and by climbing structures located in each corral. During February and March of 1994, an additional concrete drainage culvert and a 2-level elevated platform was added to each corral.

TIME FRAME AND STUDY DESIGN

The study was divided into two phases. Phase I was conducted from June 4, 1993 through August 12, 1993. During Phase I, behavioral data were collected on nine cycling females, representing three social groups (3 females observed per group). The females were placed into two categories: 1) experimental group - five females designated to receive Norplant implants at the end of Phase I; 2) control group - four females that would not receive contraception.

Data for Phase II were collected between February 22, 1994 and June 9, 1994. Study subjects for Phase II included the nine females observed during Phase I, plus three additional subjects. This resulted in observations on four social groups containing six subjects cycling without contraception (control group) and six subjects cycling while having Norplant implants (experimental group).

STUDY SUBJECTS

Table 1 gives life history data on the 12 females in this study. Criteria used for selecting the experimental subjects were: a) management need for contraception and, b) the female must have been housed in a multimale social group with at least one other cycling female not designated to receive contraception. Control group subjects were chosen as a result of their being housed in a social group with a

female in the experimental category.

The six females that received Norplant were placed on the regimen as a result of management decisions. Five of the subjects were considered adequately or overrepresented in the gene pool. The nulliparous female was housed in a social group incompatible with breeding recommendations and therefore, the decision was made to temporarily delay reproduction.

Both nulliparous females in the control group conceived during Phase II of the study: one aborted at approximately four weeks and resumed cycling (NN), the other continued to exhibit genital swelling and was retained in the study (TN). The remaining four females in the control group had all produced offspring prior to this study but may have had reduced fertility as a result of a behavioral or physiological problem; they had been cycling for several years prior to this study. Although these 6 females may not represent ideal control subjects, they all did exhibit genital swelling as well as appropriate sociosexual behaviors.

SOCIAL GROUPS

Subjects included during Phase I were housed in one of three social groups. These groups contained a total of five subjects in the experimental group and four subjects in the control group. All social groups contained 2-3 adult males,

1-2 immature males, 0-1 immature females, 1-3 acyclic females, 1-2 cycling females, and 1-2 implant candidates (cycling). There were no changes in the social composition of the groups for at least seven months prior to this study (excluding births into the groups).

The same subjects were observed during Phase II. However, changes in social composition of an additional group allowed it to be included in the study (corral 7). This group was similar in composition to the other groups and added three females to the study. One of the females had received Norplant during the same period as the other females in the study while the other two were cycling females that had been added to the group in late August 1993. Table 2 shows the social composition of all four groups.

Each social group contained at least one male of proven fertility. Births had occurred in corrals 6 and 7 just prior to the onset of the study and in corral 8 during Phase I. Pregnancies occurred in two control group females housed in corrals 7 and 8 during the study.

## PREGNANCY TESTS AND IMPLANT PLACEMENT

Pregnancy tests using the detection of chorionic gonadotropin in urine were conducted on all females at the onset of Phase I, before the experimental group received the Norplant, and at the onset and end of Phase II. Females that

received Norplant implants were also checked for pregnancy through ultrasonography of the uterus before insertion of the implants and again during immobilization required to verify that the implants had remained in place.

The experimental subjects received Norplant implants. Norplant is a progestin-only system, which contains no estrogen (Goldzieher, 1989). The Norplant system consists of six silastic tubes, 2.4 mm in diameter and 34 mm in length, each containing 36 mg of levonorgestrel, a synthetic progestin. The initial dose provided by the system in humans is about 85 μg/day; this decreases to 50 μg/day by nine months and 35 μg/day by 18 months (Wyeth Laboratories, 1990). The implants prevent pregnancy in humans of average weight for five years (Segal, 1983).

The experimental group received the Norplant implants between July 29, 1993 and August 18, 1993. Implants were inserted during the early follicular phase of the female's cycle, following a negative pregnancy test. The implants were inserted according to the protocol developed for use in humans and included in the Norplant packet (Wyeth-Ayerst Laboratories, 1990). The six implants were inserted subcutaneously into the medial surface of the upper left arm. A continuous intradermal suture pattern was used to close the skin incision. This buried suture pattern helps to prevent the chimpanzees from removing the implants. The subjects received the implants in the morning and were

returned to their social group in the afternoon of the same day.

Verification that the implants remained in place was conducted in March 1994 using manual palpation and ultrasonography. In five of the six females, all six implants were located. In one female, BR, only four implants had remained in place. Confirmation that the other two implants had indeed been removed and had not migrated internally was found during the analysis of the urinary assay data. High levels of LNG were found in the urine of two of BR's cagemates on the day following her implant placement thus indicating that the females had ingested the capsules.

GENITAL SWELLING DATA

The genital swelling of each female was scored daily using a five-point scale (0 meant no swelling; 1, 2, 3 were used to denote 1/4, 1/2, 3/4 swelling respectively; and 4 represented full swelling). For this study, swelling scores were placed into three categories: no swelling (score = 0), partial swelling (score = 1, 2, 3), and full swelling (score = 4). Genital swelling scores were recorded daily by one of three individuals, however, the same observer scored the swellings approximately 75% of the time. Reliability among scorers was assessed using Kendall's coefficient of concordance. Agreement among observers was 79.6% across 8

test days.

BEHAVIORAL DATA

Behavioral data were collected using continuous sampling on a focal animal. Each subject was observed for 15 minutes and behavioral data were recorded using the ethogram established for this study (see appendix 1). For this report, only behaviors related to sociosexual activity were analyzed (Table 5). Order of observation of groups and subjects within groups was established using a randomization table and each female was observed on most days.

