CONTROL OF OVULATION IN CYCLING MARES WITH OVARIAN STEROIDS AND PROSTAGLANDIN

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Received for publication: 7/3/80

ABSTRACT

A combined progesterone-estradiol-17β treatment was given in two experiments conducted to examine its effectiveness in controlling ovulation time in cycling mares. In the first experiment, the combined steroid (150 mg progesterone, 10 mg estradiol-17β daily for 10 days) alone or combined with prostaglandin on the first and last days of steroid treatment resulted in ovulation in 15 of 16 mares 9-13 days after last injection, 13 of them on days 10-12. A CL present prior to treatment in one mare that received no prostaglandin persisted through and for 14 days after treatment. In the second experiment the combined steroid treatment started on the first or second day of estrus blocked ovulation in only 5 of 13 mares. Thus prostaglandin is necessary at least at the end of treatment. In both experiments a total of 20 mares with no luteal function at the end of steroid treatment ovulated on days 9-13 after last injection, 18 of these on days 10-12. These results indicate that the combined steroid-prostaglandin treatment can result in ovulations in a very restricted interval with apparently a normal distribution.

ACKNOWLEDGEMENT

The investigation reported in this paper (No. 80-4-147) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director. It was supported in part by a grant from The Grayson Foundation, Inc. Prostin $F_{2\alpha}$ was furnished by The Upjohn Co., Kalamazoo, Michigan.

INTRODUCTION

It has not been possible to exercise precise control of estrous cycles and time of ovulation by modification of the luteal phase of the cycle in mares. Lysis of the cyclic corpus luteum (CL) with prostaglandin $F_2\alpha$ or its analogs results in considerable variation in the subsequent interval to ovulation (2). This variability increases with treatment on different days of the luteal phase, precluding precise control of ovulation time by the use of these products in single doses or a variety of time spaced doses.

When exogenous progesterone is given to a group of mares to inhibit ovulation for a period long enough to allow regression of all existing CL its withdrawal is followed by unpredictable intervals to ovulation. The effect of this treatment has been to reduce the interval during which ovulations occur to about one-half a normal cycle length (1). Unfortunately, the ovulations during this interval do not appear to be normally distributed but instead are distributed rather uniformly throughout the interval.

The term synchronization also has been applied to results obtained by the use of an orally active progestogen in mares (4, 5, 6). The data as presented, however, are unclear as to the length of interval during which ovulations occurred and distribution of ovulations within that interval, but seem to suggest results similar to those noted above for progesterone treatment.

Early attempts to delay uniformly the first ovulation postpartum with exogenous progesterone were only partially successful in that ovulation was delayed but by no means uniformly (3). In later studies (unpublished data, University of Kentucky), a bimodal response resulted when mares were given 100 or 200 mg of progesterone in oil solution per day from the day of foaling through day 10 postpartum (Figure 1). The bimodal pattern appeared to result because the first follicles to develop after parturition ovulated after withdrawal of progesterone in some mares and regressed in others to be replaced by new follicles that ovulated later. The means of the two modes were separated by about a week. These results suggested that it was necessary to inhibit early development of follicles during the treatment period and that this inhibition was not achieved by progesterone (100 or 200 mg/day) alone even though ovulation was prevented.

It was determined in preliminary trials that a daily treatment regime of 200 mg of progesterone combined with 10 mg of estradiol-17β in oil solution resulted in adequate inhibition of follicular development during the treatment period. More extensive trials applying this treatment from the day of foaling (day 0) through day 4 or 5 postpartum resulted in the distribution of post treatment intervals to ovulation shown in Figure 2 (unpublished data, University of Kentucky). Two characteristics suggest that this treatment in effect simply extended

the inhibition of ovarian activity imposed by steroid production by the feto-placental unit. First, the distribution of intervals from the last day of treatment to ovulation appeared to be a normal one and very similar to that for intervals from parturition to first ovulation in untreated mares foaling contemporaneously. The mean and variance of the interval from end of treatment to ovulation were 10.9 days and 5.6, respectively, compared with 10.2 days and 6.0 for the interval from foaling to ovulation in untreated mares. Second, in treated mares the average interval from parturition to ovulation (15.8 days) was prolonged by an amount of time equivalent to the period of treatment when compared to the interval from parturition to ovulation in untreated mares.

