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ESTRUS, OVULATION AND CONCEPTION FOLLOWING SYNCHRONIZATION WITH PROGESTERONE, PROSTAGLANDIN $F_2\alpha$ AND HUMAN CHORIONIC GONADOTROPIN IN PONY MARES[1]

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SUMMARY

In experiment 1, 72 pony mares were assigned randomly to an experiment of 3 × 6 factorial design (four mares/group) in which treatments were: controls, no injections (C); progesterone daily, 50 mg IM, days 0 through 18, plus HCG, 2,000 IU SC, 6 days later (P-HCG); prostaglandin $F_2\alpha$, 1.25 mg IM, days 0 and 18 only, plus HCG, 2,000 IU SC, 6 days following the last $PGF_2\alpha$ injection ($PGF_2\alpha$-HCG). Treatments were initiated on day 2 of estrus or days 1, 4, 7, 10 or 13 post-ovulation. There was no significant ($P>.05$) effect of day of cycle on which treatment began or treatment by day interaction on interval from day 18 to ovulation. Thus, data pooled over days indicated interval to ovulation was shorter ($P<.05$) for both P-HCG (8.5 ± .4 days, mean ± SE) and $PGF_2\alpha$-HCG (8.9 ± .8) treated mares than controls (12.8 ± 1.4). Although there was no difference between means for both treated groups there was greater variance ($P<.01$) in interval to ovulation following treatment with $PGF_2\alpha$-HCG than P-HCG. Mean size of largest follicle was greater ($P<.05$) in P-HCG (31.2 ± 2.7 mm) than $PGF_2\alpha$-HCG (22.7 ± 2.5 mm) treated mares; 11 of 21 P-HCG and six of 23 $PGF_2\alpha$-HCG treated mares ovulated within 2 days following HCG. Four of 21 P-HCG and six of 23 $PGF_2\alpha$-HCG treated mares ovulated before HCG treatment. Initial treatment with $PGF_2\alpha$ did not result in return to estrus or ovulation before the second $PGF_2\alpha$ injection in three of four and one of four mares which were treated on days 1 and 4 post-ovulation, respectively. Results indicate that better synchronization of ovulation occurred after the P-HCG regime than after the $PGF_2\alpha$-HCG regime.

In expeiment 2, a combination progesterone -$PGF_2\alpha$-HCG regime was followed by a single breeding (SB) or multiple breedings (MB) to determine pregnancy rate. Sixty pony mares were assigned randomly, without regard to stage of the estrous cycle, to three groups (n= 20). Controls were given no injections while treated mares received progesterone (75 mg, IM) for 10 days in addition to $PGF_2\alpha$ (1.25 mg, IM) on day 7 during progesterone treatment, and HCG (2,000 IU, SC) 5 days following progesterone. Controls were bred by artificial insemination with 10 ml fresh whole semen every other day while in estrus. Mares in one treated group (MB) were inseminated every other day during the post-treatment estrus, while another group (SB) was inseminated only once one day after HCG. Pregnancy rate following one estrous cycle was not significantly different among the three groups (controls, 64.7%; MB, 57.8%; SB, 40%). The number of inseminations per estrus and per conception, respectively, were controls, 3.9 and 6.0; MB, 3.7 and 6.4; and SB, 1 and 2.5.

(Key Words: Progesterone, Prostaglandin $F_2\alpha$, Estrus Synchronization, Conception, Mares.)

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INTRODUCTION

Control of the estrous cycle by hormonal means has been studied extensively in several farm species, but there have been comparatively few studies on the mare. Progesterone inhibits estrus, follicular growth and ovulation in mares (Loy and Swan, 1966; Van Niekerk *et al.*, 1973) while synthetic progestogens, which are active orally in other species, do not have consistent effects in the mare (Loy and Swan, 1966). Furthermore, in mares, some oral synthetic progestogens reportedly induce sporadic estrous activity (Loy and Swan, 1966; Hoppe *et al.*, 1974) although another appears to achieve a good degree of synchrony (Webel, 1975). Naturally occurring prostaglandin $F_2\alpha$ (Douglas and Ginther, 1972, 1975; Oxender *et al.*, 1974) or synthetic analogs (Allen and Rowson, 1973; Palmer and Jousset, 1975) have been used to induce premature luteolysis and early return to estrus and ovulation. However, prostaglandins are effective only after days 3 to 4 post-ovulation (Douglas and Ginther, 1975). The above treatments have primarily been used early in the breeding season (Hoppe *et al.*, 1974) or to treat problem mares, such as those in constant estrus or anestrus (Loy and Swan, 1966) or those with a persistent corpus luteum (Allen and Rowson, 1973; Kenney *et al.*, 1975). Human chorionic gonadotropin (HCG) has been shown to shorten estrus and hasten ovulation (Sullivan *et al.*, 1973) and is thus used frequently in mare breeding. The use of progesterone and/or prostaglandin $F_2\alpha$ ($PGF_2\alpha$) plus HCG for estrus control and its subsequent effects on conception in cycling mares have apparently not been studied critically.

