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THE EFFECT OF THE GONADOTROPIN-RELEASING HORMONE ANALOG, BUSERELIN, ON PREGNANCY RATES IN HORSE AND PONY MARES

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ABSTRACT

We conducted a series of trials over a four-year period on a total of 2,346 mares, to determine the effect of a single dose of the GnRH analog buserelin (20 to 40 μg im or sc) on pregnancy rates when given between 8 and 12 days after service. Although there were some statistically significant improvements in pregnancy rates in individual trials, meta-analysis of the data overall showed significant improvements at all times examined, i.e. 13 to 16, 19 to 23, 28 to 31 and 38 to 42 days after service. These results indicate that treatment of mares with 20 to 40 μg buserelin between Days 8 and 12 significantly increases pregnancy rates by approximately 10 percentage points.

Key words: mare, GnRH, pregnancy rate, meta-analysis

INTRODUCTION

Early embryonic mortality is a major cause of subfertility in several domestic species (4) including mares (9). Surveys showed average pregnancy rates to vary between 50 and 80% in mares (9). In the first few weeks of pregnancy embryonic survival is dependant on the embryo signalling its presence so that the corpus luteum is maintained. Ginther (9) has termed this the 'first luteal response to pregnancy'. In other species the period of 11 to 14 days is considered to be particularly important as this is the time when luteal function in the pregnant animal starts to diverge from that in the non-pregnant animal. In the ruminant this period is characterised by the secretion of interferon τ by the conceptus which inhibits the synthesis of the luteolysin prostaglandin F2α (PGF2α) by the endometrium (29). In the pig trophoblast,

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estrogens are thought to be the antiluteolytic factor, resulting in the secretion of PGF2α into the uterine lumen rather than into the utero-ovarian circulation (24). It is clear that the equine embryo also exerts an antiluteolytic effect via the local circulation (26) although the structural identity is unknown. However it does not appear to be due either to interferons or steroids (9). Equine chorionic gonadotrophin (PMSG; eCG), a major luteotrophic factor in the mare, is not secreted before Day 35 to 40 of pregnancy.

Evidence from cows suggests that embryonic viability and the rate of embryonic development during the first few days of pregnancy are dependant on the concentration of circulating progesterone (14). A delay in the post ovulatory rise in progesterone concentrations results in impaired embryo development (16), whereas high progesterone concentrations are associated with well developed embryos that are able to produce a strong antiluteolytic signal. In fact there appears to be a cause-effect relationship among optimal progesterone production, embryonic growth, interferon τ production and maintenance of the corpus luteum and hence pregnancy. Thus progesterone supplementation has been used empirically in mares (6) as in other species (14) to improve pregnancy rates although there is a lack of published controlled studies in intact mares.

There has been interest in the use of GnRH at or before the time of maternal recognition of pregnancy (luteal response to pregnancy) to enhance the antiluteolytic signal in early pregnancy in various species in order to reduce embryo loss (19). In numerous field studies of cattle where GnRH was given between 11 and 14 days after insemination, some reported improvements in pregnancy rate of 10 to 12% (7, 12, 27) whereas others reported no effect (10, 25). A recent meta-analysis of all published data for cattle indicated that there may be beneficial effects but that the data are heterogeneous with several interacting variables and therefore no overall interpretation could be made (22). In sheep the only two published reports show an increase in pregnancy rate (13) or litter size (3). In pigs an improvement in pregnancy rates occurred when overall herd fertility was low (21) but in a multi-site study where control farrowing rates were around 85% there was no improvement in pregnancy rate (20). However there was an increase in numbers of piglets born alive suggesting an improvement in embryonic viabilty (20), thus probably not involving an antiluteolytic effect.

In cattle and sheep GnRH treatment results in transient increases in progesterone concentrations, decreased estradiol-17ß and PGF metabolite concentrations, physiological responses consistent with maintenance of the corpus luteum (2, 15, 17). However the mechanism of the 'luteal response to pregnancy' differs among ruminants, pigs and horses. Nevertheless GnRH was shown to exert antiluteolytic and embryo-saving effects in some domestic species. Therefore it seemed reasonable to evaluate this strategy in pregnant mares.

