Use of Etonogestrel Implants to Suppress Estrous Behavior in Mares

by

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Abstract

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 T2: 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 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 estrous

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.

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List of Abbreviations

PO	Per os, orally
IM	Intramuscular
IEI	Interestrus Interval
hCG	Human Chorionic Gonadotropin
GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
IU	International Units
SEM	Standard Error of the Mean
VAC	Vitex Agnus Castus
MPA	Medroxyprogesterone
CV	Coefficient of Variation
SAS	Statistical Analysis System
LSD	Least Significant Difference
IACUC	Institutional Animal Care and Use Committee

I. Literature Review

1. Estrous Behavior

The mare is seasonally polyestrous, with estrous cycles beginning in early spring and continuing through the early fall in North America [1]. The mare's estrous cycle is approximately 21 days in length and is divided into two phases based on sexual receptivity, estrus and diestrus [2].

Estrus by definition is the period during which a mare is sexually receptive to the stallion. Behavioral estrus is induced by increasing levels of estradiol produced by the growing follicles in the absence of progesterone. The mare differs from other domestic animals in the sense that estrogen does not seem to be required for estrus behavior, but absence of progesterone is absolutely necessary [2, 3]. The average length of estrus in the mare is 6.5 days with a range between 4.5 to 8.5 days. Diestrus, the non-receptive phase of the cycle, lasts on average 14.9 days with a range of 12.2 to 16.3 days. Dominant hormones during estrus and diestrus are estrogen and progesterone respectively [4].

Estrous behavior detection is performed by a technique called "teasing" in which the mare is exposed to a stallion that is physically separated from her by a fence or a wall so they can partially interact, but the barrier provides safety for both animals and handlers. The mare's behavioral response to the stallion's presence and signals (vocalization,

nuzzling, etc) is then observed to assess her estrous status. During the "teasing" process, the mare should be exposed to the stallion for an adequate amount of time, until the observer is able to evaluate the expression of estrus. Approximately 80% of mares consistently demonstrate typical signs of estrus when they are close to ovulation; the remaining mares may not show typical behavior due to personality of the individual mare (shy, anxious, afraid, indifferent, or quiet) [1, 2].

Behavioral signs in the mare may vary in type and intensity. Different researchers have utilized different scoring assessments to make this potentially subjective observation as objective as possible [4, 5]. Posturing is the most significant sign of receptivity and involves a response with the whole body. It is characterized by an arched tail, flexed stifles and hocks, abducted rear limbs and tipped pelvis with lowering of the perineal area. The weight of the mare is primarily borne by the front legs [4]. This posture allows the mare to fully support the weight of the stallion during the breeding process. Urination is another sign related to sexual receptivity. During urination, the tail is arched and the genitalia exposed. The amount of urine that may be expelled through the vulva varies from a few drops of a viscous solution, to complete urination [4]. Clitoral winking is another component of estrous behavior and is characterized by rhythmic eversion of the labia with exposure and projection of the clitoris. It is also called clitoral or vulvar flash. The role of the clitoral wink is unknown but it has been speculated that it may increase the attention of the stallion [4].

If the mare is not receptive, she will demonstrate evasive or aggressive behavior towards the stallion by kicking, squealing, biting, holding ears back, switching the tail, moving and shaking the head, pawing and other repelling manifestations [4].

The intensity of estrous symptoms varies between animals, and ranges from undetectable to very intense. For many horse owners, this creates a problem in mares used for performance careers such as trotting, racing, dressage, and jumping. Many mares fail to perform at their best due to strong sexual behavior [6] and may become harder to manage, perform irregularly, or even appear lame during the time of estrus. This behavior is intermittent, and usually corresponds to the follicular phase of the estrous cycle when the female is receptive to the stallion. Such behavior problems may simply be deemed inappropriate by the owners, however, there may also be behavioral and physical effects of the estrogens that can adversely affect performance, such as increased joint laxity [7]. Presence of estrous behavior has been considered an important factor in reduced athletic performance. Trainers and riders of all equestrian disciplines often complain that strong estrous behavior makes mares more difficult to handle, may make them aggressive, and the presence of pain may lead to the mare performing below her potential [3]. This behavior may also be related to pain associated with growing follicles stretching the ovarian tunic, or from rupture of the follicle at ovulation. Many mares exhibit pain during ovarian palpation immediately after ovulation [7]. In a survey of American Association of Equine Practitioner (AAEP) members in 1996, 90% of the veterinarians that responded believed that the estrous cycle played a role in mare athletic performance, and only 1% believed that there was no effect on performance [3, 8].

The most determining hormonal factor in estrous behavior in the mare seems to be the absence of progesterone, and supplementing progesterone usually eliminates behavioral estrus [3, 9-12].

2. Treatments for Estrus Suppression

2.1. Non-Pharmacological Treatments

2.1.1. Ovariectomy:

Surgical removal of both ovaries will eliminate the cyclicity of mares. However, mares can exhibit signs of estrus even after ovariectomy [13]. Plasma progesterone concentrations above 1-2ng/ml will suppress behavioral estrus in mares; mares with levels below that can show signs of behavioral estrus [14-16]. Some authors believe that behavioral estrus can be explained by production of steroids (estrogens) from the adrenal gland, but others suggest that there is no effect of adrenal steroid production on estrous signs, length of estrus, or ovulation [17, 18]. The disadvantages of performing ovariectomy to suppress estrus are the facts that this procedure eliminates any future reproductive potential and the lack of effectiveness on estrus inhibition in some mares. At least 30% of ovariectomized mares continue to display some signs of behavioral estrus due to the lack of progesterone[13]. High concentrations of estrogens intensify the signs, but are not needed for estrus presentation [3]. Advantages of this treatment are that it eliminates any pain associated with the follicular phase and ovulation, and mares will respond more consistently to supplemental progesterone when needed to eliminate behavioral signs that may interfere with athletic performance.

2.1.2. Intrauterine Devices:

With the use of a 35mm intrauterine glass ball inserted within 24 hrs after ovulation, a state of false pregnancy can be induced. The goal is obtaining a prolonged luteal phase,

as occurs during pregnancy in the mare. Nie *et al* reported a prolonged luteal phase for an average of 87 days (range 76 to 109 days) during which time progesterone concentrations remained over 1ng/ml and estrous behavior was not evident. However, the prolongation of the luteal phase occurred in only 40% of the mares tested (n=12) [19]. A similar study was performed by Rivera del Alamo *et al.* in 2008. In this case, researchers used a 20mm diameter water-filled polypropylene ball instead of glass. These researchers obtained prolongation of the luteal phase in 75% of the mares (n=12), with an average luteal phase length of 57 days (range 44 to 75 days) [20].

2.1.3. Induced Pseudopregnancy:

Le Franc and Allen reported that disrupting development of the conceptus in pregnant mares beyond day sixteen can prolong the diestral phase. In this study, they showed that on average, estrus was suppressed for 82 days (range 64 to 109 days) in about 90% of the mares (10 out of 11) [21]. This technique implicates potential ethical issues as it involves aborting a viable pregnancy. It also requires the time and cost involved with cycle management, breeding, and establishing a pregnancy in these performance mares.

2.1.4. Induced Diestral Ovulation

Spontaneous diastral ovulations in the late luteal phase may cause a prolongation in the luteal phase in mares. A study performed by Hedberg *et al* in 2007 showed that the induction of a new ovulation during the luteal phase will delay the return to estrus in mares up to 90 days. Induction of ovulation was performed in mares with diestral follicles measuring greater than 30mm in diameter by using 3000 international units (IU)

of human chorionic gonadotropin (hCG) intravenously. A disadvantage of this method is that only a small percentage of mares will present with a diestral follicle large enough to respond to treatment. In this study, only three of nine mares qualified to receive the treatment in the first cycle, and a total of eight out of nine had a follicle big enough to stimulate after three cycles. This method has several limitations for its use in practice: mares may have to be followed for more than one cycle before a diestral follicle of suitable size develops, some mares may never develop a follicle large enough for ovulation induction during diestrus, and, even in presence of the suitable follicle, ovulation is not certain to occur [22].

2.1.5. Herbal Supplements:

Some supplements have a label claim for calming or modifying undesirable behavior. Valerian root has sedative-like properties and is used in humans to treat sleeping disorders and anxiety. Short term sedative and anxiolytic effects have been scientifically proven in mice [23]. Its active component (valerenic acid) acts through a mechanism similar to benzodiazepine drugs [24].

A second herbal supplement, Chaste tree berries extract (vitex agnus castus or VAC), has also been used to alter ovarian function. In humans the main use is to reduce premenstrual symptoms, particularly mastodynia [25]. Uncontrolled studies in women indicate that the extract may also have beneficial effects on other psychic and somatic symptoms of premenstrual syndrome [25]. The search for suppressive agents yielded a number of compounds (ditierpenes and clerodiadenols) with dopaminergic properties causing a suppression of prolactin [25]. A questionnaire developed to determine effects

of VAC on psychic and somatic complaints during premenstrual syndrome (depression, anxiety, cravings, and hyperhydration) showed a perceived improvement of clinical signs in 81% of patients. No serious adverse reactions were reported [26]. In horses, anecdotal reports indicate a reduction of undesirable estrous behavior using herbal supplements, but no controlled studies have been published [27].

