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Source: Journal of Wildlife Diseases, 30(2) : 241-246

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-30.2.241

Journal of Wildlife Diseases, 30(2), 1994, pp. 241–246

LEVONORGESTREL IMPLANTS AS A CONTRACEPTIVE IN CAPTIVE WHITE-TAILED DEER

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ABSTRACT: Silastic implants containing levonorgestrel (LNG) were evaluated as a contraceptive in captive white-tailed deer (*Odocoileus virginianus*). Six adult females and six female fawns received either six or nine implants in autumn. Each implant contained 36 mg of LNG. Blood was analyzed by radioimmunoassay to determine LNG release profile for 5 mo post-implantation. Serum LNG concentrations rose significantly ($P = 0.0005$) 3 days post-implantation, leveled off after 7 days, and did not change ($P = 0.5913$) during the remaining 5 mo. Mean (±SE) LNG concentrations for all months were higher ($P = 0.0377$) in adult and fawn females implanted with nine versus six rods (138.1 ± 14.4 versus 56.7 ± 12.3 pg/ml, respectively). Serum LNG levels did not differ between adults and fawns. Five of the six implanted adult females had normal estrous cyclicity; three of these five adult females became pregnant in the first year. Four implanted females (two yearlings and two adults) were monitored during a second year, and housed with a fertile buck; three of them became pregnant. We do not recommend the use of LNG in deer.

Key words: Contraception, fertility control, levonorgestrel, *Odocoileus virginianus*, white-tailed deer.

INTRODUCTION

White-tailed deer (*Odocoileus virginianus*) can become overabundant in many urban areas, nature reserves, and parks. Hunting is not a permissible management tool in many of these areas. Contraceptives may help control deer populations in these situations.

Implantable steroids have been evaluated in deer (Bell and Peterle, 1975; Matschke 1977, 1980). A limitation to the use of steroid implants in deer has been the relatively short time of effective hormone release. Ideally, a contraceptive should last the reproductive life span of the doe (Matschke, 1980). Implants could be removed if reversible contraception is desired.

Levonorgestrel (LNG) provides effective, long-term contraception in humans (Diaz et al., 1982). Diaz et al. (1982) found no pregnancies in 101 women implanted with LNG for 5 yr.

Plotka and Seal (1989) reported LNG implants were ineffective as a contraceptive in deer, but did not measure LNG concentrations. Plotka and Seal (1989) used one solid silastic rod containing 200 mg of LNG rather than dividing the dose among six rods as has been used in humans (Diaz et al., 1982). The shape and matrix of silastic implants influences steroid hormone release (Robertson et al., 1983). Therefore, our objectives were to determine blood LNG concentrations and release profiles in deer implanted with six or nine silastic rods containing LNG, and to evaluate the efficacy of this contraceptive in adult and prepubertal fawn white-tailed deer.

MATERIALS AND METHODS

We used six adult females ranging from 1.5 to 11.5 yr and six female fawns (0.5 years). Individual fawn production for these deer was not monitored previously, but pregnancy rates in our captive herd usually had been near 100% in prior years. One adult and one fawn were paired randomly and housed in 3 × 6 m stalls at the University of Georgia (UGA) Whitehall Deer Research Facility, Athens, Georgia (USA), with food and water available *ad libitum*. All deer were on a regular deworming program with 1% sterile solution of Ivermectin (Merck and Company, Inc., Rahway, New Jersey, USA) administered at 1 ml/54 kg.

We treated deer with six or nine LNG implants (Bickle et al., 1991) each of which contained 36 mg LNG (total LNG = 216 or 324 mg, respectively). The LNG was sealed inside silastic tubing (34 mm total length; 2.4 mm diameter) (Robertson et al., 1983). Implants were

placed in deer between 21 November 1990 and 28 February 1991.

