Use of etonogestrel implants to suppress estrous behavior in mares*

Ghislaine A. Dujovne,[a] Aime K. Johnson,[b] Robyn R. Wilborn,[b] Roberto A. Palomares,[c] Anne A. Wooldridge,[b] Tim D. Braden[d]

[a]Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA; [b]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL; [c]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA; [d]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL

Abstract

The objective of this study was to evaluate a human contraceptive subcutaneous implant (Implanon®) as a method to suppress behavioral estrus in mares. Twenty mares were randomly assigned to 4 groups (n=5). Group C was the control group, group T1 received one Implanon® implant (68 mg etonogestrel), group T2 received two Implanon® implants (136 mg etonogestrel), and group R was the positive control, receiving 0.044 mg/kg altrenogest daily.

Estrous behavior was evaluated twice weekly and was graded by a blinded observer. Estrous cycles were monitored during three months using progesterone levels and transrectal examinations. Interestrus interval (IEI) was measured based on both behavioral estrus (teasing scores) and serum progesterone concentration (below 1.0 ng/ml).

Mean interestrus interval ± SEM per group, based on teasing and progesterone levels, respectively, were as follows: group C 21±0.3 and 21±0.4 days; group T1: 34±8.2 and 31±6.4 days; group T2: 42±14.1 and 41±14.4 days; and group R: 111±1.3 and 48±0.9. Group T2 had an Interestrus interval twice as long as the control group, however, no statistical difference was found between groups C, T1 and T2. Group R positive control) was different from all other groups ($P<0.05$) based on teasing observations.

Etonogestrel was not consistently effective for estrus suppression in mares, however, it did produce an IEI twice as long as the negative control at the highest dose used in this study (136mg). Further studies with a higher dose would be necessary to determine whether or not etonogestrel can be used to fully suppress estrus in mares.

Keywords: Mare, estrus suppression, etonogestrel, Implanon

Introduction

Estrous behavior ("heat") is a problem in many performance mares. Animals have temperament changes and become more difficult to handle during the estrus period. Some mares show aggression, pain, and reduced performance while in heat.[1] Because of performance problems associated with estrus in mares, several treatments have been evaluated to find an ideal method for behavioral estrus suppression. Currently no single treatment has been shown to be effective, easy to use, and safe for prolonged estrus suppression. The most common and consistently effective treatments include oral altrenogest and natural progesterone injections. However, both methods necessitate repeated administration, which makes them costly and impractical in many situations. The injections can be painful and may induce swelling at the injection site leading to injection aversion or sore muscles that affect performance. Daily oral treatment with altrenogest may make the mare reluctant to be handled around the mouth and must be administered every day for complete effectiveness.[2] Other methods that have been tested with limited success include the use of intrauterine devices, repeated oxytocin injections, manual reduction of an established conceptus, herbal supplements, and induction of a diestral ovulation.[3-8] Synthetic progestins other than altrenogest (medroxyprogesterone, norgestomet, megestrol acetate), completely failed to suppress estrous behavior or maintain pregnancy in mares presumably because of a failure of these compounds to bind to the equine progesterone receptors.[9-11] There are conflicting studies

*These data were presented, in part, at the 2011 Annual Meeting of the Society for Theriogenology.

about the use of gonadotropin releasing hormone (GnRH) immunization to suppress estrous behavior. Some studies report inconsistent results while others show promising results for reducing or eliminating behavioral signs.[12-14] This treatment is currently unavailable in the United States.

Implanon® (Organon Laboratories Ltd, Science Park, Cambridge, UK) is a subcutaneous implantable contraceptive containing 68 mg of the progestin etonogestrel. This implant is formulated for long-term birth control in women.[15,16] Etonogestrel is a progestin with proven efficacy and safety in women, and also has been used in a contraceptive vaginal ring (Nuvaring®, Organon, USA Inc).[16] In women, a release rate of 25-35 µg/day of etonogestrel is required to suppress ovulation and prevent pregnancy.[17-18] Etonogestrel in Implanon® is released at an initial rate of 60 µg/day, which then decreases to 30 µg/day. This release rate results in maintaining a sufficient plasma concentration of the progestin to inhibit ovulation for up to three years in women.[17,18]

The objective of this study was to evaluate this synthetic progestin (etonogestrel) as a reliable method to suppress behavioral estrus in mares. If etonogestrel is effective at inhibiting estrous behavior in mares, the implant may provide a long-term, safe, easy, and potentially reversible treatment to suppress estrous behavior in performance mares.

Materials and methods

Twenty healthy mares between the ages of 6 and 20 years old with normal estrous cycles were used. The mares were part of the Auburn University Equine Reproduction Center teaching herd and Auburn University Animal Science Horse Center. All the animals were average size for adult horses (approximately 500 kg body weight); they were kept under similar husbandry conditions (free choice hay with grain supplementation as needed) and were housed as groups in paddocks or pasture. The study took place between June and September. Follicular growth, ovulation, and estrous behavior were monitored prior to each mare's enrollment into the study, and only animals demonstrating regular estrous cycles and normal estrous behavior in response to a teaser stallion were included. The animals were randomly allocated to 4 groups of 5 animals each using a random number generator (Research randomizer, www.randomizer.org).