The efficacy and safety of herbal products has not been scientifically tested in horses, and all active ingredients may not be known or standardized. In addition, American Horse Show Association (AHSA) regulations specifically forbid the use of many herbal or natural products that affect the performance of show horses [27].

2.2. Hormonal Treatments

2.2.1 Natural Progesterone:

A study published in 1976 first demonstrated that behavioral estrus occurs when the mare's plasma progesterone concentration is less than 2 ng/ml [3, 16]. In later studies, researchers have shown that 1 ng/ml is enough to suppress estrus in mares [14, 15, 28].

It has been shown that daily intramuscular (IM) injections of 50mg progesterone in oil administered to a diestrus mare will prevent behavioral estrus associated with the mare's next follicular phase. A larger dose of 200mg IM once dailly is required to suppress the behavior in a mare that is already in the follicular phase of the cycle [3, 29]. Other authors confirm that the use of progesterone will block estrous behavior, and doses of 0.2mg/kg (100mg for an average horse) of natural progesterone in oil on a daily basis are recommended [28]. Natural progesterone offers excellent results for suppressing behavior as it has a high affinity for its own receptors [2, 30]. Although effective,

progesterone in oil has disadvantages. Daily intramuscular injection of progesterone in oil is impractical, and is often associated with local tissue reactions at the injection site including muscle soreness which can further affect performance. Also, daily injections may lead to injection aversion in some mares [3]. A compounded long-acting injectable formulation of progesterone has become available, and a 1.5g dose once weekly can achieve serum concentrations sufficient to block behavioral estrus (>2ng/ml) [28]. This product too has been associated with local tissue reactions and muscle soreness.

2.2.2 Synthetic progesterone compounds:

The most widely used synthetic progesterone in equine practice is altrenogest, or Regumate®. Altrenogest claims to be effective in reducing behavioral estrus in 95% of mares according to the package insert. This medication (0.044 to 0.132 mg/kg orally once daily) is the most commonly used treatment to suppress problematic estrous behavior in mares [2, 7]. Altrenogest suppresses behavioral estrus in mares within 2-3 days of commencement of administration [31]. Although it controlled behavioral estrus, a 1989 study by Lofstedt and Patel showed that altrenogest may fail to completely control the equine estrous cycle because of an inability to suppress follicular development and subsequent ovulation. In this study, however, the estrous behavior was inhibited in all mares for the treatment period (20 days) [11].

Although altrenogest is very effective at inhibiting behavioral estrus, it has several disadvantages including the necessity of daily oral treatment, which in some cases make mares reluctant to be handled around the mouth. Also, daily administration makes it costly and somewhat impractical for long term use [3]. The package insert also warns

that “continued daily exposure of horse owners has potential untoward effects in humans such as disruption of the menstrual cycle, uterine or abdominal cramping, increased or decreased uterine bleeding, prolongation of pregnancy, and headaches...” (Regumate®, package insert) and thus this medication carries a significant potential human safety issue for clients.

Other synthetic progestagens have been used by equine veterinary clinicians either in an attempt to prevent abortion in pregnant mares, or to suppress the undesired estrous behavior in performance horses, however, no consistent efficacy has been scientifically proven for these compounds [2]. Wiepz (1988) found that the synthetic progesterone, norgestomet, had no effect on the estrous cycles or behavior in mares. Twelve years later, McKinnon *et al*. demonstrated that four commonly used synthetic progestagens: megestrol acetate (Ovaban, Schering-Plough Animal Health Corp, Union, NJ), medroxyprogesterone (DepoProvera, Pharmacia, Kalamazoo, Mich), hydroxyprogesterone hexanoate (Gesteron-500, Illium Veterinary Products, Smithfield, NSW, Australia), and norgestomet (Synchro-Mate B, Merial, Iselin, NJ) were not capable of maintaining pregnancy in mares in absence of endogenous progesterone [2, 27, 32, 33]. Gee *et al* injected medroxyprogesterone (MPA, Wedgewood Pharmacy, Swedesboro, NJ) weekly in mares, to evaluate the estrus suppression effects. MPA did not effectively suppress behavioral estrus or follicular activity in normal cyclic mares, and therefore is not recommended for this purpose [34].

Currently, the only effective synthetic progestagen in mares is altrenogest. The lack of efficacy of the other synthetic progestagens for the control of estrous behavior or

pregnancy maintenance in the mare is hypothesized to be due to a failure of the other compounds to bind to equine progesterone receptors [3, 7, 33].

2.2.3. Oxytocin Injections:

Administration of 60 IU of oxytocin IM twice daily on days 7 through 14 after ovulation was an efficacious method of inhibiting luteolysis and extending corpus luteum function in mares [35]. All mares in this study maintained a serum progesterone concentration greater than 1ng/ml for 30 days, and 60% of the mares maintained elevated progesterone levels until the study period ended on day 40. Estrous behavior was not monitored in this study, and all conclusions were based on serum levels of progesterone [35].

2.2.4. Immunization against GnRH:

Gonadotropin releasing hormone (GnRH) has a central role in the control of fertility and sexual behavior in mammals and thus behavioral estrus in mares. GnRH exerts this effect indirectly by controlling the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. Neutralization of GnRH can be achieved by inducing an immune response to the GnRH protein with the production of specific antibodies after vaccination with a GnRH carrier protein and an adjuvant [36]. With a two dose immunization regimen using an available GnRH vaccine, Equity® (Pfizer Animal Health, West Ryde, NSW, Australia), Elhay *et al* concluded that the vaccine was effective at inhibiting behavioral estrus for at least three months [36]. Alternatively, Dalin *et al* used GnRH conjugated with bovine serum albumin and a four

dose regimen (first three injections at 20 day intervals, and a fourth 30 days later) which produced inconsistent results for estrous behavior suppression [6]. A third study was performed using a two dose immunization protocol with a commercially available GnRH vaccine, Improvac® (CSL Limited, Australia). Improvac® contains a synthetic analogue to GnRH linked to a carrier protein and accompanied by an aqueous adjuvant. This vaccine was developed in Australia to control boar taint in intact male pigs intended for meat. The use of this vaccine in mares induced ovarian suppression in all the treated animals, with absence of ovulation for at least 5 months. However, the ovarian suppression did not always prevent the occurrence of estrous behavior because the level of progesterone was maintained under 1ng/ml in all vaccinated mares [37]. All studies reported some local tissue reaction after the injections ranging from mild local irritation to fever and lameness [6, 36, 37].

2.2.5. GnRH Antagonist:

GnRH antagonists differ from GnRH vaccines by directly inhibiting the action of GnRH, thereby inhibiting FSH and LH release and suppressing ovarian activity. The GnRH antagonist, Antarelix® (Europeptides, Argenteuil, France), has been shown to reliably postpone ovulation and increase the inter-ovulatory interval after administration in mares. In some mares, it induced a temporary cessation of all ovarian activity. However, this treatment requires frequent administration and generally results in only a short-term effect with highly variable responses between mares. The behavioral estrus signs are inconsistent due to suppressed ovarian activity, and, because of that, the use for behavioral estrus suppression is limited [37, 38].

2.2.6. GnRH Agonist:

GnRH agonists act like the mare's natural GnRH by inducing an initial rise in FSH and LH. Once this initial stimulation occurs, overstimulation of GnRH causes a downregulation of pituitary gonadotropin secretion, and induces short-term alterations in ovarian functions in mares [39]. Potent agonists of GnRH, such as deslorelin acetate, administrated through single or multiple subcutaneous implants (Ovuplant™, Fort Dodge Animal Health, Ft. Dodge, Iowa) in mares induces a prolonged period of follicular suppression [40]. Ovuplant™ is currently unavailable in the United States. Implants containing GnRH agonists have produced suppression of LH and FSH for 10 to 14 days. However, the results are inconsistent and sustained long-term ovarian inactivity was not achieved in any mare even with three implants. There is no information about the effect on estrous behavior with the follicular suppression achieved with these drugs [3, 27, 39-41].

It should be noted that the manifestation of behavioral estrus in anovulatory mares is a phenomenon that has been described after any treatment that inhibits ovarian function including vaccination against GnRH, treatment with GnRH antagonists, and ovariectomy because of the low levels of progesterone present [37].

2.2.7. Intravaginally delivered progesterone:

Progesterone-impregnated intravaginal devices such as PRID® (Progesterone Releasing Intravaginal Device, Ceva, Sanofi, France) or CIDR® (Controlled Internal Drug Release, Pfizer Animal Health: InterAg, Hamilton, New Zealand) were originally

designed to control the estrous cycle in cattle, but there are several studies that prove their effective use in mares. Progesterone Releasing Intravaginal Device (PRID®) containing 1.55g of progesterone and 15mg of estradiol benzoate and the Controlled Internal Drug Release (CIDR®) containing 1.38g of progesterone are capable of delivering sufficient quantities of progesterone to a variety of animal species, including horses, to control their estrous cycles [10, 42]. These intravaginal devices have been used successfully for estrous suppression, but these are not the method of choice in performance mares due to the potential for vaginitis or dislodgement of the device during performance [3]. All studies concerning the use of intravaginal devices in mares are for short periods of time (7 to 14 days). At this time, intravaginal devices are not a practical long-term solution for controlling behavioral estrus due to problems of device retention, relatively short action (7 to14 days) and the risk of vaginal irritation or infection induced by the presence of an intravaginal device.