All observations were made from the roof of the chimpanzee facility, therefore, these data represent only behavior exhibited while in the outdoor corrals. Observations were made when the animals had access to the den areas and when they were locked out of the den areas. A total of 355 hours of data are used in this analysis: 139 hours for Phase I and 216 hours for Phase II. Table 3 shows the number of observations per female during each Phase of the study and Table 4 provides information on the percent of observations on each female during different levels of genital swelling.

ANALYSIS AND STATISTICAL TESTS

Behaviors were placed into three categories: 1) Agonistic, 2) Affiliative, and 3) Sexual. These categories

were then subdivided by direction of behavior (i.e. given, received and mutual). Table 5 shows the behaviors analyzed in this study and the categories in which they were placed (see appendix 1 for operational definitions of the behaviors). Each 15-minute observation was summarized by frequency of behaviors recorded during that session. Frequency data were then converted to rate per hour of observation per female for each Phase of the study. Data were analyzed using SAS and Systat statistical programs.

Student's t-tests using pairwise comparisons were used to assess differences in rates of behavior between Phases I and II for females in the experimental and control groups. Differences between control and experimental groups within a Phase were assessed using Student's t-tests. Analysis of variance and Duncan's multiple range test were used to evaluate differences between social groups. Differences in variances among and between groups of females was evaluated using F-tests. Two-way analysis of variance was used to assess partner preference among the experimental group.

## RESULTS

### EXPERIMENTAL GROUP - PHASE I VS PHASE II

Overall, there was not a significant difference in the rate of sexual ($t=-1.365$, $p=.244$, $n=5$) nor affiliative ($t=0.531$, $p=.624$, $n=5$) behavior when comparing data from Phase I and Phase II. After receiving the implants, the

females did exhibit an increase in agonistic behavior ($t=2.942$, $p=.042$, $n=5$). The increase in the rate of agonistic behavior was actually an increase in the rate of submissive behavior ($t=3.28$, $p=.031$, $n=5$), as rate of aggression initiated ($t=0.52$, $p=.629$, $n=5$) and aggression received ($t=0.36$, $p=.740$, $n=5$) showed no difference between Phase I and Phase II. Table 6 gives mean rates of behaviors and t values for each category and subcategory analyzed.

When analyzing data collected only when the females were exhibiting full swelling, the difference in the rate of agonistic behavior is no longer found ($t=-0.55$, $p=.612$, $n=5$). Nor was there a difference in the rate of sexual ($t=2.07$, $p=.107$, $n=5$) or affiliative ($t=1.21$, $p=.294$, $n=5$) behavior. Table 7 provides rates and t values for all behaviors analyzed.

Grooming is often used as an indicator of affiliative interactions. When analyzing only grooming behavior, there was not a difference in rate of grooming before and after the females received the Norplant implants (Table 8). Additionally, rate of copulation and rate of male solicitation for copulation did not differ between Phases.

As noted in Tables 6, 7 and 8, several of the mean rates of behavior showed a large standard deviation. However, F-tests showed that the variance on rates of behavior during Phase I was not different from the variance of Phase II (Table 9).

INTERACTIVE PARTNERS OF EXPERIMENTAL GROUP - PHASE I VS PHASE II

As described above, rates of behavior showed little difference between Phases of the study. This could occur by the females more frequently interacting with a different category of social partner. For example, the females may have engaged in more sexual behavior with immature rather than adult males after receiving the contraceptive. To address this question, frequency of interaction with each category of social partner (Adult male, Adult female, Immature male) was compared between Phases of the study. There was not a difference in interactive partners between Phases for each of the groups of behaviors analyzed. Table 10 provides mean rates and F statistics for the three behavioral categories.

CONTROL GROUP - PHASE I VS PHASE II

There was not a difference in rate of agonistic ($t=0.642$, $p=.567$, $n=4$), sexual ($t=0.326$, $p=.766$, $n=4$) nor affiliative ($t=-0.893$, $p=.438$, $p=4$) behaviors between Phases I and II for the control group of females. Nor was there a difference in the subcategories of each of these behaviors (Table 11). This was also true for data collected on days with full swelling (Table 12). F-tests on variances found no difference in variances between Phase I and II of the study (Table 13).

EXPERIMENTAL GROUP VS CONTROL GROUP - PHASE I

Comparisons of the experimental and control groups during Phase I showed that the two groups did not differ in their rate of agonistic or affiliative behavior (Table 14). However, rate of sexual behavior was higher in the experimental group ($t=-2.685$, $p=.031$, $n=9$; and see Tables 6 and 11). The difference in rate of sexual behavior was found in both sexual behavior initiated by the focal animal and sexual behavior directed to the focal animal (Table 14). Variances between the groups did not significantly differ (Table 14).

When comparing only days in which the females had full swelling, rate of affiliative interactions did not differ (Table 14). However, both, rate of agonistic and sexual behavior was higher in the experimental group (Table 14, refer to Tables 7 and 12 for means). In the agonistic category, aggression given and aggression received were higher in the experimental group (Table 14 and refer to tables 7 and 12 for rates). In the sexual category, rate of sexual behavior initiated by the focal ($t=-2.640$, $p=.033$, $n=9$) was higher for the experimental group and sexual behavior directed to the focal approached significance ($t=2.36$, $p=.051$).

F-test on variances among females in each group did not reveal significant differences in the three categories of behaviors. However, there was a difference in the

subcategory, sexual behavior initiated by the focal animal (Table 14); the variance was greater for the control group (mean=0.229, sd=.367) than for the experimental group (mean=2.267, sd=1.489).