Treatments

Group C (control): Negative control; animals did not receive any treatment and were allowed to cycle normally.

Group T1 (Treatment Dose 1): One Implanon® implant containing 68 mg of etonogestrel.

Group T2 (Treatment Dose 2): Two Implanon® implants containing 136 mg etonogestrel.

Group R: Positive control; 0.044 mg/kg altrenogest orally once daily.

Implant application was performed using aseptic technique. The mare's movement was restricted by placing her in stocks and sedating lightly with xylazine (0.2-0.4 mg/kg iv). Two milliliters of 2% lidocaine was used as a local anesthetic at the site of insertion. The implants were introduced subcutaneously into the vulvar lips, parallel to the vulvar opening, and 5 to 10 centimeters below the dorsal vulvar commisure. Introduction was performed using the applicator provided by the manufacturer (Figure 1). For mares in group T2, one implant was applied in each vulvar lip. The removal of the implants at the conclusion of the study was performed using local anesthetic (same protocol as insertion). A 2 to 3 mm incision was made at the tip of the implant followed by gentle manual manipulation of the implant toward the incision until it was externally visible through the incision. The implant was then removed and discarded. The small incision was allowed to heal by second intention.

Altrenogest was used for the positive control group because of its proven effectiveness to suppress behavioral estrus (0.044mg/kg orally once daily).[11,19]

All treatments started in mid-diestrus, seven days after ovulation was detected by transrectal ultrasonography. The study ended with removal of the implant (groups T1 and T2) or discontinuation of altrenogest (group R) after 90 days of treatment, and mare's reproductive cycles were followed until their first ovulation after the treatment withdrawal was detected.

Data collection

Teasing. Behavioral estrus was evaluated twice weekly by an experienced observer who was blinded to the treatment groups. A teaser stallion was led to the paddock, and the mares were allowed fence line contact. If a mare did not present at the fence, she was captured with a halter and lead rope and teased individually across the fence. Behavior was scored 1 to 4 using the following scale: 1: mare completely rejects the stallion, presenting one or more of the following refusal manifestations: squealing, pawing, kicking, switching tail, holding ears back; 2: mare is indifferent to the presence of the stallion; she does not move away, but does not lift the tail or wink the clitoris (rhythmic eversion of the labia and exposure of the clitoris); 3: mare is interested in the stallion and approaches him, raising the tail, urinating and/or winking the clitoris; 4: Full estrous behavior (posture change facilitating copulation), the mare will present similar behavioral signs as score 3 (clitoris eversion (winking), elevation of the tail and urination in presence of the stallion) plus a change in the mare's posture to one that facilitates copulation (arched tail, flexed stifles and hocks, abducted rear limbs and tipped pelvis with associated lowering of the perineal area). Mares scored with a one or two were considered not in estrus, and those scored three or four were considered to be in estrus (behavior absent or present, respectively).

Progesterone. To further evaluate each mare's hormonal profile, a baseline blood sample was collected immediately prior to treatment (day seven post-ovulation), 24 and 48 hrs after treatment onset, and then weekly to evaluate plasma progesterone concentrations. The samples were processed with a progesterone radioimmunoassay (RIA) kit (Coat-a-Count®, Siemens, Los Angeles, CA) which has previously been evaluated for use in this species (intra-assay inter-assay coefficient of variation 2.46% and 17.9%).[20] Supplemental altrenogest does not affect the ability to quantify natural progesterone levels.[21]

Palpation and ultrasonography. Twice weekly examinations using transrectal ultrasonography were performed for estrous cycle monitoring and to evaluate the correlation between progesterone concentration, ovarian structures, and teasing behavior in this group of mares. An ultrasonography system (MicroMaxx®, Sonosite Inc, Bothell, WA) with an L52e transducer (5MHz) was used for all examinations.

Data analysis

Interestrus interval was calculated based on both behavioral estrus (teasing) and progesterone levels. Plasma samples with progesterone concentrations below 1.0 ng/ml, and teasing score of 3 or 4 were considered consistent with estrus. The IEI was used as an indicator of estrus suppression duration, and is counted as the time in days between periods of behavioral estrus.

Data were analyzed using the statistical software Statistical Analysis System (SAS version 9.1, SAS Institute, Cary, NC). Sample size of the groups was determined through the use of PROC POWER based on the expected means, standard deviations and a statistical power of 95%. Significant differences were identified with $p < 0.05$. Results are reported as mean +/- standard error of the mean (SEM).

Data were transformed using a logarithmic scale in order to achieve statistical assumptions (normality and constant variance). Descriptive statistics (means, SEM and coefficient of variation) were determined using PROC UNIVARIATE. In order to assess the effect of the treatment on estrus suppression, IEI were analyzed using the analysis of variance through the general linear model (PROC GLM). Comparisons among group means were performed using the least significant difference test (LSD). Pearson's correlation analysis was performed using PROC CORR.