2.2.8. Subcutaneous implants:

Various cattle implants are approved for improving weight gain and feed efficiency. Hormone-containing growth-promoting implants improve growth rate (10 to 30%), feed efficiency (5 to 15%) and carcass leanness (5 to 8%) [43]. These implants may contain progestins, estrogens, androgens, somatotropin, and/or a combination of hormones [43]. A study to test the use of Synovex-S® (Fort Dodge Animal Health, Fort Dodge, Iowa), a cattle implant containing 200mg of progesterone and 20mg of estradiol benzoate, was performed to assess the effect on behavioral estrus in mares. The authors concluded that subcutaneous insertion of Synovex-S® did not suppress behavioral signs of estrus, nor

did it block ovulation in mares. In this study, up to 80 pellets per mare (range 8 to 80 pellets) were implanted 5 days after ovulation detection [44]. The failure of this implant to control estrus is thought to be due to the lack of absorption of sufficient quantities of progesterone to suppress estrous behavior in mares [3, 44].

In conclusion of the literature review on this subject, one can state that expression of behavioral estrus, or heat, in mares can have a profound negative effect on training and performance. Clinical signs attributed to performance problems included attitude changes, tail switching, difficulty in training, squealing, excessive urination, kicking, and colic-like discomfort associated with ovulation [27]. This estrous behavior in competition mares is a real concern as it affects the horses' temperament, which then affects training and performance. Because of this, many researchers have been working to find effective treatments that inhibit behavioral estrus in mares. At this time, however, there is no single treatment available to suppress the estrous signs in mares long-term that is safe, effective, affordable, and convenient.

3. Implanon®

Implanon®[1] is a single-rod implantable contraceptive used in woman for long term birth control. This implant has been used around the world providing contraceptive protection for up to three years when inserted subcutaneously [45].

Implanon is 4cm in length and 2 mm in diameter, and consists of an ethylene vinylacetate copolymer core containing 68mg of etonogestrel, a progestin. This progestin has been used for contraception alone or in combination with ethinyl estradiol (Nuvaring, Organon USA Inc) [45].

A release rate of 25-30 μg/day of etonogestrel is required to suppress ovulation in women. This implant (Implanon) releases an initial rate of 60-70 μg/day which slowly decreases to about 30 μg/day [46]. This rate is sufficient to obtain plasma concentrations of the progestin to inhibit ovulation and provide effective contraception for up to 3 years [47].

After implant insertion, serum concentrations increase within 8 hours to concentrations associated with ovulation inhibition. Body weight is directly correlated to the levels of etonogestrel detected in serum. In a pharmacokinetic study, the highest concentrations of etonogetrel were detected in women weighing less than 50Kg (209 pg/ml) and the lowest concentrations were detected in women weighing over 70Kg (126 pg/ml) [48].

In a study performed by Funk *et al*, Implanon® was safe, highly effective (no pregnancies while etonogestrel implant was being used), and a rapidly reversible method

[1] Organon Lab Limited, Cambridge, UK

for contraception in women. No abnormalities at the implant site were noted by investigators in over 97% of the patients [45].

Advantages of etonogestrel implants over other methods of contraception include excellent safety and efficacy, rapid onset and long duration of action, and rapid return to fertility after removal. Also, the subdermal route of administration of the active progestin etonogestrel eliminates the hepatic first-pass effect, allowing lower doses to be used compared with oral administration in women [45].

II. Objective and Hypothesis

Objective

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

Hypothesis

Etonogestrel implants are able to suppress behavioral estrus in mares for a three month period at the commercially available dose (one or two 68mg implants).

III. 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 500kg body weight). They were all under similar husbandry conditions (free choice hay and 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 in 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).[2]

1. 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 68mg of etonogestrel.

[2] www.randomizer.org

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

Group R (Regumate®): Positive control, 0.044 mg/kg altrenogest (Regumate®[3]) orally once daily.

Implant application was performed using aseptic technique. The mare's movement was restricted by placing the mare 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 into the subcutaneous tissues of the vulvar lips, parallel to the vulvar opening, and five to ten centimeters below the dorsal vulvar commisure. Introduction was performed using the applicator provided by the manufacturer (Figure 1). 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 2-3mm 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.

Regumate® was used for the positive control group because of its proven effectiveness to suppress behavioral estrus (0.044mg/kg orally once a day) [12, 32].

All treatments started in mid-diestrus, seven days after ovulation was detected by transrectal ultrasonography. The study ended with removal of the implant and treatment withdrawal ninety days later, and mares were followed until their first ovulation after the treatment withdrawal.

[3] Intervet Inc, Millsboro, USA

2. Data Collection

Estrous cycles were monitored for three months (90 days) after the onset of the treatments. Data collection ended following the first detected ovulation after the withdrawal of treatment.

2.1 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 with the mares and 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 from one to four using the following scale: *One*: mare completely rejects the stallion, presenting one or more of the following refusal manifestations: squealing, pawing, kicking, switching tail, holding ears back; *Two*: mare is indifferent to the presence of the stallion; she does not move away, but does not lift the tail or wink; *Three*: mare is interested in the stallion and approaches him, raising the tail, urinating and/or winking the clitoris (rhythmic eversion of the labia and exposure of the clitoris); *Four*: Full estrous behavior (posture change facilitating copula), the mare will present similar behavioral signs that score three (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 hock, 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).

2.2 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 48hrs after treatment onset, and then weekly to evaluate plasma progesterone concentrations. The samples were processed with a Coat-a-Count® Progesterone Radioimmunoassay (RIA) kit[4] which has previously been validated for use in this species [49]. Supplemental altrenogest does not affect the ability to quantify natural progesterone levels [50].

2.3 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 mares. A MicroMaxx® ultrasonography system with an L52e transducer (5 MHz)[5] was used for all examinations.

3. <u>Data Analysis</u>

Interestrus interval (IEI) was calculated based on both progesterone levels and behavioral estrus (teasing). Mares with plasma progesterone concentrations below 1.0 ng/ml were considered to be in estrus. Mares with a teasing score of three or four were considered consistent with estrus. IEI was used as an indicator of the duration of estrus suppression and is counted as the time in days between periods of behavioral estrus as evidenced by plasma progesterone of <1ng/ml or a teasing score of 3 or 4.

[4] Siemens, Los Angeles, CA, USA
[5] Sonosite Inc, Bothell, WA, USA

3.1. Statistical analysis

Data were analyzed using Statistical Analysis System software (SAS).[6] 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 expected with $p < 0.05$.

Data were transformed using a logarithmic scale in order to achieve parametric statistical assumptions (normality and constant variance). Descriptive statistics (means, standard errors and coefficient of variation) were calculated using PROC UNIVARIATE. In order to detect the effect of the treatment on estrus suppression, interestrus intervals 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). Correlation analysis (Pearson's correlation) between IEI based on teasing and progesterone was performed using PROC CORR.

4. Animal Welfare

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

[6] Statistical Analysis System (SAS) version 9.1, SAS Institute, Cary, NC.

IV. Results

1. Clinical Observation

No abnormalities at the site of implant 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 during the first month of the study; her data was not considered for analysis.

Ovulation (detected by transrectal palpation and ultrasonography) was observed in all mares in groups C, T1 and T2. In group C, ovulation occurred regularly in all mares as expected. However, in groups T1 and T2, the 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 twenty mares returned to normal cyclicity and ovulation occurred within three to 46 days. The mean ovulation in group C, occurred 10 days after the 90 day study period (range 7 to 14). In groups T1 and T2, ovulation occurred a mean of 13 days (range 3 to 30) and 22 days (range 10-46) respectively. In group R, the mean ovulation occurred 9 days after the treatment withdrawal (range 8 to 11). No significant differences were found on the mean comparison between groups. Suggestive differences were found 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.

2. <u>Interestrus Intervals (IEI)</u>

Mean IEI based on teasing scores ± SEM was 21 ± 0.34 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 ± 6.3 days for group T1, 41 ± 14.4 days for group T2, and 48 ± 0.9 days for group R (Figure 2). Refer to Table 1 for IEI averages of each specific mare during the 90-day study period. No statistical difference was found in IEI between groups C, T1, and T2 based on teasing or progesterone levels. As expected, group R (positive control) was different from 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 (positive control) was significantly different from group C (negative control) (p=0.049). No significant differences were found between the other groups (see figures 3 through 8 to visualize progesterone concentrations for each mare in each group).

A significant difference in the response to etonogestrel was observed between mares within the same group in both T1 and T2. The coefficient of variation (CV) for group T1 was 53.07%. In group T2, the variation was even greater with a CV of 73.7%. The variation was greater in the implant groups compared with the control groups (C and R). The CV in groups C and R (negative and positive control) was 3.62% and 2.3% respectively.

2.1. Correlation

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. Pearson's correlation is a measure of dependence between two quantities. Values range from +1 indicating a perfect linear correlation (positive correlation) to -1 indicating a perfect negative linear correlation (negative correlation). If the variables are completely independent, the Pearson's correlation coefficient will be zero. In this study, the coefficient of correlation demonstrates the correlation of the IEI based on progesterone and teasing, showing no differences in the IEI as determined by each method.

V. Discussion

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) indicating some effect.