Deer were immobilized and anesthetized with xylazine hydrochloride (2 to 3 mg/kg; Mobay Corporation, Shawnee, Kansas, USA) and ketamine hydrochloride (12 to 18 mg/kg; Aveco, Inc., Fort Dodge, Iowa, USA) delivered remotely via intramuscular injection from a blowdart (Telinject, USA, Inc., Saugus, California, USA). A pretreatment blood sample was obtained on Day 0 from the jugular vein. The lateral side of the base of the neck was shaved and scrubbed with sequential cleansing using Betadine® surgical scrub (Purdue Frederick Company, Norwalk, Connecticut, USA) and 70% alcohol. An incision (3 to 5 mm) was made and the six or nine LNG implants were inserted subdermally with a 10-gauge trocar in a fan-like arrangement. The opening was closed with one or two wound clips and treated with a topical antiseptic, furazolidone (Topazone®; Norden Laboratories, Lincoln, Nebraska, USA). Sedation was reversed by yohimbine hydrochloride (0.5 to 0.8 mg/kg; half of the dose given intramuscularly, and half given intravenously; Sigma Chemical Co., St. Louis, Missouri, USA; Mech et al., 1985).

Blood samples were taken contralateral to the site of implantation. We collected blood samples 3 days after implantation, weekly for 1 mo, and monthly for 5 mo to measure serum LNG levels. Blood was obtained between 0900 and 1500 on the day of sample. Blood was allowed to clot and centrifuged at 913 × *g* for 5 min. Serum was frozen until tested.

The exact day of implantation was based on each adult female's estrous period. From October through March, which overlaps the breeding season for captive deer in Georgia (Knox et al., 1988), each adult female was checked daily for estrus using a tractable, epididyectomized male white-tailed deer. Copulation was not allowed during this period of estrus. Implantation of LNG occurred 12 to 14 days (in the luteal phase) after first estrus for each adult female. Allowing one estrous cycle before implantation assured normal cycling was occurring and provided an estimate of when the next cycle should occur. White-tailed deer estrous cycles in captivity range from 21–30 days ($\bar{x}$ = 26.2) (Knox et al., 1988). Any adult females that did not exhibit estrous behavior by 28 February were implanted at that time.

One fawn was chosen randomly for LNG implantation each time an adult female received its implants. We planned to compare estrous cycling and behavior of implanted versus untreated fawns in the next year.

Radioimmunoassay (RIA) was used to measure LNG. Levonorgestrel (D-Norgestrel) standards were obtained from Sigma Chemical Company (St. Louis, Missouri). Antiserum against LNG was donated from Schering AG, (Berlin, Germany). Tritiated and iodinated LNG were donated by the University of Southern California (USC) School of Medicine, Los Angeles, California.

Specific RIA procedures were modified from Stanczyk et al. (1975) as follows. Serum (0.5 ml) and tritiated LNG (0.1 ml) was extracted twice with 4 ml hexane each time. The hexane extract was dried under a stream of air. Samples were reconstituted with 0.1 ml ethanol, and 20 µl were evaluated. Extraction efficiency, determined by measuring the recovered ^{3}H-LNG, was 63.5% (SE = 0.006). The assay was verified by adding known amounts of LNG to control deer serum with 0, 0.2, and 1.0 ng/500 µl serum and measuring these concentrations. The samples with known amounts of LNG had a linear response. Intra-assay coefficient of variation was 4.2%. All samples were measured in the same assay. Mean for the serum blanks was 56.3 pg/ml. This value was subtracted from the results before reporting treatment values.

Following LNG implantation, any adult females or fawns that exhibited estrous behavior (continued ovarian cycling) were allowed to copulate with a fertile male. Pregnancies were determined by ultrasound (White et al., 1989).

The continuation of our LNG study involved monitoring the fawns that were implanted in 1990 and 1991 for any potential effects of LNG on puberty attainment as they matured. We removed the implants from two fawns (yearlings in fall 1991) and one adult female. These and all other female deer in the study were monitored for any potential residual effects of LNG on reproductive function, and were housed in a 1-ha pen at the Whitehall Deer Research Facility with a mature proven fertile buck when monitored for fertility.