Animal welfare

This study was performed under the regulations of the Institutional Animal Care and Use Committee (IACUC), Office of Animal Resources, Auburn University.

Results

Clinical observation

No abnormalities at the site of implant application were observed in any of the treated mares. Implant application and removal was easily performed, and mares showed no evidence of pain or discomfort during the procedure.

One mare in group R was euthanized due to a severe colic episode (unrelated to the study) during the first month of the study; her data were not considered for analysis.

Ovulation (detected by transrectal palpation and ultrasonography) was observed in all five mares in groups C, T1 and T2. In group C, ovulation occurred regularly in all mares as expected. However, in groups T1 and T2, ovulations were erratic, without an established pattern. In group R (positive control), three out of four mares ovulated. The mares that ovulated in group R also showed inconsistent ovulation intervals.

After treatment withdrawal, all mares returned to normal cyclicity, and ovulation occurred within 3 to 46 days. After the 90 day study period, ovulation in group C occurred in a mean ± SEM of 10 ± 1.1 days (range 7 to 14). In group T1 and T2, ovulation occurred in a mean of 14 ± 5.3days (range 3 to 30) and 23 ± 6.2 days (range 10 to 46), respectively. In group R, ovulation occurred in a mean of 9 ± 0.7 days after the treatment withdrawal (range 8 to 11). No significant differences were found among groups. Differences approaching significance were identified between groups C and T2 ($p=0.06$), and also between T2 and R ($p=0.06$); which may indicate an association between etonogestrel (2 implant dose) treatment removal and days to ovulation.

Interestrus interval

Mean IEI based on teasing scores ± SEM was 21 ± 0.3 days for group C, 34 ± 8.2 days for group T1, 42 ± 14.1 days for group T2 and 111 ± 1.3 days for group R. Mean IEI based on progesterone values ± SEM were as follows: 21 ± 0.4 days for group C, 31 ± 6.4 days for group T1, 41 ± 14.4 days for group T2 and 48 ± 0.9 days for group R (Fig. 2). Mean IEI of each individual mare during the 90 day study period are shown (Table). No statistical difference was found between groups C, T1 and T2 based on teasing or progesterone levels. As expected, IEI in group R (positive control) was longer than all other groups ($P<0.001$) based on teasing observations. Behavioral estrus in this group was suppressed during the entire study period.

Based on progesterone concentrations, the IEI of group R was different from group C ($p=0.049$). No significant differences were found among the other groups (Figs. 3-6).

A significant difference in the response to etonogestrel was observed among mares within the same group in both T1 and T2. The coefficient of variation (CV) for group T1 was 53%. In group T2 the variation was even greater with a CV of 73% with 3/5 (60%) of the mares experiencing a prolonged IEI (two mares showed 35 days, and one mare failed to show estrus behavior the entire study period). The other two mares in the group, however, showed no effects and experienced a normal estrus period. The variation was greater in both implant groups compared with the control groups. The CV in groups C and R (negative and positive control) was 3.6% and 2.3% respectively.

The two methods used to determine IEI (progesterone and teasing score) provided similar results. The IEI determined by teasing and progesterone levels was highly correlated ($r=0.91$). There was moderate correlation in group C ($r=0.58$), strong correlation in groups T1 and T2 ($r= 0.99$), and very low correlation in group R ($r=0.05$), as expected due the fact that erratic ovulations occurred in this group independent of the total suppression of the behavioral estrus. In this study, the correlation coefficient demonstrates the correlation of the IEI based on progesterone and teasing, showing no differences in the IEI as determined by each method.

Discussion

At the dose used in this study, etonogestrel was not effective for complete behavioral estrus suppression. However, the behavioral IEI for group T2 (136 mg etonogestrel) averaged twice as long as group C (control) indicating some clinical effect.

The use of the implant was determined to be safe in mares for at least 90 days. None of the ten treated mares in our study showed adverse reactions to the implants during the study period which implies that the implants are safe to use in the mare at least for this period of time. This is consistent with a safety study performed in humans using Implanon® implants. In that study, very few abnormalities or complications were observed at the implant site in 474 women (<3% complication rate).[22]

Ovulation was detected in 95% (18/19) of all mares, during the treatment period. Ovulation occurred at regular intervals (21 to 23 days) in the control group (group C). Irregular ovulations occurred in groups T1 and T2 which may indicate that etonogestrel, at the dose used, was unable to completely block ovulation, but did have some effect on cyclicity. Ovulation was detected in 75% (3 out of 4) of mares in group R (Regumate®), with one mare showing only a single ovulation during the entire observation period. This is consistent with previous studies that documented that altrenogest was not always able to exert predictable control over the estrous cycle in the mare and completely suppress ovulation.[23]

Group T2 displayed an IEI that was twice as long as the control group C. Although not statistically significant, this difference may be clinically relevant. The fact that the difference was not significant statistically could be explained by the high individual variation within the group T2 (CV=73%). A larger number of animals per group could have reduced this variation. Prior to this study, the sample size was determined by the PROC POWER test based on the expected means, standard deviations and a statistical power of 95%. This power was calculated based on expected means and standard deviations of previous studies where the variation was lower than that obtained in this experiment. Based on the results obtained in this study, the power was recalculated. In order to maintain a power of 95% 18 mares per group would be required. Therefore, in future studies, it seems likely that a larger sample size may be needed to reduce variation.

