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The inability of some synthetic progestagens to maintain pregnancy in the mare

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Introduction

Progesterone or synthetic progestagens are administered frequently by equine veterinary clinicians to pregnant mares with a history of pregnancy loss (Neely 1988) in the belief that such therapy is necessary to supplement inadequate production of endogenous progesterone by the *corpus luteum* (CL) or placenta (Allen 1984). Low serum progesterone concentrations have been associated with early embryonic loss in some mares (Douglas *et al.* 1985; Ginther 1985; Bergfelt *et al.* 1992), but no evidence exists to support a conclusion that primary luteal deficiency is a significant cause of early embryonic death (Allen 1984; Ginther 1992). Indeed, Irvine *et al.* (1990) showed convincingly that serum progesterone concentrations were coincidentally low in only 1 of 17 instances of spontaneous early fetal death which occurred in a large herd of Standardbred mares. Inadequate luteal function has been observed (Bergfelt and Ginther 1992) in seasonally anoestrous mares stimulated to ovulate by treatment with gonadotrophin releasing hormone (GnRH) but the resulting increase in early embryonic loss rate was apparently related to a lack of gonadotrophic support for luteal function that would not be likely to exist in normal, cycling mares in the breeding season.

Shideler *et al.* (1982) demonstrated that pregnancy could be maintained in ovariectomised mares by administering high doses of progesterone or the orally active synthetic progestagen, altrenogest, at a dose rate of 0.044 mg/kg bwt daily. However, we showed subsequently that another synthetic progestagen, hydroxyprogesterone caproate, would not similarly maintain pregnancy in ovariectomised mares when administered as a single intramuscular (i.m.) injection of 1000 mg according to the manufacturer's instructions (McKinnon *et al.* 1993). Furthermore, in a parallel study, Nobelius (1992) demonstrated that hydroxyprogesterone caproate exhibits very low binding affinity for equine endometrial progesterone receptors.

We have extended this line of enquiry in the present study by examining the abilities of 5 commercially available synthetic progestagen preparations, medroxyprogesterone acetate, hydroxyprogesterone hexanoate, norgestomet, megesterol acetate and altrenogest, to maintain pregnancy in mares following treatment with prostaglandin $F_{2\alpha}$ to induce luteolysis and so remove the endogenous source of progesterone.

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Materials and methods

The mares used in the experiment were drawn from a herd of 95 experimental mares kept in good body condition on pasture and supplemented as necessary with lucerne hay and grain. Transrectal ultrasonography was used to monitor follicle development, ovulation and presence of a *corpus luteum* in their ovaries, and to diagnose pregnancy and monitor growth or loss of the conceptus in their uteri. Fifty mares were each inseminated with 500 x 10^6 progressively motile spermatozoa when they showed oestrous behaviour in association with an ovarian follicle ≥ 30 mm in diameter (Pickett *et al.* 1987) and 25 of them conceived. When they were diagnosed pregnant by ultrasonographic recognition of the conceptus on Day 14 after ovulation they were divided randomly into 5 groups, each of 5 mares, and treated as follows, with:

Group A: 1000 mg medroxyprogesterone acetate i.m. (Promone E Aqueous Suspension)[1] every 7 days, beginning on Day 16 of pregnancy.

Group B: 500 mg hydroxyprogesterone hexanoate i.m. (Gesteron-500)[2] every 4 days, beginning on Day 16 of pregnancy.

Group C: Altrenogest *per os* (Regumate)[3] at a rate of 0.044 mg/kg, beginning on Day 16 of pregnancy for up to 10 days.

Group D: 15 mg norgestomet in the form of 5 subcutaneous slow release implants (Crestar)[4] on Day 16 of pregnancy.

Group E: 500 mg megesterol acetate *per os* (Suppress)[5] beginning on Day 16 of pregnancy for up to 10 days.