The present studies were conducted over a four-year period to determine the effect of the GnRH agonist, buserelin, on pregnancy rates in mares when given between Days 8 and 12 of pregnancy, that is just before the putative time of the first luteal response to pregnancy (9).

MATERIALS AND METHODS

General. Six trials were conducted on thoroughbred, cross-bred and pony mares in the West Midlands of England and in Wales between 1994 and 1997. Before breeding, mares were allocated into one of two groups. The mares in one group were given a single dose of the gonadotrophin-releasing hormone (GnRH) agonist buserelin (Receptal; Hoechst Roussel Vet; Milton Keynes, UK) between Days 8 and 12 after ovulation, detected by ultrasound (day of ovulation = Day 1). The mares in the other group were untreated controls. All mares were served by natural mating on the day of ovulation. Pregnancy was determined by ultrasonographic examination at predetermined intervals after ovulation. Not all mares were present for pregnancy diagnoses after the initial examination between Days 13 and 16 and fewer than 14% of mares were examined again at 40 $\pm$ 3 days.

Table 1. Summary of buserelin trials.

Trial number	Year	Number of mares		Dose (route) of buserelin (μg)	Day of Injection
		Treated	Control		
1	1994	102	102	40 im	10-11
2	1994	187	187	40 sc	8-11
3A	1995	122	122	40 sc	9
3B	1995	122	123	40 sc	11
4	1996	156	179	40 sc	9
5	1996	228	103	40 sc	10-12
6	1997	204	218	20 sc	11
		191		40 sc	11
Total	1994-1997	1312	1034	20-40	8-12

Trial 1. This trial was conducted in 1994 and included 204 thoroughbred and cross-bred mares (Table 1). Buserelin (40 μg im) was given on Days 10 or 11 after ovulation to half of the mares. Pregnancy diagnosis was performed between Days 15 and 16 and between Days 29 and 31 after ovulation.

Trial 2. This trial also was conducted in 1994 and included 374 thoroughbred and cross-bred mares (Table 1). Buserelin (40 μg sc) was given between Days 8 and 11 after ovulation to half of the mares. Pregnancy diagnosis was performed between Days 14 and 15, 19 and 23, 28 and 30 and, 38 and 42 after ovulation.

Trial 3. This trial was conducted in 1995 and consisted of two independent studies (Trial 3A and 3B) (Table 1). Trial 3A included 244 thoroughbred, cross-bred and pony mares in which half of the mares were given buserelin (40 μg sc) on Day 9 after ovulation. Pregnancy diagnosis was performed four times, between Days 13 and 16, 20 and 23, 29 and 31 and, 38 and 42 after ovulation. Trial 3B included 245 mares in which 122 of the mares were given buserelin (40 μg sc) on Day 11 after ovulation. Pregnancy diagnosis was performed at the same intervals as for Trial 3A.

Trial 4. This trial was conducted in1996 and included 335 thoroughbred and cross-bred mares (Table 1). Buserelin (40 μg sc) was given to 156 mares on Day 9 after ovulation. Pregnancy diagnosis was performed between Days 13 and 16, 20 and 23, 29 and 31 and, 38 and 42 after ovulation.

Trial 5. This trial was also conducted in 1996 (Table 1). A total of 331 thoroughbred and cross-bred mares were divided into four groups. One group of 103 mares constituted the untreated control group. The remaining 228 mares were given buserelin (40 μg sc) on either of Days 10, 11 or 12 after ovulation. Pregnancy diagnosis was performed between Days 13 and 16, 20 and 23, 29 and 31 and, 38 and 42 after ovulation.

Trial 6. This trial was conducted in 1997 and included 613 thoroughbred and cross-bred mares (Table 1). The mares were divided into three groups. The control group of 218 mares was not treated. The other two groups were given buserelin on Day 11 after ovulation. One group of 204 mares received 20 μg sc buserelin and the other group of 191 mares received 40 μg sc. Pregnancy diagnosis was performed between Days 13 and 16, 19 and 23, 28 and 30 and, 38 and 42 after ovulation. However pregnancy data are only available for the examination between Days 13 and 16.