Experiment 1 was designed to test the efficacy of progesterone-HCG or $PGF_2\alpha$-HCG in synchronizing estrus and ovulation in normally cycling pony mares. Experiment 2 was designed to determine pregnancy rate following synchronization with a progesterone – $PGF_2\alpha$-HCG treatment regime.

MATERIALS AND METHODS

Mares were of the pony type weighing approximately 150 to 250 kg and 3 to 12 years of age. Anthelmintics and vaccines were provided one month before assignment to treatment. Mares were maintained on alfalfa-grass pasture with free-choice mineralized salt and water. Although previous reproductive histories were unknown, all mares were observed to exhibit estrus and ovulate before being used. Mares were routinely teased for behavioral estrus and palpated per rectum (Ginther, 1974) to determine ovulation at least every third day or daily if in estrus or if a follicle 30 mm in diameter or larger was present. Following treatment, mares were teased and palpated daily. The stage of the estrous cycle was calculated from the day of ovulation (ovulation = day 0).

Experiment 1. During June through August, 1973, 72 mares were assigned randomly to an experiment of 3 (treatment groups) × 6 (days) factorial design with four replicates. The three treatments were: controls, no injections; progesterone daily, 50 mg intramuscular (IM) in corn oil, on days 0 through 18 plus 2,000 IU human chorionic gonadotropin (HCG)[4] subcutaneously (SC) 6 days after the last progesterone injection (P-HCG); prostaglandin $F_2\alpha$-tham salt, 1.25 mg (free acid basis) IM days 0 and 18 only followed by HCG, 2,000 IU SC, 6 days later ($PGF_2\alpha$-HCG). Treatment began on the following days of the estrous cycle; day 2 of estrus or days 1, 4, 7, 10 or 13 post-ovulation. The interval from last progesterone or $PGF_2\alpha$ treatment (day 18) to estrus and ovulation and length of post-treatment estrus were subjected to analysis of variance and Duncan's multiple range test. For controls, interval to ovulation was calculated from day 18; interval to estrus was excluded as some mares may have already been in estrus, depending on their previous cyclic pattern. Ovulations not accompanied by estrus were included in the ovulation data. If double ovulation occurred, the second ovulation was used in the calculations.

Experiment 2. In 1974, the treatment regime was shortened by combining progesterone, $PGF_2\alpha$ and HCG and the pregnancy rate following a single breeding (SB) or multiple breedings (MB) after synchronization was determined. Sixty mares were assigned randomly to three groups (20 mares/group). Control mares were given no injections while mares in the two treated groups received progesterone (75 mg, in corn oil, IM) for 10 days and $PGF_2\alpha$ (1.25 mg, IM) on day 7 during the progesterone treatment. On day 5 following the last progesterone injection, HCG (2,000 IU, SC) was given to treated mares only. Control mares

[4] Follutine, E. R. Squibb, and Sons, Inc.

were bred by artificial insemination (AI) every other day while in estrus. Treated mares were bred only once (SB) 6 days after the last progesterone injection or every other day while in estrus (MB). Semen was collected by an artificial vagina every other day from two pony stallions known to be fertile. Raw semen was filtered through cheesecloth to remove the gelatinous clot, and the remaining semen was pooled and used within 45 min of collection. The stallions were collected every 2 days for 2 weeks before semen was used for AI. Mares were inseminated intrauterine with 10 ml of fresh, undiluted semen. Behavioral estrus and ovulation were monitored and analyzed as in experiment 1. Although treatments were not begun on an assigned day of the estrous cycle, as in experiment 1, previous cycle data were known. Mares were grouped into estrus or early (days 0 to 2), mid (days 4 to 6) or late (days 9 to 15) diestrus, according to stage of the cycle on which treatment began. Pregnancy rate (number pregnant ÷ number bred during one estrus period) was based on nonreturn to estrus and palpation per rectum for a fetal bulge and ovarian changes on day 25 following last ovulation. Pregnancy was confirmed in some mares laparotomized (days 25 to 70) during a subsequent experiment; all others were palpated again at 70 to 90 days.