EXPERIMENTAL VS CONTROL - PHASE II (N=9 FEMALES)

This analysis contains only data on the original nine females in the study. There was not a difference in the rates of sociosexual behaviors exhibited by the experimental and control group during Phase II (Table 15). There was however, a difference in the subcategory, sexual behavior initiated by the focal (t=-2.53, p=.039). The experimental group initiated more sexual interactions than did the control group (experimental group, mean=0.844; control group, mean=0.319). Variances did not differ between groups (Table 15).

Analyzing only days with full swelling showed no difference in the rate of the 3 behavioral categories (Table 15). However, rate of sexual behaviors initiated by the focal approached significance (t=-2.34, p=.051), being slightly higher among the experimental group (experimental group, mean=1.652; control group, mean=0.227). Additionally, F-tests found the variance among the two groups of females to be significantly different for sexual behavior initiated by the focal (F=32.59, p=.017), mutually directed sexual behavior (F=15.92, p=.046), and affiliative behavior

directed to the focal (F=14.13, p=.027). In all cases, the variance was greater among the control group (Table 15, see Tables 7 and 12 for means).

EXPERIMENTAL VS CONTROL - PHASE II (N=12 FEMALES)

This analysis contains data on all 12 females observed during Phase II. There was not a significant difference in rate of behavior for the three behavioral categories or subcategories (Table 16). F-tests showed a difference in the variance of the two groups for the subcategory Agonistic-Submissive (F=7.78, p=.042). The variance was greater among females in the control group (see Table 16). Analysis of data collected only on days with full swelling also showed no difference in rate of behaviors exhibited by the experimental and control groups (Table 17). The variance on rate of sexually mutual behaviors was significantly greater among the control group (F=8.55, p=.034).

DIFFERENCES AMONG SOCIAL GROUPS

Rates of sociosexual behaviors in the three categories did not differ among the four social groups (Table 18). However, rate of behavior in the subcategory, agonistic behavior directed to the focal, was significantly higher in one corral (F=4.15, p=.047). Table 18 provides rates of behavior by social group and statistical results.

**DISCUSSION**

The paucity of significant differences found in the rates of sociosexual behavior exhibited by females before and after receiving implants is important. These data indicate that female chimpanzees can be placed on Norplant contraceptive implants and retain the expression of much of their normal affiliative and sexual behavior. However, it should be emphasized that the females with Norplant implants in this study continued to exhibit genital swelling that reached maximal tumescence. These results may reflect this genital swelling response. Not all females with Norplant implants continue to exhibit genital swelling (Bettinger, in press; also see chapters 2 and 3) and therefore may not produce the same behavioral results.

The increase in the rate of submissive behavior shown by the females after insertion of implants may reflect the fewer number of days they exhibited maximal genital swelling (Chapters 3). Bloomsmith et al., (1992) reported that females in their study (some of which were also included in this study), showed significantly less submissive behavior when fully tumescent. Other studies have reported similar results (Yerkes, 1939b; Goodall, 1986). It is interesting to note however, that the same result was not found in the other behaviors analyzed.

Perhaps the lack of a significant difference in the rates of affiliative and sexual behaviors reflect the fact

that although the females exhibited fewer days of full swelling preceding detumescence, ovulation may have occurred in five of the six females on the Norplant regimen (Chapters 2 and 3). Although female chimpanzees exhibit full swelling for approximately 11-12 days before ovulation, frequency of copulation is highest during the periovulatory period (Elder, 1938; Yerkes, 1939a; Tutin, 1979; Coe et al., 1979; Allen, 1981; Wallis, 1982; Goodall, 1986; Hasegawa and Hiraiwa-Hasegawa, 1990; Shefferly and Fritz, 1992; Wallis, 1992). The behavioral data provide additional support for the hypothesis that the luteal phase increase in PdG concentrations was from ovulation, not luteinization of the follicle (Chapter 2).

The increase in copulatory frequency during this period suggests that chimpanzees are responding behaviorally, whether male or female initiated, to the physiological conditions present immediately preceding ovulation. If this is the case, the rate of affiliative and sexual behaviors would be most strongly influenced by behaviors exhibited during the periovulatory period.

The data do provide evidence that the rate of sexual behaviors did decrease, although not significantly, after the females were placed on the Norplant regimen. Data collected during Phase I showed that the experimental group exhibited a higher rate of sexual behavior, and specifically, sexual behaviors initiated by and directed to

them. However, when comparing these same females during Phase II, the difference in rate of sexual behavior was not significant between the groups. Comparisons made between Phase I and Phase II for each group of females, showed that rate of behavior did not differ between Phases.

Reviewing rate data show that during Phase II, the experimental group exhibited slightly lower rates of sexual behavior while the control group exhibited slightly higher rates of sexual behavior when compared to Phase I. Although the experimental group continued to initiate significantly more sexual interactions than did the control group, sexual behavior directed toward them was no longer significantly higher than sexual behavior directed toward the control group.

Although individual variation cannot be addressed statistically, variation within groups of females was tested. F-tests found that variation within the experimental and control groups did not differ between phases of the study. However, variances between the groups was significantly different for some behaviors and variances were higher among females in the control group.

When using all 12 females in the analysis, there is not a difference in the rate of any of the behaviors. Therefore, data on experimental and control group females were combined and analyzed with respect to social group. The only significant difference was found in the rate of aggression

received by the females. Females in corral 7 received more aggression than did females in the other corrals. Two of the females in this group had been introduced just 5 months prior to inclusion in this study. The increased aggression they received may reflect their short tenure within the group.