Statistical analysis. Data were analyzed for the effect of GnRH treatment on pregnancy rate within each trial. Then in order to derive an overall estimate of the effect of buserelin across the individual trials, pregnancy data were combined from all trials to form one treated and one control group for each time interval after service. Statistical analyses were conducted on combined data as follows: (i) Trials 1 to 6 for the proportions of mares diagnosed pregnant between Days 13 and 16; (ii) Trials 2 to 5 for the proportions of mares diagnosed pregnant between Days 19 and 23; (iii) Trials 1 to 5 for the proportions of mares diagnosed pregnant between Days 28 and 31; (iv) Trials 2 to 5 for the proportions of mares diagnosed pregnant between Days 38 and 42.

The proportion of mares diagnosed pregnant (pregnancy rate) was defined as the number of mares confirmed to be pregnant at a given time divided by the total number of mares served, i.e.those diagnosed as pregnant plus those not pregnant at the current

and all preceding time intervals. The data in each comparison were combined and analyzed using the Mantel-Haenszel test (1).

Within Trial 6, the data for control mares and mares treated with either 20 or 40 μg buserelin were compared for statistically significant differences using chi-squared analysis for differences between observed proportions in two independent groups (1).

Statistical tests were performed using the EpiInfo-Version 6 (5) computer software. In addition to the chi-squared tests, the results were expressed as the associated relative risk and odds ratio quantifying the increased likelihood of pregnancy in treated mares relative to untreated control mares.

RESULTS

The results of pregnancy diagnoses for the individual trials are shown in Table 2.

Trials 1 to 5. For Trial 1 the proportions of mares pregnant in each group on Day 15 to 16 and Day 29 to 31 after ovulation are shown in Figure 1a. Representation of the percentage of mares pregnant after Days 15 and 16, the "overall proportion of mares pregnant" was expressed as follows: the number of mares confirmed to be pregnant at the given time interval, divided by the total number of mares diagnosed either pregnant or not pregnant at all preceding time intervals (Table 2).The overall proportions of mares pregnant in each group between Days 13 and 16, 19 and 23, 27 and 31 and, 37 and 43 after ovulation in Trial 2 are shown in Figure 1b. The cumulative pregnancy data from Trials 3A and 3B are shown in Figure 1c. The pregnancy data for Trial 4 are shown in Figure 1d. Data from all treated groups in Trial 5 were combined and compared to the control group (Figure 1e).

Trial 6. Pregnancy data from Trial 6, Days 13 to 16 are shown in Figure 2. Chi-squared analysis showed a significant difference ($P = 0.004$) between the pregnancy rates of mares treated with 20 μg and the controls. Similarly the pregnancy rates of mares treated with 40 μg and the controls were also significantly different ($P = 0.03$). The pregnancy rates of mares treated with either 20 or 40 μg were not significantly different from each other ($P = 0.52$).

All trials combined. The chi-squared data, probabilties, odds ratios and relative risks of pregnancy between treated and control mares in the individual trials are shown in Table 3. Comparison of the odds ratios for the individual trials showed that they were homogeneous, therefore potential confounding variables such as breed and route of injection did not have any effect on outcome of treatment. The results from

Table 2. Results of pregnancy diagnoses in buserelin trials.