The purpose of this study was to determine whether a combined progesterone-estradiol-17β treatment might be used to exercise a greater degree of control of ovulation time in cycling mares by shortening the range of time over which ovulations occurred following treatment, and by normalizing the distribution of ovulations within that time range. The study was also conducted to evaluate possible problems that might be encountered in this procedure.

MATERIALS AND METHODS

Mares used were of mixed light horse breeding. They were estimated to weigh between 400 and 550 kg and were 4 to 15 years of age. They were maintained on a mixed grass-legume pasture and were given a small amount of oats daily. All were in good condition prior to and throughout the experimental period.

Experiment 1. Twenty-four mares were tested for estrus daily with a teaser stallion. Rectal palpation of ovaries was done daily during estrus or when follicles 25 mm or larger were present, and at least every third day at other times. Following establishment of normal estrous cycles mares were assigned randomly, irrespective of stage of cycle, to one of three treatment groups. All treatments, given intramuscularly (IM), were started on May 29 and were: Group 1 (control), 3 ml of cottonseed oil (CSO) daily for 10 days; Group 2, 150 mg progesterone and 10 mg estradiol-17β dissolved in 3 ml CSO daily for 10 days; Group 3, the same steroid treatment as Group 2 but 10 mg of prostaglandin $F_2\alpha$ (Prostin $F_2\alpha$, The Upjohn Company) were given IM on day 1 and day 10 of the treatment period. Teasing and examination per rectum were continued until 6 days after the post treatment ovulation. Intervals from last injections to estrus and to ovulation and length of estrus were examined by analysis of variance.

Experiment 2. Thirteen mares used in this experiment had a normal estrous cycle prior to use in this trial or were in early estrus following a diestrus of normal length after a previous ovulation. Teasing and rectal examinations were done as in Experiment 1. Treatment consisted

of IM injection of 200 mg of progesterone and 13.2 mg of estradiol-17β in CSO daily for 5 days beginning on day of estrus when a follicle of 25 mm or larger was first palpated. Treatment began on the first or second day of estrus in 12 estrous periods and on the third day in one. On treatment days 6 through 10 the dosage was reduced to 150 mg of progesterone and 10 mg of estradiol-17β. Teasing and rectal examinations were continued through the first ovulation after treatment.

RESULTS AND DISCUSSION

Experiment 1. Mares in Groups 2 and 3 exhibited unusual responses to the teaser stallion within 24 hours after receiving their first steroid treatment. Most displayed some signs of estrus such as winking of the vulvar labia and raising tails. These signs, however, were accompanied by a generally agitated behavior and none would accept the stallion. As treatment continued, response to the teaser became more uniformly that of diestrus and all mares rejected the stallion's advances. Mares in Group 1 showed no unusual behavior patterns.

Ovarian follicular activity was inhibited during the 10-day steroid treatment period in 14 of 16 mares in Groups 2 and 3. One mare (Group 3) was in the second day of estrus at the beginning of treatment. A 45 mm follicle present at that time continued to develop and ovulated 5 days after the onset of estrus (day 4 of treatment). The second mare (also Group 3) was six days post-ovulation at the beginning of treatment. A follicle first palpable on the fifth day of treatment continued to grow to about 45 mm by three days post treatment but failed to ovulate. Subsequently a follicle on the opposite ovary progressed to ovulation 9 days post treatment. Two additional mares in Group 2 developed follicles of about 25 mm during treatment but these regressed prior to the end of treatment. In no instance did a follicle palpated during treatment in Groups 2 and 3 ovulate after treatment.

