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Induction of ovulation with buserelin in jennies: In search of the minimum effective dose

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ABSTRACT

The aim of this study was to evaluate the minimum dose of buserelin acetate (buserelin) able to induce ovulation between 24 and 48 h from treatment (positive response) in estrous jennies. Jennies were studied during a total of 172 estrous cycles: ovarian activity was routinely monitored by ultrasound; when the dominant follicle reached a diameter of 33 ± 1 mm, estrous jennies were treated by subcutaneous administration of different doses of buserelin, 3.3 mg ($N = 11$), 1.5 mg ($N = 21$), 0.8 mg ($N = 12$), 0.4 mg ($N = 16$), 0.2 mg ($N = 13$), 0.1 mg ($N = 16$), 0.04 mg ($N = 14$), 0.02 mg ($N = 16$), or employed as controls ($N = 53$). Single jennies ($P = 0.0001$) and GnRH dose ($P = 0.003$) significantly affected ovulation rates. Ovulation rates between 24 and 48 h of each treated group, except for the 0.02 mg group, was higher than in the control group ($P < 0.05$). The minimum dose of buserelin effective to induce ovulation in estrous jennies was 0.04 mg.

1. Introduction

Induction of ovulation enhance efficiency of assisted reproduction techniques in domestic animal species, including the donkey (Camillo et al., 2010; Rota et al., 2012). Classically, induction of ovulation in a short predictable time (24–48 h) is easily obtained by hCG both in mares (Day, 1939; Barbacini et al., 2000) and jennies (Carluccio et al., 2007) but it is well known that the effectiveness of hCG is reduced by successive injections (Duchamp et al., 1987). GnRH agonists deslorelin acetate (deslorelin, 1.5 or 2.25 mg, implant, Meinert et al., 1993) and buserelin acetate (buserelin, 6 mg, Levy and Duchamp, 2007) resulted in the mare in ovulation rates between 24 and 48 h similar to hCG. More recently it was reported as low doses, up to 0.5 mg, of both deslorelin and buserelin were able to induce ovulation in mares (Lindholm et al., 2010). Another GnRH agonist, Lecirelin acetate (100 μg, Carluccio et al., 2007), was effective in inducing ovulation in jennies. The aim of this study was to evaluate the minimum dose of buserelin effective to induce ovulation in estrous jennies.

2. Materials and methods

One-hundred-seventy-two estrus cycles of 12 Amiata jennies (age: 2–16 years; weight: 265–298 kg), maintained in paddocks and fed with hay ad libitum at the Veterinary Sciences Department of the University of Pisa, were monitored daily by ultrasound. At the evidence of a follicle of 33 ± 1 mm (day 0 = D0) the jennies in estrus were randomly assigned to treatment ($N = 119$ cycles) or control groups (C1, untreated, $N = 39$ cycles; C2, injected

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Table 1
Ovulation rates 0–24, 24–48 and more than 48 h after the sc administration of different doses of buserelin and in control groups in jennies.

Buserelin dose (mg)	Ovulation time		
	0–24 h	24–48 h	>48 h
Untreated (C1)[a]	5/39 (12.8%)	6/39 (15.4%)	28/39 (71.8%)
Saline (C2)[a]	1/14 (7.1%)	2/14 (14.3%)	11/14 (78.6%)
0.02[a]	1/16 (6.25%)	5/16 (31.25%)	10/16 (62.5%)
0.04[b]	1/14 (7.1%)	10/14 (71.4%)	3/14 (21.4%)
0.1[b]	1/16 (6.25%)	12/16 (75%)	3/16 (18.75%)
0.2[b]	2/13 (15.4%)	8/13 (61.5%)	3/13 (23.1%)
0.4[b]	0/16 (0%)	13/16 (81.25%)	3/16 (18.75%)
0.8[b]	1/12 (8.3%)	8/12 (66.7%)	3/12 (25.0%)
1.5[b]	3/21 (14.3%)	12/21 (57.1%)	6/21 (28.6%)
3.3[b]	0/11 (0%)	9/11 (81.8%)	2/11 (18.2%)

[a,b] Different superscripts within columns means significative difference ($P < 0.05$).