The use of the implant was determined to be safe in mares for at least 90 days. None of the 10 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 this study, very few abnormalities or complications were observed at the implant site in 474 women (<3% complication rate) [45].

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 [11].

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 (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 were the variation was lower than the one we obtained in this experiment. Therefore, in future studies, it seems likely that a larger sample size may reduce the effect of variation.

As expected group R (positive control) was significantly different from 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, 12, 32, 34].

When IEI was calculated based on progesterone concentrations, only groups C and R were significantly different (positive and negative control groups). This is because, as previously mentioned, altrenogest failed to completely control the estrous cycle and ovulation. Progesterone concentrations in mares receiving altrenogest may be unpredictable, rising over and decreasing below 1 ng/ml without being accompanied by estrous behavior, indicating ovulations are occurring [11].

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 supplemental progesterone 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 we exclude the Group R from the correlation test, the coefficient of correlation 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). In humans, the maximum serum concentrations detected were 813 pg/ml four days after implant insertion, and declined to 156pg/ml at the end of the three years [48]. In humans, body weight was related to overall serum concentrations of etonogestrel where the highest levels were found in women weighing less than 50kg (~200pg/ml) and the lowest concentration was found in women weighing over 70kg (~150pg/ml) [48]. Based on this information, the expected concentrations in the horse should be five to ten times less than the serum levels obtained in women, considering an average women weight of 65kg compared with a 500kg body weight in the mare. In this study we were using a dose of 0.13 to 0.27mg/kg, compared with 1.05 mg/kg used in the commercial dose for women (assuming an average body weight of 65kg).

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 animals (group C), we speculate that etonogestrel in the correct dose may suppress estrous behavior more consistently. Further studies with a more weight appropriate dose (525 mg per 500kg

horse) and a higher 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.

VI. Conclusions

The high correlation of the two parameters to determine IEI (teasing and progesterone) may validate 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. The use of the implant was determined to be safe in mares for at least 90 days.

References

[1] Crowell-Davis SL. Sexual behavior of mares. Hormones and Behavior 2007;52(1):12-17.

[2] McKinnon AO, Squires EL, Vaala WE, Varner DD. Equine Reproduction, Second ed. Wiley-Blackwell, West Sussex, UK, 2011.

[3] Pryor P, Tibary A. Management of Estrus in the Performance Mare. Clinical Techniques in Equine Practice 2005;4(3):197-209.

[4] Ginther OJ. Reproductive biology of the mare: basic and applied aspects Equiservices Cross Plains, WI, 1992.

[5] Górecka A, Jezierski TA, Sloniewski K. Relationships between sexual behaviour, dominant follicle area, uterus ultrasonic image and pregnancy rate in mares of two breeds differing in reproductive efficiency. Animal Reproduction Science 2005;87(3-4):283-93.

[6] Dalin AM, Andresen Ø, Malmgren L. Immunization against GnRH in mature mares: antibody titres, ovarian function, hormonal levels and oestrous behaviour. Journal of Veterinary Medicine Series A 2002;49(3):125-31.

[7] Hinrichs K. Irregularities of the estrous cycle and ovulation in mares (Including seasonal transition). In: Robert Youngquist WT Current Therapy in Large Animal Theriogenology. St Louis: Saunders, 2007. p. 150-52.

[8] Jorgensen JS, Vivrette S, Correa M, Mansmann RA. Significance of the estrous cycle on athletic performance in mares. American Association of Equine Practitioners Proceedings 1996;42:98-100.

[9] Squires EL. Use of progestins in open and pregnant mares. Animal Reproduction Science 1993;33(1-4):183-93.

[10] Pohl O, Buff S, Garnier F, Guérin P, Lefranc AC. Pharmacokinetic analysis during intravaginal progesterone-releasing devices (PRID®/CIDR®) application in the mare. Pferdeheilkunde 2009;25(2):105-10.

[11] Lofstedt RM, Patel JH. Evaluation of the ability of altrenogest to control the equine estrous cycle. Journal of the American Veterinary Medical Association 1989;194(3):361.

[12] Daels PF, McCue PM, DeMoraes MJ, Hughes JP. Persistence of the luteal phase following ovulation during altrenogest treatment in mares. Theriogenology 1996;46(5):799-811.

[13] Hooper RN, Taylor TS, Varner DD, Blanchard TL. Effects of bilateral ovariectomy via colpotomy in mares: 23 cases (1984-1990). Journal of the American Veterinary Medical Association 1993;203(7):1043-46.

[14] Hawkins DL, Neely DP, Stabenfeldt GH. Plasma progesterone concentrations derived from the administration of exogenous progesterone to ovariectomized mares. Journal of Reproduction and Fertility Supplement 1979(27):211-16.

[15] Loy RG, Swan SM. Effects of exogenous progestogens on reproductive phenomena in mares. Journal of Animal Science 1966;25(3):821-6.

[16] Nett TM, Pickett BW, Seidel GE, Voss JL. Levels of luteinizing hormone and progesterone during the estrous cycle and early pregnancy in mares. Biology of Reproduction 1976;14(4):412-15.

[17] Watson ED, Hinrichs K. Adrenal production of sex steroids in the mare. Theriogenology 1989;32(6):913-19.

[18] Dalin AM, Hedberg Y, Alexanderson K, Kindahl H. Tetracosactid (ACTH) treatment of mares in estrus-effect on estrous signs, ovulation and hormone levels. Theriogenology 2002;58(2-4):515-18.

[19] Nie GJ, Johnson KE, Braden TD, Wenzel JGW. Use of an intra-uterine glass ball protocol to extend luteal function in mares. Journal of Equine Veterinary Science 2003;23(6):266-73.

[20] Rivera del Alamo MM, Reilas T, Kindahl H, Katila T. Mechanisms behind intrauterine device-induced luteal persistence in mares. Animal Reproduction Science 2008;107(1-2):94-106.

[21] Lefranc A. Nonpharmacological suppression of oestrus in the mare. Equine Veterinary Journal 2004;36(2):183-85.

[22] Hedberg Y, Dalin AM, Santesson M, Kindahl H. A preliminary study on the induction of dioestrous ovulation in the mare–a possible method for inducing prolonged luteal phase. Acta Veterinaria Scandinavica 2006;48(1):12-18.

[23] Chow NK, Fretz M, Hamburger M, Butterweck V. Telemetry as a tool to measure sedative effects of a valerian root extract and its single constituents in mice. Planta Medica 2011;77(8):795-803.

[24] Murphy K, Kubin ZJ, Shepherd JN, Ettinger RH. Valeriana officinalis root extracts have potent anxiolytic effects in laboratory rats. Phytomedicine 2010;17(8-9):674-78.

[25] Wuttke W, Jarry H, Christoffel V, Spengler B, Seidlova-Wuttke D. Chaste tree (Vitex agnus-castus)-pharmacology and clinical indications. Phytomedicine 2003;10(4):348-57.

[26] Loch EG, Selle H, Boblitz N. Treatment of premenstrual syndrome with a phytopharmaceutical formulation containing Vitex agnus castus. Journal of Women's Health & Gender-based Medicine 2000;9(3):315-20.

[27] McCue PM. Estrus suppression in performance horses. Journal of Equine Veterinary Science 2003;23(8):342-44.

[28] Vanderwall DK, Marquardt JL, Woods GL. Use of a compounded long-acting progesterone formulation for equine pregnancy maintenance. Journal of Equine Veterinary Science 2007;27(2):62-66.

[29] Perkins NR. Equine reproductive pharmacology. The Veterinary Clinics of North America Equine practice 1999;15(3):687-704.

[30] Ganjam VK, Kenney RM, Flickinger G. Effect of exogenous progesterone on its endogenous levels: biological half-life of progesterone and lack of progesterone binding in mares. Journal of Reproduction and Fertility Supplement 1975(23):183-88.

[31] Hodgson D, Howe S, Jeffcott L, Reid S, Mellor D, Higgins A. Effect of prolonged use of altrenogest on behaviour in mares. The Veterinary Journal 2005;169(1):113-15.

[32] 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(1):181-93.

[33] McKinnon AO, Lescun TB, Walker JH, Vasey JR, Allen WR. The inability of some synthetic progestagens to maintain pregnancy in the mare. Equine Veterinary Journal 2000;32(1):83-85.

[34] Gee EK, DeLuca C, Stylski JL, McCue PM. Efficacy of medroxyprogesterone acetate in suppression of estrus in cycling mares. Journal of Equine Veterinary Science 2009;29(3):140-45.

[35] Vanderwall DK, Rasmussen DM, Woods GL. Effect of repeated administration of oxytocin during diestrus on duration of function of corpora lutea in mares. Journal of the American Veterinary Medical Association 2007;231(12):1864-67.

[36] Elhay M, Newbold A, Britton A, Turley P, Dowsett K, Walker J. Suppression of behavioural and physiological oestrus in the mare by vaccination against GnRH. Australian Veterinary Journal 2007;85(1 2):39-45.

[37] Imboden I, Janett F, Burger D, Crowe MA, Hässig M, Thun R. Influence of immunization against GnRH on reproductive cyclicity and estrous behavior in the mare. Theriogenology 2006;66(8):1866-75.

[38] Watson ED, Pedersen HG, Thomson SRM, Fraser HM. Control of follicular development and luteal function in the mare: Effects of a GnRH antagonist*. Theriogenology 2000;54(4):599-609.

