

New Pharmacological Treatments for Equine Reproductive Management

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Introduction

Loy (1970) reported that only 55% of mares bred annually produce live foals. Recent data from *The Jockey Club (2006)* indicate that only 37,025 of 64,123 (57.7%) of thoroughbred mares bred during 2005 produced live foals. This is considerably lower than foaling rates of 71 to 85%, which are reported on farms where extensive reproductive management is used. Overall, reproductive management has not improved much in the last 30 years. The long estrus period, with ovulation at any time from 1 to 10 days after the beginning of estrus, has made reproductive management of cyclic mares time-consuming, expensive and most importantly, inefficient. Furthermore, the confusion associated with the long and variable transition from anestrus to cyclicity in mares greatly magnifies the complexity of efficient reproductive management for this category of mares.

There is a need to develop and capitalize on controlled breeding programs for the horse industry based on new advances in the understanding of cost effective hormonal control of reproduction in mares and stallions. Success of such programs will be attributable in large part to recent advances in biodegradable controlled release drug delivery systems that will allow single administration products to replace prolonged daily treatment protocols (Burns, 1999; Rathbone et al., 2000). These formulations will reduce labor and the associated handling stress to the animals and producers and offer veterinarians an important means of maintaining effective compliance rates on farms with wide varieties of management systems. In this paper, we will discuss traditional and new treatments used for reproductive management in modern equine breeding programs.

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Treatments For Management of Seasonally Anestrous Mares

As long as the breed associations continue to use January 1st as the official birth date for foals born the same season, there will be economic incentives to breed mares as early as possible. Therefore, treatment protocols for the induction of ovulation in anestrous or transitional mares will be highly valued.

Progestins - In 1973, Van Niekerk et al., reported that daily treatment with 100 mg of progesterone for 7 days blocked estrus during treatment followed by a normal estrus with ovulation. Studies with the synthetic progestin altrenogest (Webel and Squires 1982; Squires et al., 1983;) suggested that most successful responses occur in transitional mares treated after mid March (northern hemisphere) and in mares with at least moderate sized follicles (>20 mm). Taylor et al., (1982) reported satisfactory control of ovulation in late transitional mares that had been maintained under increased photoperiod for at 60 days with daily progesterone and estradiol treatment.

It is inconvenient and time consuming to administer altrenogest, progesterone or progesterone and estradiol to mares daily. Recently, Burns et al., (1999) evaluated a new controlled release preparation ***P*** + consisting of microspheres made from a biocompatible, biodegradable polymeric excipient poly (DL-lactide) designed to deliver in a single injection the entire dose of progesterone and estradiol at a controlled rate for a duration of 12 to 14 days. This formulation resulted in accurate control of fertile ovulations in late transitional mares with follicles > than 30 mm. More recently, it was shown that treatment with a single 2 mL dose (600 mg) using BioRelease P4 LA 300 results in good control of ovulation in transitional mares with follicles between 20 mm and 30 mm. Ovulation also occurred in non-responding “lighted” mares that had been lighted >50 days but had follicles < 15mm prior to treatment. In this later group, 57% of the P4 LA 300 mares ovulated within 2 weeks following treatment compared to 7% in control mares (Burns et al 2007).

Although progestin treatment given daily or as a single injection in a controlled release formulation appears to be of little value in deep anestrous mares, such treatments appear to be very useful in managing mares through the transitional phase when follicles greater than 20 to 25 mm are present. Such treatments may also be of benefit in non-responding “lighted” mares with little follicular activity.

GnRH / GnRH Analogs - The ability of GnRH or its analogs to stimulate LH release has led to their use for ovulation induction in anovulatory mares using frequent injections (Bailey and Douglas, 1977; Bergfelt and Ginther, 1992), pulsatile infusion (Johnson, 1987; Becker and Johnson, 1992), continuous infusion (Hyland et al., 1987) or slow release implants (Harrison et al 1990; Meyer et al 1990; Mumford et al 1994). Similar to progestins or progesterone plus estradiol, most successful responses to treatment occur as the time of year advances or the diameter of the largest follicle at the initiation of treatment increases.

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In addition, GnRH treatment of mares with small follicles (<15 mm) works about one third of the time and is associated with a high rate of early pregnancy loss (58%) due to inadequate luteal function (Bergfelt and Ginther, 1992). Furthermore, recent reports of suppression of follicular growth in cyclic mares with commercial deslorelin implants point out the complexity of working in this area and the need for careful pharmacodynamic research in GnRH/GnRH analog product development.

Pituitary Extracts – Stimulation of follicular growth and ovulation can be achieved in anovulatory pony mares within 3 weeks using equine pituitary extracts (Douglas et al., 1974). Coy et al. (1999) reported that administration of equine pituitary extracts (25 mg total protein, intramuscularly, once daily) resulted in a higher percentage of transitional mares (8 of 9, 89.9%) ovulating in response to EPE administration than mares in deep anestrus (2 of 9, 22.2%). The mean interval from onset of treatment to ovulation for the transitional mares was 11.8 ± 5.0 days. Recently, a commercial, lyophilized, reagent preparation that contains eFSH activity equivalent to 25 mg B-FSH-ReF-001 Standard, manufactured by Bioniche Animal Health USA, Inc., Athens, GA 30601 has become available. Niswender et al. (2003) demonstrated that administration of the commercial equine FSH twice daily to transitional mares resulted in 8 of 10 mares ovulating after approximately 5 days of injection. The use of eFSH treatment offers breeders a new safe and effective means of hastening the first ovulation of the year in transitional mares.

