

ENDOCRINE DIAGNOSTICS IN THE BROODMARE: WHAT YOU NEED TO KNOW ABOUT PROGESTINS AND ESTROGENS

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INTRODUCTION

Diagnostic endocrinology in equine practice has been routinely used for over 20 years. By far the most common assay is for progesterone. It is important to distinguish between progesterone, progestins and progestagens since the difference is not recognized by most veterinary practitioners. It should be noted that the metabolites of pregnenolone (P5) and progesterone (P4) are usually referred to as progestagens. Progestins will be used in this paper to refer to progesterone and its metabolites. It is recognized that the assay used in the present study may also measure some fraction of pregnenolone and therefore a more correct term may have been progestagens.

The assay data presented in this manuscript were generated by radioimmunoassay using the same source of kits (Diagnostic Products Corporation, Los Angeles, California) by BET Reproductive Laboratories in Lexington, Kentucky, USA. All serum samples were assayed in duplicate and were from mares of various breeds submitted to the lab by veterinarians throughout the USA. This lab routinely performs over 120 assays per day for progestins in broodmares during the peak of the breeding season and 25 assays per day for estrogens between November and March.

The purpose of this paper is to provide both a data set for levels of progestins and estrogens from a non-research mare population during pregnancy and examine their relationship in mares with suspected placentitis.

The radioimmunoassay kits used are for progesterone and estradiol -17 β . It should be noted that the antibody used in this progesterone kit cross reacts with progesterone metabolites and thus the kit can be used to measure progestins as well as progesterone. Similarly, the antibody used in the estradiol kit cross reacts with both conjugated and nonconjugated estrogens therefore data presented in this study are discussed as total estrogens

PROGESTINS

In the pregnant broodmare progesterone is present only through 150 days of pregnancy and thereafter the predominant progestins are 5 α -pregnanes and 17 α -hydroxy progesterone. In 1975 Holtan (1) reported that progesterone became very low dropping to levels below 2ng/ml by 180 days of pregnancy remained low until 30 days prior to parturition at which time levels rose dramatically until the mare foaled. By 1990, Holtan and co-workers (2) found that by using more specific assay techniques including gas chromatography and mass spectrometry, progesterone was not detectable during the last half of pregnancy however progestins were detectable during this time and consisted predominantly of 5 α -pregnanes. These progestins were actually what was rising prior to parturition. Recently it has been suggested that 5 α -pregnane-3, 20-dione (5 α DHP) may be more biologically important than progesterone in late gestation in the

mare (3). This importance is related to its greater binding by uterine P4 receptors than P4 or other progestagens and that its concentration rises during the last 20 to 30 days of gestation falling rapidly during the last few days before birth (4). It is also a major precursor for other progestagens. Interestingly, 5 α DHP concentrations were elevated by exogenous Regumate (altrenogest) but not by P4 in oil in late gestation in mares (5). Species crosses such as in jennies pregnant with a hinny fetus have significantly higher serum progesterone than horse mares carrying a horse fetus. Mares pregnant with a mule fetus appear to have progesterone concentrations similar to mares pregnant with a horse fetus (6). Progestin data in this paper are from mares carrying horse fetuses.

Assaying serum levels has generated all data in the literature regarding progestins in mares. However, currently in human monitoring programs, especially in post-menopausal women, the focus is on salivary levels of progesterone and other steroids. The hypothesis is that a salivary level may represent the delivery of the hormone to tissue membranes whereas serum concentrations measure the quantity of hormone not yet delivered to the tissue (7). Apparently, salivary progestins have not been measured in mares and compared to serum concentrations.

ESTROGENS

Since 1930 it has been known that pregnant mare urine and blood had very high levels of estrogenic substances (8). In 1971, the first paper describing urine tests for pregnant mares was reported (9). It is now known that serum and urinary estrogens during pregnancy in the mare exhibit identical patterns. Estrogens during pregnancy in the mare, like progestins, take many forms. Basically, they are either conjugated to sulfates (example estrone sulfate) and represent the bound fraction or they are unbound free estrogens such as estrone and estradiol. Terqui and Palmer (10) reported a 100 times greater concentration of conjugated estrogens than free estrogens. This subject has been previously reviewed (6). In general, the predominant estrogens during pregnancy in mares in order of magnitude are estrone, equilin, equilenin, estradiol-17 β and estradiol-17 α . A report describing the use of plasma equilin and estrone in the assessment of fetoplacental function in the mare appeared in 1977 (11). Subsequently in 1988 a report from the University of California at Davis team lead by Stabenfeldt and Hughes (12) data on the use of serum estrone sulfate as an indicator of fetal demise in horses was published. BET Labs has been routinely performing assays of total estrogens using the DPC estradiol-17 α kit to both assess pregnancy status and fetal viability after 100 days of pregnancy since 1985 for the field veterinarian. It should be remembered that this particular assay system does not have adequate sensitivity to distinguish between ovarian estrogens and fetal-placental estrogens before day 100.