Trial	Group	Initial number of mares	Number mares pregnant / total number mares examined[a] (%)			
			Days 13 to16	Days 19 to 23	Days 28 to 31	Days 38 to 42
1	Control	102	68 / 102 (67)		63 / 102 (62)	
	Treated	102	74 / 102 (73)		71 / 102 (70)	
2	Control	187	100 / 187 (54)	85 / 179 (47)	63 / 160 (39)	12 / 110 (11)
	Treated	187	107 / 187 (57)	97 / 177 (55)	68 / 152 (45)	17 / 101 (17)
3 (A and B)	Control	245	141 / 245 (58)	133 / 240 (55)	96 / 210 (46)	28 / 143 (20)
	Treated	245	162 / 245 (66)	141 / 228 (62)	104 / 193 (54)	34 / 124 (27)
4	Control	179	102 / 179 (57)	84 / 163 (52)	62 / 144 (43)	17 / 100 (17)
	Treated	156	107 / 156 (69)	86 / 138 (62)	58 / 111 (52)	9 / 64 (14)
5	Control	103	60 / 103 (58)	35 / 81 (43)	23 / 70 (33)	4 / 51 (8)
	Treated	228	148 / 228 (65)	130 / 215 (60)	107 / 194 (55)	20 / 107 (19)
6	Control	218	111 / 218 (51)			
	Treated (20 μg)	204	131 / 202 (65)			
	Treated (40 μg)	191	116 / 188 (62)			

[a] The number of mares determined to be pregnant at the current time interval divided by the total number of mares of known pregnancy status including all mares found to be not pregnant at all previous time intervals. The number in parentheses is the percentage of mares diagnosed pregnant.