Necropsy of any implanted deer that died was preformed by University of Georgia, College of Veterinary Medicine, Diagnostic Laboratory (Athens, Georgia).

All data were analyzed using SAS (Statistical Analysis System, SAS Institute, Cary, North Carolina, USA). We used a factorial design with two levels of implants, and two ages, and an implant-by-age interaction term. Individual animals served as experimental blocks to test for differences over time in serum LNG concentrations.

RESULTS

Our method of implanting LNG rods was rapid, easy, and resulted in no procedural mortality. Once an animal was im-

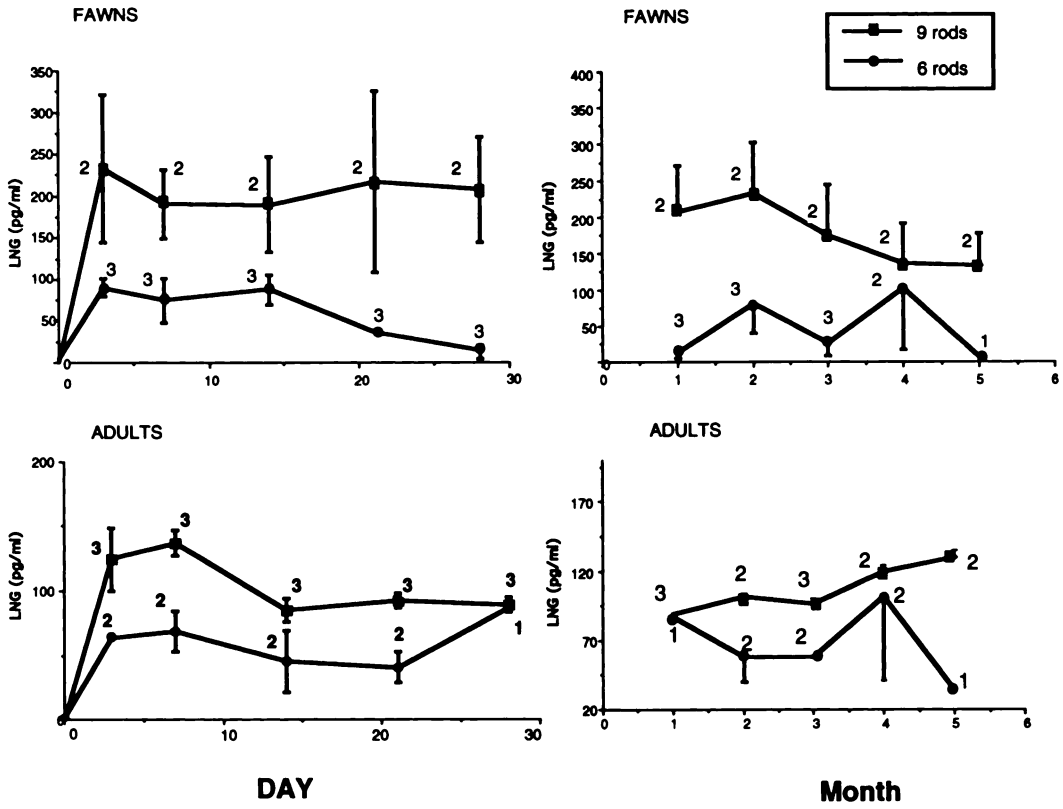

FIGURE 1. Mean and SE (vertical bars) serum levonorgestrel (LNG) concentrations for Days 0 to 28 and Month 1 to 5 post-implantation in captive adult and fawn female deer treated with either six or nine LNG rods during the 1990 to 1991 breeding season. The numbers at each data point are the number of deer sampled.

mobilized, implantation was finished in about 15 min.