MATERIALS AND METHODS

Data for progestins and total estrogens were taken from results of assays of serum samples submitted to BET Labs during one year using the DPC kits described above. These data are used to provide the graphic display of these two hormones during pregnancy in the mare and represent a widely available methodology for clinical use. Thus, the data represent normal concentrations of these two hormones during pregnancy and can serve as reference

values for both researchers and clinicians. Appropriate data analyses were used to detect statistical differences.

RESULTS

Data for mean progestin concentrations during the first 30 days of pregnancy in mares are shown in Table 1. As can be seen from the table, most samples for progestin determinations are taken around the time of the first ultrasound examination between days 14 and 18 post-ovulation.

Table 1. Mean progestin concentrations during the first 30 days of pregnancy

<i>Days of pregnancy</i>	<i>No. of mares</i>	<i>Mean</i>	<i>S.E.M.</i>
3	2	7.35	0.45
4	4	4.42	1.62
5	39	6.94	0.45
6	30	7.01	0.62
7	46	6.48	0.45
8	5	8.40	1.4
9	2	5.75	1.50
10	21	5.23	0.54
11	25	6.28	0.59
12	36	5.40	0.44
13	28	5.37	0.41
14	123	5.45	0.25
15	238	6.6	0.19
16	116	5.23	0.30
17	73	4.82	0.34
18	85	5.2	0.36
19	36	6.61	0.82
20	42	4.91	0.43
21	33	4.99	0.51
22	16	3.94	0.67
23	30	6.17	0.59
24	19	4.45	0.76
25	22	6.54	1.70
26	11	5.28	1.33
27	6	4.48	0.83
28	27	4.59	0.61
29	10	3.89	0.82
30	67	4.95	0.46

Placentitis is the single greatest cause of pregnancy loss, stillbirth and premature foals in the USA (13,14). It is the authors experience that endometritis is the greatest cause of low progesterin concentrations during the first 30 days of pregnancy. Data in table 2 compare pregnancy rates in mares with low vs. normal progesterin levels on day 12 post-ovulation. Rates were almost double when progesterins were above 2.5ng/ml. Approximately 85% of mares that had low concentrations of progesterins at day 12 were positive for pathogenic bacterial when uterine lavage effluents were cultured (15).

Table 2. Relationship between serum progesterin (P4) concentrations on day 12 post- ovulation and pregnancy rates.

<i>Serum P4 concentrations</i>	<i>No. of mares</i>	<i>Pregnancy rates (%)</i>
P4 > 2.5 ng/ml	50	71 ^a
P4 < 2.5 ng/ml	25	37 ^b

Means with different superscripts are different.

Fig. 1. illustrates serum concentrations throughout days 5 to 90 of pregnancy in a total field sample of 1695 mares. The pattern of progesterins is similar to that reported in Ginther's review from research populations of small numbers of mares. As would be expected, progesterins rise after formation of accessory corpora lutea around day 45 of pregnancy. The range of progesterins throughout pregnancy as denoted by the two bold lines in the figure was between 4 and 10 ng/ml. It therefore can be assumed that values outside this range are abnormal for clinical diagnostic purposes.