Dopamine Antagonist – In recent years, treatment with dopamine D2-antagonists such as domperidone and sulpiride have been shown to induce cyclicity in anovulatory mares in some studies (Besognet et al., 1996,1997; Brendemuehl and Cross, 2000) but not in others (McCue et al. 1999; Donadeu and Thompson, 2002). As with progestins or GnRH / GnRH analogs, it appears that the treatment is most effective in transitional mares, mares in locations with mild winter climates (Brendemuehl and Cross, 2000) or mares that have received 28 days of increased photo-stimulation (Nagy et al 1999). Daels et al (2000) has also reported that sulpiride treatment of mares maintained indoors in locations with cold climates is more effective than in mares maintained outdoors at the same location.

The mechanism of action by which dopamine antagonists stimulate follicular activity in the mare is poorly understood. The similar effects of domperidone which does not cross the blood brain barrier and sulpiride which does, argues against a central /hypothalamic site of action for the D2 antagonist. Treatment of anovulatory mares with D2 antagonists does not alter acute secretion of LH and FSH (Nequin et al. 1993b; Aurich et al., 2000) and Brendemuehl and Cross (2000) found no effects on FSH after domperidone treatment but both LH and estrogen conjugate levels were significantly increased by 28 days of treatment.

Observations that dopamine antagonists stimulate prolactin secretion and that exogenous prolactin given to anovulatory mares can stimulate growth of large follicles in deeply anestrus mares (Nequin et al., 1993a) and hasten the occurrence of the first ovulation in anestrus mares (Thompson et al., 1997) suggests that the dopamine antagonist could be

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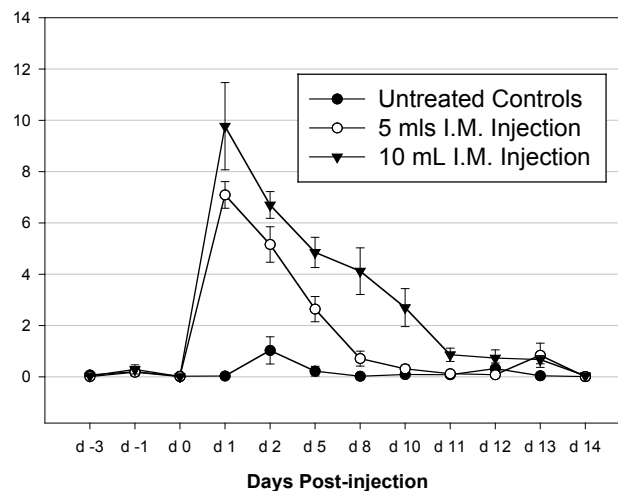
acting through prolactin at the ovarian level. Recently, Kelly et al., (2006) reported on the use of domperidone or sulpiride for stimulating prolactin secretion in cyclic and deeply anestrous non-lighted mares. Both dopamine antagonists can be used in combination with an estrogen to greatly enhance prolactin secretion. The combination of estradiol benzoate and sulpiride was also effective at inducing ovulation in deeply anestrous non-lighted mares. Mares receiving the estradiol + sulpiride ovulated 45 days earlier than mares receiving sulpiride alone, 29 days vs 73.6 days. The combination of estradiol pretreatment followed by dopamine D2-antagonist treatment may have great potential for the treatment of deep anovulatory mares.

Treatments For Reproductive Management of Cyclic Mares

Progestins - Control of estrus has been achieved by extending the luteal phase with exogenous progesterone. Progestins, either as injectable or oral forms, have been used to regulate estrus in transitional (Webel and Squires, 1982), post-partum (Loy et al., 1975); and cycling mares (Holton et al, 1977; Squires et al., 1979; Squires et al., 1992).

Recently, several new compounded controlled release progestin formulations have become available to veterinarians by prescription. The formulation BioRelease P4 LA 150 (BET Pharm, Lexington, Ky. www.Betpharm.com) has been shown to keep mean blood levels above 2 ng/ mL for approximately 10 days (Bingle et al., 2003) as shown below (Graph courtesy of Dr. Bea Bingle). Vanderwall et al., (2003) has also demonstrated that the formulation is effective at maintaining pregnancy between days 18 and 45 when given every 7 days to pregnant mares injected with cloprostenol to remove endogenous luteal function.