with saline, $N = 14$ cycles). Treatment consisted of subcutaneous administration of the following doses of buserelin (Suprefact®, Sanofi-Aventis, Milan, Italy): 3.3 mg ($N = 11$), 1.5 mg ($N = 21$), 0.8 mg ($N = 12$), 0.4 mg ($N = 16$), 0.2 mg ($N = 13$), 0.1 mg ($N = 16$), 0.04 mg ($N = 14$), 0.02 mg ($N = 16$). Buserelin was injected at 6 pm (hour 0) of D0 and treated jennies were monitored at hours 14, 24, 38, 42, 48, 62 and every 24 h until ovulation. In the control groups, jennies were either not treated and monitored once a day for ovulation (C1, $N = 39$ cycles) or injected at 6 pm (hour 0) of D0 with 1 ml of saline sc and monitored with the same schedule of treated jennies (hours 14, 24, 38, 42, 48, 62 and every 24 h until ovulation, C2, $N = 14$ cycles). The response to the treatment was evaluated as positive when ovulation occurred between 24 and 48 h after buserelin administration. Between 6 and 8 days after ovulation, jennies were treated with 3 mg of the PGF2α analogue alfaprostol (Gabbrostim®, CEVA VETEM S.p.a., AG) to induce luteolysis (Blanchard et al., 1999; Carluccio et al., 2008).

The results were analyzed with the software StatGraph Centurion XVI.

The ovulation rates occurring earlier than 24 h, between 24 and 48 h and later than 48 h following buserelin or saline injection (treatment and C2 groups) or from the evidence of a follicle of 33 ± 1 mm (C1 group), the distribution of ovulations between 24–38, 39–42 and 43–48 h after treatment, and the mean diameter of follicles before ovulation, were analyzed by ANOVA (GLM), including in the model both the effects of treatment and jenny.

Table 2
Time of ovulation (mean ± sd, h) after sc administration of different doses of buserelin or 1 ml of saline in jennies.

Buserelin dose (mg)	Cycles	Mean ± sd (h)
Saline (C2)	14	83.6 ± 31.9[a]
0.02	16	85.9 ± 49.1[a]
0.04	14	50.7 ± 29.2[b]
0.1	16	49.1 ± 25.9[b]
0.2	13	51.9 ± 30.4[b]
0.4	16	52.3 ± 24.9[b]
0.8	12	56.7 ± 34.2[b]
1.5	21	52.5 ± 29.6[b]
3.3	11	51.1 ± 23.9[b]

[a,b] Different superscripts within columns means significative differences ($P < 0.05$).

One-way ANOVA was used to analyze the effects of the mean time to ovulation between GnRH treatment groups and C2, and of the inter-ovulatory interval between a treatment-control cycle and a control–control cycle. Differences between jennies in positive responses were evaluated by Fisher LSD comparison.

Binary logistic regression has been performed to evaluate the effect of the single jenny and GnRH dose on ovulation rate between 24 and 48 h where the GnRH dose and jenny with the highest response rates were used as reference values.

The effect of repeated administrations of GnRH was analyzed by Chi Square test for trend, comparing the ovulation rates between 24 and 48 h in the following cycle groups: cycles 1–3, cycles 4–6, cycles 7–9, cycles 10–13. Control cycles and cycles in which jennies were treated with the

Table 3
Preovulatory follicle diameter (mean ± sd, mm) after sc administration of different doses of buserelin or 1 ml of saline in jennies.

Buserelin dose (mg)	Cycles	Mean ± sd (mm)
Saline (C2)	14	43.1 ± 4.6[d]
0.02	16	40.2 ± 4.8[b,c,d]
0.04	14	38.4 ± 3.2[a,b]
0.1	16	38.7 ± 4.4[a,b]
0.2	13	36.4 ± 5.5[a,b]
0.4	16	38.4 ± 5.4[a,b]
0.8	12	37.1 ± 5.4[a,b,c]
1.5	21	37.1 ± 4.9[a,b]
3.3	11	36.6 ± 5.4[a]

[a,b,c,d] Different superscripts within columns means significative differences ($P < 0.05$).

Table 4
Distribution of ovulation in jennies ovulating between 24–38, 39–42 and 43–48 h after a sc administration of different doses of buserelin; $P > 0.05$.

Buserelin dose (mg)	Cycles	Ovulation time		
		24–38 h	39–42 h	43–48 h
0.02	5	2 (40%)	3 (60%)	0
0.04	10	6 (60%)	4 (40%)	0
0.1	13	4 (30.1%)	7 (53.8%)	2 (15.4%)
0.2	8	3 (37.5%)	4 (50%)	1 (12.5%)
0.4	13	4 (36.4%)	5 (45.4%)	4 (36.4%)
0.8	8	4 (50%)	3 (37.5%)	1 (12.5%)
1.5	12	3 (25%)	6 (50%)	3 (25%)
3.3	9	3 (33.3%)	6 (66.7%)	0
Total	78	29 (37.2%)	38 (48.7%)	11 (14.1%)