ELSEVIER

Animal Reproduction Science 40 (1995) 305–321

ANIMAL REPRODUCTION SCIENCE

Endogenous prostaglandin secretion during cloprostenol-induced abortion in mares

Peter F. Daels [a,*], Hussni Mohammed [a], S.M.E. Montavon [b], G.H. Stabenfeldt [b], J.P. Hughes [b], Kristina Odensvik [c], H. Kindahl [c]

[a] *Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*

[b] *Department of Population Medicine, School of Veterinary Medicine, University of California, Davis, CA 95616, USA*

[c] *Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden*

Accepted 7 July 1995

Abstract

Repeated administration of prostaglandin is the treatment of choice for the termination of pregnancy in mares more than 40 days pregnant. Even though it is well documented that PGF-2α or analogue needs to be administered every 12–24 h for successful induction of abortion, little is known about the underlying endocrine changes and the mechanism by which abortion occurs. The aim of this study was to characterize the changes in PGF-2α, progesterone and estrogen secretion during prostaglandin-induced abortion. Six mares, 82–102 days pregnant, were treated daily with 250 μg cloprostenol, blood was collected at 1-h intervals until fetal expulsion and pregnancy examination was performed daily. Four mares, 92–97 days pregnant, received no treatment but were subjected to the same hourly blood collections and daily genital examinations described for cloprostenol-treated mares for 3 days. Mean time from first cloprostenol administration until fetal expulsion was 48.6 ± 5.6 h and required 2.8 ± 0.2 cloprostenol administrations. In all mares, progesterone concentrations decreased in a near linear manner after the first cloprostenol administration and were invariably low (1.3 ± 0.2 ng ml^{-1}, mean $\pm$ SEM) at the time of fetal expulsion. Mean estrogen secretion remained unchanged until 5 h before fetal expulsion and then decreased rapidly to non-pregnant levels. Endogenous PGF-2α secretion rate increased with each cloprostenol administration and culminated in sustained PGF-2α secretion which persisted until fetal expulsion was completed. From these results we conclude that cloprostenol-induced abortion is

* Corresponding author. Tel: +1–607–2533845; Fax: +1–607–2533055; E-mail: pfd1@cornell.edu.

SSDI 0378-4320(95)01429-2

associated with endogenous PGF-2α secretion, fetal expulsion coincides with sustained PGF-2α secretion and low progesterone concentrations and plasma estrogen concentrations remain unchanged until hours before fetal expulsion.

Keywords: Mares; Prostaglandin; Cloprostenol; Abortion

1. Introduction

Veterinarians are occasionally requested to induce abortion in mares (e.g. twin pregnancy, unwanted matings). In most domestic species, methods for termination of pregnancy are based on the elimination of the source of progesterone. During the first 40 days of gestation in mares, pregnancy is maintained by progesterone secreted by the maternal corpus luteum and administration of a single luteolytic dose of prostaglandin F-2α (PGF-2α) or analogue is sufficient to cause complete regression of the corpus luteum and terminate the pregnancy (Douglas et al., 1974; Squires et al., 1980; Lofstedt, 1986; Ginther, 1992; Squires and Bosu, 1993).

Around Day 40 of gestation, chorionic girdle cells develop into endometrial cups which secrete equine chorionic gonadotropin (eCG) from around Day 40 until Days 100–150 of gestation (Allen and Stewart, 1993). The luteotropic action of eCG is believed to stimulate continued steroid secretion by the primary corpus luteum and the development of secondary (supplementary) corpora lutea on the maternal ovaries (Ginther, 1992; Allen and Stewart, 1993). After 2 months of gestation, the feto-placental unit contributes to the circulating pool of progestins (mainly 5α-pregnanes) and estrogens (mainly estrone sulphate). From approximately Day 70 of gestation, the maternal corpora lutea are no longer necessary for maintenance of pregnancy, even though maternal corpora lutea continue to secrete progesterone until Days 100–150 of gestation. Eight out of 14 mares remained pregnant following ovariectomy on Day 70 of pregnancy (Holtan et al., 1979). All of five mares ovariectomized on Day 20 and treated with a progestogen (altrenogest) until Day 70 remained pregnant even though exogenous progestogen supplementation was discontinued (Daels et al., 1990). In another study, four pregnant mares, in which the corpus luteum had regressed due to experimentally induced endotoxemia, remained pregnant after termination of altrenogest supplementation on Day 70 of gestation despite the absence of active luteal tissue on the maternal ovaries (Daels et al., 1991).

After the second month of gestation, multiple administrations of prostaglandin, either at 12- or 24-h intervals, are required to induce abortion in mares (Lofstedt, 1986; Voller et al., 1991; Ginther, 1992; Squires and Bosu, 1993). In one report, a single injection of a luteolytic dose of a prostaglandin analogue on Days 70 and 77 of gestation did not result in abortion in eight of eight pregnant mares even though progesterone concentrations dropped to levels less than 2 ng ml^{-1} in some mares (Squires et al., 1980). The same authors reported that prostaglandin administered every 12 ($n = 8$) or 24 ($n = 8$) h starting on Day 70 terminated pregnancy in all mares (Squires et al., 1980). The interval from first injection to abortion was 4–5 days regardless of whether prostaglandin was administered once or twice daily. In a more recent report, four mares were administered

a luteolytic dose of prostaglandin F-2α (PGF-2α) on Days 65 and 75 of gestation while receiving daily altrenogest supplementation (Voller et al., 1991). Mares remained pregnant and PGF-2α administration caused only partial regression of the maternal luteal tissue, as indicated by a transient decrease in progesterone concentrations. In the same study, five pregnant mares received daily altrenogest supplementation and were injected every 5 days with a luteolytic dose of prostaglandin F-2α. In these mares, the primary corpus luteum regressed after the first PGF-2α administration on Day 10 of pregnancy. However, secondary corpora lutea did not consistently regress after PGF-2α administration and it was concluded that PGF-2α failed to induce complete luteolysis due to the presence or development of immature secondary corpora lutea which were unresponsive to PGF-2α. It is also possible that the failure to induce complete luteolysis is due to the luteotropic effects of eCG. It has been reported that in-vitro luteinized human granulosa cells pre-incubated with high concentrations of hCG fail to respond to the luteolytic agent, cloprostenol (McNatty et al., 1975; Michael and Webley, 1991; Webley et al., 1991). More recently, it has been demonstrated that administration of hCG to non-pregnant rhesus monkeys completely abolishes the luteolytic effect of PGF-2α in vivo (Auletta and Kelm, 1994). Taken together these results suggest that hCG secreted by the conceptus may help in the protection of the gravid corpus luteum against the luteolytic effects of PGF-2α. A similar mechanism could exist in the pregnant mare, where high levels of eCG may be exerting an antiluteolytic influence on the maternal corpora lutea, inhibiting the luteolytic effect of PGF-2α.