A significant ($P = 0.0005$) rise in LNG concentrations occurred three days post-implantation (Fig. 1). Concentrations of LNG leveled off after 1 wk and did not change over the 5-mo period ($P = 0.59$). Levonorgestrel concentrations were higher ($P = 0.037$) in fawn and adult females implanted with nine versus six rods. Mean (±SE) LNG concentrations (pg/ml) over the 5 mo were 138.1 ± 14.4 and 56.7 ± 12.3 pg/ml, for the adult and fawn females implanted with nine or six rods, respectively. There was no interaction between LNG dose and time. Concentrations of LNG did not differ ($P = 0.42$) between fawns versus adults.

Reproductive effects of LNG varied for each adult female. Following implantation, one adult female continued cycling but never became pregnant, two adult females became pregnant on first breeding, and one adult female became pregnant on her third breeding. Two adult females with nine implants gave birth after normal gestation and displayed no complications during parturition. One adult female with six implants became pregnant but died 106 days after conception.

All females that had implants removed became pregnant the next year as yearlings or adults. The four implanted females examined during the second year became pregnant except for one yearling that was implanted with nine rods.

Unexpected side effects from the LNG implants appeared during spring 1991. Five deer with six implants displayed health problems. These deer had reduced

food intake in April and May. Despite all attempts to induce feeding with succulent natural browse and fruit, one doe became severely emaciated and died on 25 May 1991. At the time of death she was pregnant with one fetus (106 days old). Two adults and two fawns also became emaciated. We monitored food intake and removed implants from these deer. All recovered except for one adult female which died on 7 July 1991. From necropsy, primary pathological findings were acute gastrointestinal hemorrhage, and abdominal fat necrosis.

DISCUSSION

The success of LNG as a contraceptive in humans is related to the sustained hormone release for at least 5 yr (Diaz et al., 1982). Serum levels of LNG are high the first month after implantation, then decline to a relatively constant level thereafter (Moore et al., 1978). We observed a similar release profile of LNG in our deer (Fig. 1).

Alvarez et al. (1983) found stable plasma levels of LNG in humans at about 400 pg/ml for ≤6 yr after implantation. Deer LNG concentrations in the six and nine rod groups (56.7 and 138.1 pg/ml, respectively) were much lower than in humans. However, the biological activity of contraceptive steroids may vary widely between species because of differing physiology and metabolic pathways (Phillips et al., 1987; Goodman, 1989).

Levonorgestrel implants can cause infertility in humans either through alteration of ovarian cycling or by its effects on cervical mucous or uterine muscular activity (Roy et al., 1984). The contraceptive effect of LNG may be achieved partly by inhibition of ovulation (Croxatto et al., 1982). However, infertility in LNG-treated women may occur without disruption of ovulation (Brache et al., 1990). Brache et al. (1990) found that patterns of ovarian response in women treated with LNG varied from no luteal activity and a decrease in ovarian cycles to nearly normal cycles. Alvarez et al. (1986) confirmed ovulation in two LNG users by laparotomy, but believed infertility was achieved by abnormal endocrine profiles. Levonorgestrel users had lower follicle stimulating hormone and luteinizing hormone peaks, and lower mid-luteal stage progesterone levels when compared to controls (Alvarez et al., 1986).

Although these physiological effects may have operated in our deer, three of the five adult females that cycled normally in the 1990–91 breeding season became pregnant. Of the deer remaining implanted for the 1991-92 breeding season, only one female implanted with nine rods as a fawn was infertile. Additionally, pregnant adult females with implants were able to complete normal gestation and developed no complications during parturition. Thus, we believe that LNG has limited effect as a progestogen in white-tailed deer at the doses tested.