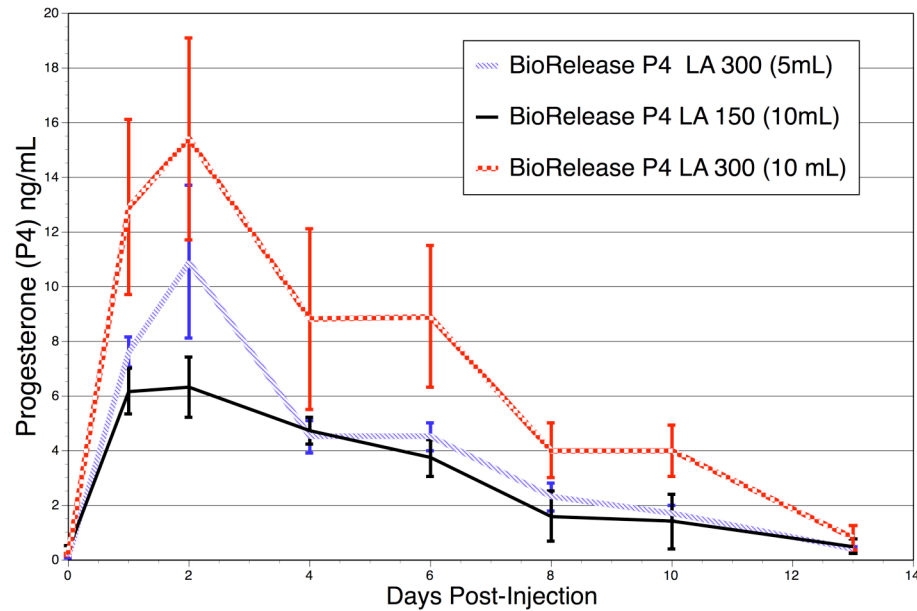
**Progesterone (P4) Concentrations
After BioRelease P4 LA 150 Injection**



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Burns, et.al., (2007) has recently conducted studies with a more concentrated progesterone formulation Biorelease P4 LA300 (www.Betpharm.com) that produced similar blood levels to the earlier P4 LA 150 formulation but requires only half the injection volume as shown below.



Storer et al., 2007 recently evaluated several new compounded controlled release injectable altrenogest formulations using either the BioRelease liquid delivery system or the longer acting Lactide-co-glycolide microparticle (MP) systems (www.Betpharm.com). Results are summarized in the table below.

Days to Estrus & Ovulation following Lutalyse & Sustained Release Progestin Treatment			
Formulation	N	Days to estrus	Days to ovulation
LA 150 1.5 mL	6	12.7 ± 0.42 ^b	17.2 ± 0.75 ^b
LA 150 3 mL	6	15.8 ± 1.51 ^b	21.8 ± 0.54 ^b
MP 500	6	32.8 ± 4.80 ^c	34.7 ± 3.72 ^c
Medroxyprogesterone	6	6.2 ± 1.38 ^a	11.3 ± 2.14 ^a
Controls	7	3.9 ± 0.51 ^a	8.3 ± 1.17 ^a
Means ± SE; means with different superscripts differ (P < 0.05)			

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All injectable altrenogest formulations were effective at extending the interovulatory interval and delaying estrus. The altrenogest MP 500 formulation had the greatest inhibitory effect. This formulation may be beneficial for the performance horse by inhibiting estrus behavior for a period of 30 d and could be administered repeatedly as desired to maintain reproductive quiescence or anestrus behavior. The altrenogest LA150/1.5 mL was also very effective and would be valuable when shorter periods of estrus suppression (12 to 14 days) are desired such as in transitional mares to establish normal cycles or for estrus and ovulation synchronization programs where the degree of estrus and ovulation compare favorably with daily altrenogest treatment (Squires et. al., 1992; or progesterone + estradiol treatments Burns et. al., 1993. Lastly, the results confirm the observations of McKinnon et. al., 2000 that medroxyprogesterone acetate treatment is not effective for the suppression of estrus or ovulation in mares.

The results from a recent preliminary study also suggest that weekly treatment with BioRelease Altrenogest (225 or 450 mg) are very biocompatible and effective at maintaining pregnancy in prostaglandin treated mares for a period of at least 9 days with no significant differences between the two doses studied (Morrow and Burns 2007). However, based on our extensive clinical experience with BioRelease P4 LA 150 or 300 for pregnancy maintenance in recipient mares we would recommend the use of natural progesterone BioRelease formulations until additional larger studies with BioRelease Altrenogest LA 150 have been completed. Since progestin-treated non-cyclic mares are frequently used as embryo recipients in large commercial programs, the new once weekly controlled release formulations are advantageous in such programs because of the reduced labor and the associated handling stress to the animals and producers. Furthermore, such formulations offer veterinarians an important means of maintaining effective compliance rates on farms with wide varieties of management systems.

GnRH / GnRH Analogs - Induction or hastening of ovulation in mares has been a major goal of researchers for many years. The discovery that human chorionic gonadotropin given to mares would shorten estrus and hasten ovulation (Loy and Hughes, 1966; Sullivan et al., 1973; Voss et al., 1975) led to its widespread use, even though repeated usage is associated with decreased response and antibody formation (Roser et al., 1979; Wilson et al., 1990). The ability of GnRH (Ginther and Wentworth, 1974) or its analogs (Squires et al., 1983; Harrison et al., 1991) to stimulate LH release led to research into their use for induction of ovulation, as their small size renders them less antigenic. However, a single injection of GnRH or its analogs produced inconsistent results (Irvine et al., 1975; Wallace et al., 1977; Ginther, 1992), and injections every 12 hours, although effective (Harrison et al., 1991), are generally considered impractical.

