General discussion

The clinical effect of combining progesterone, estradiol 17beta and prostaglandins is remarkably good and in the experience of the author and others 1,2 is an effective way to control the estrous cycles of mares.

In contrast to ruminants, most mares only have one follicle wave during an estrous cycle2b and unless treatment includes a hormone to control follicle growth, the time of ovulation will not be controlled when treatment ends. This is not a problem in ruminants because of the frequent follicle waves that occur in those species, always providing a follicle that is fairly close to maturation when progestogen treatment is withdrawn. In mares by contrast, if progestogen treatment is stopped when a small follicle is present, it could take up to two weeks for that follicle to mature and ovulate. If a large follicle is present, the mare could ovulate in one or two days! This is why it is so important to control follicle growth if one wishes to control the equine estrous cycle.

Estradiol is considered to have a modest suppressive effect on the release of FSH in cycling mares. 2-9 Therefore estradiol 17beta can control follicle development in mares. When it is used together with progestagens, estradiol 17beta may cause even greater suppression of LH than with progestagens alone,10 but this has not yet been demonstrated on mares.

Once it has been established that a mare is normal, non-pregnant and cycling, progesterone and estradiol 17beta (P&E) are given intra-muscularly for ten days to suppress follicle growth of the smaller follicles in the ovaries. As mentioned earlier, the value of progesterone in this role is questionable. In fact, this author believes that P&E might work just as well to suppress follicle growth in the absence of progesterone. However, this still theory needs to be tested.

In general, follicle development is well controlled by P&E and by the tenth day of treatment any large follicles originally present have ovulated or regressed. Only small follicles3 (few greater than 22mm in diameter) are present in the ovaries.

After the last injection of P&E, follicles grow quite predictably and when an injection of 3000 IU of hCG is given on the eighth day after the end of P&E treatment i.e. on day 18 after the start of the P&E treatment, most ovulations occur late in the evening of day 9 or early in the morning of day 10. Therefore, the time from the beginning of treatment to ovulation is 19.5 days.

Interestingly, the growth of already-large follicles is not suppressed during P&E treatment and these large follicles often ovulate during the first few days of treatment, forming corpora lutea. It is for this reason that a luteolytic dose of prostaglandin is administered on the last day of P&E treatment. In fact (Robert Loy personal communication) some ovulations occur as late as day...
Notes

10 during P&E treatment. Therefore a few mares ovulate so late during P&E treatment that the corpora lutea formed after those ovulations are not mature enough to be susceptible to prostaglandin-induced luteolysis at the end of P&E treatment. That fact leads the author to suggest the prostaglandins also be given at the time of the hCG injection on day 8 after the end of P&E treatment. In fact, first the injection of prostaglandin traditionally given on the tenth day of P&E treatment may not even be required because corpora lutea in mares do not last for more than 14 days anyway. In other words, any luteal tissue present in the ovaries at the onset of the ten day P&E treatment (or formed during the first few days of treatment) would have undergone luteolysis by the time ovulation occurred on day 19.

We have used P&E to control estrus and ovulation effectively for at least 23 years. Its use has been associated with normal conception rates in both foaling and barren mares. 

Treatment Failures
Occasional treatment failures and concerns are reported from time to time. Failures may be associated with:

Mares that are not yet cycling properly when treatment commences

We believe this is a significant problem in northern regions where mares may have their first ovulations as late as April or even May. It is essential to demonstrate that a mare is cycling before treatment starts. This can be done by demon-

Under-dosing
We suggest that the dose for draft mares be increased accordingly and that packaging should allow for wastage.

Under dosage may also be a problem in cold weather because when P & E is kept in a cold barn, the steroids will precipitate out of the oil carrier and mares will be under dosed. For that reason, P & E should be kept warm at all times.

Failure of luteolysis at the end of P&E treatment.
As mentioned earlier, some mares ovulate so late during P&E treatment that the corpora lutea formed as a result of these ovulations are not yet susceptible to prostaglandin-induced luteolysis at the end of P&E treatment. For this reason, an additional prostaglandin injection should be given at the time of the hCG injection on day 8 after the end of P&E treatment.

Injection sites and technique
It is apparent that many horse owners are not aware that intramuscular injections of any sort should be given well above the lateral vertebral processes in the neck. Failure to do so can result in swellings such as the one shown here. In two mares that failed to respond to P&E treatment we saw the same swellings in

Failures are associated with:
1. Mares not cycling
2. Under-dosing
3. Failure of luteolysis
both mares when they were delivered to our clinic for breeding. Subsequent treatment with the same bottle of P&E in the same mares and an unrelated draft mare produced no swellings. We suspect that the hormonal preparation may pool in these swellings, producing lower systemic concentrations than those required for effective treatment. Together with swellings due to lack of injection hygiene, this experience has taught us that it is unwise to presume that horse owners are familiar with proper intramuscular injection techniques.