The results of this study show, that at least initially, Norplant has little impact on sociosexual behavior. However, over the lifetime of an individual, they may have a much greater impact. Placing a female on a contraceptive regimen for an extended period of time has the potential of drastically altering her normal life history pattern. The majority of a wild female chimpanzee's reproductive life is spent in an acyclic state, resulting from either pregnancy or lactation. Tutin (1980) reported that on average, a female chimpanzee experiences 19 cycles before her first conception, followed by an average of 4.5 cycles between successive pregnancies. Wallis and Lemmon (1986) calculated that the average female chimpanzee will experience 46 cycles (not including genital swelling exhibited during pregnancy) in her lifetime.

Average cycle length for the females in this study was 38 days (Chapter 3). Placing a female on Norplant for five years would add an additional 49 genital swelling cycles to her reproductive life. This would more than double the number of cycles she would normally experience.

Additionally, many of the females for which contraceptives are deemed necessary may get pregnant in fewer than the 4.5 cycles reported by Tutin (1980). Indeed, two females in this study had repeatedly conceived during their first post-partum cycle. For these females, the number of genital swelling cycles they experience is increased even more substantially than for other females.

Female chimpanzees that continue to exhibit genital swelling after being placed on a contraceptive regimen will experience a larger number of genital swelling cycles than they normally would in their lifetime. Understanding how this affects female social interactions will be important for captive chimpanzee management in the future. Male behavior will also need to be evaluated: do males show less interest in these females as they continue to exhibit genital swelling on a long-term basis? Another concern pertains to aggression among males. If several females within a group are on a contraceptive regimen at the same time, will the genital swellings of these females elicit as much aggressive behavior in males as do swellings of females cycling without contraception? There are many questions yet to be addressed before we can understand the long-term effect this new management practice may have upon behavior of captive chimpanzees.

**CONCLUSIONS**

1) Norplant can provide an effective form of contraception for facilities wishing to maintain normal levels of sociosexual behavior within their group.

2) Individual chimpanzees may respond differently to the Norplant regimen. Although significant differences were not found in rates of affiliative and sexual behavior of the females on Norplant as a group, individual variation may be significant.

3) Placing females on a contraceptive regimen can significantly increase the number of genital swelling cycles they experience in their lifetime. Additional data are needed to document the long-term effects contraceptives may have on social group dynamics.

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TABLE 1. History of the 12 cycling female chimpanzees in this study

| Female | Corral | Age (Yrs.) | Parity | Implant date |
|---|---|---|---|---|
| Experimental group: | | | | |
| MS | 4 | 9 | 0 | 8/11/93 |
| GR+ | 7 | 25 | >2 | 7/29/93 |
| BR | 4 | 26 | >2 | 7/29/93 |
| BT | 8 | 28 | >2 | 8/11/93 |
| BD | 6 | 29 | >2 | 8/18/93 |
| GG | 6 | 30 | >2 | 7/29/93 |
| Control group: | | | | |
| TN* | 8 | 10 | 0 | -- |
| NN+@ | 7 | 10 | 0 | -- |
| PP+ | 7 | 26 | >1 | -- |
| MR | 8 | 27 | >1 | -- |
| PL | 6 | 29 | >2 | -- |
| US | 4 | 29 | >2 | -- |

*TN became pregnant immediately prior to the onset of Phase II. She continued to show genital swelling and was retained in the study.

+These females were not included in Phase I of the study.

@This female became pregnant during Phase II but aborted after approximately two weeks.

TABLE 2. Composition of the four social groups included in this study

| Corral number | Phase | Adult males | Adult females* | Immature males | Immature females | Total |
|---|---|---|---|---|---|---|
| 4 | I | 2 | 1/4/0 | 1 | 0 | 8 |
| 4 | II | 2 | 1/2/2 | 1 | 0 | 8 |
| 6 | I | 3 | 1/3/0 | 2 | 1 | 10 |
| 6 | II | 3 | 1/1/2 | 2 | 1 | 10 |
| 8 | I | 2 | 3/3/0 | 2 | 1 | 11 |
| 8 | II | 2 | 3/2/1+ | 2 | 1 | 11 |
| 7 | II | 3 | 1/2/1 | 1 | 1 | 9 |

*Females listed by acyclic/cyclic/implanted.

+One female in the cycling group was pregnant but frequently exhibited genital swelling.

TABLE 3. Total number of 15-minute observations on each female during Phases I and II of the study

| Subject | Phase I | Phase II |
|---|---|---|
| Experimental group: | | |
| BR | 61 | 72 |
| MS | 71 | 72 |
| BD | 74 | 72 |
| GG | 50 | 72 |
| BT | 60 | 72 |
| GR | - | 72 |
| Control group: | | |
| US | 56 | 72 |
| PL | 64 | 72 |
| MR | 53 | 72 |
| TN | 67 | 72 |
| PP | - | 72 |
| NN | - | 72 |