RESULTS

Experiment 1. The main effect of treatment was significant ($P<.05$) with no effect of day on which treatment was begun (day 2 of estrus or days 1, 4, 7, 10 and 13 post-ovulation) or treatment x day interaction of end points measured. Data were, therefore, pooled for main effect of treatment (control, P-HCG or $PGF_2\alpha$-HCG) and are shown in table 1. Three mares did not ovulate following P-HCG treatment and one mare in the $PGF_2\alpha$-HCG group was treated at the wrong time so were excluded from the data. Interval from end of treatment (day 18) to ovulation was shorter ($P<.05$) in mares of both treated groups as compared to controls. Although there was no difference between means of the treated groups, there was greater variance ($P<.01$) in the $PGF_2\alpha$-HCG treated group. There was no significant difference in interval from treatment to the first day of estrus for P-HCG or $PGF_2\alpha$-HCG treated mares or total length of post-treatment estrus between all three groups. The frequency of post-treatment ovulations is shown in figure 1. If one considered breeding only on day 6 post-treatment 11 of 21 (52.3%) P-HCG and six of 23 (26%) $PGF_2\alpha$-HCG treated mares ovulated on day 7 or 8. If a period of 4 days is considered, (days 6 through 9), 15 of 21 (71.4%) P-HCG and 10 of 23 (43%) $PGF_2\alpha$-HCG treated mares ovulated during this time. Some mares in both the P-HCG (four mares) and $PGF_2\alpha$-HCG (six mares) treated groups ovulated before HCG was given.

Three mares did not return to estrus or ovulate following P-HCG treatment while three others ovulated without exhibiting estrus. Progesterone effectively suppressed estrus, follicular development and ovulation except for two of four mares which ovulated when treatment

TABLE 1. EFFECT OF PROGESTERONE-HCG OR $PGF_2\alpha$-HCG ON SYNCHRONIZATION OF ESTRUS AND OVULATION IN MARES (EXPERIMENT 1)

Item	Controls	Treated[a]	
		P-HCG	$PGF_2\alpha$-HCG
No. mares	24	21	23
Length of estrus	6.7 ± .5[b]	5.4 ± .5[b]	6.3 ± .6[b]
Interval to estrus[d]	. . .	4.0 ± .3[b]	4.0 ± .6[b]
Interval to ovulation	12.8 ± 1.4[b]	8.5 ± .4[c]	8.9 ± .8[c]
Inter-ovulatory interval[e]	24.2 ± .6[b]	35.3 ± 1.7[c]	36.3 ± 1.5[c]

[a]Progesterone (50 mg, IM) days 0 through 18. $PGF_2\alpha$ (1.25 mg, IM) day 0 and 18 only. Treated groups also received HCG (2,000 IU, SC) on day 6 post-treatment. Controls received no injections.

[b,c]Mean ± SE. Means within a row with different superscripts are different ($P<.05$).

[d]Normal dispersion of cyclic activity within the control group precludes this calculation.

[e]Excludes ovulations following first $PGF_2\alpha$ treatment.

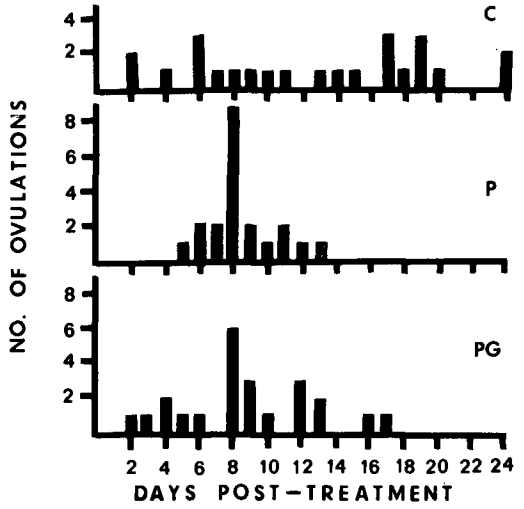

Figure 1. Frequency distribution of mares ovulating following progesterone (P, 50 mg/day, IM) for 18 days or $PGF_2\alpha$ (PG, 1.25 mg, IM) on days 0 and 18. Treated mares received HCG (2,000 IU, SC) day 6 post-treatment. Controls (C) received no injections.

was begun on day 2 of estrus; both mares had follicles >20 mm diameter when treatment began. Two mares had bilateral ovulations 1 and 2 days apart.