Unexplained failures
P&E works very well in most mares (perhaps 90% plus) but it certainly is not infallible. In some cases, we simply do not understand the reason for failure of P&E treatment.

Concerns about follicle size after the end of P&E treatment
Veterinarians occasionally report that small follicles (34 to 36 mm) are present on the day that semen is to be shipped in. Consequently, they are concerned over the follicle size and the potential for failure of ovulation that evening.

One thing to remember is that follicles may actually be bigger than they appear on ultrasound because simple measurements of diameter ultrasound are not accurate estimates of true follicle size. In most cases, follicles do in fact ovulate on cue on the evening of day 9.5 especially if uterine starburst is evident or fading on ultrasound examination.

In a small number of cases, ovulation will occur on days 10 to 11 but these still result in pregnancies when cooled semen is used. However, very few pregnancies will result when ovulation is delayed and frozen semen is used.

The predictability of ovulation
The ability of P&E to control the time of ovulations is usually excellent.

On one occasion when ovulations were controlled for the use of frozen semen, we found that 13 of 14 ovulations occurred between day 9 and 10 after the end of P&E treatment. In another case, where an owner had never inseminated a mares before but was instructed on how to do so by phone, 9 of 11 mares conceived to a single insemination by shipped semen after P&E treatment! We have also found that ovulation occurs quite predictable in the evening of day 9 after the end of P&E treatment and consequently use it as our standard approach for the timing of insemination with frozen semen; only performing single post-ovulation inseminations at less than 4 hours after ovulation.

When frozen semen is to be used, we usually schedule ovulations for a Wednesday evening. One clinician will ultrasound the mare for the first half of the night and a colleague for the latter half; every four hours. As soon as ovulation is detected, the semen is thawed and the mare inseminated. We use a similar approach for cooled-transported semen but of course, do not perform examinations every four hours! In the latter case, semen is merely inseminated as soon as it arrives that evening.

Because most stud farms collect and ship out semen on a Mon-Wed-Fri schedule (some do not, so check ahead!) this works out well for all parties involved when cooled semen is used. Stud owners especially enjoy the predictability of knowing when they will have to ship semen well in advance of shipping time.

Of course, ovulations can be scheduled for any day of the week when frozen-thawed semen is used.

Does one have to start P&E at a specific time of the estrous cycle?
Owners often ask if they have to know where the mare is in her estrous cycle before they begin P&E treatment. The
answer of course is no; that is one reason why P&E is so useful.

Using P&E before foal heat.
We have little experience in the use of P&E in pre-foal heat mares (where the hypothalamic-pituitary-ovarian axis may not be functioning efficiently) therefore we are reluctant to recommend its use in such cases.

Using P&E in conjunction with light treatment.
Some practitioners used P&E in conjunction with lighting programs and claim good results but because we seldom use light treatments, we have no data in that regard.

Steroid concentrations after IM injection of P&E
This author measured steroid concentrations (unpublished data) in four ovariectomized mares using single doses of P&E.

After IM injection of P&E using a standard dose of P&E in cottonseed oil in 4 ovariectomized mares, serum estradiol 17beta concentrations in all the mares rose above 150pg/ml between hour one and eight post injection with a mean peak of 350 pg/ml and substantial variation between animals. In the same mares, serum progesterone concentrations rose to maximum of 14.8 ng, 4.9 ng, 2.6 ng and 3.1 ng/ml between 6 and 8 hours after injection. Again, there was considerable variation in uptake and maximum concentration after a IM injection.

Interestingly, no mare had progesterone concentrations over 2 ng/ml after 8 hours post injection. This is remarkable because P&E treatment works so well when given every 24 hours, yet serum steroid concentrations are only above 2ng/ml (often considered to be a “minimal physiological serum concentration for luteal phase mares) for a short period of time. Perhaps avid steroid binding to tissue sites is the reason why P&E is effective when given at the recommended frequency.

Alternate routes for P&E administration

Oral administration of P&E
This author has done controlled trials on both ovariectomized and anestrous mares (unpublished data) using single and double doses of P&E via the oral route using regular and paste formulations of P&E. These studies were motivated by the fact that many owners have an aversion to injections needed for P&E treatment.

Using progesterone as a marker, serial blood samples for up to 24 hours after oral administration revealed that P&E is rapidly catabolized by the liver and does not appear in the systemic circulation. That finding contradicts anecdotal comments of those who say that P&E appears to be effective via the oral route.