Even though LNG has progestational activity, it is structurally related to testosterone (Goodman, 1989). Phillips et al. (1987) compared action and effectiveness of progestogens used in contraceptives in rats and rabbits. Each progestogen varied with route of administration, species tested, and effect examined. In that study, LNG was the most androgenic of all progestogens tested. High anti-ovulatory effects of LNG administered orally or intramuscularly occurred in the rabbit. However, ovulation in the rat was greatly affected by oral but not subcutaneous LNG administration. Based on these differences between species, routes of administration, and physiological effects, we believe it is difficult to extrapolate contraceptive methods from one animal model to another.

Metabolism of LNG in deer may limit its progestogen activity. Further research may be necessary to determine LNG interaction with other hormones in white-tailed deer to evaluate the health effects. However, LNG implants as used in our

study would not be effective in controlling reproduction in white-tailed deer.

We observed possible health problems in deer using LNG implants. This finding is different from humans where minimal side effects have been reported (Roy et al., 1984). Deer normally undergo voluntary food restriction in the winter but increase intake during the milder temperatures and longer days of spring (Warren et al., 1981). Voluntarily reduced food consumption was seen in five deer even in the spring. Two of these deer eventually died. Three of them recovered and increased their food consumption after removal of implants. Body weight changes in humans treated with LNG implants have been reported to increase or decrease depending on the individual (Moore et al., 1978). Perhaps feeding behavior in deer is affected by synthetic steroids or their metabolites. Feed intake should be monitored closely in any future studies. The health problems we observed also may have been related to steroid effects on the immune system (Joshi et al. 1971). Future research with implanted synthetic steroids should also consider possible immunological effects.

In conclusion, implantation of LNG rods in deer was a rapid and simple procedure. We observed a significant release of LNG after subcutaneous implantation. However, LNG was not effective as a contraceptive at the levels we detected in adult white-tailed deer. Implantation of LNG did not appear to affect puberty attainment. Levonorgestrel levels detected in this study did not affect the length of gestation or parturition. Therefore, we do not recommend the use of LNG in white-tailed deer. Additionally, any future studies with implantable steroids should evaluate potential health effects.

ACKNOWLEDGMENTS

This work was supported by the U.S. National Park Service (Southeast Region subagreement Number 4, Cooperative Agreement Number 5000-9-8020), and by McIntire-Stennis Project (Number GEO-0030).

We thank the personnel at the Fort Dodge Laboratories for their generous donation of the LNG implants. Staff at Schering Ag (Berlin, Germany) kindly supplied the anti-LNG for RIA analysis. Dr. F. Z. Stanczyk and C. Grozinger of Livingston Laboratory of USC Medical Center provided invaluable help and donated the radiolabelled LNG for RIA analysis. Also much appreciation goes to Drs. R. R. Kraeling, G. B. Rampacek and S. Leischan, L. Taras, and B. Johnston, of USDA Russell Research Laboratories (Athens, Georgia) for their assistance and expertise in the RIA analysis. Thanks also is needed for all the student volunteers who freely gave time and assistance in sample collection.

LITERATURE CITED

ALVAREZ, F., V. BRACHE, A. FAUNDES, E. D. B. JOHANSSON, V. ODLIND, AND H. NASH. 1983. Levonorgestrel plasma levels during continuous administration with different models of subdermal implants. Contraception 27: 123–130.

———, ———, A. S. TEJADA, AND A. FAUNDES. 1986. Abnormal endocrine profile among women with confirmed or presumed ovulation during long-term NORPLANT® use. Contraception 33: 111–119.

BELL, R. L., AND T. J. PETERLE. 1975. Hormone implants control reproduction in white-tailed deer. Wildlife Society Bulletin 3: 152–156.

BICKLE, C. A., J. F. KIRKPATRICK, AND J. W. TURNER, JR. 1991. Contraception in striped skunks with Norplant® implants. Wildlife Society Bulletin 19: 334–338.

BRACHE, V., F. ALVAREZ-SANCHEZ, A. FAUNDES, A. S. TEJADA, AND L. COCHON. 1990. Ovarian endocrine function through five years of continuous treatment with NORPLANT® subdermal contraceptive implants. Contraception 41: 169–177.