In Group 1, follicular development appeared to be normal and reflected the stage of the estrous cycle as established prior to the start of treatment. Ovulation occurred in two mares during the treatment period. Following the treatment period mares in Group 1 returned to estrus within 14 days and all had ovulated by 19 days after the end of treatment. Estrus lasted 6.5 days and ovulation occurred 0.9 days before the end of estrus.

Termination of a 10-day treatment with the combined progesterone-estradiol-17β preparation resulted in a return to estrus within 6 days and ovulation within 12 days in 7 of 8 mares in Group 2. Estrus was of normal length (7.4 days) and ovulation occurred on the average 1.0 days before the end of estrus. Based upon the stages of the cycle at the start of treatment in this group (days 4-12 after ovulation), all CL should have ceased function or have been very near the end of normal life span at treatment end. This appeared to be true except for

one mare. In this single instance the CL present prior to treatment persisted through 14 days post treatment (plasma progesterone >1 ng/ml, total CL life span 39 days) at which time 10 mg $PGF_2\alpha$ given IM resulted in estrus within 48 hours and ovulation within 96 hours. Data from this mare were excluded from statistical examination. It is assumed that if $PGF_2\alpha$ had been given to this mare on the first and last days of steroid treatment (as in Group 3), it would have resulted in estrus and ovulation closer to those of other mares in Group 2.

The end of treatment in Group 3 was followed by return to estrus within 8 days and ovulation within 13 days in all 8 mares. Estrus was of normal duration (6.5 days) and ovulation occurred 0.5 days before the end of estrus.

Analysis of variance found no difference among group means for intervals from last injection to estrus or ovulation or for length of post treatment estrus.

The effects of treatments on control of ovulation time are summarized in Table I. In mares the control of estrus per se has relatively little practical value due to its length, variability and the uncertain relationship of its onset to the time of ovulation. Ovulation, on the other hand, represents as precise a point in time as in any other species. Thus control of estrus becomes less relevant and control of ovulation even more critical as practical matters in equine breeding management. The precision of control of ovulation is determined by the interval of time during which ovulations occur and their distribution within that interval. The mean time from the end of a treatment to ovulation in a group of mares says little about the degree of control achieved unless a description of the distribution of ovulations about the mean is provided.

The decreased ranges and variances of intervals from the end of treatments to ovulation in Groups 2 and 3 (Table I) combined with increased ranges and variances in intervals from ovulation prior to end of treatments to ovulation after treatments in these same groups constitute a qualitative definition of "control" of ovulation. The quantitative aspects of this control are a matter of interpretation of the data in Table I.

Experiment 2. It has been difficult to prevent ovulation when treatments that are normally effective if begun during diestrus were started during estrus (1, 6, unpublished data, the University of Kentucky). In Experiment 1 the only mare in which steroid treatment began during estrus ovulated during treatment. Experiment 2 was conducted to provide more information concerning steroid treatment started early in estrus when a developing follicle was present.

The treatment imposed in this study prevented ovulation of follicles present at the start of treatment in only 5 of 13 mares. In

these 5 mares, ovulation of new follicles occurred 11 days after treatment in 3 mares and 12 days after treatment in the others. Table II shows the individual response patterns of mares that ovulated during treatment. Ovulations during treatment occurred as early as the second and as late as the tenth day. Most occurred within normal intervals after the onset of estrus.

In 6 of 8 mares ovulating during treatment, intervals from the end of treatment to next ovulation ranged from 9 to 16 days while 2 did not ovulate again during the experimental period (Table II). The effect of exogenous steroid treatment at the time of ovulation and for variable times afterward had inconsistent effects on CL function. Corpus luteum life span appeared shortened as indicated by a short interovulatory period in two instances (Mare B and Mare C), unaffected in four (Mares A, D, G and H), and prolonged in two (Mares E and F). In the latter two, plasma progesterone levels were elevated for at least 26 and 25 days after treatment.

The net effect of initiating steroid treatment during estrus was to decrease the precision of control of ovulation time after treatment. The factors involved in the decreased precision were 1) whether ovulation occurred during treatment, 2) the time during treatment that ovulation occurred, and 3) the duration of function of luteal tissue formed at that ovulation.