The question remains as to how multiple administration of PGF-2α or analogue results in abortion in mares more than 2 months pregnant. From previous work it appears that complete elimination of functional corpora lutea might not be the only reason for abortion since mares no longer need luteal progesterone production for maintenance of pregnancy (Holtan et al., 1979; Daels et al., 1991). It is possible that abortion is the result of a combination of declining progesterone levels and more direct effects of PGF-2α on the gravid uterus. Madej and co-authors examined the endocrine changes during prostaglandin-induced abortion in two mares, 158 and 173 days pregnant (Madej et al., 1987). Mares were treated with 10 mg PGF-2α at 12-h intervals until fetal expulsion. Within 3 h after each injection, an increase in PGF-2α metabolite concentrations were detected, possibly reflecting a combination of increasing circulating exogenous and endogenous PGF-2α concentrations. Abortion coincided with a peak of PGF-2α metabolite concentrations. However, because of the use of native PGF-2α in this study it was difficult to distinguish between exogenous and endogenous PGF-2α. Based on the duration of the increase in PGF-2α metabolite concentrations before fetal expulsion and the interval between PGF-2α administration to abortion, it was concluded that fetal expulsion likely coincided with a surge in endogenous PGF-2α secretion.

The goal of the present study, was to characterize in detail the endocrine changes which occur during prostaglandin-induced abortion in pregnant mares that no longer require the maternal corpora lutea for maintenance of pregnancy. In this experiment, we set out to determine the endogenous contribution to the circulating PGF-2α metabolite pool during the period of induction and at the time of fetal expulsion. Plasma progesterone secretion profiles were characterized and their relationship with the time of fetal expulsion examined. Fetoplacental estrogen secretion, a marker for fetal viability,

was also characterized and the critical time at which estrogen concentrations deviate from normal was calculated.

2. Materials and methods

2.1. Animals

Ten pregnant mares of light-horse type (five Thoroughbreds, two Standardbreds, two Quarterhorses and one Arabian) ranging between 5 and 16 years of age (mean 9.7 years) were used for this study. Mares had no previous history of subfertility or pregnancy loss and were judged to be reproductively sound as determined by palpation and ultrasonography per rectum of the reproductive tract, vaginal examination and normal reproductive cyclicity. Mares were bred either by artificial insemination or natural cover and the date of ovulation was established by daily examination of the ovaries during estrus. After conception, pregnancy was diagnosed at regular intervals before the experiment and daily during the experiment. Six mares, 82, 85, 90, 97, 97 or 102 days pregnant on Day 1 of treatment (mean $\pm$ SEM: 92.2 ± 3.2), were randomly assigned to the treatment group (prostaglandin analogue injections). Four mares, 92, 94, 95, 97 days pregnant (94.5 ± 1), did not receive any treatment but were subjected for 3 days to the same hourly sampling protocol and pregnancy examination schedule as mares in the treatment group.

2.2. Treatment and sample collection

Mares in the treatment group ($n = 6$) were treated at 24-h intervals with the PGF-2α analogue cloprostenol until the fetus was expelled. Cloprostenol (250 μg, Estrumate, Haver/Diamond Scientific, Shawnee, KS 66201) was administered intramuscular at 08:00 h and administration was repeated daily until the expelled fetus was detected in the stall or could no longer be detected in the reproductive tract of the mare. Pregnancy examination was performed daily by ultrasonography per rectum (Aloka 210, 5 MHz linear transducer, Corometrics Medical Systems Inc., North Wallingford, Connecticut).

Blood samples were collected for determination of progesterone, estrogen conjugate and 15-keto-dihydroprostaglandin metabolite (PGFM) from all mares. Each mare was fitted with an indwelling catheter in the jugular vein the day before the first cloprostenol administration and catheters were flushed with heparinized saline after each sampling. Six hourly blood samples were collected the day before the first cloprostenol administration to establish baseline concentrations. Starting at the time of the first cloprostenol administration hourly blood samples were collected until 6 h after expulsion of the fetus. Blood samples were collected from the untreated mares at 1-h intervals for 3 days. Blood was collected into heparinized tubes (Monoject Vacutainers, Sherwood Medical, St. Louis, MO) and chilled immediately. Plasma was separated and stored at -20°C until analyzed.

2.3. Endocrine analysis

2.3.1. Estrogen conjugate

Plasma concentrations of estrogen conjugates were determined using a direct radioimmunoassay without extraction (Daels et al., 1990). Tritiated estrone-3-sulfate (New England Nuclear, Boston, MA) was used for tracer and the antibody (R-583) was produced in rabbits and was directed against estrone-3-glucuronide which had been conjugated to bovine serum albumin. For the assay, plasma samples were diluted 1:200 in Tris buffer and 0.05 ml was assayed. The limit of sensitivity was 0.36 ng ml^{-1} plasma. The inter-assay coefficient was 9% around 25% binding and 10% around 60% binding; intra-assay coefficient of variation was 9%.

2.3.2. Progesterone

Plasma progesterone concentrations in ether-extracted samples were determined by enzyme immunoassay (Munro and Stabenfeldt, 1984). The reported cross-reactivity for the antiserum is 11α-hydroxyprogesterone 21.4%, 5α-pregnane-3,20 dione 29.5% and less than 0.5% for other steroids tested. The inter- and intra-assay coefficient of variation were 9% and 11%, respectively.

2.3.3. Prostaglandin

Prostaglandin-F2α secretion was monitored by measurement of the 15-keto-dihydro-PGF2α metabolite (PGFM) in unextracted plasma samples by radioimmunoassay (Granstrom and Kindahl, 1982). The antibody cross-reacts with 15-keto-PGF2α (16.0%), 13, 14-dihydro-PGF2α (4.0%), and with 15-keto-13, 14-dihydro-PGE2 (1.7%). The relative cross-reactivity between the antibody and cloprostenol was less than 0.1% (Kindahl et al., 1980a,b; Lindell et al., 1980). The detection limit of the assay is 10 pg ml^{-1} of plasma. The interassay coefficient of variation was 14%, and the intra-assay coefficient of variation ranged between 6.6% and 11% at different points on the standard curve.