All the mares were given a single i.m. injection of 5 mg $PGF_{2\alpha}$ (Lutalyse)[6] on Day 18 of pregnancy (i.e. 2 days after commencing progestagen administration) to induce luteolysis of the primary *corpus luteum*. The mares were blood sampled daily and the serum assayed subsequently for progesterone concentration using an amplified enzyme-linked immunoassay

TABLE 1: Pregnancy losses after treatment with synthetic progestagens

Experimental group	No. mares	Treatment (commenced on Day 16 after ovulation)	Mean (± s.d.) interval from $PGF_{2\alpha}$ administration to abortion (days)
A	5	1000 mg medroxyprogesterone acetate every 7 days (i.m.)	4.2 ± 2.6
B	5	500 mg hydroxyprogesterone hexanoate every 4 days (i.m.)	3.4 ± 1.1
C	5	0.044 mg/kg altrenogest daily (*per os*)	All remained pregnant to Day 30
D	5	15 mg norgestamet once (s.c.)	2 ± 0
E	5	500 mg megesterol acetate daily (*per os*)	3 ± 1.5

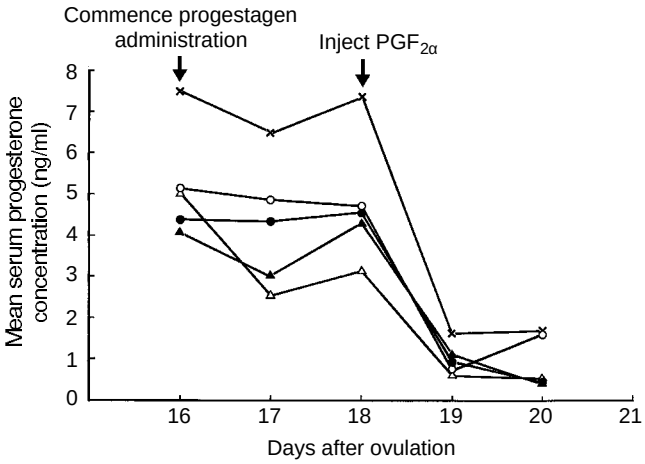

Fig 1: Mean serum progesterone concentrations measured in 5 groups of pregnant mares (n = 5 mares per group) treated with one of 5 different synthetic progestagens from Day 16 after ovulation, with a single i.m. injection of $PGF_{2\alpha}$ given on Day 18 to induce luteolysis of the primary corpus luteum. ●—● Group A, *1000 mg medroxyprogesterone acetate i.m. every 7 days; x—x* Group B, *500 mg hydroxyprogesterone hexanoate i.m. every 4 days;* ▲—▲ Group C, *0.044 mg/kg altrenogest orally once daily;* o—o Group D, *15 mg norgestomet s.c. once only;* Δ—Δ Group E, *500 mg megesterol acetate orally once daily*.

(AELIA), developed and validated for horse serum by Allen and Sanderson (1987). The mouse monoclonal anti-progesterone serum used in the assay (Stanley *et al.* 1986) was shown to exhibit <1% cross-reactivity with medroxyprogesterone acetate and altrenogest, but it was not tested against the 3 other progestagens used in this study.

Daily blood sampling was continued until either abortion occurred, or a fetus with a detectable heartbeat was found to be still present in the uterus on Day 24 after ovulation. Ultrasonography was also performed daily to monitor the continuation or otherwise of pregnancy, the occurrence of oedematous folds in the endometrium as an indication of the onset of oestrus, the time from $PGF_{2\alpha}$ treatment to pregnancy loss if this occurred, and the interval from $PGF_{2\alpha}$ to the next ovulation. The times to pregnancy loss and ovulation and the concentrations of progesterone in peripheral serum were compared between the groups.

