

GRANT EXPENDITURE RESPONSIBILITY REPORTS

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FOR RESEARCH BY
AUBURN UNIVERSITY
INTO THE
EFFECTIVENESS OF A
HUMAN CONTRACEPTIVE
SUBDERMAL IMPLANT
(IMPLANON©) ON
ESTRUS SUPPRESSION IN MARES



LAY SUMMARY OF THE PROJECT

Effectiveness of a human contraceptive implant (Implanon®) on estrus suppression in mares

Ghislaine Dujovne

Undesirable behavior during estrus or "heat" can be a problem in performance mares. Many animals show temperament changes and become more difficult to handle which reduces their performance. Some mares show aggression or signs of pain around the time of ovulation. Because of these behavioral problems, many treatments have been evaluated to suppress estrus in the mare. However, no long-acting treatment (months or years) has been shown thus far to be safe and effective for suppression of behavioral estrus.

The objective of this study was to evaluate the use of Implanon®, a human birth control subdermal implant containing etonogestrel (a synthetic progestogen), as a long-term alternative to inhibit unwanted estrous behavior in mares. This implant provides up to three years of effective birth control in women. We designed an experiment using 4 groups of 5 healthy mares each between the ages of 6 and 20 years, with normal previous reproductive cycles and heat behavior. The groups were as follows: *Group C*: Negative control group, no hormonal treatment; *Group T1*: one Implanon® implant; *Group T2*: two Implanon® implants; and *Group R*: Positive control group, Altrenogest, a known effective synthetic progestogen (Regumate®) orally once daily.

Behavioral signs of estrus were evaluated by exposure to a stallion twice weekly and observation by an experienced observer who didn't know which mare was given what treatment (blinded observer). Also, the mare's reproductive cycle was followed for 3 months via transrectal palpation and ultrasonography performed by a veterinarian, and weekly blood samples to measure hormone levels (progesterone).

The interestrus interval (IEI), the time elapsed from one behavioral estrus to the next, was measured based on behavioral estrus signs and hormone levels (progesterone below 1.0 ng/ml).

Average interestrus interval per group based on teasing and progesterone levels respectively were as follows: Group C (negative control) 21.2 and 21.7 days; Group T1 (one implant): 34.5 and 31.4 days; Group T3 (two implants): 42.7 and 41 days. For Group R (Regumate), behavioral estrus was suppressed during the entire study period, as expected, with an interestrus interval of 111.25 days based on teasing, and 48 days based on progesterone levels. No statistical difference was found between groups C, T1 and T2; but group R (Regumate) was significantly different from all other groups based on teasing. The estrus determination by teasing and progesterone levels was highly correlated, which may validate the behavioral observation as a reliable method to determine estrus.

Conclusions.

No statistical difference was identified between the control group and the Implanon® treated animals; however, a clinically significant difference was observed between groups C and T2. In Group C (negative control, no treatment), the animals exhibited estrus behavior every 21 days (normal cycles) compared to Group T2 (2 implants) where the mares exhibited estrus every 42 days (double of the average for this species). The fact that this clinical difference was not significant for the statistical analysis could be explained by the high variability between mares within the same treatment group. For example, two animals responded to treatment with a

full suppression of estrous behavior (no behavior during the 3 months of observation), while three others had no response at all (normal cycles of 21 days).

The use of two Implanon® implants containing etonogestrel was not consistently effective for estrus suppression in the mare. We suspect the amount of hormone contained in two implants (total of 136mg) was not adequate to fully suppress cyclicity and behavior in an animal as large as a mare. Future studies with a higher dose would be necessary to determine if etonogestrel can be used to fully suppress estrus in mares.

Acknowledgments.

Thank you to the US Equestrian Federation for the financial support that allowed us to perform this study.

Use of etonogestrel implants to suppress estrus behavior in mares

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The objective of this study is to evaluate a synthetic progestin (etonogestrel) as a reliable method to suppress behavioral estrus in mares.

Healthy mares between the ages of 6 and 20 years with normal estrous cycles were randomly assigned to 4 groups (n=5). Group C was the control group (no treatment), group T1 received one Implanon® subdermal implant (68mg etonogestrel), group T2 received two Implanon® implants (136 mg etonogestrel), and group R was the positive control, receiving 0.044 mg/kg altrenogest orally daily (Regumate®).

Behavioral estrus response to teasing with a stallion was evaluated twice weekly by a blinded observer. Estrous cycles were monitored for three months by weekly progesterone levels and twice weekly transrectal examinations. Interestrus interval (IEI) was measured based on both behavioral estrus (teasing scores) and plasma progesterone concentration (below 1.0 ng/ml).