2.4. Statistical analysis

Mean progesterone concentrations at the time of fetal expulsion (based on samples taken immediately before and after fetal expulsion) were compared with the mean pretreatment concentrations to account for treatment and to the mean concentrations in the untreated mares taken at the time immediately before and after the mean interval which elapsed in the treated mares between the cloprostenol administration and fetal expulsion. Significant differences were evaluated using Student t-test.

To determine the time point at which mean estrogen concentrations start to decline in the treated mares, a step-wise linear regression was used. For the analysis, the period during which estrogen concentrations remained unchanged and the period during which levels were declining were determined arbitrarily and linear regression was calculated for each period separately. A range of time points around the point of deflection was chosen and regression analysis for the two periods was performed based on each specific cut-off point. Each regression model was evaluated for goodness-of-fit using the

respective coefficient of determination. The process was repeated for each time point and the time point at which the maximal coefficient of determination was found was considered the onset of decline.

The effect of cloprostenol on endogenous PGF-2α secretion was analyzed by calculating the sum of hourly PGFM concentrations divided by the total number of h (pg ml^{-1} h^{-1}) during the 24 h interval following each cloprostenol administration or the time interval from last cloprostenol administration to fetal expulsion. The results of these calculations represent in essence the mean plasma PGFM concentrations for a given interval but for simplicity are referred to as hourly secretion rate. The daily mean PGF-2α secretion rate for each mare for each day was used as a single data point and effect of day and treatment were analyzed by ANOVA and significant differences between means were evaluated using Tuckey's multiple comparison.

3. Results

3.1. Outcome of pregnancy

All untreated mares remained pregnant and carried a normal foal to term. Mares treated with cloprostenol expelled their fetus on the average after 48.6 ± 5.6 h (mean ± SEM) or 2.8 ± 0.2 cloprostenol injections (Table 1). Some mares showed varying transient clinical symptoms of abdominal discomfort in the hours after the second and third cloprostenol injection. During the hours preceding fetal expulsion all mares showed signs of abdominal discomfort, ranging from restlessness and looking at flank to frequent laying down and rolling. In all mares, the fetus and complete fetal membranes were expelled without complications. In four mares, the fetus was expelled with the fetal membranes still intact. The appearance of the fetus at the time of expulsion suggested that the fetus likely died immediately before or during expulsion. In one mare (No. 7546), expulsion of a live fetus was observed. None of the mares had clinical signs of endometritis or metritis, including vaginal discharge, fever or loss of appetite, following the induced abortion.

Table 1
Outcome of pregnancy

Mare (ID No.)	Age	Days pregnant	Time to abortion (h)	Number of cloprostenol admin.
8524	15	82	47	3
7526	16	85	49	3
8503	12	90	50	3
7546	10	97	39	2
319	14	97	51	3
7572	9	102	56	3

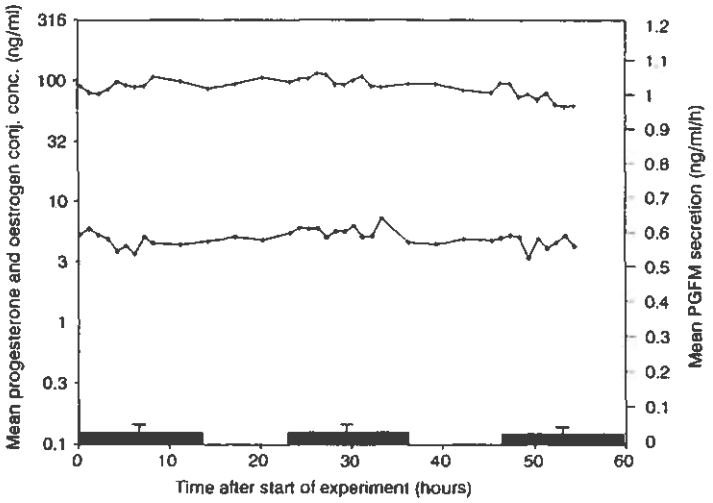

Fig. 1. Daily mean PGF-2α secretion rate (mean + SEM) (solid bars), progesterone (♦) and estrogen (+) concentrations for untreated mares. Secretion rate was calculated over each 24 h of sample collection.

3.2. Progesterone secretion

Plasma progesterone concentrations remained unchanged in untreated mares (Fig. 1). In the treated mares, plasma progesterone concentrations declined following the first cloprostenol administration (Figs. 2a–2f). The rate of decline appeared to be correlated with the pretreatment progesterone concentrations (range 3–23 ng ml^{-1}) and all profiles converged to similar low levels at the time of fetal expulsion (Fig. 3). Mean plasma progesterone concentration at the time of fetal expulsion was 1.3 ± 0.2 ng ml^{-1} (mean $\pm$ SEM), based on the sample taken immediately before and after fetal expulsion. Mean progesterone concentration at the time of fetal expulsion was significantly ($P < 0.05$) lower than pretreatment mean concentrations in treated mares (11.5 ± 1.1 ng ml^{-1}) and mean concentration at the equivalent time (28–29 h after onset of sampling) in untreated mares (4.2 ± 0.5 ng ml^{-1}).

3.3. Estrogen secretion

Mean estrogen concentrations remained constant over the three observation days in the untreated mares (Fig. 1) and from the first cloprostenol administration until 5 h before fetal expulsion in the treated mares (Fig. 4). During this period, the secretion pattern in untreated and treated mares were parallel as determined by analysis of the regression coefficient ($P < 0.05$). Starting at 5 h before fetal expulsion, mean estrogen concentrations started to decrease in a near linear manner.