[39] Johnson CA, Thompson Jr DL, Kulinski KM, Guitreau AM. Prolonged interovulatory interval and hormonal changes in mares following the use of Ovuplant (TM) to hasten ovulation. Journal of Equine Veterinary Science 2000;20(5):331-36.

[40] Johnson CAM, S L; Thompson Jr, D L. Effects of multiple GnRH analogue (deslorelin acetate) implants on cyclic mares. Theriogenology 2002;58(2-4):469-71.

[41] Farquhar VJ, McCue PM, Nett TM, Squires EL. Effect of deslorelin acetate on gonadotropin secretion and ovarian follicle development in cycling mares. Journal of the American Veterinary Medical Association 2001;218(5):749-52.

[42] Rathbone MJ. Conceptual and commercially available intravaginal veterinary drug delivery systems. Advanced Drug Delivery Reviews 1997;28(3):363-92.

[43] Preston RL. Hormone containing growth promoting implants in farmed livestock. Advanced Drug Delivery Reviews 1999;38(2):123-38.

[44] McCue PM, Lemons SS, Squires EL, Vanderwall DK. Efficacy of Synovex-S® implants in suppression of estrus in the mare. Journal of Equine Veterinary Science 1997;17(6):327-29.

[45] Funk S, Miller MM, Mishell DR. Safety and efficacy of Implanon (TM), a single-rod implantable contraceptive containing etonogestrel. Contraception 2005;71(5):319-26.

[46] Olsson SE, Odlind V, Johansson E. Clinical results with subcutaneous implants containing 3-keto desogestrel. Contraception 1990;42(1):1-11.

[47] Wenzl R, van Beek A, Schnabel P, Huber J. Pharmacokinetics of etonogestrel released from the contraceptive implant Implanon®. Contraception 1998;58(5):283-88.

[48] Huber J. Pharmacokinetics of Implanon®: An integrated analysis. Contraception 1998;58(6):85S-90S.

[49] Ball BA, Wilker C, Daels PF, Burns PJ. Use of progesterone in microspheres for maintenance of pregnancy in mares. American Journal of Veterinary Research 1992;53(8):1294-97.

[50] Squires EL, Heesemann CP, Webel SK, Shideler RK, Voss JL. Relationship of altrenogest to ovarian activity, hormone concentrations and fertility of mares. Journal of Animal Science 1983;56(4):901-10.

Appendix (Figures and Tables)

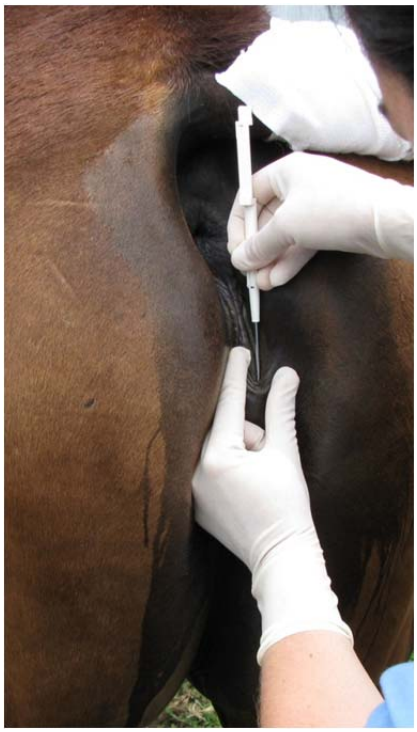

Figure 1. Implant application. The implant is being placed in the right vulvar lip using the applicator provided by the manufacturer. Local anesthesia was used prior to implant placement.

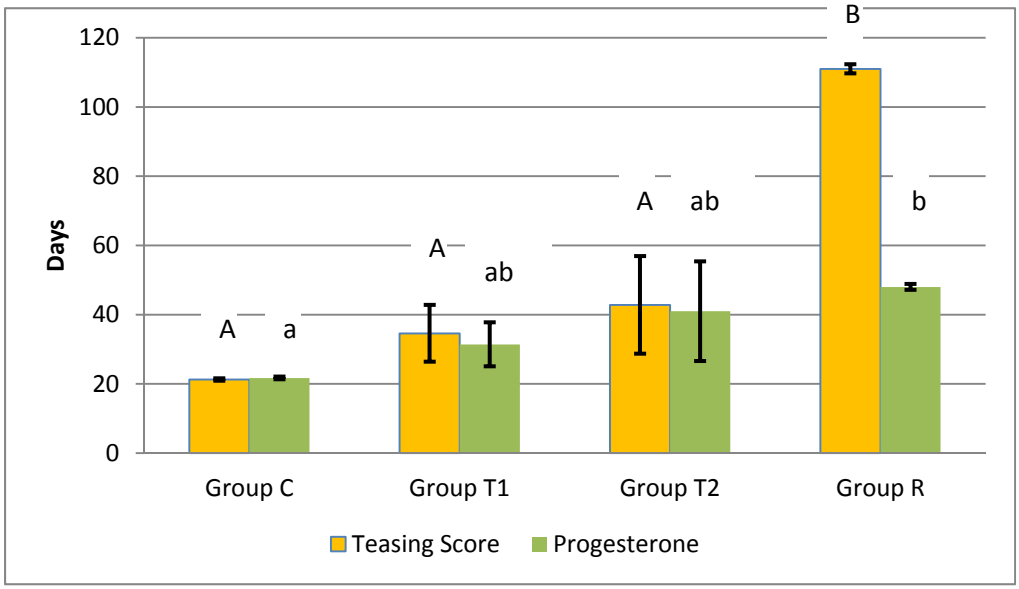

Figure 2. Interestrus interval based on teasing scores and progesterone levels ± SEM. Different letters indicate significant differences between groups ($p<0.05$). Uppercase letters compare IEI based on teasing score and lowercase letters based on progesterone levels.

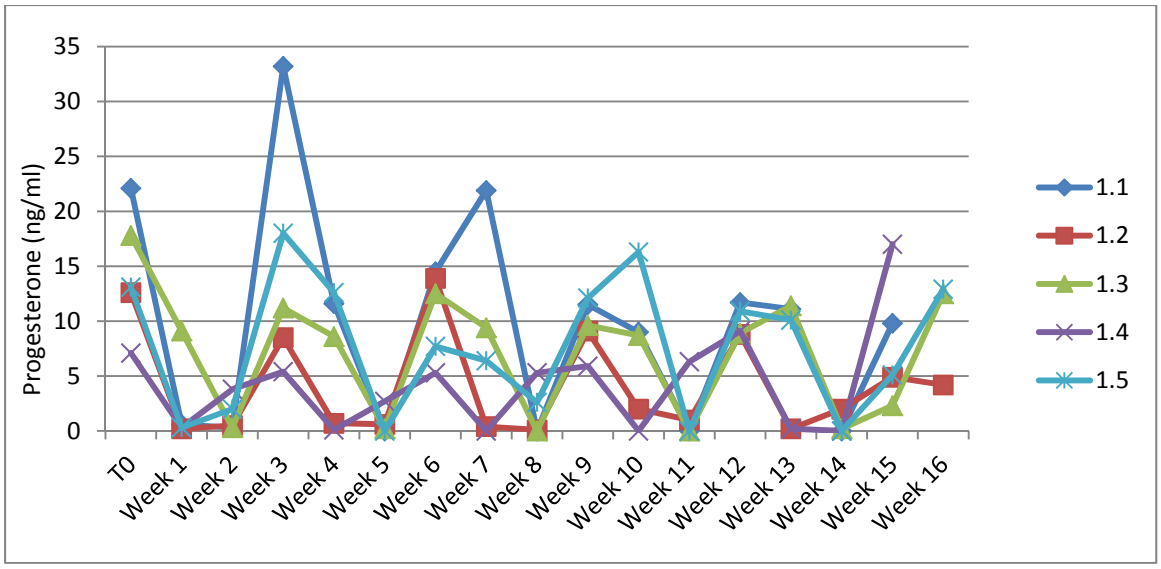

Figure 3. Progesterone concentration in animals within the Control Group (no treatment)

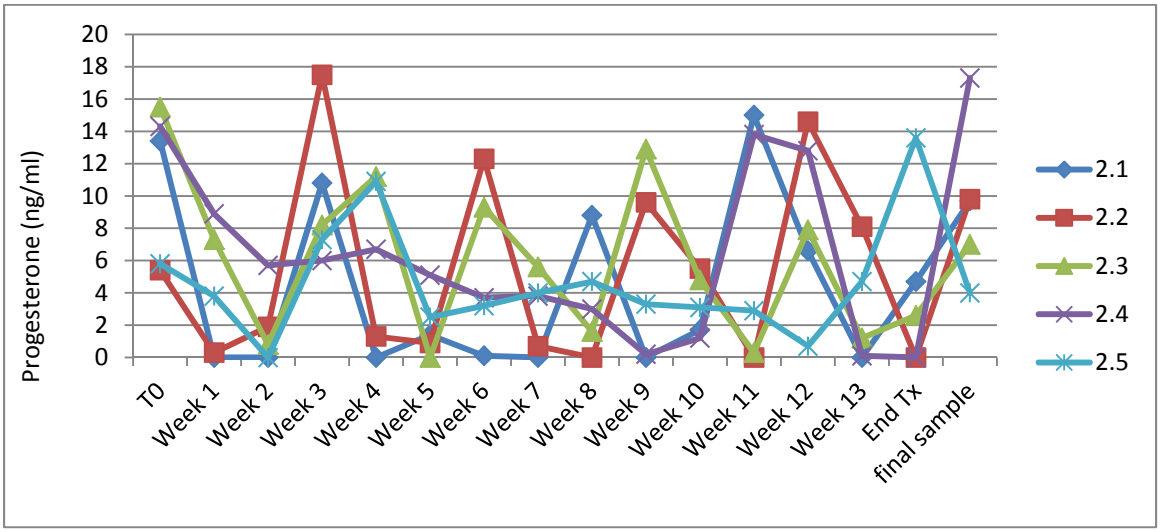

Figure 4. Progesterone concentration in animals within the One Implant Group (T1). Progesterone concentrations after treatment withdrawal are not shown on this graph for comparison purposes.