As expected, group R (positive control) had an IEI significantly longer than all other groups. Estrous behavior in these mares was completely suppressed during the entire study period which confirms altrenogest as a reliable positive control for suppressing behavioral estrus. This is consistent with several previous studies where altrenogest effectively suppressed estrous behavior in mares.[9,11,19,24]

When IEI was calculated based on progesterone concentrations, only groups R and C were significantly different (positive and negative control groups, respectively). This is because, as previously mentioned, altrenogest failed to completely suppress ovulation. Progesterone concentrations in mares receiving altrenogest may be unpredictable, sometimes rising over or decreasing below 1 ng/ml without being accompanied by estrous behavior, indicating ovulations are occurring.[23]

The difference between IEI based on teasing and progesterone concentrations in group R (111 days versus 48 days, respectively) can be attributed to the fact that behavioral estrus is suppressed in mares receiving altrenogest even in the presence of follicular growth or ovulation. This explains the low correlation (r=0.05) between the two parameters (IEI based on teasing scores and progesterone levels) in group R when compared with the general correlation of teasing to progesterone (r=0.91). If the Group R is excluded from the correlation test, the correlation coefficient increases to r=0.98, almost perfect correlation.

Etonogestrel determination in plasma was not possible in our study mares due to technical limitations to measure the small concentrations of the drug expected in circulating blood (picograms/ml). In women, the maximum serum concentrations detected were 813 pg/ml four days after implant insertion, and declined to 156 pg/ml at the end of the three years.[25] In that study, body weight was related to overall serum concentrations of etonogestrel where the highest levels were found in women weighing less than 5 0kg (≈200 pg/ml) and the lowest concentration was found in women weighing over 70 kg (≈150 pg/ml).[25] Based on this information, it is possible to speculate that the expected concentrations of etonogestrel in the horse should be five to ten times less than the serum levels obtained in women, considering an average woman's weight of 65 kg compared with a 500 kg body weight in the mare. This study used a dose of 0.13 to 0.27 mg/kg, compared with 1.05 mg/kg used in the commercial dose for women (assuming an average body weight of 65 kg). For this study we chose a lower dose in order to make this treatment financially comparable with daily altrenogest administration during a period of 90 days,

knowing that a higher dose would be more adequate but not financially practical for estrus suppression use in mares based on the commercial cost of the individual implants.

Based on previous information, the fact that behavioral estrus was completely suppressed in one of the study mares throughout the treatment period, and IEI was twice as long in the T2 (two implant) group compared with the control mares, we speculate that etonogestrel in the correct dose may suppress estrous behavior more consistently. Further studies using a weight-adjusted dose (525 mg per 500 kg horse) and a greater number of animals per group to reduce the effect of individual variability are warranted to determine whether or not etonogestrel is a reliable alternative for estrus suppression in the mare.

Conclusions

The high correlation of the two parameters to determine IEI (teasing and progesterone) validates the teasing score as a reliable method to detect estrus in mares. This may only be valid if the teasing is performed by an experienced observer and the mare normally exhibits estrus signs in presence of a stallion.

At the dose used in this study, etonogestrel was not effective for complete behavioral estrus suppression. However, the behavioral IEI for group T2 (136mg etonogestrel) was twice as long as group C (control) which suggests that future studies with a higher dose are warranted to determine whether or not etonogestrel can be used to fully suppress estrous behavior in mares.

Acknowledgments

The authors thank the United States Equestrian Federation (USEF) for their financial support; Ms. Marti McCoy and Dr. Barbara Schmidt for their assistance with mare handling during this project; and Dr. Sue Duran, Auburn University Large Animal Pharmacy, for her assistance in obtaining the implants. The USEF had no involvement in the study design, data collection, or preparation of this report.