Recently, deslorelin, a potent GnRH analogue delivered via Ovuplant™, a short term release implant (Jochle and Trigg, 1994) or via SABER Mate E which uses the SABER Delivery System, (Burns et al., 1997; Fleury et al., 1999) have been shown to consistently advance ovulation within 48 hours in estrus mares having follicles 30 to 40mm in diameter.

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However, in its first two years of commercial use, anecdotal reports suggest that mares with Ovuplant-induced ovulation can experience delayed return to estrus and a prolonged interovulatory interval. Delayed return to estrus and prolonged interovulatory intervals have also been reported after PGF_{2α}-induced luteal regression commonly used in embryo transfer programs when Ovuplant was used (Johnson et. al., 2000).

In a non-controlled field study, Morehead and Blanchard (2000) reported that mean interovulatory intervals were longer for Ovuplant treated mares than for untreated or hCG treated mares ($P < 0.01$). Eighty percent (80%) of Ovuplant treated mares had interovulatory intervals of 18 to 25 days, and 19% had interovulatory intervals > 25 days. Ninety seven percent (97%) of untreated or hCG treated mares had interovulatory intervals of 18 to 25 days, and none had interovulatory intervals > 25 days. More Ovuplant treated mares had extended (> 25 days) interovulatory intervals than hCG or non-treated mares ($P < 0.05$). It was concluded that in this group of central Kentucky Thoroughbred mares, it appeared that factors such as season (month) and management (farm) had only minor effects on the incidence of extended interovulatory intervals following use of Ovuplant.

In contrast, studies (Burns et al., 1997, 1999a, 2000; Fleury et al., 1999,) with SABER Mate E revealed no extended interovulatory intervals, even at higher 3X, 5X and 10 X doses. The reason for the difference in response of mares to Ovuplant or SABER Mate E may be related to the deslorelin release rate in the two products. The SABER formulation released nearly twice as much deslorelin in the first 24 hours compared to Ovuplant (Burns et. al., 1997). Moreover, blood levels of deslorelin in mares given the SABER formulation had returned to base line by 24 hours post treatment, while blood levels of deslorelin in Ovuplant-implanted mares remained elevated compared to the saline group at 24 and 36 hours ($P < .05$) (Burns et al., 2000). This suggests that continued deslorelin release past 24 hours might increase interovulatory intervals and lead to hyposcretion of LH and FSH as observed by Johnson et al., (2000). In support of this concept, Farquhar et al (2001) have observed that if the Ovuplant implants were removed at 48 hours post implantation the increased interovulatory interval in mares short-cycled with PGF_{2α} could be prevented.

In recent years, a new compounded deslorelin formulation containing 1.5 mg per/mL has become available to veterinarians by prescription. The formulation BioRelease deslorelin (BET Pharm, Lexington, Ky. (www.Betpharm.com) has been reported to effectively induce ovulation in cyclic mares with follicles $>$ than 35 mm. Burns, (2007) recently reviewed six previous studies conducted to compare the effectiveness of a new drug delivery system (BioRelease) for administering the GnRH analogue and the effects of dose on interval to ovulation and fertility in mares (see tables below). A total of 1004 ovulatory mares from 4 controlled studies and 2 respective field studies were used. One of four doses (0.5 1.0, 1.5 or 2.0 mg) of deslorelin were given intramuscularly as a single injection.

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Table1. Ovulation Data			
TREATMENTS (mg)	Hours to Ovulation	Mares Ovulating by 48 hours (%)	
CONTROLS			
0mg	100	1 /29	7%
0 mg (n=12) Fleury et al 2004	104 (30 to 35mm)	NA	
0 mg (n=12) Fleury et al 2004	81 (>35 mm)	NA	
Ovuplant Berezowsk 2003	45.6	61/66	92.6%
hCG Berezowsk 2003	52.8	24/29	83%
hCG Brei and Burns 2005 2500 IU-IV	51	33/37	89%
hCG KY Field Study 2006 2500 IU-IV	NA	68/85	80%
BioRelease DES Formulations			
0.5 mg (n=12) Fleury et al 2004	49.0 (30 to 35mm)	NA	
0.5 mg (n=12) Fleury et al 2004	45.8 (>35 mm)	NA	
0.75 mg Kolling and Allen 2005	41.9	138/139	99%
1.0 mg Fleury et al 2004	44.8 (30 to 35mm)	NA	
1.0 mg Fleury et al 2004	40.8 (>35 mm)	NA	
1.0 mg Fleury et al 2003	44.1	23/25	92%
1.5 mg Berezowsk 2003	48.0	16/17	94%
1.5 mg Fleury et al 2003	49.8	25/27	93%
1.5 mg Kolling and Allen 2005	44.0	192/196	98%
1.5 mg Brei and Burns 2005	47.7	106/110	96%
1.5 mg KY Field Study 2006	NA	335/428	78%
2.0 mg Fleury et al 2003	48.7	23/25	92%
1.5 mg AVERAGE		674/778	86.6%
OVER ALL AVERAGE		858/967	88.7%

Controls were injected with saline hCG, Ovuplant or CPE (Equine Pituitary Extract, INRA). All injections were given during estrus when ovarian follicles had reached a diameter of 30 to 40 mm as determined by ultrasound. The interval from injection to ovulation was also determined by ultrasound. The percent of mares ovulating within 48 hours of injection were compared among the groups in some studies. Only 7% of saline control mares ovulated within 48 hrs and averaged 80 to 104 hours. In comparison 88.7% of mares in the treated groups ovulated within 48 hours.