Transdermal administration of P&E
In humans, it has been shown that steroids can be applied topically for therapeutic purposes. Therefore we used four ovariectomized mares in a cross-over trial (unpublished data) and compared serum estradiol and progesterone concentrations after no treatment (controls), after IM injection of 150 mg progesterone and 10 mg estradiol 17beta and after applying 150 mg progesterone and 10 mg estradiol 17beta or 300 mg of progesterone and 20 mg of estradiol 17beta (all steroids dissolved in benzyl alcohol and cottonseed oil) on a 20x20 cm patch of shaved skin on the rump of each mares. Serum concentrations of both steroids were measured by RIA on samples taken q 2 hrs.

Serum concentrations of both progesterone and estradiol 17beta were typical after intramuscular injection but no uptake of the steroids was evident in the serum when they were applied topically.
It is possible that larger doses may have increased transdermal uptake of P&E but the doses used in this study were 15 to 20 times as high/unit body weight as those used to achieve effective serum levels in humans. Therefore transdermal steroid treatment in mares does not appear to be feasible or may only work at extremely high topical doses.

**Intra-uterine and intravaginal administration**

This author performed a trial on ovariecatomized mares (unpublished data) using single doses of P&E via intrauterine and intravaginal routes.

Using serum progesterone as a marker, serial blood samples taken up to 24 hours revealed that P&E is rapidly absorbed from both the uterus and vagina. Absorption profiles resemble those generated by IM injection of P&E.

Peak serum concentrations of progesterone by both routes was attained between 0.5 and 8 hours, varying from 6 to 22 ng/ml. These data indicated that P&E can also be administered via either of these alternate routes. Subsequent to these findings, we also studied (unpublished) ovariecatomized mares treated with intrauterine devices for P&E delivery. These devices were made from SilasticR (MDX-4-4210 elastomer from Dow Corning) and measured 5 mm x 90 mm. The IUD matrix and steroid weighed 3.8 g and contained 1.78 g of progesterone and 123 mg of estradiol 17 beta. A nylon pull-string 40 cm in length was be attached for easy withdrawal.

IUDs were inserted into four ovariecatomized mares with the intention of measuring progesterone, estradiol 17beta and PGFM. However, all mares had expelled their IUDs by day six after insertion. Prior to the ejection of the IUDs, the maximum serum progesterone concentration was very low, varying from 0.4 to 1.7 ng/ml on day one or two after insertion.

**Intravaginal sponges**

Dr. Eric Palmer (personal communication) has used polyurethane 40 mm x 50 mm sponges impregnated with estradiol plus altrenogest or P&E. They provided control over ovarian activity in mares when they were inserted into the vagina. However, the sponges caused vaginitis and in many cases, were difficult to remove because of vaginal adhesions.

This author has also performed several trials with intravaginal sponges (using sponges coated with Silastic dispersion and non-coated sponges) and has also used some novel intravaginal devices. All these devices suffered from one of two problems i.e. premature loss/ejection from the vagina or severe post-withdrawal vaginitis accompanied by a transient but foul smelling discharge. It was evident that the surface characteristics of sponges were responsible for this irritation because irritation was virtually absent when the sponges were coated with a thin layer of SilasticR MDX-4-4210 elastomer. Unfortunately, this coating allowed premature expulsion of sponges.

Some practitioners have reported using CIDR intravaginal devices (InterAg, Hamilton NZ) in mares with excellent retention and few side effects. However, CIDRs only contain progesterone and are not effective for accurate control of the estrous cycle.

**Traditional application of P&E**

The usual doses of progesterone and estradiol 17beta for a 500 kg mare are 150 mg of progesterone and 10 mg of estradiol 17beta respectively. In the case of draft mares, 1.5 to 2 time this dose should be given, depending on the weight of the mare. A mixture of both steroids is given intramuscularly once daily.

**Estradiol cypionate (ECP) or estradiol benzoate must not be used in place of unconjugated estradiol 17 beta** because both are catabolized com-
Notes paratively slowly and will cause delayed and erratic returns to estrus.

The following table illustrates a typical application of P&E. In this case, ovulation has been scheduled for a Wednesday evening because semen is commonly collected and shipped on Mondays, Wednesdays and Fridays.

### Typical use of P&E in a broodmare

**Method**
Use 15 ml of Benzyl alcohol and warm it to 40°C to facilitate dissolving of the steroids. Dissolve 5 g of progesterone and 330 mg of estradiol 17beta in the benzyl alcohol.

Pour the dissolved steroids into a sterile 100 ml vial and add 85 ml of cottonseed oil to make the total volume 100 ml. Cap the vial and withdraw 35 ml of the mixture.

In a sterile area and using a device that can exert considerable pressure on a syringe (one can be made from a bar clamp) push the mixture through a 0.22 micron “Millipore” filter so that the filtrate drips into a 35 ml vial. Cap the 35 ml vial, label it appropriately.