TABLE 4. Percent of observations on each subject during different levels of genital swelling

| | Phase I | | | Phase II | | |
|---|---|---|---|---|---|---|
| Subject | Flat | Partial | Full | Flat | Partial | Full |
| Experimental group: | | | | | | |
| BR | 59.0 | 8.2 | 32.8 | 34.7 | 48.6 | 16.7 |
| MS | 60.6 | 8.4 | 31.0 | 51.4 | 11.1 | 37.5 |
| BD | 60.8 | 4.1 | 35.1 | 40.3 | 22.2 | 37.5 |
| GG | 62.0 | .0 | 38.0 | 69.4 | 11.1 | 19.4 |
| BT | 78.4 | 3.3 | 18.3 | 20.8 | 52.8 | 26.4 |
| GR | - | - | - | 34.7 | 30.6 | 34.7 |
| Control group: | | | | | | |
| US | 60.7 | 17.9 | 21.4 | 52.8 | 29.2 | 18.0 |
| PL | 82.8 | 15.6 | 1.6 | 55.6 | 33.3 | 11.1 |
| MR | 41.5 | 7.6 | 50.9 | 44.4 | 31.9 | 23.6 |
| TN | 35.8 | 25.4 | 38.8 | 9.7 | 58.3 | 32.0 |
| PP | - | - | - | 63.9 | 1.4 | 34.7 |
| NN | - | - | - | 48.6 | 20.8 | 30.6 |

TABLE 5. Behaviors included in each of the behavioral categories and subcategories analyzed for this study.

| CATEGORY | SUBCATEGORY | BEHAVIORS INCLUDED |
|---|---|---|
| AGONISTIC BEHAVIOR: | | bite, charge, chase, crouch, display, extend hand, flee, grab, head bob, hit, kick, pant-grunt |
| (agg) | Aggression Given by the Focal | bite, charge, chase, display, grab, hit, or kick another chimpanzee |
| (agr) | Aggression Received by the Focal | crouch, flee, and behaviors included above directed to the focal. |
| (sub) | Submissive to Another | extend hand, head bob, pant grunt |
| AFFILIATIVE BEHAVIOR: | | approach, follow, groom, play, stare/watch, touch |
| (act) | Focal Acts to Another | approach, follow, groom given, stare/watch, and touch |
| (rec) | Directed to the Focal | other approaches focal, other follows focal, other grooms focal |
| (mut) | Mutual | focal and other groom simultaneously, play with another |
| SEXUAL BEHAVIOR: | | copulate, genital rub, inspect, masturbate, mount, present, solicit |
| (act) | Focal Acts to Another | inspect, masturbate, mount, present, solicit |
| (rec) | Directed to the Focal | inspect, mount, solicit |
| (mut) | Mutual | copulate, genital rub |

TABLE 6. Results of Student's t-tests and rates of behavior of five female chimpanzees (experimental group) for all days before and after receiving Norplant implants (mean rate ± sd per hour of observation)

| Category (Subcategory) | Phase I | | Phase II | | t |
|---|---|---|---|---|---|
| | mean | sd | mean | sd | |
| Agonistic | .585 | .313 | .767 | .361 | 2.94* |
| (agg) | .345 | .157 | .411 | .323 | .52 |
| (agr) | .166 | .130 | .200 | .155 | .36 |
| (sub) | .074 | .061 | .156 | .091 | 3.28* |
| Affiliative | 15.415 | .484 | 16.744 | 6.916 | .53 |
| (act) | 8.408 | 5.078 | 9.711 | 6.383 | .82 |
| (rec) | 3.339 | 1.388 | 4.166 | 1.296 | 1.11 |
| (mut) | 3.668 | 1.229 | 2.867 | 1.693 | .68 |
| Sexual | 3.556 | 1.116 | 2.888 | 1.140 | 1.37 |
| (act) | 1.140 | .645 | .844 | .371 | 1.74 |
| (rec) | 1.783 | .366 | 1.511 | .640 | 1.32 |
| (mut) | .631 | .572 | .522 | .413 | .35 |

*p<.05

TABLE 7. Results of Student's t-tests and rates of behavior of five female chimpanzees (experimental group) for days with full swelling, before and after receiving Norplant implants (mean rate ± sd per hour of observation)

| Category | Phase I | | Phase II | | |
|---|---|---|---|---|---|
| (Subcategory) | mean | sd | mean | sd | t |
| Agonistic | .089 | .366 | 1.105 | .912 | .55 |
| (agg) | .673 | .276 | .366 | .394 | .46 |
| (agr) | .180 | .112 | .498 | .692 | 1.07 |
| (sub) | .036 | .081 | .240 | .220 | 1.81* |
| Affiliative | 18.546 | 6.562 | 14.681 | 8.373 | 1.21 |
| (act) | 9.267 | 6.896 | 8.245 | 5.044 | .59 |
| (rec) | 6.932 | 3.783 | 4.432 | 3.069 | 1.15 |
| (mut) | 2.347 | 1.752 | 2.004 | 1.688 | .24 |
| Sexual | 8.888 | 2.407 | 5.714 | 4.535 | 2.07 |
| (act) | 2.267 | 1.489 | 1.652 | 1.184 | 1.77 |
| (rec) | 4.712 | 2.550 | 2.398 | 2.030 | 1.67 |
| (mut) | 1.908 | 1.494 | 1.664 | 1.738 | .31 |

*p<.05

TABLE 8. Results of Student's t-tests and rates of selected behaviors for five female chimpanzees (experimental group) on all days before and after receiving Norplant implants (mean rate ± sd per hour of observation)

| Behavior | Phase I | | Phase II | | |
|---|---|---|---|---|---|
| | mean | sd | mean | sd | t |
| Groom total | 7.661 | 3.455 | 9.611 | 3.961 | .81 |
| groom given | 4.214 | 2.708 | 5.044 | 3.858 | .57 |
| groom rec'd | 2.032 | .930 | 2.533 | 1.353 | .81 |
| groom mutual | 1.415 | 1.638 | 2.033 | 1.195 | .50 |
| Copulation | .536 | .453 | .456 | .426 | .28 |
| Male solicit sex Focal | .432 | .247 | .322 | .173 | 1.13 |