3.4. Prostaglandin secretion

In the untreated mares, plasma PGFM concentrations and secretion rate remained at basal levels throughout the 3 day observation period (Fig. 1). Hourly PGFM concentra-

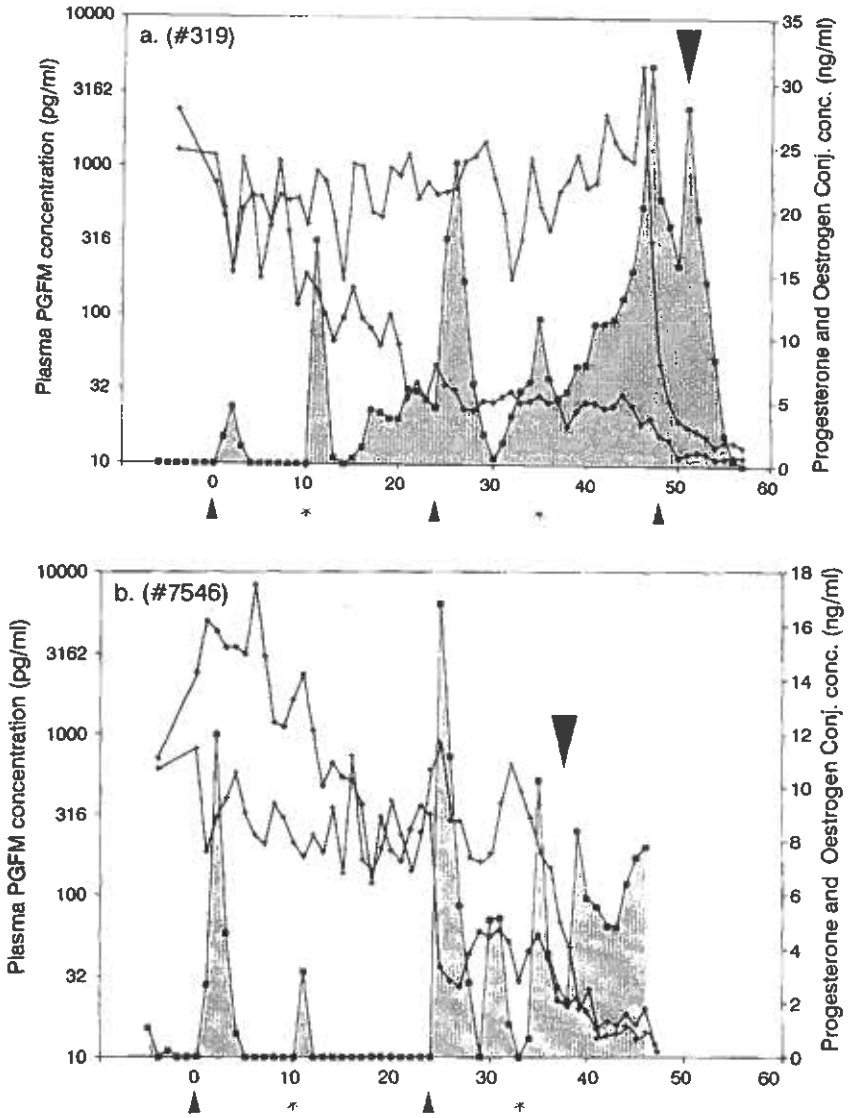

Fig. 2. Plasma PGFM (■), estrogen (+) and progesterone (◆) concentrations in pregnant mares treated with cloprostenol (250 μg cloprostenol) at 24-h intervals. The arrows along the *x*-axis indicate the time of cloprostenol administration (first administration occurred at Time 0). Asterisk along *x*-axis indicate time of reproductive examination per rectum. Solid arrow (pointing down) indicates time of fetal expulsion. (a) Mare 319; (b) Mare 7546; (c) Mare 8503; (d) Mare 7572; (e) Mare 8524; (f) Mare 7526.

tions varied between < 10 and 52 pg ml^{-1}. Daily mean PGFM secretion rates were 31 pg ml^{-1} h^{-1}, 31 pg ml^{-1} h^{-1} and 27 pg ml^{-1} h^{-1} on Days 1, 2 and 3, respectively, and no significant differences ($P < 0.05$) were observed between days.

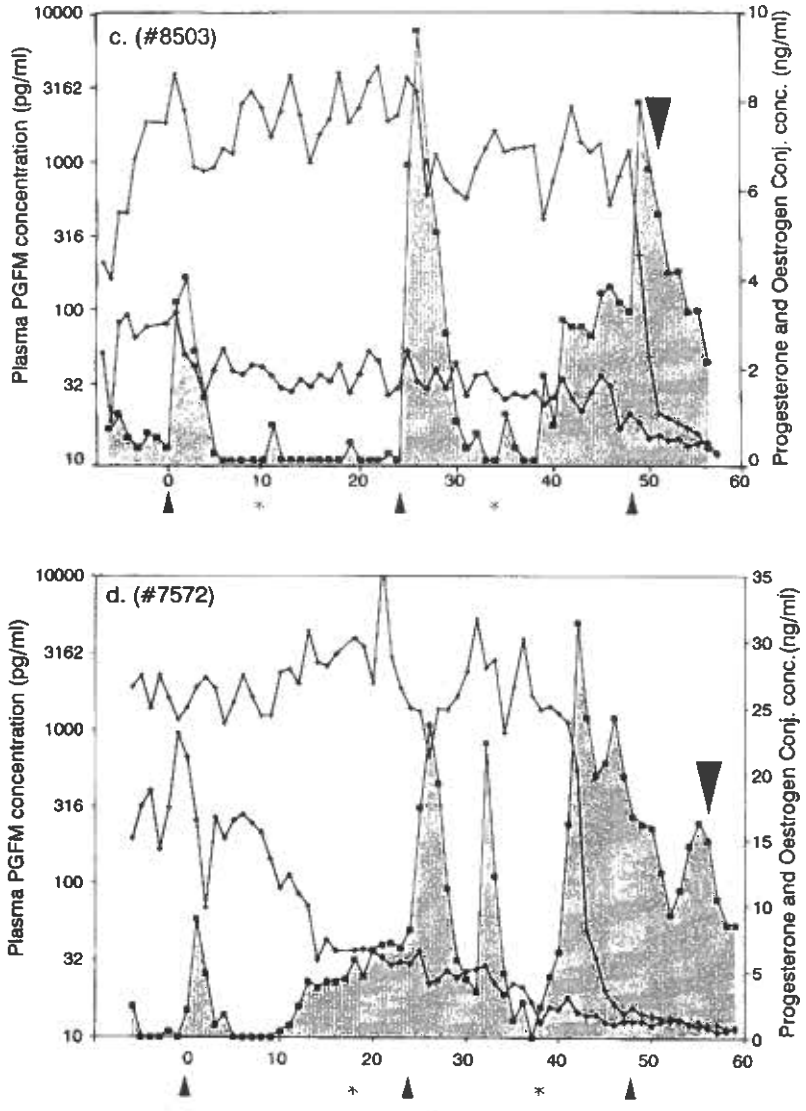

Fig. 2 (continued).