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There were no observed differences related to dose in the three studies that examined dose. The 1.0 mg dose was as effective as the 2.0 mg dose in one study, 0.75 was similar to 1.5 mg in another and 0.5 was as effective as 1.0 in another. In that study mares bred with follicles 30 to 35 mm ovulated 5 hours later than mares with follicles > 35 mm. Although not statistically examined mares treated in a large clinical field study seemed not to respond as well as mares in the controlled studies with a 78% ovulation response within 48 hours compared to 92 to 99% in the other studies. Mares in this study included all mares bred over the entire breeding season and probably included some transitional mares. Lower responses (80%) with hCG were also observed in this study. Our clinical experience over the last four years strongly suggest lowered efficacy when treating late transitional mares prior to the establishment of normal cycles for all ovulatory induction drugs. Recently, McKinnon (2006) has suggested that higher ovulatory rates were observed using 1.5 mL (2.25 mg BioRelease deslorelin) than with the standard 1 mL 1.5 mg dose early in the year. This observation deserves additional study.

In the two studies that examined fertility (Table 2) the BioRelease deslorelin formulations appeared to consistently achieve higher fertility 13%; compared to hCG in the field study and 15 to 30% higher embryo recovery rates in the other study.

Table 2 Mare Fertility Data	
TREATMENTS	Embryo Recovery Rates (ER) or Pregnancy Rate/(PR) Cycle
CONTROLS	
CPE (Crude Equine Pituitary Extract LH 20mg)	37.5% 15/40 ER
Ovulplant Kolling and Allen 2005	53% 26/49 ER
hCG Kolling and Allen 2005 3000 IU-IV	55% 44/80 ER
hCG <i>Kentucky Field Trial Study</i> 2500 IU-IV	49% in 85 Mares Bred Total Cycles Not reported PR
BioRelease DES Formulations	
TREATMENTS (DES mg)	Embryo Recovery Rates (ER) or Pregnancy Rate/(PR) Cycle
0.75 mg Kolling and Allen 2005	68.5 % 63/92 ER
1.5 mg Kolling and Allen 2005	69.2 % 126/182 ER
1.5 mg <i>Kentucky Field Trial Study</i>	62.0 % in 428 Mares Bred Total Cycles Not reported PR

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These results demonstrate that BioRelease Deslorelin can be used to advance fertile ovulation in estrous mares. No adverse reactions were reported in any of the six studies.

Estrogens

Several studies have indicated that the susceptibility of the equine uterus to endometritis is influenced by ovarian steroids. Mares under estrogen influence are more capable of eliminating uterine infection than mares under the influence of progesterone (Ganjam, et. al., 1982; Evans et al., 1986, 1987). In addition, treatment with daily injections of 15mg estradiol benzoate, initiated early in estrus and continuing until day 6 post-ovulation appeared to assist in eliminating bacterial endometritis from subfertile mares with a history of susceptibility to repeated uterine infections (Bracher et. al., 1991). This suggests that estrogen treatment may improve chances for conception in mares susceptible to endometritis. Treatment of mares with estradiol (5 to 10 mg/day) during estrus results in enhanced uterine tone following ovulation, especially in mares with enlarged baggy reproductive tracts (Burns, 1993). Cook et. al., (1991) reported that estradiol microsphere treatment with doses as high as 100 mg had no effect on fertility in mares during treatment (75% vs 75% in vehicle treated controls). Kelly et al., (2006) reported that 10 mg of estradiol benzoate given every other day was able to stimulate prolactin and LH in anovulatory and cyclic mares when combined with sulpiride or domperidone. This dose may be of clinical significance in that it had no suppression of follicular growth or other cyclic endpoints as are observed with higher estrogen doses (Burns and Douglas, 1981).

Douglas (2004) reported higher foaling rates in estrogen + antibiotic treated mares assumed to be affected by placentitis compared to similar mares receiving only antibiotics (see table)

<i>Group</i>	<i>Number of mares</i>	<i>Mean ± SD progestins at first exam (ng/mL)</i>	<i>Mean ± SD estrogen at first exam (pg/mL)</i>	<i>% Live foal rate</i>
No estrogens	20	19.3 ± 5.0 ^a	600 ± 200 ^a	20 ^a
# Estrogen Treatment	50	21.0 ± 6.3 ^b	660 ± 191 ^b	70 ^b
Controls	70	8.7 ± 1.4 ^b	1800 ± 256 ^b	87 ^c

Estradiol cypionate (ECP) or estradiol 17 β at a dose rate of 10 to 20 mg sid or bid was given intramuscularly.