Following the first injection of $PGF_2\alpha$ there was apparently no effect when given during estrus, but one of four (day 1 diestrus) and three of four (day 4 diestrus) mares returned to estrus and ovulated. All other mares apparently responded to the first $PGF_2\alpha$ treatment except one mare treated on day 10 diestrus. This mare remained in estrus until after the second $PGF_2\alpha$ injection. One mare had bilateral ovulations following the second $PGF_2\alpha$ injection.

Experiment 2. The effects of the combination progesterone-$PGF_2\alpha$-HCG treatment regimen are shown in table 2. Three mares in the men are shown in table 2. Three mares in the control group did not exhibit estrus after assignment to treatment and one mare in the treated, multiple breeding group was in estrus 34 days so treatment estrus in the three groups of mares was not different (P>.05) and interval from end of treatment to estrus (4.2 *vs* 4.6 days) or ovulation (8.9 *vs* 10.4 days) between the treated groups of mares was not different (P>.05) indicating a lack of effect between single or multiple breedings (table 2). There was no difference (P>.05) in pregnancy rate between controls (64.7%), treated, single breeding (40%), or treated, multiple breeding (57.8%).

The number of inseminations per estrus and per conception, respectively, were 3.9 and 6 for controls, 1 and 2.5 for treated, single breeding, and 3.7 and 6.4 for treated, multiple breeding mares (table 2).

The frequency of ovulations post-treatment is shown in figure 2. Six of nine mares which ovulated on day 7 and one of two each on days 8 and 9 (48 and 72 hrs post-insemination) conceived to one service on day 6. Cumulative percentage of ovulations for treated, single breeding mares was 45% by day 7, 65% by day 9 and 85% by day 11 post-treatment. For treated, multiple breeding mares ovulations were 15.8% by day 7, 52.6% by day 9 and 78.9% by day 11. Four treated, multiple breeding mares ovulated 18 days after progesterone withdrawal and all four conceived. One of these mares was in estrus 18 days while another was in estrus 12 days, and therefore, were bred 9 and 6 times, respectively.

All mares in Experiment 2 were assigned to begin treatment on the same calendar day irrespective of the stage of their estrous cycle. However, previous cycle data were known and thus comparisons of stage of the estrous cycle when treatment began could be made. There was no difference (P>.05) in interval from end of treatment to estrus or ovulation or length of post-treatment estrus in the treated mares. Therefore data from these two groups of mares

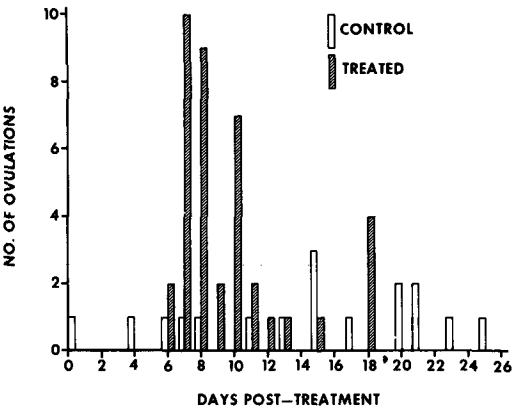

Figure 2. Frequency distribution of mares ovulating following treatment with progesterone (75 mg, IM) for 10 days and $PGF_2\alpha$ (1.25 mg, IM) day 7 during, and HCG (2,000 IU, SC) day 5 following, progesterone treatment. Controls received no injection.