In the treated mares, plasma PGFM concentrations before the first cloprostenol administration were similar to those observed in untreated mares and varied between < 10–32 pg ml^{-1} (Figs. 2a–2f). Following the first cloprostenol administration (Day 1), mean PGF-2α secretion rates in treated and untreated mares were similar ($P > 0.05$) (Fig. 5). Mean PGF-2α secretion rate in treated mares was significantly ($P < 0.05$) higher than in untreated mares on Days 2 and 3. Mean PGF-2α secretion rate increased

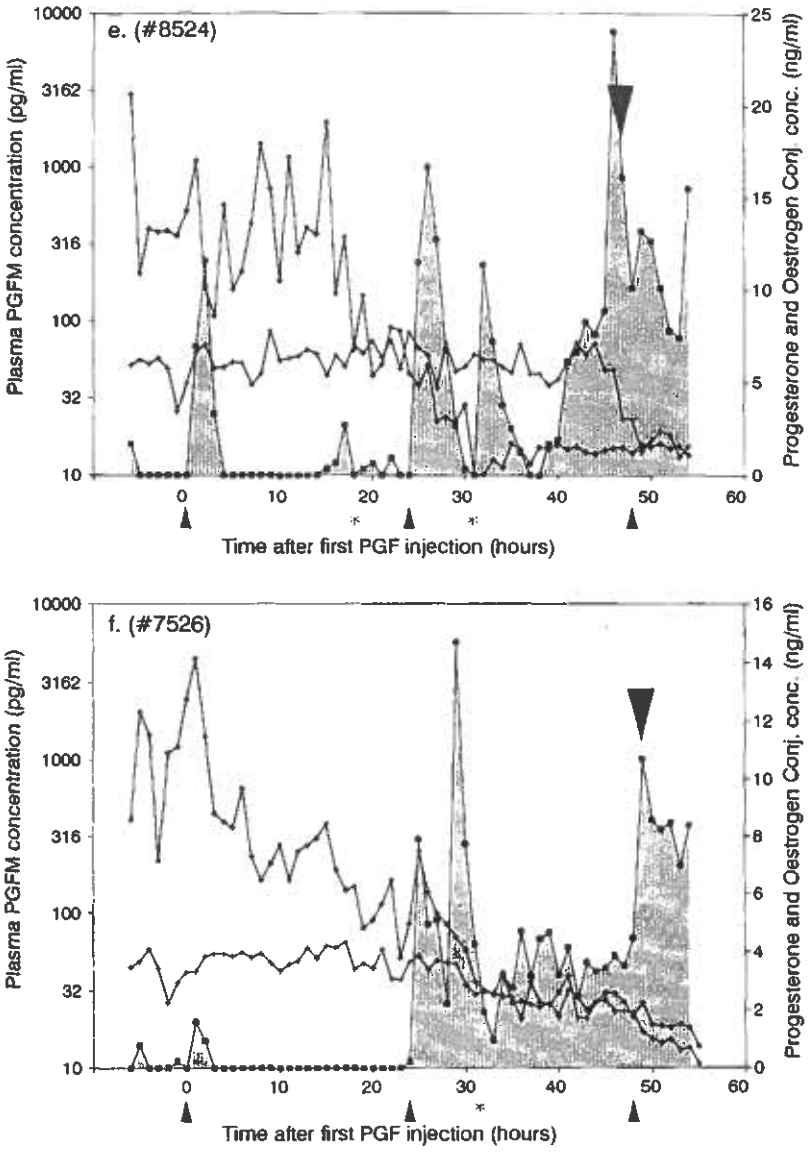

Fig. 2 (continued).

with each subsequent cloprostenol administration and was significantly ($P < 0.05$) higher on Days 2 and 3 when compared with Day 1 (Fig. 5). Individual PGFM secretion rate increased in three out of four mares on Day 3 but mean PGFM secretion rate was not significantly ($P > 0.05$) higher on Day 3 when compared with Day 2. During the treatment, both cloprostenol administration and examination per rectum caused increases in plasma PGFM concentrations (Figs. 2a–2f). The magnitude of the individual re-

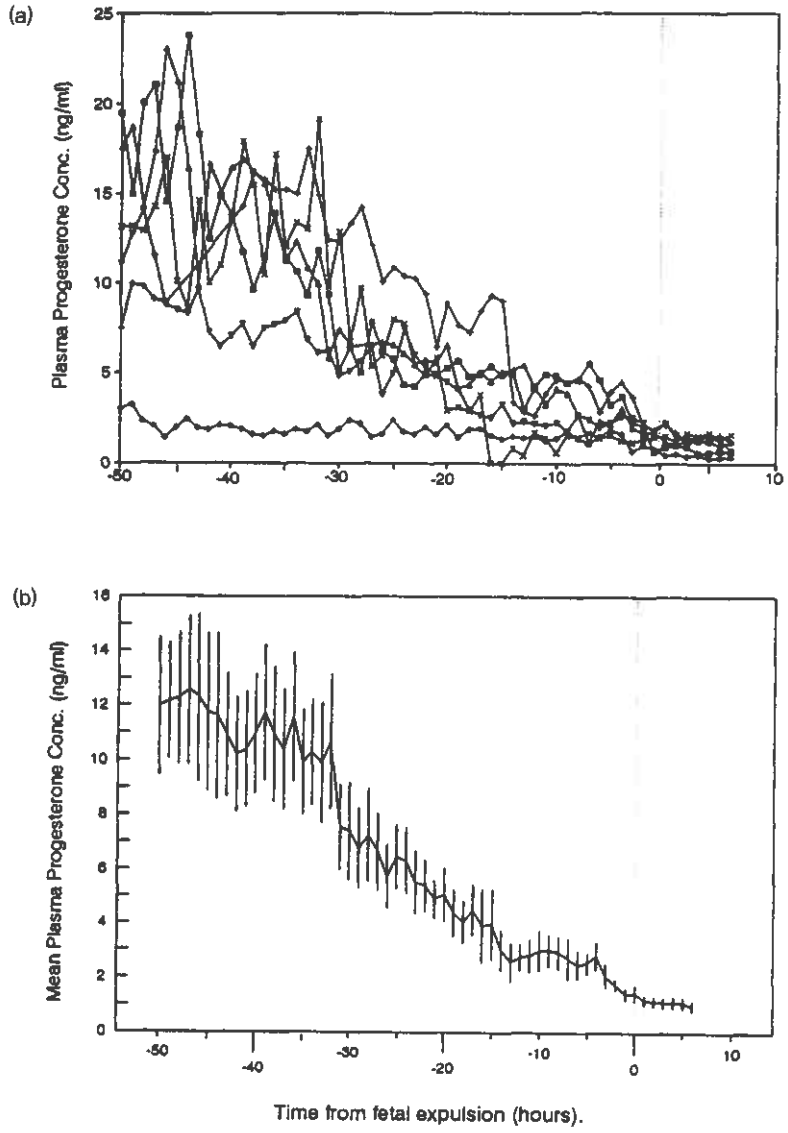

Fig. 3. Individual (a) and mean (b) plasma progesterone concentrations (mean ± SEM) in cloprostenol-treated mares. Data are aligned according to time of fetal expulsion (Time 0). Mares 319 (■), 7526 (▼), 7546 (+), 7572 (▲), 8503 (◆) and 8524 (×).