We dispense 35 ml vials as a single treatment regime for a 500 kg mare for ten days (10 days x 3 ml per day = 30 ml plus 5 ml for wastage). Our cost is approximate $Can 40.00 per ten day treatment. Compounding pharmacies

<table>
<thead>
<tr>
<th>MONDAY</th>
<th>TUESDAY</th>
<th>WEDNESDAY</th>
<th>THURSDAY</th>
<th>FRIDAY</th>
<th>SATURDAY</th>
<th>SUNDAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start P&amp;E on the first Saturday that follows after 14 days post foaling. If mare is not pregnant, wait until first ovulation of season has been confirmed.</td>
<td>First day of P&amp;E treatment</td>
<td>Day 2 of P&amp;E treatment</td>
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<tr>
<td>Day 3 of P&amp;E treatment</td>
<td>Day 4 of P&amp;E treatment</td>
<td>Day 5 of P&amp;E treatment</td>
<td>Day 6 of P&amp;E treatment</td>
<td>Day 7 of P&amp;E treatment</td>
<td>Day 8 of P&amp;E treatment</td>
<td>Day 9 of P&amp;E treatment</td>
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<tr>
<td>Day 10 of P&amp;E treatment</td>
<td>Day 1 post P&amp;E</td>
<td>2 post P&amp;E</td>
<td>Day 3 post P&amp;E</td>
<td>Day 4 post P&amp;E</td>
<td>Day 5 post P&amp;E</td>
<td>Day 6 post P&amp;E</td>
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<tr>
<td>Also give PG</td>
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<tr>
<td>Day 7 post P&amp;E</td>
<td>Day 8 post P&amp;E</td>
<td>Day 9 post P&amp;E</td>
<td>Day 10 post P&amp;E</td>
<td></td>
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</tr>
<tr>
<td>Inform stud of semen required on Wednesday is using shipped semen.</td>
<td>Give 3000 I.U. hCG IV or IM. between 8-10am</td>
<td>Ship semen. AI when it arrives or US q 4 hrs if using frozen semen.</td>
<td>Confirm ovulation &amp; schedule pregnancy diagnosis.</td>
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</tr>
</tbody>
</table>

Note that the GnRH analog deslorelin (“Ovuplant®” Fort Dodge) may be used instead of hCG.
will provide a somewhat more expensive product but even so, it is a very inexpensive treatment.

**Adverse reactions to P&E**

Using doses of 3 ml of P&E over many hundreds of injections, we and our colleagues in the field have seen no adverse effects attributable to treatment. However, a small number of owners (7 in approximately 23 years of use) have reported swelling at injection sites. This is discussed on the second page of this article as well. In that regard, one should educate clients on correct injection sites and technique.

**Commercial sources of P&E**

As a practitioner, one may not be able to purchase the reagents needed to manufacture P&E in your own practice. If a local pharmacy cannot formulate P&E for you, P&E can be purchased from one of the following sources.

Summit Veterinary Pharmacy, 25 Furbacher Lane, Aurora, Ontario. L4G 6W3. Contact: Mr Matthew Wren. Phone (toll free): 1-866-794-7387. mwren@svprx.ca.

Summit sells two concentrations of P&E. Request the formula containing 3.3 mg of estradiol and 50mg of progesterone per ml.


Centaur labs produces a more concentrated product that we do (75 mg of progesterone and 5mg of estradiol 17 beta per ml) which can precipitate out more easily in cool weather. Order the product about 3 weeks in advance to allow time for it to be formulated.

You will notice that Centaur Labs places two warning labels on the bottles; i) “Potential carcinogen”. Due to the well known effects of estrogens on mammmary and cervical cancer in women. Certainly, there is no evidence to suggest that P&E is carcinogenic in mares when used normally.

ii) “For experimental purposes only”. This is essentially true. P&E has not gone through the rigorous testing required by regulatory authorities in Canada (or anywhere else in the world) so you, the practitioner are liable for adverse reactions. However, P&E is used in very large amounts in Kentucky and my colleagues and I have use it for about 30 working years without seeing any serious side effects.

Laboratoires Denis Giroux Inc 332, Boulevard. Daniel-Johnson, Saint-Pie Quebec J0H 1W0 Canada. Phone: 1-888-888-7979. Fax: 1-800-223-0666. E-mail: giroux@gsg-net.qc.ca


Pharmaceutical Specialties, Baton Rouge, LA. Phone: 1-877-835-4755, e-mail: mbourg@eatel.net.

Please let the author know (lofstedt@upei.ca) if you have an alternate supplier that can be added to this list, especially in the UK, Europe or anywhere outside of North America

**Selected references**


12. Lofstedt, R.M. and Ireland, W. P. 2000 Measuring follicles and Other Sphere-like Structures