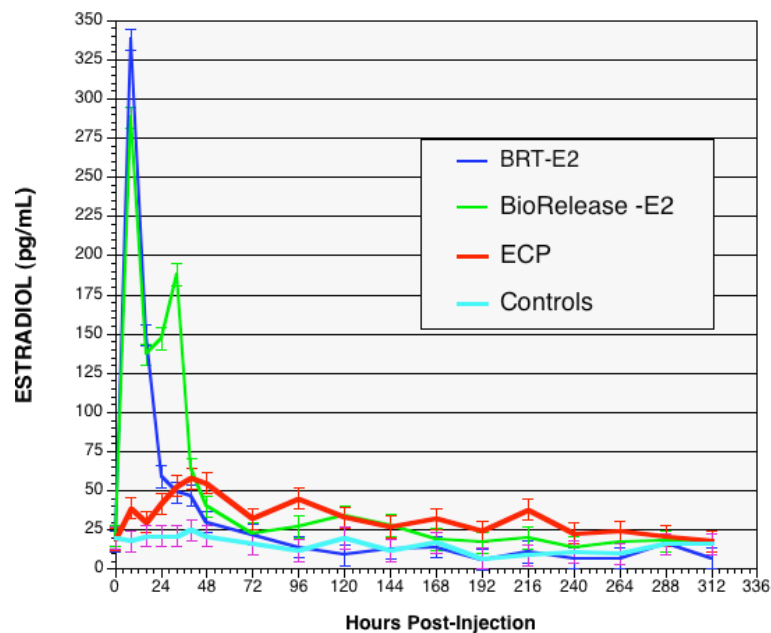
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Total estrogens were higher and progestins were lower ($P < 0.05$) in controls delivering live foals than in mares suspected to have placentitis. Means within a column with different superscripts differ significantly. As would be expected, mares in the two placentitis groups had higher progestins and lower estrogens than controls. Mares receiving estrogen therapy had higher live foal rates than mares receiving no estrogen therapy. Both placentitis groups had lower live foal rates than controls

It was also reported that mares presenting with an estrogen level of 300 pg/ml or less had less than a 15% live foal rate irrespective of what therapy was initiated. It was noted that in general serum samples do not always reflect a significant elevation in estrogens in mares treated with 10 to 20 mg of estrogens IM suggesting that higher doses of exogenous estrogens may be indicated.

Pinto and Burns (2004) compared various estrogens in terms of serum estradiol concentrations following a single IM injection of a 50mg dose of Biorelease Estradiol cypionate (ECP) vs. two different E2-17 β formulations delivered in a proprietary long acting vehicle (www.BetPharm.com). Results are shown below.



The results illustrate that serum concentrations of E2-17 β were ten-fold higher in mares treated with BioRelease E2-17 β compared to BioRelease Estradiol cypionate treatment. Moreover, concentrations were elevated above 50 pg/mL for either one or two days depending on the formulation used. The BioRelease estradiol formulation at doses between 20 and 100 mg /day (based on estradiol blood levels) along with appropriate antibiotic therapy appears to be clinically effective in pregnant mares with placentitis.

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Pituitary Extracts / eFSH - The lack of a commercially available source of equine pituitary extract (EPE) or purified equine FSH has been a major factor in the high cost of equine embryo transfer programs. Because embryo recovery per cycle from a donor mare is approximately 50% per cycle and pregnancy rate per embryo transferred is approximately 50 to 70% there is a 25 to 35% chance of obtaining a pregnancy from the donor each cycle. However, if multiple ovulations could be induced, which result in 2 or 3 embryos collected per cycle, then the efficiency of embryo transfer would be greatly improved.

As mentioned earlier, a commercial, lyophilized, reagent preparation that contains eFSH activity equivalent to 25 mg B-FSH-ReF-001 Standard, manufactured by Bioniche Animal Health USA, Inc., Athens, GA 30601 has become available. In a preliminary evaluation study, Alverenga and Squires (2003) reported an embryo recovery rate of 50% from mares during the non-treated control cycle. However, after eFSH treatment of the same mares, 12 of 16 mares (75%) provided an embryo. The average number of embryos was 1.9, which is nearly a 4-fold increase in embryo recovery. Embryo recovery was 50% per ovulation.

In a second experiment (Niswender et al., 2003a), mares were treated with eFSH (12.5 mg) plus hCG or Ovuplant. The mares treated with eFSH and hCG had 3.4 ovulations and 1.8 pregnancies per mare vs 1.8 ovulations and 0.8 pregnancies for mares treated with eFSH and Ovuplant. The Control mares provided 1.1 ovulations and 0.6 pregnancies per mare. A significantly greater number of pregnancies per mare was noted when hCG was used to induce ovulation in combination with eFSH. In summary, the commercial preparation of eFSH from Bioniche Inc. appears to function well using 12 mg injected twice daily plus hCG used to induce multiple ovulations.