References

1. Hinrichs K: Irregularities of the estrous cycle and ovulation in mares (including seasonal transition). In: Youngquist RS, Threlfall WR, editors. Current therapy in large animal theriogenology. 2nd edition. St Louis: WB Saunders; 2007. p. 150-152.
2. Pryor P, Tibary A: Management of estrus in the performance mare. Clin Tech Equine Pract 2005;4:197-209.
3. Nie GJ, Johnson KE, Braden TD, et al: Use of an intra-uterine glass ball protocol to extend luteal function in mares. J Equine Vet Sci 2003;23:266-273.
4. Rivera del Alamo MM, Reilas T, Kindahl H, et al: Mechanisms behind intrauterine device-induced luteal persistence in mares. Anim Reprod Sci 2008;107:94-106.
5. Vanderwall DK, Rasmussen DM, Woods GL: Effect of repeated administration of oxytocin during diestrus on duration of function of corpora lutea in mares. J Am Vet Med Assoc 2007;231:1864-1867.
6. Lefranc A: Nonpharmacological suppression of oestrus in the mare. Equine Vet J 2004;36:183-185.
7. McCue PM: Estrus suppression in performance horses. J Equine Vet Sci 2003;23:342-344.
8. Hedberg Y, Dalin A, Santesson M, et al: A preliminary study on the induction of dioestrous ovulation in the mare-a possible method for inducing prolonged luteal phase. Acta Vet Scand 2007;48:12-18.
9. Gee EK, DeLuca C, Stylski JL, et al: Efficacy of medroxyprogesterone acetate in suppression of estrus in cycling mares. J Equine Vet Sci 2009;29:140-145.
10. McKinnon AO, Lescun TB, Walker JH, et al: The inability of some synthetic progestagens to maintain pregnancy in the mare. Equine Vet J 2000;32:83-85.
11. Wiepz GJ, Squires EL, Chapman PL: Effects of norgestomet, altrenogest, and/or estradiol on follicular and hormonal characteristics of late transitional mares. Theriogenology 1988;30:181-193.
12. Dalin AM, Andresen Ø, Malmgren L: Immunization against GnRH in mature mares: antibody titres, ovarian function, hormonal levels and oestrous behaviour. J Vet Med A 2002;49:125-131.
13. Imboden I, Janett F, Burger D, et al: Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. Theriogenology 2006;66:1866-1875.
14. Elhay M, Newbold A, Britton A, et al: Suppression of behavioural and physiological oestrus in the mare by vaccination against GnRH. Aust Vet J 2007;85:39-45.
15. Glasier A: Implantable contraceptives for women: effectiveness, discontinuation rates, return of fertility, and outcome of pregnancies. Contraception 2002;65:29-37.

16. Croxatto HB, Mäkäräinen L: The pharmacodynamics and efficacy of Implanon® 1: An overview of the data. Contraception 1998;58:91-97.
17. Wenzl R, van Beek A, Schnabel P, et al: Pharmacokinetics of etonogestrel released from the contraceptive implant Implanon®. Contraception 1998;58:283-288.
18. Makarainen MD: Ovarian function during the use of a single contraceptive implant: Implanon compared with Norplant. Fertil Steril 1998;69:714-721.
19. Daels PF, McCue PM, DeMoraes MJ, et al: Persistence of the luteal phase following ovulation during altrenogest treatment in mares. Theriogenology 1996;46:799-811.
20. Ball BA, Wilker C, Daels PF, et al: Use of progesterone in microspheres for maintenance of pregnancy in mares. Am J Vet Res 1992;53:1294-1297.
21. Squires EL, Heesemann CP, Webel SK, et al: Relationship of altrenogest to ovarian activity, hormone concentrations and fertility of mares. J Anim Sci 1983;56:901-910.
22. Funk S, Miller MM, Mishell DR: Safety and efficacy of Implanon (TM), a single-rod implantable contraceptive containing etonogestrel. Contraception 2005;71:319-326.
23. Lofstedt RM, Patel JH: Evaluation of the ability of altrenogest to control the equine estrous cycle. J Am Vet Med Assoc 1989;194:361-364.
24. Squires EL: Use of progestins in open and pregnant mares. Anim Reprod Sci 1993;33:183-193.
25. Huber J: Pharmacokinetics of Implanon®: An integrated analysis. Contraception 1998;58:85-90.

Table. Average IEI of each mare based on 90-day observation period; IEI was calculated based on twice weekly teasing observations and once weekly progesterone samples.

Treatment Group	Interestrus Interval (days)		
	Mare	Teasing	Progesterone
Group C	C.1	21.75	22.75
	C.2	20	21
	C.3	21.75	21
	C.4	21	21
	C.5	21.75	22.75
Group T1	T1.1	21.6	21
	T1.2	21.75	21
	T1.3	21	21
	T1.4	49	47
	T1.5	59.5	47
Group T2	T2.1	21	21
	T2.2	24.5	21
	T2.3	35	30.7
	T2.4	98	98
	T2.5	35.3	34.3
Group R	R.1	110	49
	R.2	111	45.5
	R.3	115	49
	R.4	109	49