GENERAL DISCUSSION

The use of progesterone and estradiol-17β resulted in more precise control of ovulation than has been reported when progestogens alone were used provided, 1) no ovulation occurred during treatment, and 2) CL were not functional past the end of treatment. The interval over which post treatment ovulations occurred under these conditions was 5 days (days 9 through 13 after treatment) with 18 of 20 ovulations occurring on days 10, 11 and 12 (Experiments 1 and 2 combined). Condition 1) above is difficult to achieve when treatment is begun during estrus and one workable solution would be to extend the combined steroid treatment to 15 days so CL formed by ovulation during the first part of treatment (when most do occur) would be susceptible to lysis by $PGF_2\alpha$ given the last day of steroid treatment. Prostaglandin treatment the final day of steroid treatment would be expected also to lyse CL that might otherwise persist following the steroid treatment as occurred in one mare in Group 2 of Experiment 1 and in two mares in Experiment 2. Persistence of CL has also been observed following long term progesterone treatment (unpublished data, University of Kentucky).

The lack of precision of control of ovulation time resulting from treatment with progesterone alone does not occur only when treatment is

initiated during estrus (1; unpublished data, University of Kentucky). The interval during which ovulations occur after treatment may approximate half a cycle length in mares in which treatment began during the luteal phase, with ovulations fairly uniformly distributed throughout that interval. One result of such a distribution is that at a given time following steroid withdrawal, some follicles may be susceptible to the ovulation advancing effects of HCG and others not, as suggested by the data of Holtan, Douglas and Ginther (1).

Progesterone treatment (and, presumably, progestogens generally) does not inhibit follicular development uniformly. A given dosage may result in inhibition at fairly early stages in some instances with only the latest stages affected in others. In extreme instances only terminal stages of development, including ovulation, are blocked. Thus when progesterone given to block ovulation is withdrawn in a group of mares, follicles in a wide range of developmental stages may exist resulting in ovulation of more mature follicles in a short time and a much longer time to ovulation in cases of the most immature follicles.

Combined progesterone and estradiol-17β treatment appeared to result in inhibition of follicular development at more uniformly early stages based upon ovarian palpation, conceivably at a fairly specific developmental stage. Withdrawal of this treatment resulted in a narrower interval during which ovulations occurred and an apparently more normal distribution of ovulations within that interval.

The results of these trials should not be extrapolated to treatment with progestogens other than progesterone combined with estrogens other than free estradiol-17β in solution in oil. Neither should these results be extrapolated to treatment in other than mares cycling during mid-breeding season.

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TABLE I. EFFECTS OF OVARIAN STEROIDS AND $PGF_2\alpha$ ON CONTROL OF OVULATION (OV) TIME IN MARES (EXPERIMENT 1).

		OV, Days Post-Treatment			Interval, last OV before end of treatment to post-treatment OV		
Group	n	Mean	Range	S^2	Mean	Range	S^2
1	8	11.5	5-19	24.8	20.6	19-24	3.1
2	7	11.1	10-12	1.1*	27.8	23-36	23.1**
3	8	10.9	9-13	1.6*	25.5	19-32	24.8**

*Less than Group 1, P <.01

**Greater than Group 1, P <.02

Equality of Variance (S^2) tested by two tailed F test.

TABLE II. RESPONSE PATTERNS OF INDIVIDUAL MARES THAT OVULATED (OV) DURING COMBINED PROGESTERONE-ESTRADIOL-17β TREATMENT BEGUN DURING EARLY ESTRUS (EXPERIMENT 2).

	OV During Treatment			
Mare	Day of treatment	After onset of estrus	OV, days Post treatment	Interval from treatment OV to post treatment OV
A	2	3	9	17
B	10	11	14	14
C	8	11	13	15
D	5	6	16	21
E	6	6	-*	-*
F	7	7	-*	-*
G	4	5	12	18
H	5	6	14	19

*CL formed at OV during treatment continued to function following treatment. See text.