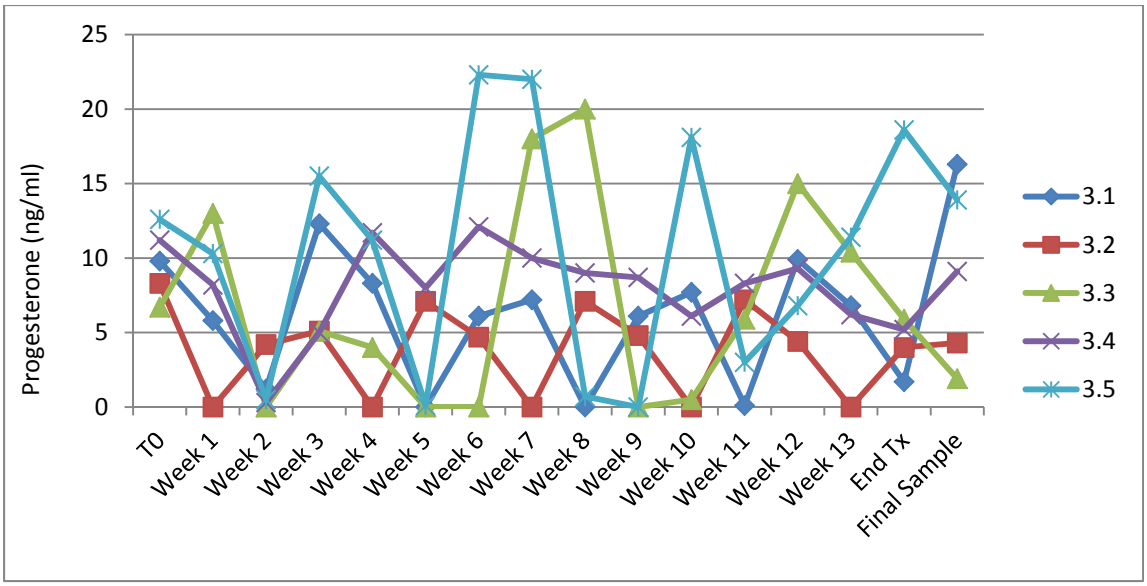

Figure 5. Progesterone concentration in animals within the Two Implant Group (T2). Progesterone levels after treatment withdrawal are not shown on this graph for comparison purposes.

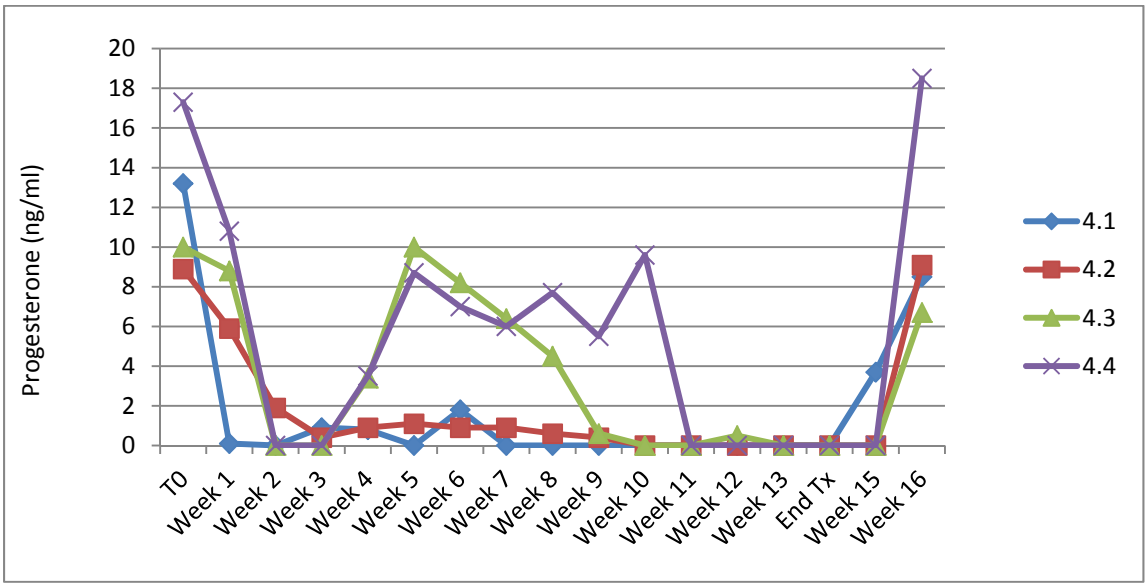

Figure 6. Progesterone concentrations in animals within the Regumate Group (R).

Figures 7a,7b,7c,7d,7e. Progesterone concentrations in individual mares from One Implant Group (T1).