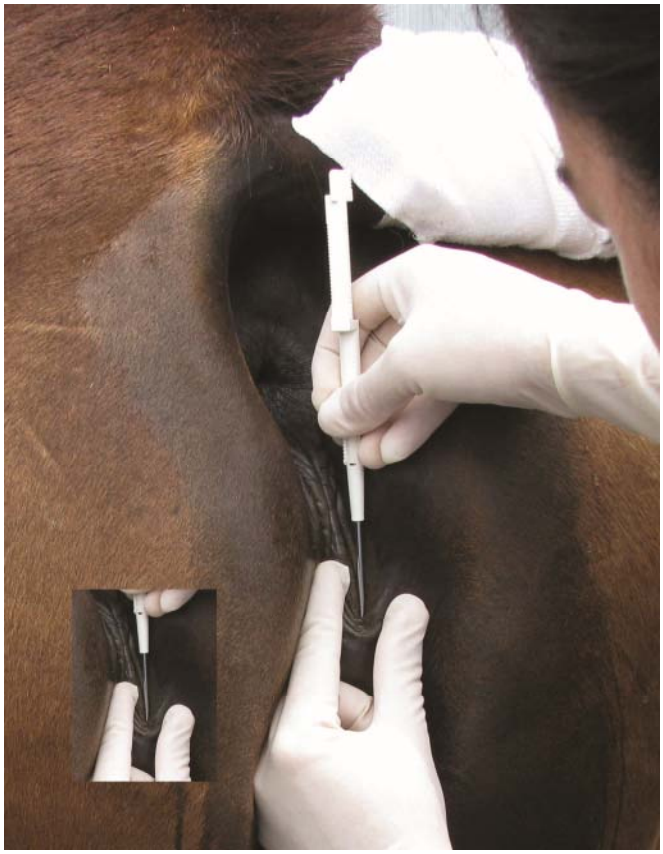

Figure 1. Implant application. The implant is being placed in the right vulvar lip using the applicator provided by the manufacturer. Local anesthesia was used prior to implant placement.

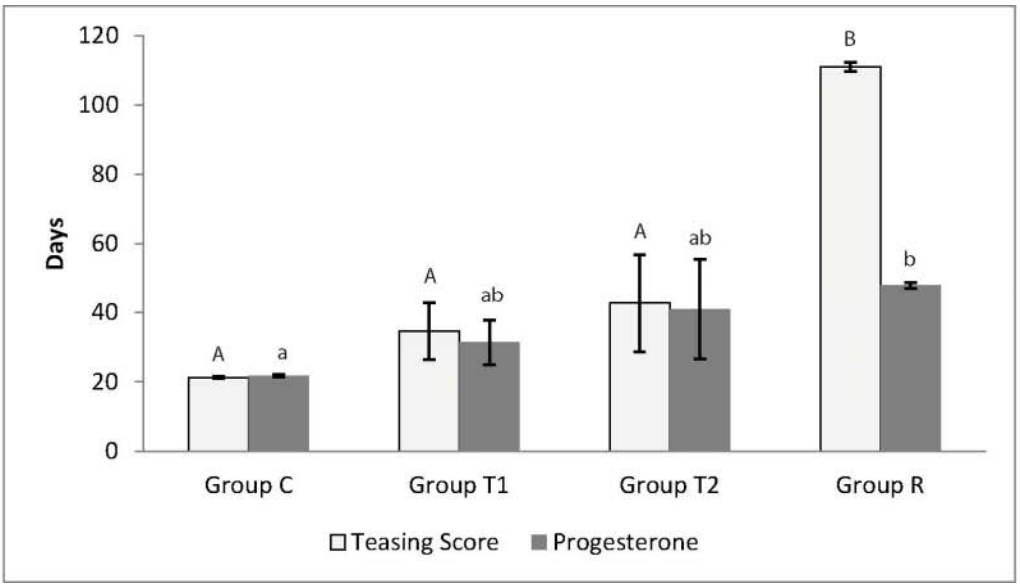

Figure 2. Interestrus interval based on teasing scores and progesterone levels ± SEM. Different letters indicate significant differences between groups ($p<0.05$). Uppercase letters compare IEI based on teasing score and lowercase letters based on progesterone levels.