CROXATTO, H. B., S. DIAZ, M. PAVEZ, P. MIRANDA, AND A. BRANDEIS. 1982. Plasma progesterone levels during long-term treatment with levonorgestrel silastic implants. Acta Endocrinology 101: 307–311.

DIAZ, S., M. PAVEZ, P. MIRANDA, D. N. ROBERTSON, I. SIVIN, AND H. B. CROXATTO. 1982. A five-year clinical trial of levonorgestrel silastic implants (NORPLANT®). Contraception 25: 447–456.

GOODMAN, A. L. 1989. The biochemistry of oral contraceptive steroids. Seminars in Reproductive Endocrinology 7: 199–204.

JOSHI, U. M., S. S. RAO, S. J. KORA, S. S. DIKSHIT, AND K. D. VIRKAR. 1971. Effect of steroidal contraceptives on antibody formation in the human female. Contraception 3: 327–333.

KNOX, W. M., K. V. MILLER, AND R. L. MARCHINTON. 1988. Recurrent estrous cycles in

white-tailed deer. Journal of Mammalogy 69: 384–386.

MATSCHKE, G. H. 1977. Fertility control in white-tailed deer by steroid implants. The Journal of Wildlife Management 41: 731–735.

———. 1980. Efficacy of steroid implants in preventing pregnancy in white-tailed deer. The Journal of Wildlife Management 44: 756–758.

MECH, D. L., G. D. DEL GIUDICE, P. D. KARNS, AND U. S. SEAL. 1985. Yohimbine hydrochloride as an antagonist to xylazine hydrochloride-ketamine hydrochloride immobilization of white-tailed deer. Journal of Wildlife Diseases 21: 405–410.

MOORE, D. E., S. ROY, F. Z. STANCZYK, AND D. R. MISHELL. 1978. Bleeding and serum d-norgestrel, estradiol and progesterone patterns in women using d-norgestrel subdermal polysiloxane capsules for contraception. Contraception 17: 315–328.

PHILLIPS, A., D. W. HAHN, S. KLIMEK, AND J. L. MCGUIRE. 1987. A comparison of the potencies and activities of progestogens used in contraceptives. Contraception 36: 181–192.

PLOTKA, E. D., AND U. S. SEAL. 1989. Fertility control in female white-tailed deer. Journal of Wildlife Diseases 25: 643–646.

ROBERTSON, D. N., I. SIVIN, H. A. NASH, J. BRAUN, AND J. DINH. 1983. Release rates of levonorgestrel from silastic® capsules, homogenous rods and covered rods in humans. Contraception 27: 483–495.

ROY, S., D. R. MISHELL, JR., D. N. ROBERTSON, R. M. KRAUSS, M. LACARRA, AND M. J. DUDA. 1984. Long-term reversible contraception with levonorgestrel-releasing silastic* rods. American Journal of Obstetrics and Gynecology 148: 1006–1013.

STANCZYK, F. Z., M. HIROI, U. GOEBELSMANN, P. F. BRENNER, M. E. LUMKIN, AND D. R. MISHELL. 1975. Radioimmunoassay of serum d-norgestrel in women following oral and intravaginal administration. Contraception 12: 279–298.

WARREN, R. J., R. L. KIRKPATRICK, A. OELSCHLAEGER, P. F. SCANLON, AND F. C. GWAZDAUSKAS. 1981. Dietary and seasonal influences on nutritional indices of adult male white-tailed deer. The Journal of Wildlife Management 45: 926–936.

WHITE, I. R., W. A. C. MCKELVEY, S. BUSBY, S. SNEDDON, AND W. J. HAMILTON. 1989. Diagnosis of pregnancy and prediction of fetal age in red deer by real-time ultrasonic scanning. 1989. The Veterinary Record 124: 395–397.

Received for publication 31 December 1992.