TABLE 9. Results of F-tests on variances of behaviors exhibited by five female chimpanzees (experimental group) before and after receiving Norplant implants

| Category (Subcategory) | F | p |
|---|---|---|
| Behavior: | | |
| Agonistic | 1.326 | .791 |
| (agg) | 4.251 | .190 |
| (agr) | 1.416 | .744 |
| (sub) | 2.221 | .459 |
| Affiliative | 2.042 | .506 |
| (act) | 1.580 | .668 |
| (rec) | 1.416 | .744 |
| (mut) | 1.896 | .551 |
| Sexual | 1.045 | .967 |
| (act) | 3.020 | .310 |
| (rec) | 3.053 | .305 |
| (mut) | 1.940 | .537 |

TABLE 10. Results of t-tests and rate of interaction by partner category for five female chimpanzees (experimental group) before and after receiving Norplant implants

| | Phase I | | | Phase II | | | |
|---|---|---|---|---|---|---|---|
| Partner | Adult male | Adult female | Immature male | Adult male | Adult female | Immature male | t |
| Behavior: | | | | | | | |
| Agonistic | .251 | .194 | .072 | .367 | .111 | .178 | .72 |
| Social | 3.306 | 7.615 | 3.824 | 4.744 | 9.656 | 1.680 | 1.64 |
| Sexual | 1.514 | .799 | 1.019 | 1.343 | .655 | .859 | .02 |

TABLE 11. Results of Student's t-tests and rates of behavior of four female chimpanzees (control group) for all days during Phases I and II of the study (mean rate ± sd per hour of observation)

| Category | Phase I | | Phase II | | |
|---|---|---|---|---|---|
| (Subcategory) | mean | sd | mean | sd | t |
| Agonistic | .445 | .155 | .528 | .234 | .64 |
| (agg) | .335 | .102 | .375 | .209 | .37 |
| (agr) | .094 | .102 | .111 | .064 | .33 |
| (sub) | .016 | .031 | .042 | .053 | 1.72 |
| Affiliative | 17.968 | 6.339 | 16.181 | 7.528 | .89 |
| (act) | 9.417 | 5.291 | 7.833 | 4.133 | .64 |
| (rec) | 5.049 | 3.700 | 6.222 | 3.138 | 1.02 |
| (mut) | 3.501 | 2.240 | 2.125 | .907 | 1.15 |
| Sexual | 1.682 | .931 | 1.861 | .842 | .33 |
| (act) | .332 | .227 | .319 | .200 | .17 |
| (rec) | 1.071 | .441 | 1.444 | .800 | .90 |
| (mut) | .278 | .383 | .097 | .115 | 1.11 |

TABLE 12. Results of Student's t-tests and rates of behavior of four female chimpanzees (control group) for days with full swelling during Phases I and II of the study (mean rate ± sd per hour of observation)

| Category | Phase I | | Phase II | | |
|---|---|---|---|---|---|
| (Subcategory) | mean | sd | mean | sd | t |
| Agonistic | .115 | .231 | .617 | .678 | 1.43 |
| (agg) | .115 | .231 | .266 | .215 | 2.08 |
| (agr) | .000 | .000 | .274 | .440 | 1.25 |
| (sub) | .000 | .000 | .076 | .153 | 1.00 |
| Affiliative | 14.296 | 7.290 | 19.799 | 14.679 | .62 |
| (act) | 8.888 | 7.056 | 5.887 | 2.745 | .87 |
| (rec) | 4.105 | 2.893 | 10.984 | 11.536 | 1.51 |
| (mut) | 1.302 | 2.227 | 2.928 | 1.743 | 1.04 |
| Sexual | 2.326 | 2.407 | 3.207 | 2.510 | .45 |
| (act) | .229 | .367 | .227 | .207 | .01 |
| (rec) | 1.385 | 1.286 | 2.286 | 2.233 | .88 |
| (mut) | .712 | .891 | .318 | .436 | 1.07 |

TABLE 13. Results of F-tests on variances of behaviors exhibited by four female chimpanzees (control group) during Phases I and II of this study

| Category (Subcategory) | F | p |
|---|---|---|
| Agonistic behavior | 2.281 | .515 |
| (agg) | 4.204 | .269 |
| (agr) | 2.522 | .467 |
| (sub) | 2.897 | .406 |
| Affiliative behavior | 1.410 | .784 |
| (act) | 1.638 | .694 |
| (rec) | 1.390 | .792 |
| (mut) | 6.106 | .171 |
| Sexual behavior | 1.222 | .873 |
| (act) | 1.288 | .840 |
| (rec) | 3.291 | .354 |
| (mut) | 11.154 | .078 |

TABLE 14. Results of Student's t-tests and F-tests comparing rates of behaviors and variances between experimental (n=5) and control (n=4) groups during Phase I of the study

| | All days | | Days with full swelling | |
|---|---|---|---|---|
| Behavior | t | F | t | F |
| Agonistic | .81 | 4.11 | 3.65** | 2.52 |
| (agg) | .11 | 2.35 | 3.22** | 1.43 |
| (agr) | .90 | 1.64 | 3.17** | -- |
| (sub) | 1.71 | 3.84 | .88 | -- |
| Affiliative | .69 | 1.72 | .92 | 1.23 |
| (act) | .29 | 1.09 | .08 | 1.05 |
| (rec) | .97 | 7.11 | 1.23 | 1.71 |
| (mut) | .14 | 3.32 | .79 | 1.61 |
| Sexual | 2.69* | 1.44 | 3.81 | 1.24 |
| (act) | 2.36* | 8.12 | 2.64 | 16.49* |
| (rec) | 2.65* | 1.45 | 2.36 | 3.93 |
| (mut) | 1.05 | 2.26 | 1.40 | 2.81 |