Prostaglandins - Estrus can be controlled by shortening the luteal phase with single or multiple injections of prostaglandin F₂ α (Douglas and Ginther, 1972,1974; Squires et al., 1981). Prostaglandins are probably the most widely used drug for control of the mare's cycle. However, because of the many other tissues sensitive to PGF₂ α , side effects including sweating, diarrhea, pelvic muscle spasms and increased heart rate are frequently associated with prostaglandin treatment. Such side effects often cause client concern over the safety and comfort of their mares even though serious side effects are rare. In an attempt to reduce the side effects Irving et al., (2000) demonstrated that the effective dose of Lutalyse (Pharmacia Upjohn, Kalamazoo, MI) could be reduced 10 fold if given as 2 injections 24 hours apart. Two treatments resulted in luteolysis in 10 out of 10 mares and side effects were greatly reduced: heart rate change (P< 0.0001); sweating (P=0.0014 and pelvic muscle spasms (P=0.0002). The authors concluded that by mimicking the endogenous release pattern of PGF₂ α secretion with repeated surges at 24 hours intervals, the effectiveness of the very low 0.5 mg doses was greatly enhanced and treatment side effects were significantly reduced.

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Alvarenga et al., (1998) examined the luteolytic effectiveness of using low or micro doses of PGF_{2α} injected at the (BAI HUI) acupuncture point. This point is located at the sacral lumbar space and is frequently used to treat ovarian disturbances in Veterinary Acupuncture. The results indicated that Lutalyse, (Pharmacia Upjohn, Kalamazoo, MI) given at a very low micro dose of 0.5 mg (one tenth the conventional recommended dose) administered at the BAI HUI acupuncture point was equally effective at inducing luteolysis in mid-luteal phase mares as the conventional 5 mg i.m.dose. Although not critically examined, the micro dose of PGF_{2α} administered at the (BAI HUI) acupuncture point also appeared to reduce treatment related side effects.

Recently, a new compounded prostaglandin formulation containing Fluprostenol (50 ug /mL) has become available to veterinarians by prescription. Clinical impressions on the product's effectiveness appear to be similar to Lutalyse in over 600 mares treated in Texas and negative side effects were not observed in treated mares.

Equimune (MCWE) - The inflammatory reaction to sperm and cellular detritus following breeding or insemination is considered a normal physiological reaction by the mare's endometrium. Normal mares are able to clear this inflammation within 48hrs, become pregnant and carry a full term pregnancy; other mares (susceptible mares) frequently fail to resolve these processes and fail to become pregnant. This condition appears to become chronic in many susceptible mares. LaBlanc et al. (1995) noted that the vaginal exudate from these mares does not always contain bacteria. This condition has been explained as a lack of lymphatic drainage, due to defective myometrial contractions. Recently, Fumuso et al. (2000) examined the effect of 1500 mcg of *Mycobacterium* Cell Wall Extract given intravenously, (Equimune® I.V. BIONICHE Animal Health) on post-breeding uterine inflammation in both normal and susceptible mares. The results indicated administration of Equimune significantly reduced the post-AI inflammation, in both normal mares and susceptible mares and Equimune treatment dramatically reduced persistent inflammation during diestrus, in susceptible mares.

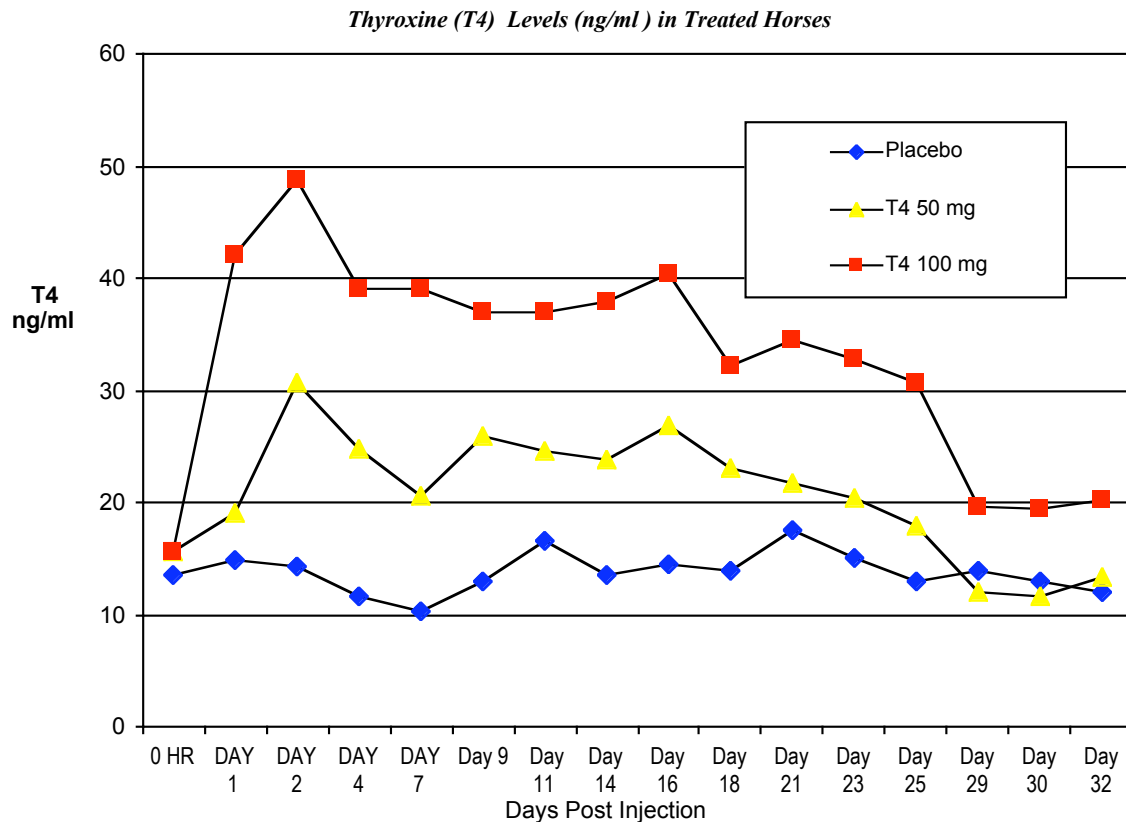
Thyroid Hormones – Thyroxine (T4) - An accurate figure for the incidence of equine hypothyroidism is difficult to estimate but has been reported to be high, especially in young horses and broodmares (Irvine and Evans 1975, Chen and Riley 1981). Lowe et al., (1974) failed to observe reproductive problems in thyroidectomized mares. However, many practitioners continue to report clinical improvements in fertility after thyroid hormone supplementation. Nachreiner and Hyland (1993) advocate using baseline Total T3 and T4 as the most economical and practical diagnostic approach and suggest 5 to 10 mg L-thyroxine per 500 KG body weight per day as a good starting dose if thyroxine replacement is initiated. Because many non-thyroid-related illnesses and drugs can depress thyroid hormone levels, care should be taken to rule out false diagnosis of hypothyroidism. Drugs affecting thyroid hormone levels include phenylbutazone and glucocorticoids, management practices such as stall confinement, shipping or a sudden change in routine can also suppress T4 levels.