sponses, although highly variable, tended to increase over time. The peak PGFM concentrations occurred after the second cloprostenol administration even though four out of six mares aborted after the third cloprostenol administration (Table 2). In five out of six mares, highest PGF-2α secretion rate was observed on the day of fetal expulsion

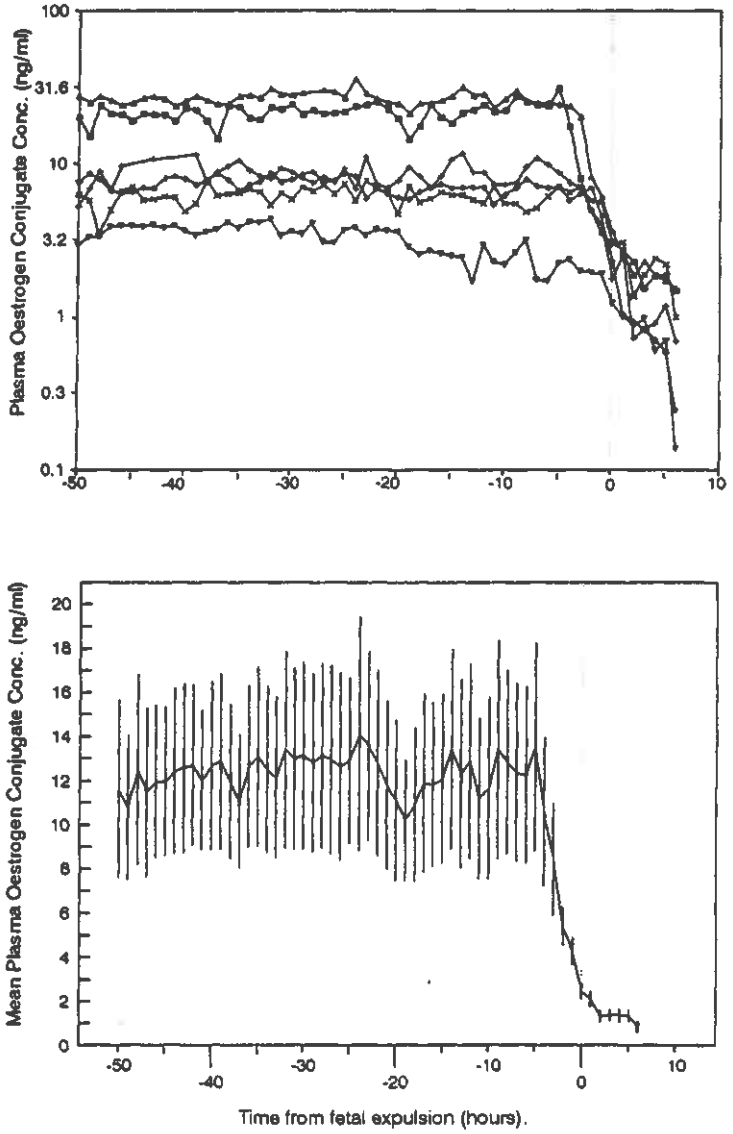

Fig. 4. Individual (a) and mean (b) plasma estrogen concentrations (mean ± SEM) in cloprostenol-treated mares. Data are aligned according to time of fetal expulsion (Time 0). Mare 319 (■), 7526 (▼), 7546 (+), 7572 (▲), 8503 (♦) and 8524 (×).

(Table 2). Fetal expulsion was preceded by a sustained prostaglandin secretion and plasma PGFM concentrations were higher than 30 pg ml^{-1} starting 4–18 h before fetal expulsion (Figs. 2a–2f). In one mare (Mare No. 7526), the onset of the pre-expulsion PGF-2α surge appeared to be triggered by the cloprostenol administration (Fig. 2f).

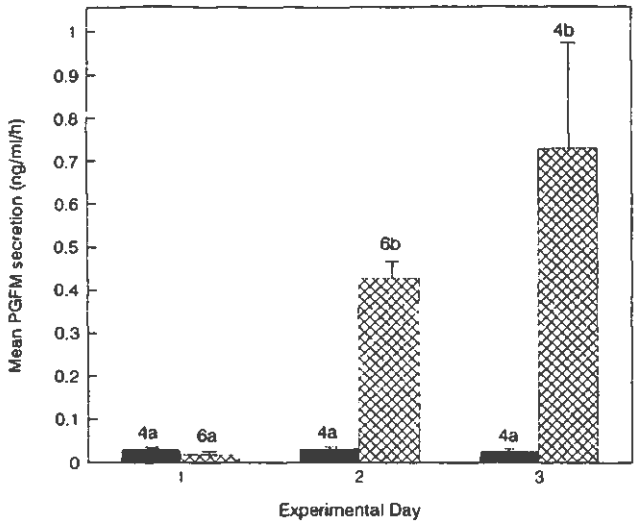

Fig. 5. Daily mean PGF-2α secretion rate (mean + SEM) for untreated (solid bars) and cloprostenol-treated (hatched bars) mares. Secretion rate was calculated over the 24 h following each cloprostenol injection or from cloprostenol injection until fetal expulsion for the treated mares and over each 24-h period for untreated mares. Numbers above the SEM bars indicate number of mares included and different letters indicate significant differences ($P < 0.05$).

However in the remaining five mares, the pre-expulsion PGF-2α surge appeared to have occurred spontaneously and thus there was no direct correlation to the last cloprostenol injection.

4. Discussion

It appears that the PGFM concentrations measured in this experiment are not a direct reflection of the concentration of cloprostenol or its metabolites in the blood circulation. We have previously demonstrated in cattle that the cross-reactivity of the antibody used for this study is less than 0.1% for cloprostenol and immunoreactivity with cloprostenol metabolites was not observed in these studies (Kindahl et al., 1980a,b; Lindell et al., 1980). In the present study, PGFM concentrations remained low in most mares following the first cloprostenol administration and increased on subsequent days, suggesting an endogenous source.