*p<.05
**p<.02

TABLE 15. Results of Student's t-tests and F-tests comparing rates of behaviors and variances between experimental (n=5) and control (n=4) groups during Phase II of the study

| Behavior | All days | | Days with full swelling | |
|---|---|---|---|---|
| | t | F | t | F |
| Agonistic | 1.14 | 2.39 | .89 | 3.35 |
| (agg) | .19 | 2.37 | .45 | 3.35 |
| (agr) | 1.06 | 5.85 | .56 | 2.47 |
| (sub) | 2.20 | 2.95 | 1.25 | 2.04 |
| Affiliative | .12 | 1.18 | .66 | 3.07 |
| (act) | .51 | 2.39 | .83 | 3.38 |
| (rec) | 1.35 | 5.86 | 1.24 | 14.13* |
| (mut) | .78 | 3.49 | .81 | 1.07 |
| Sexual | 1.48 | 1.84 | .98 | 3.26 |
| (act) | 2.53* | 3.46 | 2.34+ | 32.59* |
| (rec) | .14* | 1.56 | .18 | 1.31 |
| (mut) | 1.97 | 13.01 | 1.49 | 15.92* |

*p<.05
+p=.051

TABLE 16. Results of Student's t-tests and rates of behavior for experimental (n=6) and control (n=6) groups for observations on all days (mean rate ± sd per hour of observation)

| | Phase I | | Phase II | | | |
|---|---|---|---|---|---|---|
| Behavior | mean | sd | mean | sd | t | F |
| Agonistic | .787 | .327 | .750 | .641 | .13 | 3.85 |
| (agg) | .361 | .313 | .287 | .223 | .47 | 1.97 |
| (agr) | .259 | .201 | .333 | .426 | .39 | 4.50 |
| (sub) | .167 | .086 | .130 | .240 | .36 | 7.78* |
| Affiliative | 18.398 | 7.394 | 15.713 | 6.454 | .67 | 1.31 |
| (act) | 9.648 | 5.712 | 7.843 | 4.113 | .63 | 1.93 |
| (rec) | 5.306 | 2.918 | 5.269 | 3.021 | .02 | 1.07 |
| (mut) | 3.444 | 2.072 | 2.602 | 1.258 | .85 | 2.71 |
| Sexual | 2.889 | 1.021 | 2.657 | 1.443 | .32 | .47 |
| (act) | .759 | .392 | .778 | .800 | .05 | 4.16 |
| (rec) | 1.657 | .675 | 1.694 | .756 | .09 | 1.25 |
| (mut) | .472 | .389 | .185 | .280 | 1.47 | 1.93 |

*p=.042

TABLE 17. Results of Student's t-tests, F-tests and rates of behavior for experimental (n=6) and control (n=6) groups for observations during full swelling

| | Phase I | | Phase II | | | |
|---|---|---|---|---|---|---|
| Behavior | mean | sd | mean | sd | t | F |
| Agonistic | 1.027 | .838 | .855 | .838 | .36 | 1.00 |
| (agg) | .332 | .362 | .177 | .216 | .90 | 2.81 |
| (agr) | .469 | .624 | .475 | .525 | .02 | 1.41 |
| (sub) | .227 | .199 | .203 | .367 | .14 | 3.40 |
| Affiliative | 18.047 | 11.139 | 19.013 | 12.165 | .14 | 1.19 |
| (act) | 9.058 | 4.931 | 7.060 | 4.738 | .72 | 1.08 |
| (rec) | 6.226 | 5.183 | 8.626 | 9.654 | .54 | 3.47 |
| (mut) | 2.763 | 2.396 | 3.327 | 1.533 | .49 | 2.44 |
| Sexual | 5.562 | 4.074 | 4.784 | 3.302 | .36 | 1.52 |
| (act) | 1.404 | 1.221 | 1.074 | 1.391 | .44 | 1.30 |
| (rec) | 2.665 | 1.930 | 3.286 | 2.056 | .54 | 1.14 |
| (mut) | 1.493 | 1.610 | .424 | .550 | 1.54 | 8.55* |

*p=.034

TABLE 18. Results of analysis of variance and rates of behavior by social group (corral) during Phase II of the study (n=12 females)

| Corral | 4 | 6 | 7 | 8 | F |
|---|---|---|---|---|---|
| Behavior: | | | | | |
| Agonistic | .889 | .722 | 1.093 | .370 | 1.28 |
| (agg) | .537 | .370 | .111 | .278 | 1.61 |
| (agr) | .204 | .185 | .704* | .093 | 4.15* |
| (sub) | .148 | .167 | .278 | .000 | 1.48 |
| Affiliative | 18.370 | 16.889 | 18.741 | 14.222 | .22 |
| (act) | 11.852 | 8.130 | 8.352 | 6.648 | .55 |
| (rec) | 4.278 | 6.833 | 5.907 | 4.130 | .56 |
| (mut) | 2.241 | 1.926 | 4.481 | 3.444 | 1.72 |
| Sexual | 2.611 | 2.778 | 3.815 | 1.889 | 1.50 |
| (act) | .519 | .889 | 1.241 | .426 | 1.23 |
| (rec) | 1.648 | 1.722 | 2.259 | 1.074 | 1.87 |
| (mut) | .444 | .167 | .315 | .389 | .27 |

*p=.048

# CHAPTER V

# CONCLUSIONS

The data presented from this study indicate that Norplant effectively prevented pregnancy in the seven chimpanzees during the first year of use. Ovarian hormone data on two females indicated that follicular development was suppressed in the cycle analyzed during Norplant treatment. Five females exhibited concentrations of $E_1G$ and PdG indicative of follicular and luteal development. The data suggest that ovulation may have occurred in these females, however, without direct observation of a corpus luteum on the ovary, ovulation can only be inferred.