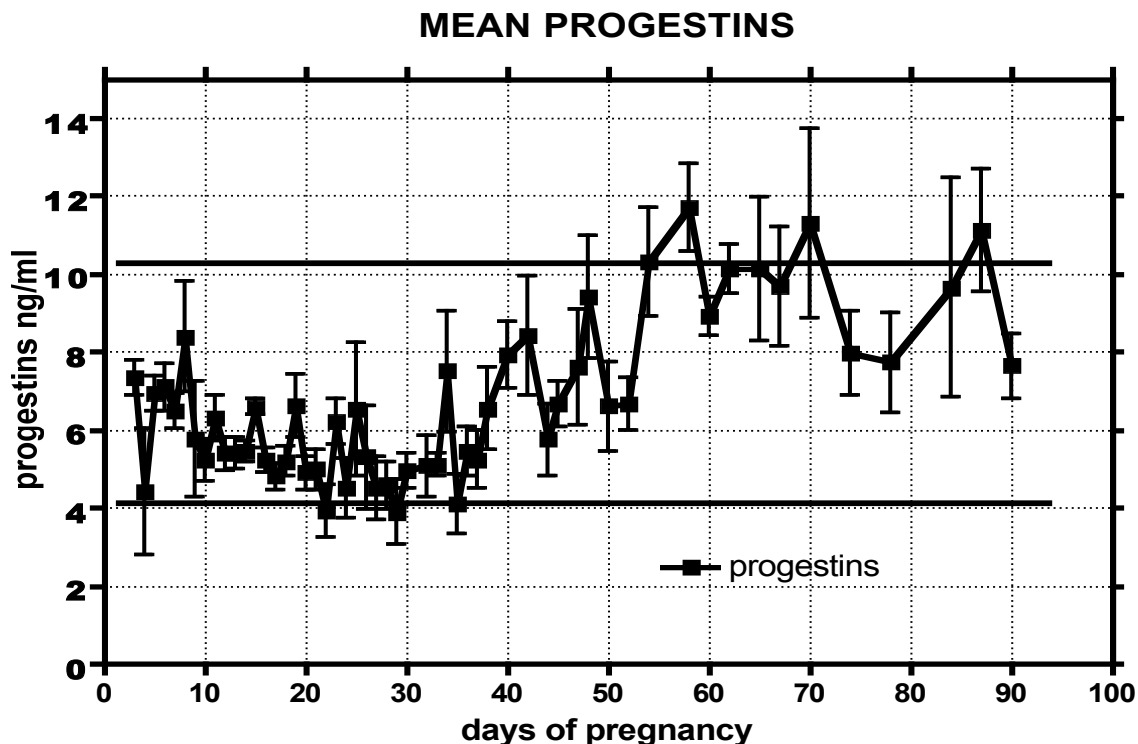


Fig. 1. Mean and SD serum concentrations of progestins during the first 90 days of pregnancy (n=1695 mares).

Progestin concentrations between days 90 and 340 of pregnancy are shown in figure 2. Progestins range between 4 and 10 ng/ml until day 300. Concentrations begin to increase at about 180 days of pregnancy reaching a peak mean of approximately 26 ng/ml by day 340. This likely reflects the increased production of 5α DHP and other 5α pregnanes. These patterns using the DPC radioimmunoassay kits are similar to those reported by others using radioimmunoassay in university laboratories and by other methods such as gas chromatography (6).