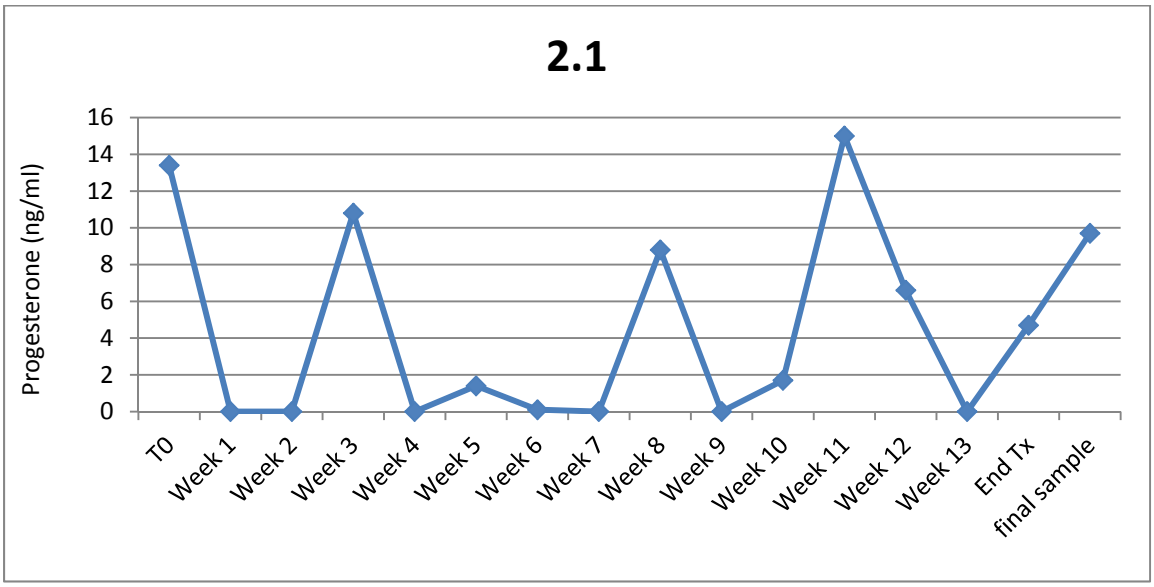

Fig 7a

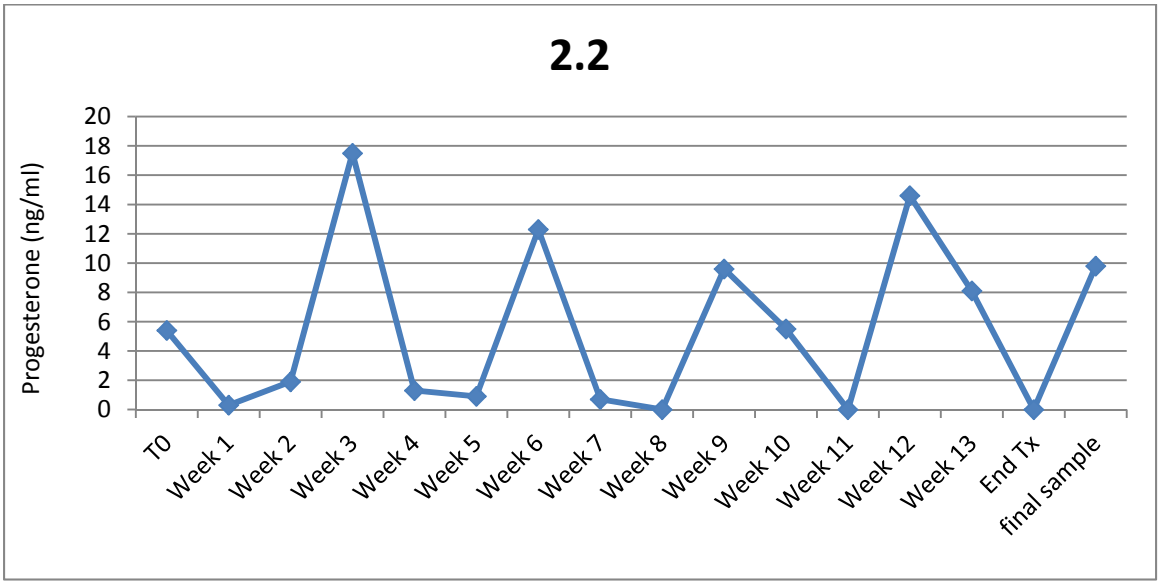

Fig 7b

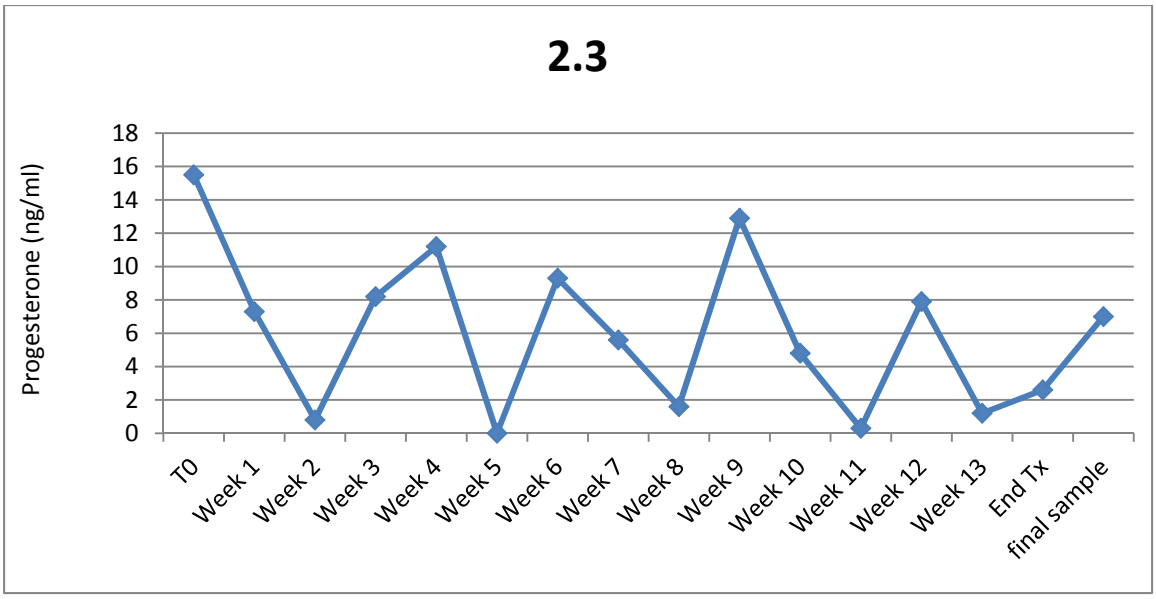
2.3
Progesterone (ng/ml)
18
16
14
12
10
8
6
4
2
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
End Tx
final sample

Fig 7c

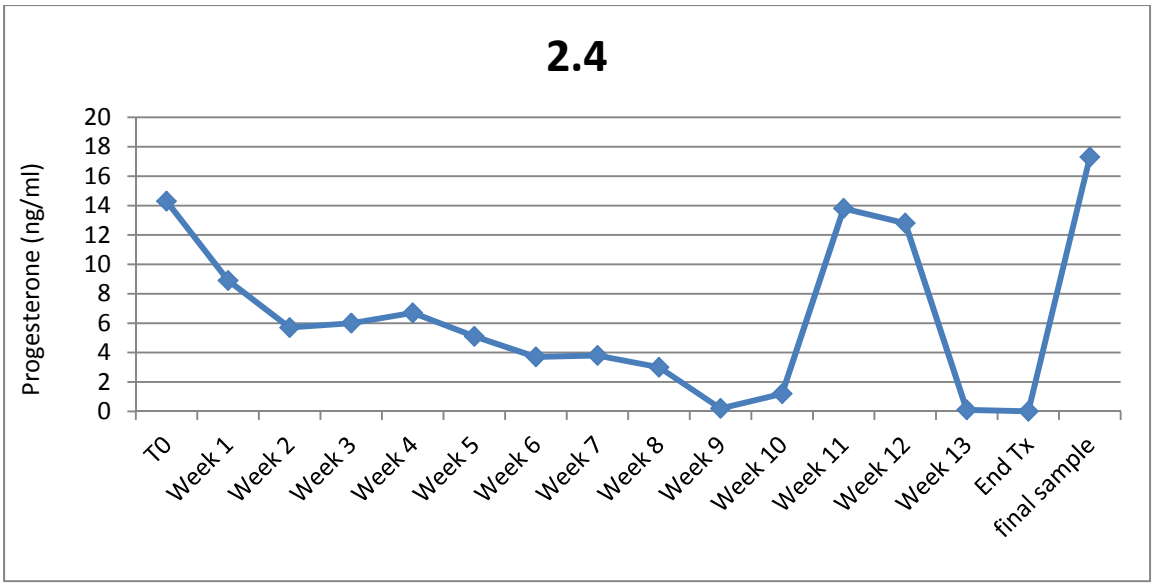
2.4
Progesterone (ng/ml)
20
18
16
14
12
10
8
6
4
2
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
End Tx
final sample

Fig 7d

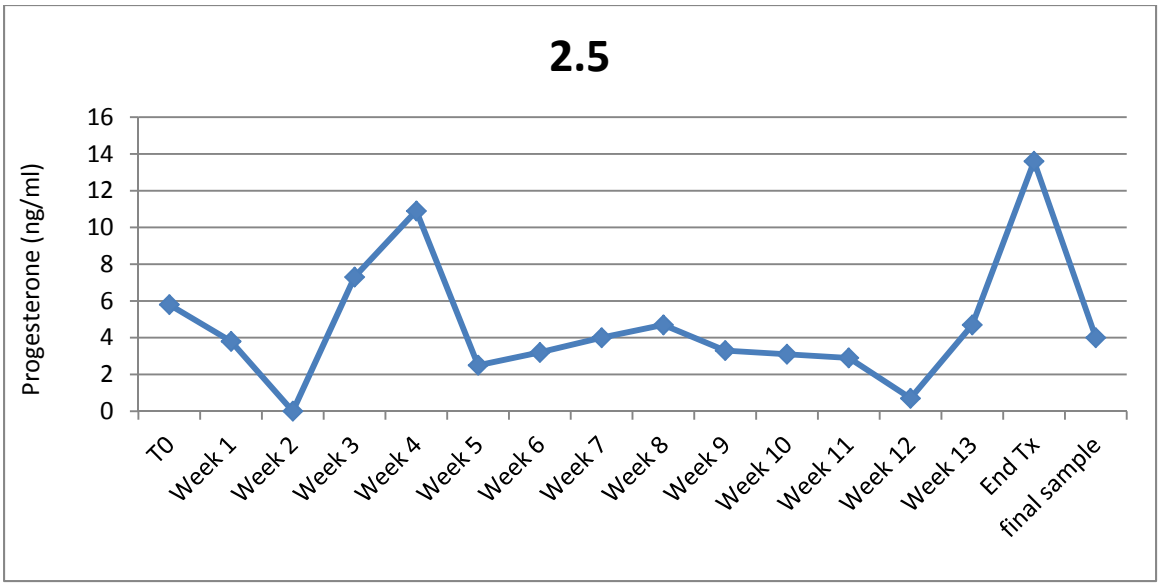

Fig 7e

Figures 8a,8b,8c,8d,8e. Progesterone concentrations in individual mares from Two Implant Group (T2).