Although one nulliparous female did not resume genital swelling after insertion of Norplant implants, the remaining females continued to exhibit cyclic genital swelling, including a phase of maximal tumescence. However, genital swelling cycles exhibited more intrafemale and interfemale variation. Mean cycle length did not differ between natural cycles and cycles during Norplant treatment, but, the

subjects did exhibit more days with partial swelling and fewer days with full swelling.

Analysis of behavioral data indicated that rates of affiliative and sexual interactions during Norplant treatment, did not differ from rates during natural cycles. The females that received implants exhibited an increase in submissive behavior which may reflect the fewer number of days per cycle they had maximal swelling. Rates of behavior for the experimental (implant) group was not significantly different from that of the control group.

Results of this study provide valuable information for managers of captive chimpanzees. In the majority of the females, Norplant provided effective contraception without suppressing genital swelling or inhibiting sociosexual behavior. However, the results of this study provide only initial information. Additional data are needed on the longterm effects of Norplant treatment on chimpanzees. The high rate of cycles presumed to be ovulatory six months following insertion of implants suggests that chimpanzees may be less responsive to the contraceptive action of levonorgestrel than are humans. The longevity of effectiveness must be determined before we can assume that Norplant will prevent pregnancy for five years in chimpanzees as it does in humans.

APPENDIX 1.

**CHIMPANZEE ETHOGRAM**

Operational definitions of behaviors recorded during this study. Direction of behavior was always indicated. * denotes behaviors analyzed for this thesis.

| CODE | BEHAVIOR | DEFINITION |
|---|---|---|
| AGC* | Aggressive contact | When one individual hits, kicks, bites, threatens another group member. Name partner. |
| APP* | Approach | One individual deliberately walks or runs toward another group member. Name partner. |
| AV* | Avoid/flee | One animals walks or runs away from an approaching group member. |
| CART | Carry/hold infant | Walking or sitting while supporting an immature group member. Name partner. |
| CH* | Chase | One individual runs after another group member. State if aggressive or play. Name partner. |
| CL | Climb | Traveling up or down from vertical structures. |
| COPRO | Aberrant fecal | Eating, manipulating, examining feces. |
| COP* | Copulation | A male mounts and thrusts a female. Name partner. |
| DIS* | Display | Individual is usually piloerect with shoulders hunched, running across exhibit, may hit or kick an inanimate object, run past or charge another individual. Pant hoots often accompany this behavior. Name others involved. |
| EAT | Eat | Consuming food. |

| | | |
|---|---|---|
| EXXP | Examine, explore, manipulate object | Examining or manipulating an object. Includes next building and using enrichment devices. Name object. |
| FOLL* | Follow | One individual is deliberately walking behind another. Name partner. |
| FOR | Forage | Actively searching for food/vegetation to consume. |
| GEN* | Genital Rub | Female rubs her genitalia against another female's genitalia. |
| GR_* | Groom | Picking through hair or at skin, removing debris with hand or mouth. Includes: groom self (grs); groom other (grg), receiving grooming (grr) and groom mutual (grm). |
| HOLD | Hold | Securely grasping an object with hand. |
| IDLE | Idle | Not exhibiting active behavior. Includes sitting, laying, staring into space. |
| INSP* | Inspect | An animal sniffs or probes the genitalia of another individual. Name partner. |
| LOB | Look out bars | An animal is actively watching an activity or individual through the bars. |
| LOC | Look out corral | Individual climbs to an elevated area corral and watches an activity or another individual outside of the enclosure. |
| MAST* | Masturbate | Self stimulation of the genitalia. Indicate if animal is watching another individual while masturbating. |
| MNT* | Mount | One individual mounts another but thrusting does not occur. Name partner. |

| | | |
|---|---|---|
| NUR | Nurse | Offspring (usually) is suckling a female. Indicate partner. |
| OOV | Out of view | Focal animal is out of sight. |
| PH | Pant hoot | The loud vocalization typical of chimpanzees. |
| PG* | Pant grunt | A series of short guttural grunts given by a subordinate animal to a higher ranking animal. Indicate partner. |
| PL* | Play | Includes wrestling, tickling, chasing and gymnastics. May be accompanied by a play face and "laughing". Indicate if alone or social. Name partner. |
| PR* | Present | Female may crouch with genitalia directed to a male or merely approach and orients genitalia in the male's line of vision. Indicate if sexual or submissive. Name partner. |
| SOL* | Solicit sex | An animal approaches another and solicits sexual behavior by presenting, head bobbing, swaying, penile display. Also includes attention getting behavior exhibited toward another social group. Name partner. |
| SUB* | Submissive | Includes head bobbing, pant grunting, crouching. |
| TOC* | Touch/ embrace | One individual extends hand or arms and gently makes contact with another group member. Indicate partner. |
| TR | Travel | Walking from one area to another area. |
| WA* | Watch/ stare | One individual is actively watching another group member, often within a few inches of one another. |

# VITA

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