The outcome of pregnancy and the time from first cloprostenol administration to fetal expulsion are in agreement with previous reports (Squires et al., 1980; Lofstedt, 1986; Voller et al., 1991; Ginther, 1992; Squires and Bosu, 1993). All fetuses were alive less than 24 h before fetal expulsion as determined by the presence of fetal cardiac motion during the daily ultrasonographic examinations. No changes in the size or shape of the fetal sac, ultrasonographic appearance of allantoic fluid or position of the conceptus were noticed during subjective ultrasonographic examinations. Plasma progesterone

Table 2
Endogenous PGFM concentrations during PGF-induced abortion

	Total PGF2α secretion (area under curve) until fetal expulsion	Mean hourly PGF2α secretion rate total per hour (pg ml^{-1} h^{-1})	Maximum PGFM concentration for 24 h period (pg ml^{-1})
Mare No. 8524			
Day 1	331 pg ml^{-1} per 24 h	13	248
Day 2	10878 pg ml^{-1} per 24 h	453	7651
Day 3			
Mare No. 7526			
Day 1	255 pg ml^{-1} per 24 h	11	20
Day 2	7089 pg ml^{-1} per 24 h	295	5711
Day 3	530 pg ml^{-1} per 1 h	530	1011
Mare No. 8503			
Day 1	332 pg ml^{-1} per 24 h	13	167
Day 2	10482 pg ml^{-1} per 24 h	437	7440
Day 3	2987 pg ml^{-1} per 2 h	1493	2510
Mare No. 7546			
Day 1	1084 pg ml^{-1} per 24 h	45	1000
Day 2	8017 pg ml^{-1} per 15 h	535	6403
Day 3			
Mare No. 319			
Day 1	606 pg ml^{-1} per 24 h	25	321
Day 2	8163 pg ml^{-1} per 24 h	340	4882
Day 3	2206 pg ml^{-1} per 3 h	735	2565
Mare No. 7572			
Day 1	319 pg ml^{-1} per 24 h	13	59
Day 2	12276 pg ml^{-1} per 24 h	511	4959
Day 3	1326 pg ml^{-1} per 8 h	165	274

concentrations decreased steadily following the first cloprostenol injection in all mares. Even though progesterone concentrations at the onset of the study varied widely, ranging from 3 to 23 ng ml^{-1}, progesterone concentrations at the time of fetal expulsion were all similarly low with minimal variation among mares (1.3 ± 0.2 ng ml^{-1}). Estrogen concentrations in cloprostenol-treated mares did not change significantly until only hours before fetal expulsion and the onset of the decline in estrogen concentrations appeared to coincide with the onset of the sustained secretion of PGF-2α. The observation that estrogen secretion did not change until only hours before fetal expulsion agrees with our clinical impression that the fetus remained viable during the pre-expulsion period. We propose that myometrial contractions during the days before fetal expulsion, when progesterone concentrations were still elevated, were poorly coordinated and transient. During the sustained PGF-2α secretion in the hours before fetal expulsion, when progesterone concentrations were low, myometrial contractions became more coordinated. As myometrial contractions became more effective the placenta separated from the endometrial surface resulting in an acute drop in circulating estrogen levels and finally expulsion of the fetus. Thus it appears that plasma estrogen concentrations, although a direct reflection of placental function and fetal viability has little

predictive value for the diagnosis of impending, spontaneous abortion and changes indicative of impending abortion do not occur until active expulsion of the fetus.

The results of this study indicate that prostaglandin-induced abortion at the end of the first trimester is associated with significant increases in endogenous PGF-2α secretion. The association between the sustained PGF-2α secretion and fetal expulsion suggests that PGF-2α is secreted by the uterus. Prostaglandin secretion was highest during fetal expulsion and subsided almost immediately after fetal delivery in some but not all mares. Whereas it is possible that in the early stages of the abortions PGF-2α may have been secreted by the fetal placenta, this appears unlikely during the final stages of abortion. Plasma PGFM concentrations remained elevated at a time when declining estrogen concentrations suggest a loss of contact between placenta and endometrium.

The question remains as to what triggers fetal expulsion. The generic model for the onset of labor in mammalian species includes many factors which each play a specific role (for review see Thorburn, 1991; Challis and Lye, 1994). Before the onset of labor, oxytocin and prostaglandin receptors and myometrial gap junctions are present at low levels. The absence of gap junctions is responsible for the high input resistance of the myometrial smooth muscle and poor coordination of uterine contractions. At parturition, the appearance of large numbers of gap junctions significantly enhances electrical coupling within the myometrium and has a critical impact on the ability of the myometrium to develop the synchronous high amplitude contractions of labor. The number of receptors for oxytocin increase at the onset of labor, resulting in an increased responsiveness to pituitary oxytocin. Oxytocin activates myometrial contractions through direct activation of the myometrial cells and through stimulation of prostaglandin secretion. Prostaglandin and oxytocin are both potent stimulators of myometrial contractions. In addition, prostaglandin might also enhance gap junction formation resulting in increased coordination, and interact with the oxytocin system to enhance the effect of both agonists. Although the relative role of progesterone and estrogen during the initiation of parturition is unclear and differs between species, it is generally accepted that during pregnancy progesterone has an inhibitory effect on gap junction formation, oxytocin receptor formation, uterine prostaglandin secretion and pituitary oxytocin release whereas estrogen has opposing effects. At parturition, the onset of labor is not only due to increased stimulant production but rather a switch in the balance of stimulatory and inhibitory agents. In our experiment, progesterone concentrations decreased following cloprostenol administration whereas estrogen concentrations remained unchanged resulting in a shift in the progesterone-to-estrogen ratio. The decreased progesterone-to-estrogen ratio provides an environment that is more favorable for labor-associated changes such as increased numbers of gap junctions and oxytocin receptors. In addition to inducing conditions that are conducive to uterine contractions (gap junctions and altered steroid environment), cloprostenol may also stimulate pituitary oxytocin secretion which stimulates endogenous PGF-2α secretion and uterine contractions. In non-pregnant mares, PGF-2α administration stimulates (pituitary) oxytocin secretion (P. Daels, personal observation, 1993) and myometrial contractions (Cadario et al., 1994; Troedsson et al., 1994). Thus it is possible that spontaneous PGF-2α secretion in the hours before fetal expulsion is provoked by a shift in progesterone-to-estrogen ratio resulting in an increased myometrial sensitivity to oxy-

tocin which resulted in stimulation of uterine PGF-2α secretion and myometrial contractions. This hypothesis is supported by the results of ongoing experiments in our laboratory which indicate that progesterone inhibits endogenous PGF-2α and maintains pregnancy.