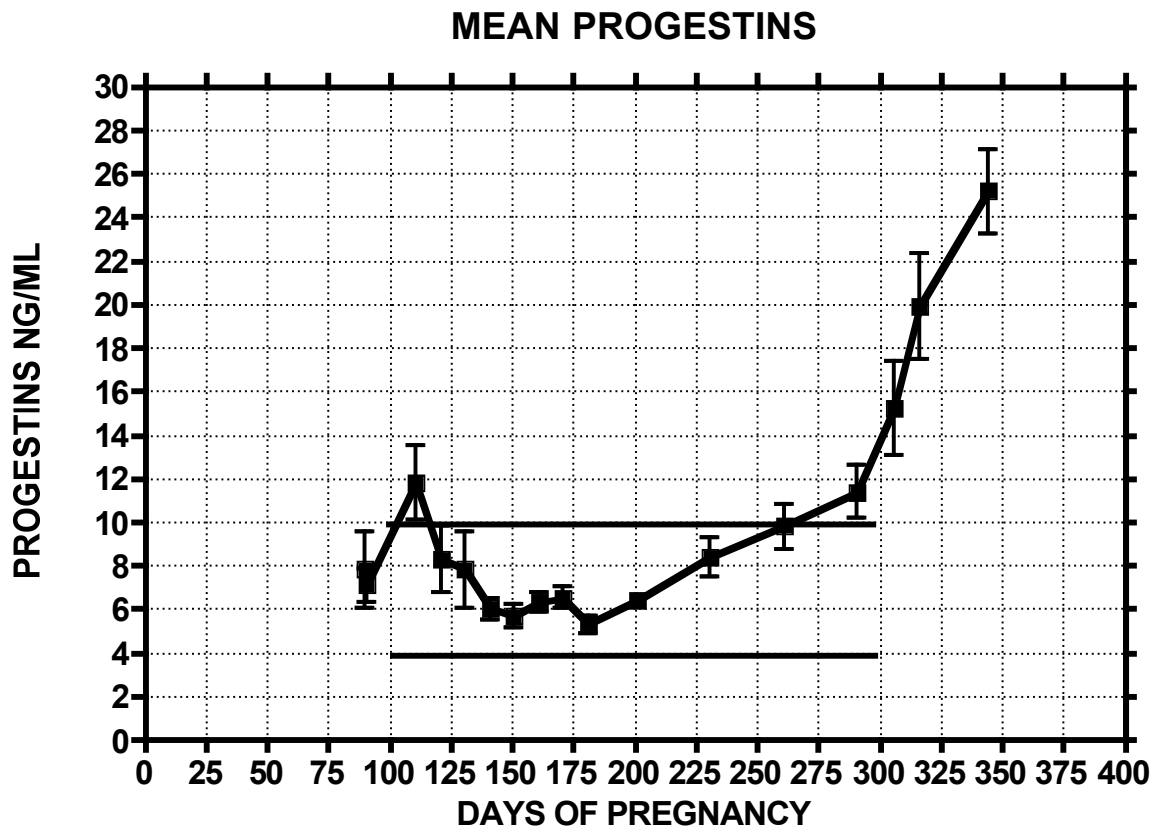


Fig 2. Mean and SD serum concentrations of progestins during days 90 to 340 of pregnancy (N=1,000 mares).

The mean concentrations of estrogens in mares pregnant between days 100 and 340 are shown in Fig. 3. It should be noted estrogen concentrations are reported in pg/ml whereas progestins are reported in ng/ml. After day 150 of pregnancy, mean concentrations remain above 1,000 pg/ml until approximately day 310 at which time estrogens fall below this level and are approximately 500 pg/ml at 340 days. In the present study, estrogens peak at about day 180 and begin to decline thereafter similar to results for various estrogens reported by others (6). The majority of the estrogens measured by the DPC assay kit are likely conjugated estrogens especially estrone sulfate.

After day 150, estrogen concentrations below 1,000 pg/ml are often associated with placentitis. Data for mares that were suspected to be affected by placentitis based on clinical findings by the