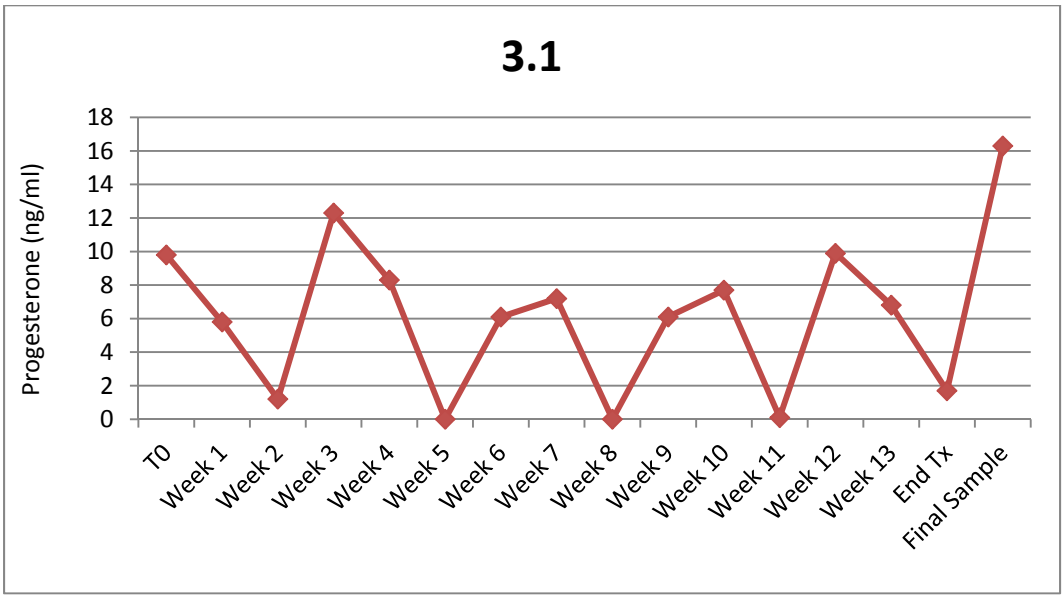

Fig 8a

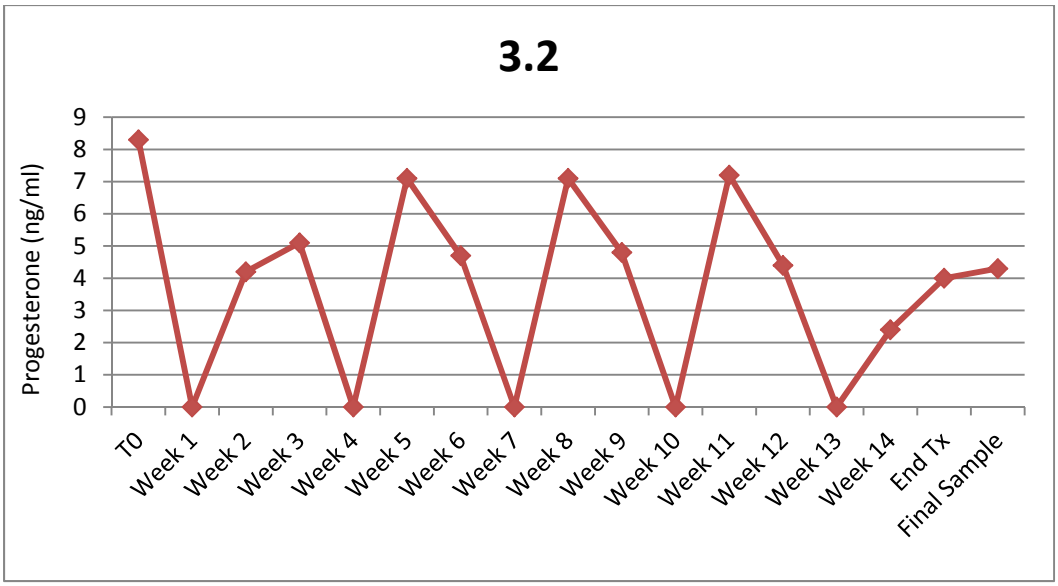
3.2
Progesterone (ng/ml)
9
8
7
6
5
4
3
2
1
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
Week 14
End Tx
Final Sample

Fig 8b

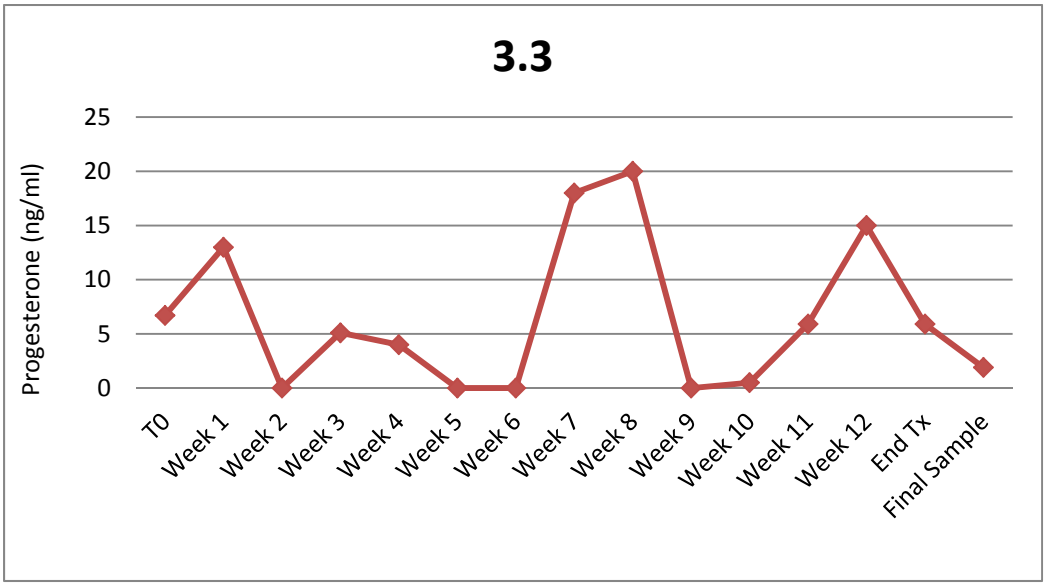
3.3
Progesterone (ng/ml)
25
20
15
10
5
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
End Tx
Final Sample

Fig 8c

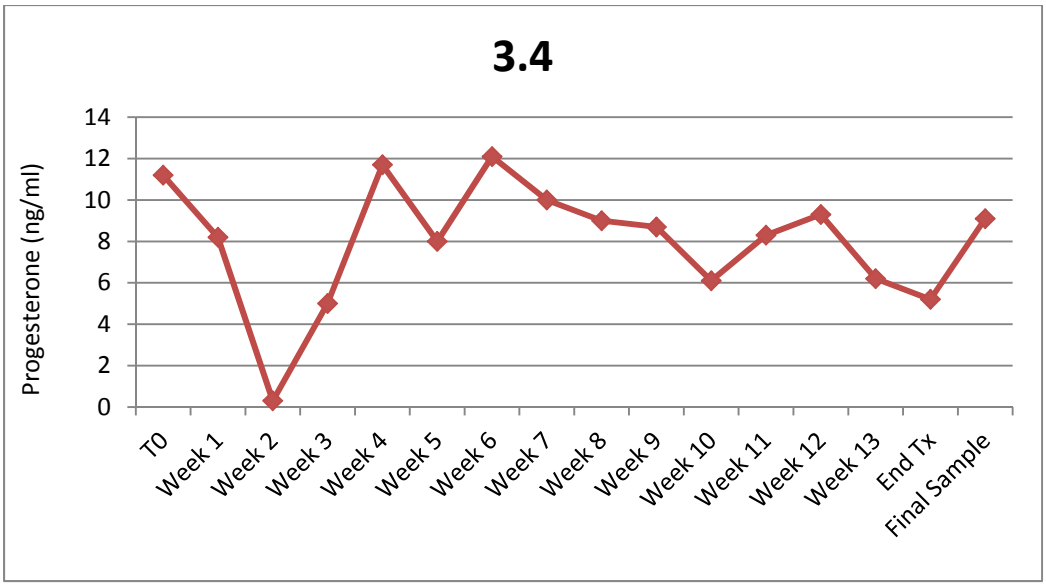
3.4
Progesterone (ng/ml)
14
12
10
8
6
4
2
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
End Tx
Final Sample

Fig 8d

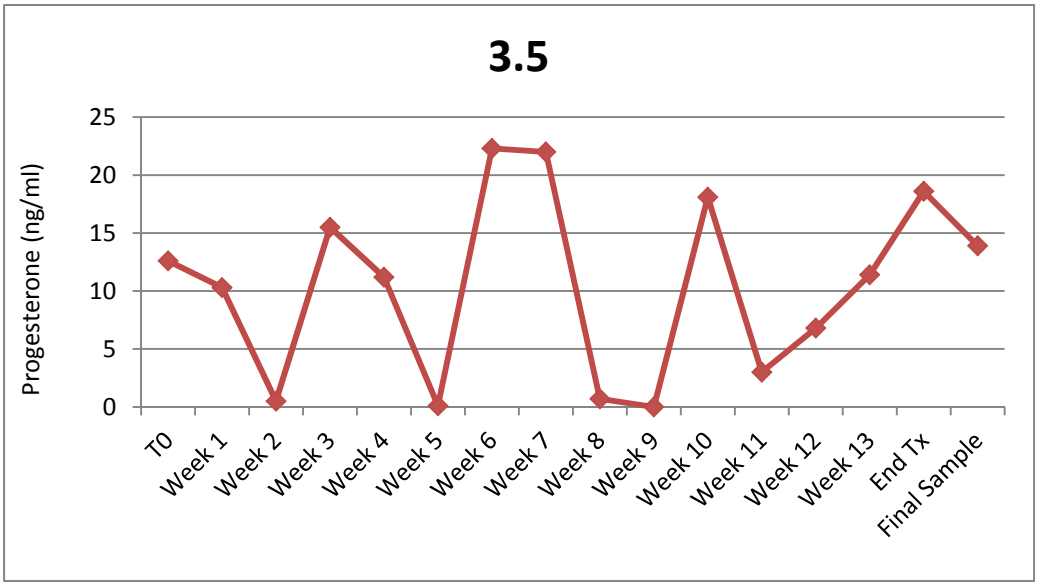
3.5
Progesterone (ng/ml)
25
20
15
10
5
0
T0
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Week 7
Week 8
Week 9
Week 10
Week 11
Week 12
Week 13
End Tx
Final Sample

Fig 8e

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

Table 1.

Average interestrus interval (IEI) of each mare based on 90 days observation period. IEI was calculated based on twice weekly teasing observations and once weekly progesterone samples.