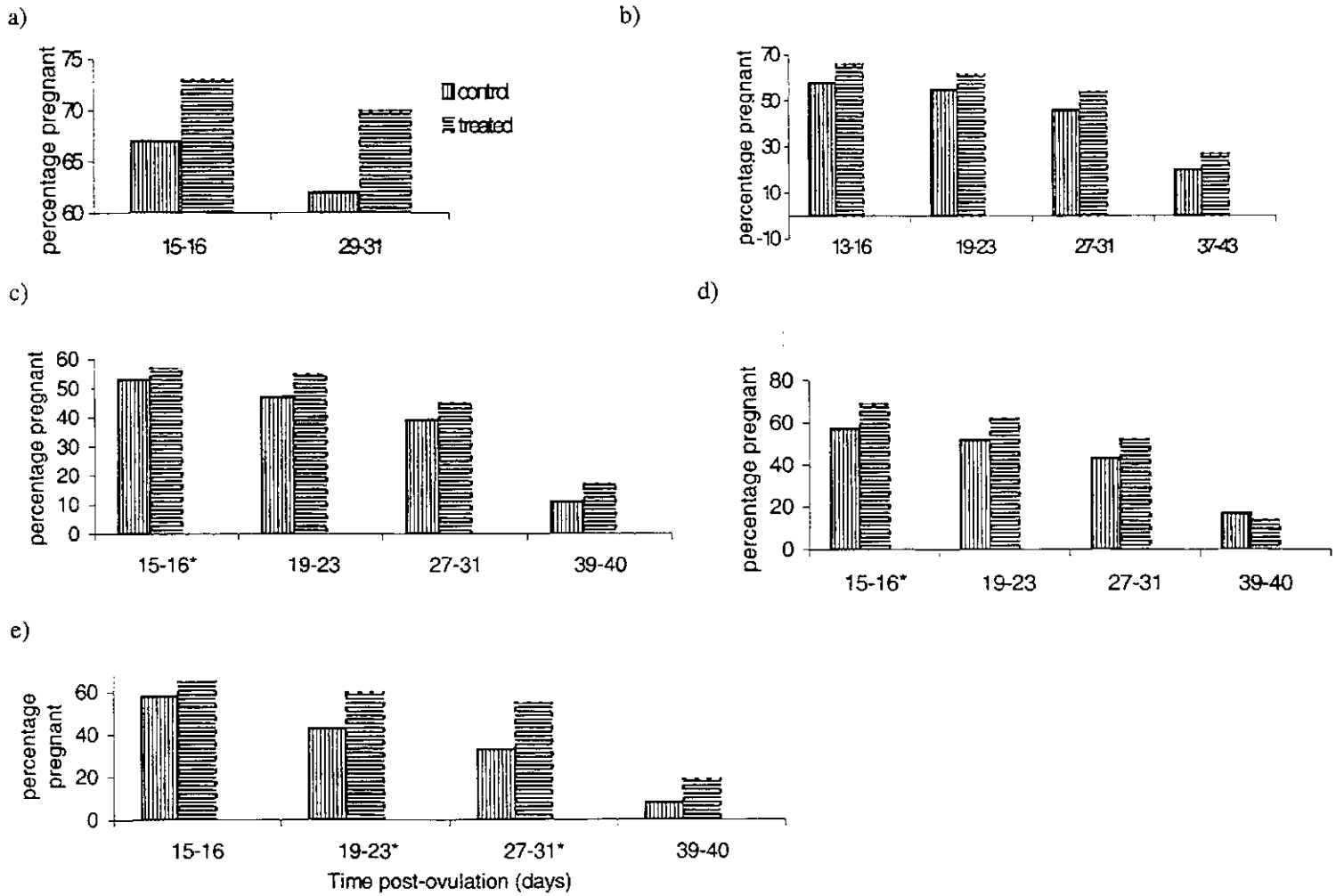

Figure. 1. Comparison of pregnancy rates between buserelin treated and untreated control mares at various times after ovulation / service in Trials 1 to 5 (Figures1a, b, c, d, e respectively). * indicates significant difference between groups ($P < 0.05$).

Table 3. Chi-squared tests for significant differences in pregnancy rate between treated and control mares at each time interval in each study.

		Time after ovulation (days)			
Trial	Statistic	13 to 16	19 to 23	28 to 31	38 to 42
1	Chi-squared ($\chi 2$)	0.83	nd	1.39	nd[a]
	Significance (P)	0.36	nd	0.24	nd
	Odds ratio (OR)	1.32	nd	1.42	nd
	Relative risk (RR)	1.09	nd	1.13	nd
2	$\chi 2$	0.53	1.91	0.92	1.56
	P	0.47	0.17	0.34	0.21
	OR	1.16	1.34	1.25	1.65
	RR	1.07	1.15	1.14	1.54
3 (A and B)	$\chi 2$	3.81	1.99	2.69	2.29
	P	0.05	0.16	0.10	0.13
	OR	1.44	1.30	1.39	1.55
	RR	1.15	1.12	1.18	1.40
4	$\chi 2$	4.79	3.54	2.13	0.25
	P	0.03	0.06	0.14	0.61
	OR	1.65	1.56	1.45	0.80
	RR	1.20	1.21	1.21	0.83
5	$\chi 2$	1.35	7.10	10.23	3.16
	P	0.25	0.01	0.001	0.08
	OR	1.33	2.01	2.51	2.70
	RR	1.11	1.40	1.68	2.38
6	$\chi 2$	8.90	nd	nd	nd
	P	0.003	nd	nd	nd
	OR	1.67	nd	nd	nd
	RR	1.24	nd	nd	nd

[a]Not determined

the Mantel-Haenszel analysis of combined data from Trials 1 to 6 on Days 13 to16 after ovulation showed a highly significant difference ($P < 0.0001$) between the percentage of mares pregnant in the treated group and the percentage of mares pregnant in the control group. All other comparisons using the Mantel-Haenszel test, detected a significant difference in proportions pregnant between the treated and control groups of mares. The corresponding P values of these tests are shown in Table 4.

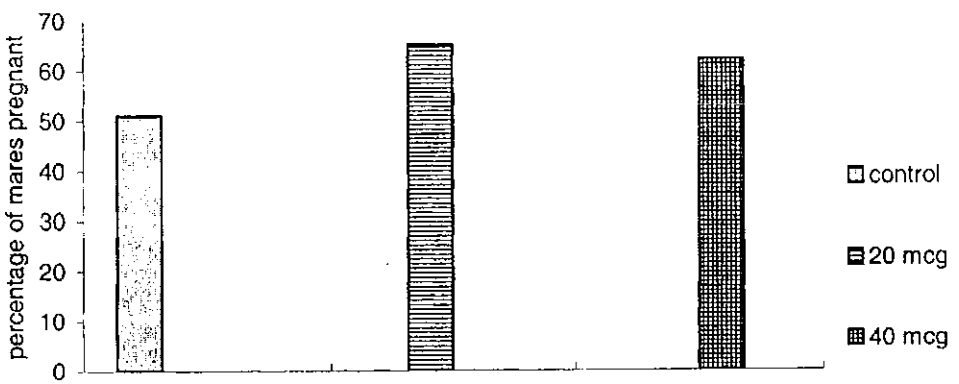

Figure 2. Proportion of mares pregnant out of the total number of mares tested for pregnancy on Days 13 to 16 after ovulation in Trial 6.