Results

Pregnancy losses

All 5 mares in each of *Groups A, B, D* and *E* aborted between 2 and 8 days after the administration of $PGF_{2\alpha}$ on Day 18 after ovulation, whereas all 5 mares in *Group C* given altrenogest before and after $PGF_{2\alpha}$ treatment remained pregnant (Table 1). Ten of the 20 mares that aborted (50%) lost their conceptus on Day 20, 3 on Day 21, 3 on Day 22, 2 on Day 23, 1 on Day 24 and 1 on Day 26, to give a mean (± s.d.) time to pregnancy loss of 3.3 (± 1.7) days. Seventeen of the 20 aborting mares (85%) exhibited endometrial oedema, which was first detected ultrasonographically prior to abortion in 14 of them (82%), on the day of abortion in one mare and on the day after abortion in the other 2 mares. Two of the *Group C* mares given altrenogest developed mild endometrial oedema but did not abort.

Progesterone profiles

Serum progesterone concentrations in all mares had fallen to <2 ng/ml on the day after $PGF_{2\alpha}$ administration (Day 19 after ovulation) and remained very low on the following days (Fig 1). There were no differences in serum progesterone concentrations between any of the groups of mares, either before or after administration of $PGF_{2\alpha}$, thereby indicating that the anti-progesterone serum used in the assay probably showed negligible cross-reactivity against any of the progestagens administered. One mare in *Group E* exhibited incomplete luteolysis although nonetheless lost the conceptus at the expected time. All mares in *Groups A, B, D* and *E* had returned to oestrus and ovulated within 11 days after $PGF_{2\alpha}$ administration and the mean (± s.d.) interval from $PGF_{2\alpha}$ to ovulation was 9.2 ± 1.6 days.

Discussion

This experiment demonstrated convincingly that none of the 4 progestagens, medroxyprogesterone acetate, hydroxyprogesterone hexanoate, norgestomet and megesterol acetate, when administered to pregnant mares at the dose rates recommended by their respective manufacturers, can maintain pregnancy between Days 18 and 30 after ovulation in the absence of endogenous progesterone secreted by a viable *corpus luteum*. It is therefore reasonable to conclude that the same progestagens will be unlikely to be any more efficacious if administered later in pregnancy, especially after mid-gestation when the placenta becomes the sole source of endogenous progesterone in equine pregnancy (Holtan *et al.* 1975; Shideler *et al.* 1982; Knowles *et al.* 1994).

The progestagens used in the experiment are commonly administered to horses by equine veterinary clinicians, either in an attempt to prevent abortion in pregnant mares or to suppress sexual behaviour in performance horses. The dose rates and routes and frequency of administration were those recommended by the manufacturers and it is unfortunately not possible to distinguish whether the abortions occurred as a result

of insufficient administration of active compound or, more probably, failure of the progestagen to bind adequately to endometrial progesterone receptors in the treated animals. Altrenogest was used as a positive control in the experiment since we had shown previously that this particular progestagen is able to maintain pregnancy in ovariectomised mares (McKinnon *et al.* 1993). This earlier trial also showed that hydroxyprogesterone caproate is unable to maintain pregnancy in ovariectomised mares which may have been the reason why the same synthetic molecule was repackaged under the new name of hydroxyprogesterone hexanoate. This renaming process was not known to us when the present experiment began.

Despite the lack of efficacy data to support the administration of any of these 4 synthetic progestagens to mares to prevent abortion, they are used widely in equine stud veterinary practice for this purpose. The unequivocal results of this study demonstrate convincingly that the practice should cease forthwith.

Acknowledgement

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Manufacturers'addresses

[1]Pharmacia and Upjohn Pty. Ltd., Rydalmere, NSW, Australia.
[2]Illium Veterinary Products, Smithfield, NSW, Australia.
[3]Hoechst Rousell Vet, Melbourne, Victoria, Australia.
[4]Intervet (Australia) Ltd., Castle Hill, NSW, Australia.
[5]Jurox Pty Ltd, Silverwater, NSW, Australia.
[6]Upjohn Limited, NSW, Australia.

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