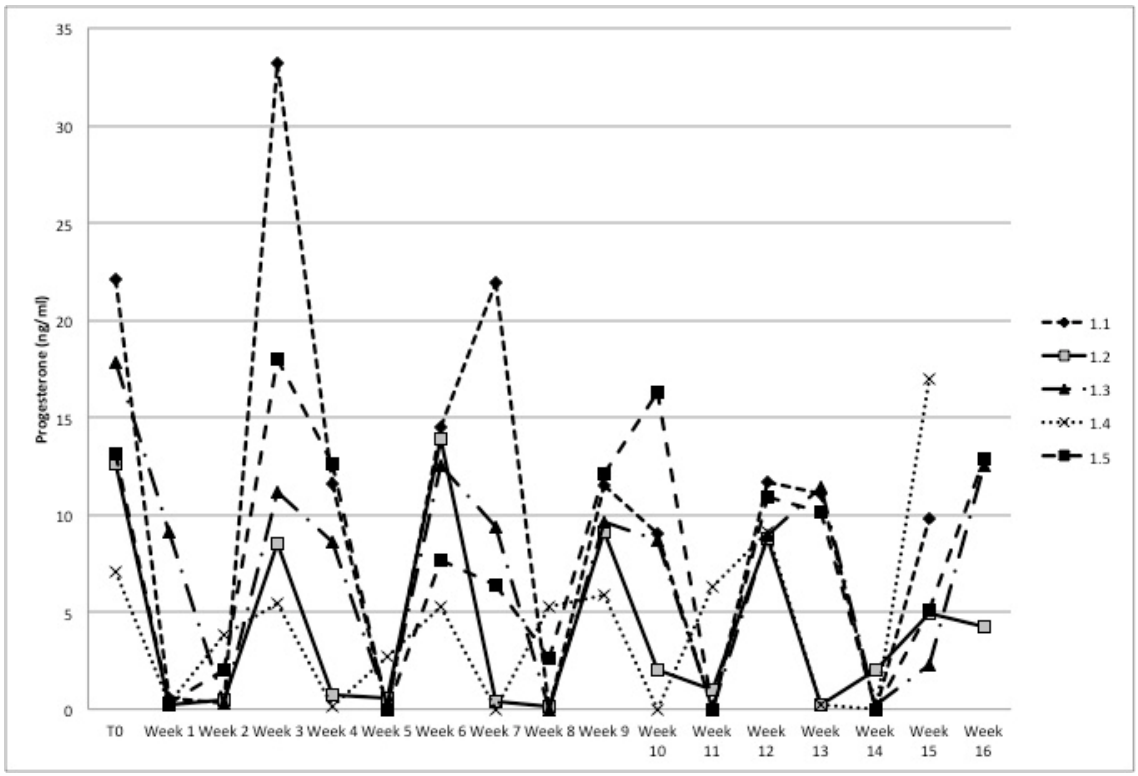

Figure 3. Progesterone concentration in animals within the control group (no treatment).

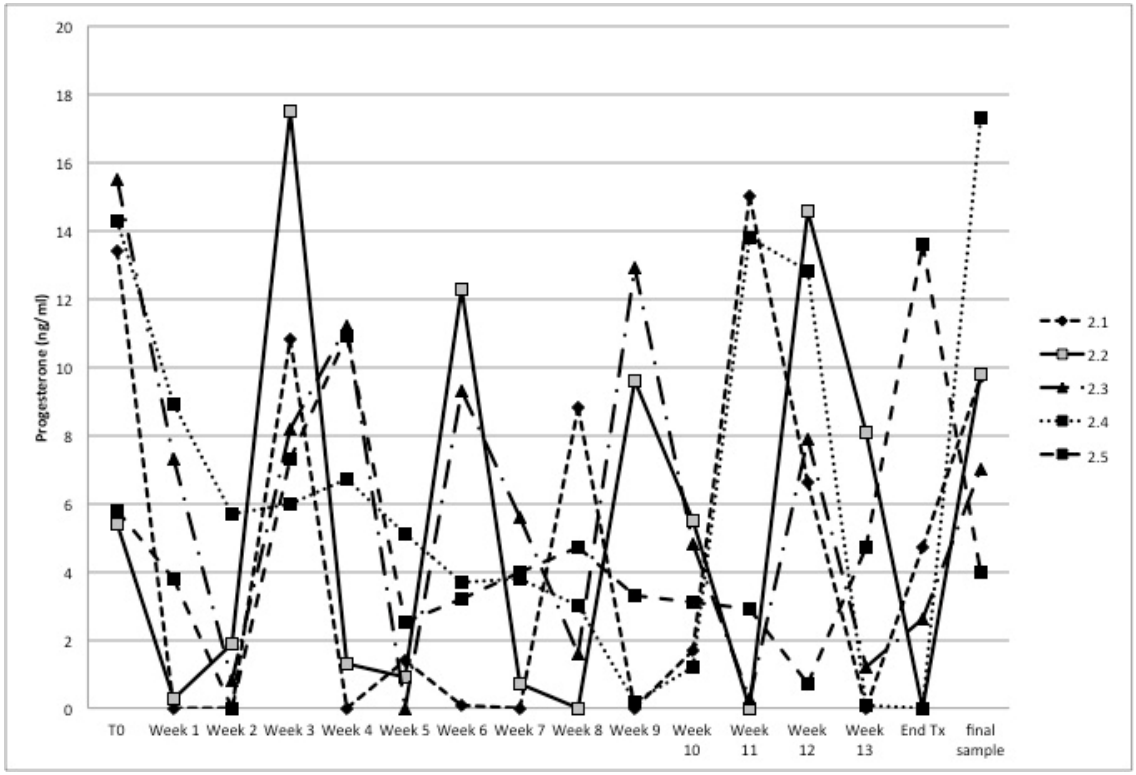

Figure 4. Progesterone concentration in animals within the one implant group (T1).