Table 4. Mantel-Haenszel analysis of combined data from Trials 1 to 6 for significant differences in pregnancy rates between treated and control mares at each time interval.

	Time after ovulation (days)			
	13 to16	19 to 23	28 to 31	38 to 42
$\chi 2$ [a]	17.50	12.03	13.01	3.90
P [b]	<0.0001	0.0005	0.0003	0.0483
OR	1.44	1.47	1.46	1.49
RR[c]	1.16	1.19	1.22	1.39

[a]Mantel-Haenszel summary chi-squared

[b]Mantel-Haenszel weighted odds ratio for all strata

[c]Mantel-Haenszel weighted relative risk of pregnancy after treatment with buserelin.

Note: Relative risk and odds ratio values greater than one indicate that treated mares were more likely to be pregnant than were untreated mares.

DISCUSSION

Results of Trials 1 and 2 were previously reported in the literature (23) but were included in the current analysis for the purpose of completeness of the data set. The above results indicate that treatment with 20 or 40 μg buserelin between Days 8 and 12 after ovulation and service, significantly increases the overall pregnancy rate by up to 10% in mares. Furthermore the difference between pregnancy rates in treated and control mares was significant at each time interval monitored i.e. until Days 38 to 42 after ovulation. We know of no other similar published studies in the mare.

Differences were detected in pregnancy rates between treated and control mares as early as Days 13 to 16 after ovulation, some time before luteolysis occurs. Therefore the treatment must have exerted some effect on embryo survival other than by inhibiting luteolysis, i.e. in the period before luteolysis. An attractive explanation would be a mechanism involving a luteotrophic effect, enhancing progesterone production and hence embryo viability, either from the extant corpus luteum or through the induction of accessory corpora lutea. Embryonic growth rate and survival appears to be correlated with circulating progesterone concentrations in the cow (14). However we have no direct evidence that such a mechanism occurs in mares. Indeed

in a recent study where non-pregnant mares were injected with with 40 μg buserelin on Day 9 after ovulation, there was no consistent increase in progesterone concentrations or any evidence of secondary ovulation or follicle luteinization (11).

Circulating progesterone concentrations are lower on Day 12 in non-pregnant mares than in pregnant mares, 2.5 ng/mL being thought to be the critical distinction between normal luteal function and dysfunction (6). There are few controlled studies where progesterone has been supplemented in early pregnancy except in ovariectomized mares (28).

Data from the cow shows that GnRH treatment during the luteal phase induces accessory corpora lutea (30). However enhancement of progesterone production was shown to be transient in related studies (8, 15). An interesting observation from some field studies in polytocous species, the sheep and pig, showed that treatment with GnRH between Days 11 and 14 of pregnancy increases litter size at birth rather than pregnancy rate per se (3, 20). Therefore this effect cannot be operating via prevention of luteolysis (which would manifest as an effect on pregnancy rate) but rather through an effect on embryo viability within a litter. Further information on the effects of exogenous GnRH on follicular development and luteal function would probably help to answer these questions.

As is often the case with field data, in each trial a number of animals were lost to follow-up examinations. However there was no overall evidence of bias between treatment groups. The repeated pattern of the results over four breeding seasons suggests a robust effect. Thus we believe that improved pregnancy rates through the administration of buserelin are likely to be real and evident under practical field conditions. Indeed results from additional studies during 1998 and 1999 on over 1000 more mares confirmed the present results with both 20 and 40 μg doses (JR Newcombe, unpublished data).

Further trials using buserelin in mares are indicated to determine the optimal dose and timing of treatment and to confirm effects on numbers of foals born. However even larger sample sizes may be necessary to ensure adequate power of the statistical tests.

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