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Also, dietary factors such as excessive carbohydrates and endophyte infected tall fescue pastures or pastures containing goitrogenic factors such as thiocyanates and perchlorates can cause problems. Diets high or low in iodine can alter thyroid levels and selenium deficiency alters conversion of T4 to T3. Lastly, chronic conditions such as Cushings disease, laminitis and gastric ulcers can lead to lowered T4 levels.

Recently, we have investigated the possibility of using biodegradable lactide-glycolide microparticles to deliver thyroxine for a period of thirty days. Preliminary results (see below) suggest that a 30 day controlled release T4 formulation is possible.



Cushing's Disease – Pergolide, Cyproheptadine or Trilostane - Equine Cushing's Disease (ECD), also known as pituitary pars intermedia dysfunction (PPID) is one of the most common equine endocrinopathies of older horses and ponies. The disease results from the loss of dopaminergic inhibition of the pars intermedia of the pituitary resulting in increased synthesis and secretion of pro-opiomelanocortin derived hormones leading to a state of hyperadrenocorticism. Clinical signs include hirsutism, muscle wasting, polyuria, polydipsia, laminitis, lethargy, pseudolactation, decreased fertility, and loss of immune resistance to infections (Love 1993; Douglas 1999).

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Diagnosis of PPID can be confirmed by numerous methods including an overnight dexamethanone suppression test, plasma ACTH levels, plasma glucose and insulin levels (Beach, 1999; Donaldson et al., 2002). Douglas (1999), recommends a test that examines the cortisol rhythm along with Insulin and T4 (2 samples 8 to 10 hours apart. No grain is given within 4 hours before either sample (protocol at www.betlabs.com). This test is based on observations that the normal diurnal cortisol rhythm is lost in horses with PPID (Dybdal et al 1994). More recent data suggest a combination of the low dose dex and the cortisol rhythmicity test may be more powerful in confirming the presence of PPID.

Proposed treatments for ECD include the dopaminergic agonists pergolide or the serotonin antagonist cyproheptidine (Dybdal 1997). Donaldson et al (2002) reports that pergolide appears to be more effective than cyproheptidine for treating ECD. Recently, McGowan and Neiger (2003) have reported that Trilostane, a competitive 3-beta hydroxysteroid dehydrogenase inhibitor can be used to control clinical signs of ECD in horses without side effects. In practice, a combination of pergolide and cyproheptidine is more effective than either drug used alone. Trilostane is not readily available in the USA.

Summary and Conclusions

The future offers significant opportunities to improve reproductive performance within the equine industry. This review has attempted to address traditional and new pharmacological treatments for reproductive management in mares, with special reference to the benefits of new biodegradable drug delivery systems. The benefits include the ability to integrate the administration of estrous control products around farm and veterinary schedules, the reduction of distress to the animals by decreasing the number of times they must be handled and the financial benefits realized from effective hormone therapy. Effective hormone therapy requires selection of an appropriate drug delivery system or treatment, which should be based on the drugs pharmacokinetic and pharmacodynamic properties to achieve the best results and avoid potential negative side effects. The success of any therapeutic or production drug is dependant on the compliance of farm personnel for administering prescribed treatments to obtain successful results. Veterinarians and breeders will find that biodegradable drug delivery systems offer an important means of maintaining effective compliance rates on farms with wide varieties of management systems.

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