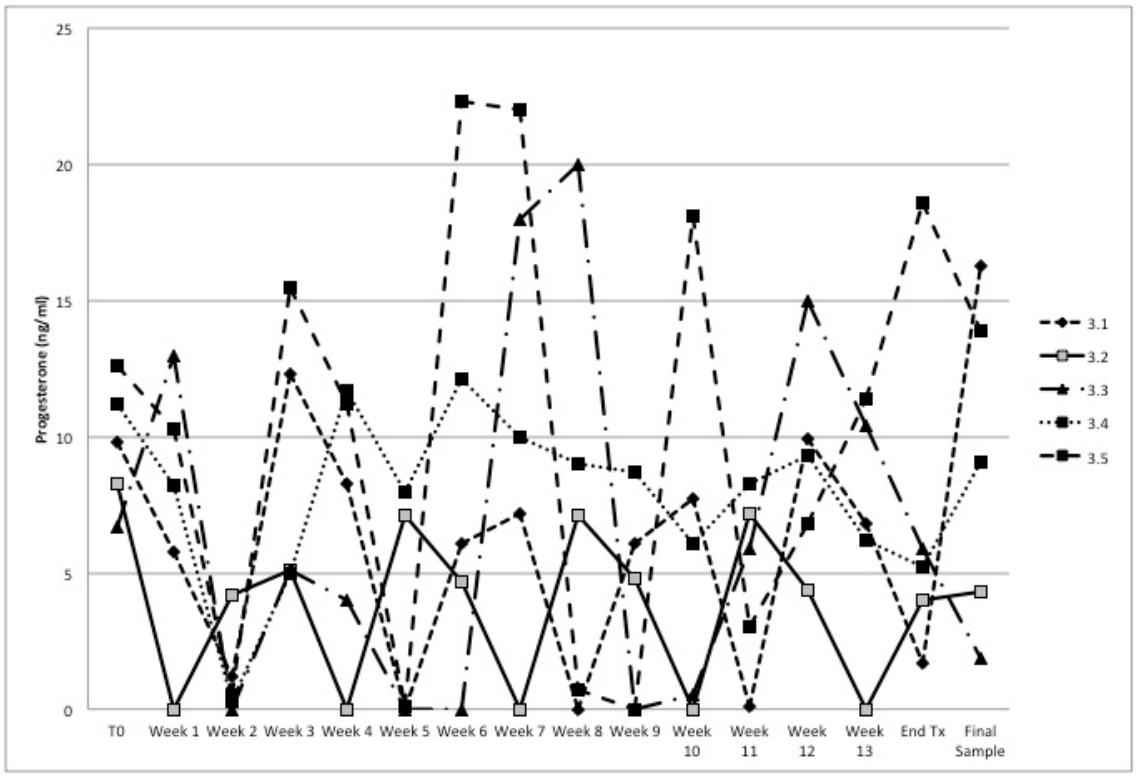

Figure 5. Progesterone concentration in animals within the two implant group (T2).

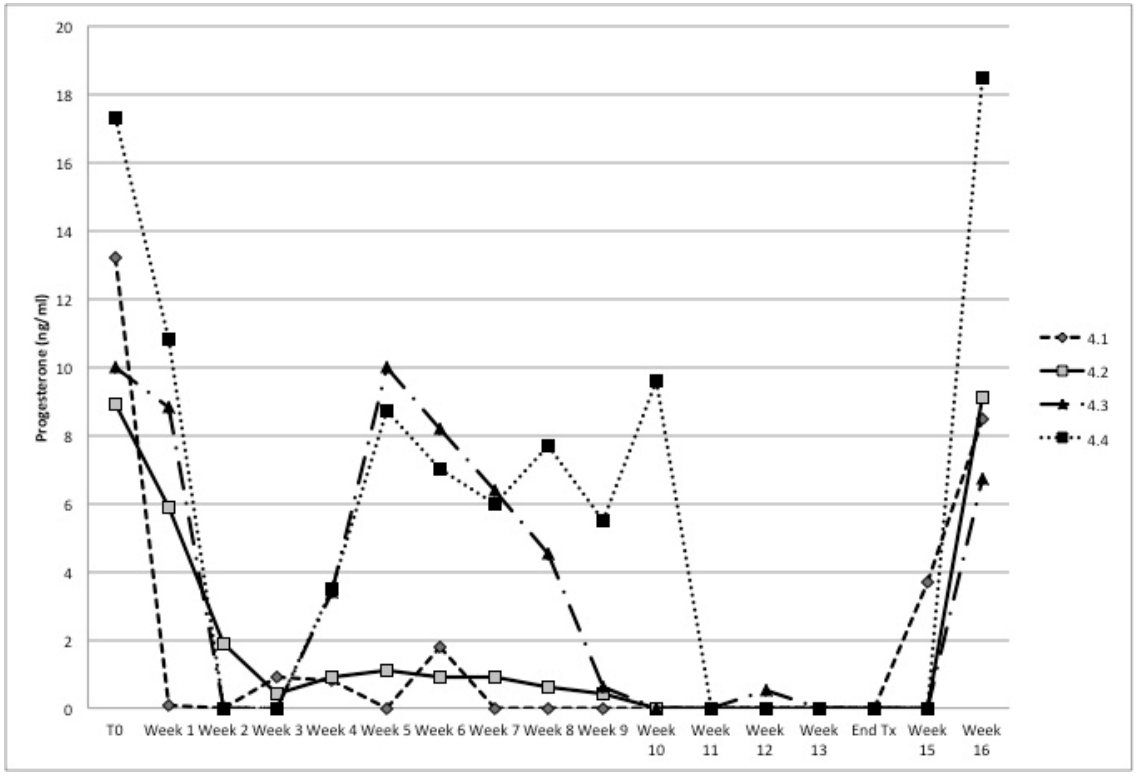

Figure 6. Progesterone concentrations in animals within the altrenogest group (R).