In conclusion, our data demonstrate that cloprostenol-induced abortion is associated with significant secretion of endogenous PGF-2α secretion. Fetal expulsion coincides with sustained endogenous PGF-2α secretion. Fetal expulsion and PGF-2α secretion uniformly occur at a time when progesterone concentrations have reached low levels. Estrogen conjugate concentrations do not change significantly during the days preceding abortion suggesting that the fetus remains viable until close to the time of fetal expulsion.

Acknowledgments

We thank Coralie Munro for assistance with progesterone and estrogen analysis, the staff at the Equine Research Laboratory for technical assistance and Betty Hansen for assistance with data analysis and manuscript preparation. This research was supported in part by the Equine Research Laboratory (University of California-Davis), the Harry M. Zweig Memorial Fund for Equine Research (Cornell University) and the Swedish Council for Forestry and Agricultural Research.

References

Allen, W.R. and Stewart, F., 1993. Equine chorionic gonadotropin. In: Equine Reproduction. Lea & Febiger, Philadelphia, pp. 81–96.

Auletta, F.J. and Kelm, L.B., 1994. Mechanisms controlling corpus luteum function in the rhesus monkey (*Macaca mulatta*): inhibitory action of hCG on luteolysis induced by PGF-2α. J. Reprod. Fertil., 102: 215–220.

Cadario, M.E., Thatcher, M.J. and LeBlanc, M.M., 1994. Relationship between prostaglandin and uterine clearance of radiocolloid in the mare. Proceedings of the Sixth International Symposium on Equine Reproduction, 7–13 August 1994, Caxambu, Brazil, pp. 117–118 (abstract).

Challis, J.R.G. and Lye, S.J., 1994. Parturition. In: Knobil, E., Neill, J.D. (Editors), The Physiology of Reproduction (Vol. 2). Raven Press, New York, pp. 985–1032.

Daels, P.F., Shideler, S., Lasley, B.L., Hughes, J.P. and Stabenfeldt, G.H., 1990. Source of oestrogen in early pregnancy in the mare. J. Reprod. Fertil., 90: 55–61.

Daels, P.F., Stabenfeldt, G.H., Hughes, J.P., Odensvik, K. and Kindahl, H., 1991. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. Am. J. Vet. Res., 52: 282–288.

Douglas, R.H., Squires, E.L. and Ginther, O.J., 1974. Induction of abortion in mares with prostaglandin F2-α. J. Anim. Sci., 39: 404–407.

Ginther, O.J., 1992. Reproductive Biology of the Mare: Basic and Applied Aspects. Equiservices, Cross Plains, WI.

Granstrom, E. and Kindahl, H., 1982. Species differences in circulating prostaglandin metabolites. Relevance for the assay of prostaglandin release. Biochim. Biophys. Acta, 713: 555–569.

Holtan, D.W., Squires, E.L., Lapin, D.R. and Ginther, O.J., 1979. Effect of ovariectomy on pregnancy in mares. J. Reprod. Fertil., 27 (Suppl.): 457–463.

Kindahl, H., Edqvist, L.-E. and Lindell, J.-O., 1980a. On the control of prostaglandin release during the bovine estrous cycle. Adv. Prostaglandin Thromboxane Res., 8: 1351–1355.

Kindahl, H., Lindell, J.-O. and Edqvist, L.-E., 1980b. On the control of prostaglandin release during the bovine estrous cycle. Effect of cloprostenol injections. Nord. Vet. Med., 32: 467–473.

Lindell, J.-O., Kindahl, H. and Edqvist, L.-E., 1980. Prostaglandin induced early abortion in the bovine. Clinical outcome and endogenous release of prostaglandin F-2α and progesterone. Anim. Reprod. Sci., 3: 289–299.

Lofstedt, R.M., 1986. Termination of unwanted pregnancy in the mare. In: D.A. Morrow (Editor), Current Therapy in Theriogenology 2. W.B. Saunders, Philadelphia, pp. 715–717.

Madej, A., Kindahl, H., Nydahl, C., Edqvist, L.-E. and Stewart, D.R., 1987. Hormonal changes associated with induced late abortions in the mare. J. Reprod. Fertil., 35 (Suppl.): 479–484.

McNatty, K.P., Henderson, K.M. and Sawers, R.S., 1975. Effects of prostaglandin F2alpha and E2 on the production of progesterone by human granulosa cells in tissue culture. J. Endocrinol., 67: 231–240.

Michael, A.E. and Webley, G.E., 1991. Prior exposure to gonadotrophins prevents the subsequent antigonadotrophic actions of cloprostenol by a cyclic AMP-dependent mechanism in cultured human granulosa cells. J. Endocrinol., 131: 319–325.

Munro, C. and Stabenfeldt, G.H., 1984. Development of microtitre plate enzyme immunoassay for the determination of progesterone. J. Endocrinol., 101: 41–49.

Squires, E.L. and Bosu, W.T.K., 1993. Induction of abortion during early to midgestation. In: A.O. McKinnon and J.L. Voss (Editors), Equine Reproduction. Lea & Febiger, Philadelphia, pp. 563–566.

Squires, E.L., Hillman, R.B., Pickett, B.W. and Nett, T.M., 1980. Induction of abortion in mares with Equimate: effect on secretion of progesterone, PMSG and reproductive performance. J. Anim. Sci., 50: 490–495.

Thorburn, G.D., 1991. The placenta, prostaglandins and parturition a review. Reprod. Fertil. Dev., 3: 277–294.

Troedsson, M.H.T., Liu, I.K.M., Ing, M. and Pascoe, J., 1994. Smooth muscle electrical activity in the oviduct and the effect of oxytocin, PGF-2α and PGE-2 on the myometrium and the oviduct of the cycling mare. Proceedings of the Sixth International Symposium on Equine Reproduction, pp. 113–114 (abstract).

Voller, B.E., Parry-Weeks, L.C. and Holtan, D.W., 1991. The effect of Regu-Mate, a synthetic progestin, on early pregnancy maintenance, conceptus growth, and corpora lutea development in pregnant pony mares. J. Equine. Vet. Sci., 11: 46–50.

Webley, G.E., Richardson, M.C., Given, A. and Harper, J., 1991. Preincubation of human granulosa cells with gonadotrophin prevents the cloprostenol-induced inhibition of progesterone production. Mum. Reprod., 6: 779–782.