TABLE 2. EFFECT OF PROGESTERONE, $PGF_2\alpha$ AND HCG ON SYNCHRONIZATION OF ESTRUS AND OVULATION AND ON PREGNANCY RATE IN MARES (EXPERIMENT 2)

Item	Controls	Treated[a]	
		Single breeding	Multiple breeding
No. mares	17	20	19
Length of estrus	7.3 ± .6[b]	5.7 ± .6	7.4 ± .8
Interval to estrus[c]	. . .	4.2 ± .3	4.6 ± .7
Interval to ovulation	14.2 ± 1.8	8.9 ± .5	10.4 ± 1.0
Inter-ovulatory interval	23.6 ± .6	25.5 ± 2.0	27.5 ± 1.6
No. pregnant	11	8	11
Percent pregnant[d]	64.7	40.0	57.8
No. inseminations per estrus	3.9	1	3.7
No. inseminations per pregnancy	6.0	2.5	6.4

[a]Treatment; progesterone (75 mg, IM) for 10 days plus $PGF_2\alpha$ (1.25 mg, IM) on day 7 during, and HCG (2,000 IU, SC) 5 days after, progesterone treatment. Controls received no injections.

[b]Mean ± SE (where indicated). No differences ($P>.05$) between groups for length of estrus, interval to estrus or ovulation, inter-ovulatory interval and percent pregnant.

[c]Normal dispersion of cyclic activity within the control group precludes this calculation.

[d]Percent pregnant = number pregnant ÷ number bred during one post-treatment estrus only.

were pooled and grouped according to stage of the estrous cycle when treatment was begun (i.e., estrus, early, mid or late diestrus). As in Experiment 1, stage of the cycle when treatment was begun had no effect ($P>.05$) on the end points examined. Pregnancy rates seemed to be greater when treatment began in mid or late diestrus although numbers were small.

Determination of pregnancy by palpation per rectum of a turgid, tubular uterus with a fetal bulge on day 25 was verified in many mares laparotomized on day 25, or later, in a subsequent experiment. Mares not laparotomized were palpated again at 70 to 90 days to further verify pregnancy; no indication of early embryonic resorption or abortion was detected.

Discussion

The difficulty in determining the optimal time to breed mares is well known. The practical advantages associated with estrus control of mares on breeding farms to reduce or eliminate teasing and palpation and more precisely schedule breeding makes synchronization a promising tool. Techniques previously tried usually had some undesirable aspects such as severe side effects and refusal of feed with methallibure (First, 1973), inconsistent or no response to some orally active progestogens (Loy and Swan, 1966; Hoppe *et al.*, 1974) or the prerequisite of a functional corpus luteum (CL) with the use of $PGF_2\alpha$ (Douglas and Ginther, 1975; Oxender *et al.*, 1974). Thus the present study, even though requiring daily injections, seemed promising. A different delivery system, such as feeding, implants, or pessaries could be adopted.

The interval from withdrawal of progesterone to estrus (4.0 ± .3 days) and ovulation (8.5 ± .4 days) in Experiment 1 (table 1) is similar to other reports using progesterone (Loy and Swan, 1966; Van Niekerk *et al.*, 1973) but appears to be slightly shorter than after methallibure (First, 1973). The lack of a significant day effect indicates treatment beginning at any stage of the cycle did not affect subsequent interval to ovulation. Thus such a treatment regime could be used without prior knowledge of the stage of cycle. Even though better synchronization would be desirable after progesterone withdrawal 52.3% ovulated on days 7 or 8, and 71.4% on days 6 through 9, which would make breeding once, or twice, during this time feasible.

Estrus, follicular development and ovulation were effectively suppressed with 50 mg progesterone per day. There was no evidence of follicular cysts after progesterone treatment as reported by Loy and Swan (1966) or incidence of estrus or ovulation during treatment as with methallibure (First, 1973). Large follicles (20

to 30 mm dia.), if present at the beginning of treatment, usually regressed and did not ovulate.