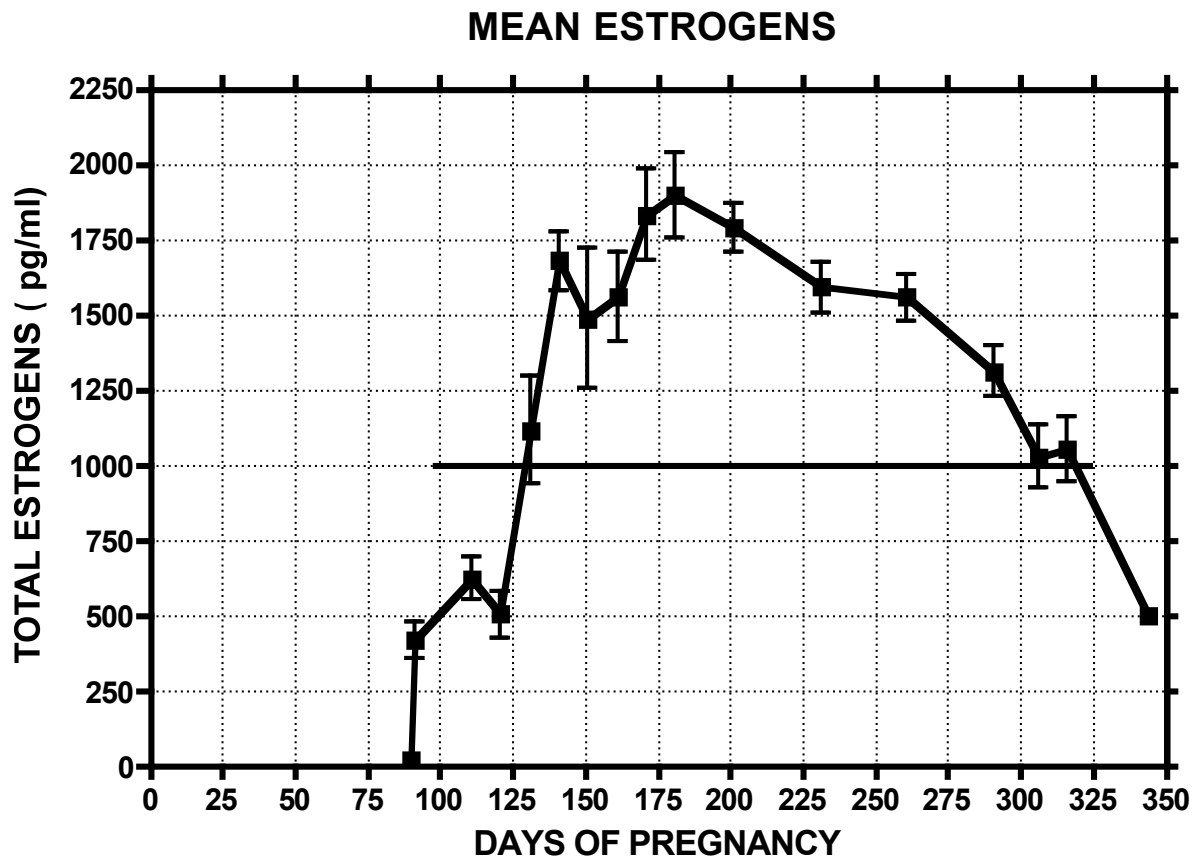


Fig. 3. Mean and SD of total estrogens after 100 days of pregnancy (n=695 mares). Normal values between day 150 and 300 exceed 1000 pg/ml.

attending veterinarian are presented in Tables 2 and 3. Hormonal results are based on a single blood sample taken at various times during the day.

Table 2. Relationship between progestins and total estrogens during days 150 to 280 of pregnancy in mares which aborted due to placentitis and mares which delivered live foals. Data are from a single blood sample from each mare.

<i>Group</i>	<i>Number of mares</i>	<i>Progestins ng/ml</i>	<i>Total estrogens pg/ml</i>
Aborting	45	20.1 ± 4.9 ^a	350 ± 150 ^a
Live foal	60	6.5 ± 1.1 ^b	1790 ± 683 ^b

It is important to recognize that between days 150 and 280 of pregnancy, placentitis usually results in higher progestins and lower estrogens when compared to normal mares. Thus, a single serum hormonal assay for these two hormones can add valuable information to enable the

attending veterinarian to confirm the presence of placentitis and indicate that both antibiotic and hormonal therapy may be needed.

Since a consistent finding in aborting mares diagnosed with placentitis was low estrogens, a group of mares suspected to have placentitis were treated either with estradiol cypionate (ECP) or Estradiol-17 β . All mares were given antibiotics deemed appropriate by the attending veterinarian. No progestins were given. Controls received no treatments and delivered live foals. Results are shown in Table 3.

Table 3. Progestins, estrogens and live foal rates in mares assumed to be affected by placentitis versus controls. All mares were between 160 and 280 days pregnant. Hormonal results are from a single blood sample taken on initial examination. The two groups with placentitis were treated with systemic antibiotics and with or without estrogens. Either estradiol cypionate (ECP) or estradiol 17 β at a dose rate of 10 to 20 mg sid or bid was given intramuscularly.

<i>Group</i>	<i>Number of mares</i>	<i>Mean \pm SD progestins at first exam (ng/ml)</i>	<i>Mean \pm SD estrogen at first exam (pg/ml)</i>	<i>% Live foal rate</i>
No estrogens	20	19.3 \pm 5.0 ^a	600 \pm 200 ^a	20 ^a
ECP or E217B	50	21.0 \pm 6.3 ^a	660 \pm 191 ^a	70 ^b
Controls	70	8.7 \pm 1.4 ^b	1800 \pm 256 ^b	87 ^c

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 has been the author's experience 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 should be 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 and suggests that higher doses of exogenous estrogens may be indicated.

A recent study was completed by Pinto and Burns (16) which compared various estrogens in terms of serum estradiol concentrations following a single IM injection of a standard 50mg dose

of ECP vs. two different E2-17 β formulations delivered in a proprietary long acting vehicle (BioRelease Technologies, Lexington, Ky, USA). Results are shown in Fig. 4.