Mean IEI per group, based on teasing and progesterone levels respectively, were as follows: group C (control) 21±0.3 and 21±0.4 days (± SEM); 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 T1 had an IEI twice longer than 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, and estrus behavior in this group was suppressed during the entire study period. Based on progesterone levels, IEI was different only between groups R and group C.

The IEI determined by teasing and progesterone levels were highly correlated ($r=0.911$). The high correlation between teasing and progesterone levels may validate teasing score as a reliable tool to determine estrus in mares by an experienced observer in animals with regular estrus behavior.

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

Acknowledgments: Thank to US Equestrian federation for the financial support.



COLLEGE OF VETERINARY MEDICINE

DEPARTMENT OF PATHOBIOLOGY

Dear Dr. Dujovne,

Thank you for your submission of a scientific abstract for the 2010 Auburn Phi Zeta Research Emphasis Day on Wednesday, November 10, 2010.

Your submission, Effectiveness of a human contraceptive implant (Implanon®) on estrus suppression in mares, was accepted for platform presentation.

The presentation will be 10 min long, followed by a 5 min discussion period. Please prepare the presentation as a PC Powerpoint file, and bring your file on memory stick by 10 min prior to the start of your session of the platform presentations in the morning or afternoon on Wednesday, November 10, 2010, in the Overton Auditorium of the Joy Goodwin Rudd Student Center.

For your presentation time and accuracy of your abstract, please consult the attached Proceedings of the 2010 Phi Zeta Research Emphasis Day.

I also invite you as presenter to attend the Phi Zeta Banquet with no cost to you on 6:30 pm at the Auburn Hotel & Conference Center. We will have a tasty New Orleans style buffet dinner. Please send a brief return email by the end of next week if you will be able to join us at the banquet.

On behalf of all Phi Zeta members, I want to thank the presenters and their co-investigators and mentors for the participation in the Phi Zeta Research Day.

Bernhard Kaltenboeck

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Effectiveness of a human contraceptive implant (Implanon®) on estrus suppression in mares

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Introduction.

Excessive estrus behavior is a commonly reported problem in performance mares. Many animals show temperament changes and become more difficult to handle which reduces their performance during the estrus period. Because of these behavioral problems, many treatments have been evaluated to suppress estrus in the mare, but currently, no single treatment has been shown effective and safe for prolonged suppression. The objective of this study is to evaluate Implanon® (human contraceptive implant) as a reliable method to suppress behavioral estrus in mares.

Methods.

Healthy mares between the ages of 6 and 20 years with normal estrous cycles were randomly allocated to 4 groups of 5 animals each. Group 1: negative control (no treatment), Group 2: one Implanon® implant containing 68mg etonogestrel, Group 3: two Implanon® implants, 136 mg etonogestrel; and Group 4: positive control, 0.044 mg/kg altrenogest (Regumate®) orally once daily.

Behavioral estrus response to teasing with a stallion was evaluated twice weekly by an experienced observer who was blinded to the treatments. Estrous cycles were followed for 3 months with weekly progesterone levels and twice weekly examinations via transrectal ultrasonography. The interestrus interval (IEI) was measured based on both behavioral estrus (teasing) and progesterone levels (below 1.0 ng/ml). Data were analyzed using ANOVA (Least Significant Difference) and Correlation tests using the Statistical Analysis System version 9.1 (SAS Institute, Cary, NC).

Results.

Mean IEI per group, based on teasing and progesterone levels respectively, were as follows: Group 1 (control) 21.2 and 21.7 days; Group 2: 34.5 and 31.4 days; Group 3: 42.7 and 41 days. For Group 4, the estrus behavior was suppressed during the entire study period, with an IEI of 111.25 days based on teasing and 48 days based on progesterone levels. No statistical difference was found between groups 1, 2 and 3; but group 4 (positive control) was significantly different from all other groups ($P < 0.05$). The IEI determined by teasing and progesterone levels were highly correlated with an $R = 0.911$, with a lower correlation in group 4 (Regumate®).

Conclusions.

No statistical difference was identified between the control group and the Implanon® treated animals; however, a clinically significant difference was seen in Gp 3 (2 implants) with double the IEI compared with Gp 1. This could be explained by the high variability between individuals within the same group, especially in Gp 3. The high correlation between teasing and progesterone levels validates teasing as a reliable tool to determine estrus in mares by an experienced observer. At this dose etonogestrel was not effective for estrus suppression. Future studies with a higher dose would be necessary to determine whether or not etonogestrel can be used to fully suppress estrus in mares.

Acknowledgments.

Thank to US Equestrian Federation for the financial support; Ms. Marti McCoy and Dr. Barbara Schmidt for assistance with the mares.