The use of two injections of $PGF_2\alpha$ 18 days apart was designed to induce luteolysis if a CL was present at the first injection and theoretically all CL should then be approximately 10 to 19 days old at the time of the second injection. The mean interval from treatment to ovulation, 10 to 12 days reported by Douglas and Ginther (1975), would preclude shortening the interval between the two $PGF_2\alpha$ injections much less than 18 days as some CL may not be more than 4 days old at the time of the second injection. In addition, mares in estrus, or days 1 to 4 of diestrus, and not responding to the first treatment may have CL 16 to 22 days old at second injection and thus natural luteolysis may have begun. This may have accounted for the greater variation ($P<.05$) in interval to ovulation, 8.9 ± .8 days after $PGF_2\alpha$-HCG *vs* 8.5 ± .4 days after P-HCG. Another explanation of the greater variation following $PGF_2\alpha$-HCG may be that the interval from luteolysis, either naturally occurring or induced, to subsequent ovulation is due to variability between mares and that the interval is more consistent within the same mare. Examination of the intervals from the first *vs* the second $PGF_2\alpha$ injection (figure 1) indicates a signficiant correlation ($r = .59$, $P<.05$). That is, mares responding with a shorter interval following the first injection had similar intervals to ovulation following the second $PGF_2\alpha$ injection; those mares having longer intervals following the first had similar intervals following the second $PGF_2\alpha$ injection. Synchronization following both treatments in Experiment 1 was based on the theory of progesterone withdrawal, either from an exogenous (P-HCG) or endogenous ($PGF_2\alpha$-HCG) source but a significant difference in variances may indicate somewhat different mechanisms are involved. Only 23% of the $PGF_2\alpha$-HCG treated mares ovulated on days 7 or 8, and 43% on days 6 through 9 as compared to 52 and 71%, respectively, for the same time periods following P-HCG treatment.

The use of HCG in this study was designed to hasten ovulation, as reported by Sullivan *et al.* (1973) and First (1973). The effect of HCG, although not critically tested, may have contributed to the better synchronization of ovulation due to larger ($P<.05$) follicles being present at the time of HCG treatment in P-HCG (31.2 ± 2.7 mm dia.) than in $PGF_2\alpha$-HCG (22.7 ± 2.5 mm dia.) treated mares. After the completion of the present experiment, Palmer and Jousset (1975) reported on the use of a similar prostaglandin-HCG sequence using a synthetic prostaglandin analog. Their two prostaglandin treatments were 14 days apart, each followed 6 days later by HCG. Approximately 66% of the mares ovulated, as determined by an increase in plasma progesterone, within 5 to 7 days of the last prostaglandin injection. Conception rates following this treatment were not determined.

In Experiment 1, the long treatment regime may have effectively lengthened inter-ovulatory interval. In mares which were in mid-diestrus at the beginning of treatment (e.g., day 13), an 18-day treatment plus 9 days to subsequent ovulation would allow a 40-day inter-ovulatory interval, and thus allow fewer estrous periods per season. In Experiment 2, the combination progesterone-$PGF_2\alpha$ treatment was designed to reduce this interval.

The intervals from treatment to estrus and treatment to ovulation in Experiment 2 were similar to Experiment 1, but the mean inter-ovulatory interval was shortened from 36 days to 26 days, closer to that of the controls (24 days). There was no difference between interval from treatment to ovulation in Experiment 2 whether single or multiple breedings were done. Cumulative percentage for all treated mares ovulating was 30.8% by day 7, 59% by day 9 and 82% by day 11, indicating two breedings 48 hr apart would have covered over 80% of the ovulations. One mare became pregnant when ovulation occurred 72 hr following a single breeding.

The reduction in pregnancy rate following synchronization treatment in cattle (Woody and Pierce, 1974) was not evident in the present study with mares. Pregnancy rates after using the same multiple breeding regime for controls (64.7%) and one treated group (57.8%) were not different ($P>.05$). Even in the treated group of mares receiving only a single breeding, the pregnancy rate was 61.5% if one considers only those mares ovulating within 72 hr of breeding. It therefore appears that the low overall pregnancy rate for treated, single breeding mares (40%) was not due to hormone treatment but lack of synchrony of ovulation. Following synchronization of mares with methallibure, First (1973) reported no reduction in pregnancy rate although side effects made this treatment undesirable. Following progesterone

treatment of problem mares, Van Niekerk *et al.* (1973) reported 75 to 80% pregnancy rate. Following an 18-day treatment with an orally active progestogen, Webel (1975) reported that nine of 17 (53%) control and 10 of 26 (38%) treated mares conceived. However, in the latter study the breeding regime was not stated.

That a reasonable degree of synchronization was achieved (Experiments 1 and 2) without significant reduction in pregnancy rate (Experiment 2) suggests such treatments may be incorporated into breeding farm management programs. If approximately 80% of the ovulations following treatment could be covered by two services, without teasing or palpation, there would likely be a savings in labor and stallion usage.

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