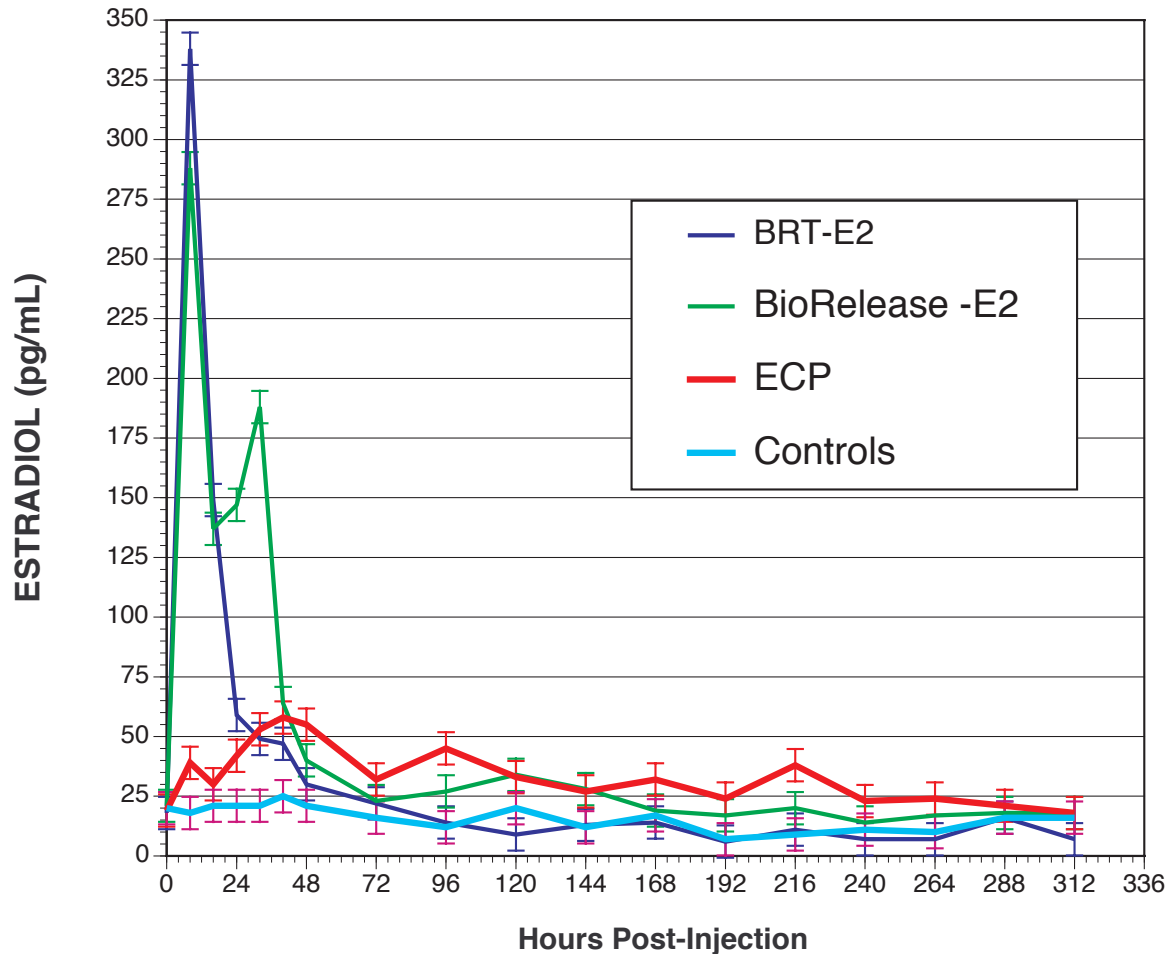


Fig. 4. Patterns of Estradiol-17 β (E2-17 β) concentrations following a single IM injection of ECP vs. long acting E2-17 β (BRT-E2 and BioRelease-E2).

Results illustrate that serum concentrations of E2-17 β were ten-fold higher in mares treated with long acting E2-17 β compared to ECP treatment. Moreover concentrations were elevated above 50 pg/ml for either one or two days depending on the formulation used. These long acting preparations have not been tested in mares with placentitis.

In studies using intra-cervical inoculations with bacteria to induce ascending placentitis in the mare, plasma progesterin concentrations rose in mares developing chronic placentitis and in mares after surgery (17). However, when the same authors measured only estradiol 17 β (a non conjugated estrogen) and not total estrogens, the plasma concentrations were not affected by placentitis. If the authors in the experimental model study had measured both conjugated and non-conjugated (total) estrogens, it is likely they would have seen the same drop in estrogens as observed in the field samples assayed in the present study.

In conclusion, serial serum assays using the DPC radioimmunoassay kits for progesterone and estradiol 17 β can be used by both researchers and clinicians to assess fetal well being after 100 days of gestation in the mare.

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