Comparison of the effects of eFSH and deslorelin treatment regimes on ovarian stimulation and embryo production of donor mares in early vernal transition

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Abstract

The objective was to compare the effects of eFSH and deslorelin treatment regimes on ovarian stimulation and embryo production of donor mares in early spring transition. Starting January 30th, mares kept under ambient light were examined by transrectal ultrasonography. When a follicle ≥25 mm was detected, mares were assigned to one of two treatment groups, using a sequential alternating treatment design. In the eFSH group, mares ($n = 18$) were treated twice daily with eFSH (12.5 mg im) until they achieved a follicle ≥35 mm; hCG was given 36 h later. In the deslorelin group, mares ($n = 18$) were treated twice daily with deslorelin (63 μg im) until a follicle ≥35 mm was detected, and then they were given hCG. Estrous mares were inseminated with fresh semen. Eight days after ovulation, embryo recovery attempts were performed. In each group, 14/18 (78%) mares ovulated following the eFSH or deslorelin treatment regimes. The mean (95% CI) interval from treatment initiation to ovulation was 8.2 d (7.3, 8.9) and 7.2 d (6.2, 8.1) in the eFSH and deslorelin groups, respectively. In the eFSH group, the number of ovulations was significantly higher (mean ± S.E.M.; 3.4 ± 0.4 vs. 1.1 ± 0.1 ovulations), and more embryos were recovered (2.6 ± 0.5 vs. 0.4 ± 0.2 embryos/recovery attempt). We concluded that eFSH and deslorelin treatment regimes were equally effective in inducing ovulation in early transitional mares, within a predictable time of treatment; however, the eFSH regime increased the number of ovulations and embryos recovered per mare.

1. Introduction

Mares are seasonal, polyestrus, long-day breeders. During the winter, the function of the mare's hypothalamic–pituitary–ovarian axis changes; GnRH secretion is minimal, and follicular waves cease in the majority of mares [1,2]. Following winter solstice, anestrous mares gradually obtain ovulatory competence during a prolonged phase called vernal transition. This transition phase is characterized by a series of stages or events characterized by increased GnRH and gonadotropin secretion, resurgence of follicular development, estrous behavior and, finally ovulation [2,3]. During the early transitional phase, the number of follicles with a diameter ≥20 mm in the mare's ovaries increases, and ovaries usually contain several developing and atretic follicles [4,5]. During the late transition phase, most mares develop

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1–3 anovulatory follicular waves, each characterized by a large dominant follicle ($\geq$35 mm); follicles continue to emerge and regress until one is ultimately recruited to be the ovulatory follicle [4,5]. Increasing daylight length plays the major role in the resurgence of ovulatory competence, and the mean month that mares experience the first ovulation of the breeding season depends on the geographical latitude at which they live, genetics, nutrition, climate, and other environmental factors [2,3,5–7].

Successful regimes to stimulate ovarian cyclicity in mares to overcome winter anestrus and/or the prolonged transitional phase is of interest to the horse breeding industry, as economic pressures exist to produce foals early in the year. This has become even more pronounced in the last three decades, since the use of embryo transfer and other technologies to improve embryo production has grown and become more attractive. Many horse owners would like to recover embryos from their performance mare early in the year, before the show season; others would like to have their mare conceive early in the year, after she has already produced a few embryos. Various therapeutic strategies to advance the first ovulation of the year have been investigated. In addition to artificial photoperiod [8], hormonal strategies employed include the use of GnRH and GnRH analogs [1,9–13], progesterone and progestins [14,15], hCG [16], prostaglandins [17], prolactin [18], dopamine antagonists [19], equine pituitary extract [20,21], and more recently, equine FSH (eFSH) [22,23]. However, information on efficacious and practical hormonal treatment regimes for early transitional mares used for embryo transfer, with a predictable interval to ovulation, embryo production, and continuation of cyclicity, is still limited.

The objective of this study was to determine and compare the efficacy of deslorelin (GnRH agonist) and eFSH treatment regimes in donor mares in early spring transition. We investigated the effects of two treatment protocols on folliculogenesis, interval to first ovulation, ovulation rate, ovulation synchrony, embryo recovery rate, and continuation of cyclicity. We hypothesized that both treatment regimes would stimulate follicular growth and ovulation in early transitional mares, within only a few days of treatment; however, we anticipated that the eFSH treatment would result in higher number of ovulations and higher embryo recovery rate.

2. Materials and methods

2.1. Animals and reproductive tract examinations

This study was performed between January and June 2006, in the research facility of the University of Saskatchewan in Canada, which is located at 52°07′ latitude in the Northern Hemisphere. Thirty-six mares, Quarter Horse/Percheron cross type, ages 3–10 y, with a body condition score of at least 5 out of 9, were used for this study. They had no signs of systemic disease or lameness, and had good perineal conformation. Mares were kept under ambient light in outdoor paddocks with sheds, and were maintained on alfalfa and grass hay, with access to water and trace-mineralized salt, in accordance with the University of Saskatchewan's Institutional Animal Care and Use Committee.

Transrectal palpation and ultrasonographic examinations of the reproductive tracts were performed starting on January 30th, 2006. Uterine and cervical tone were palpated and separately scored from 1 to 4 (1 – soft, 2 – moderately soft, 3 – moderately toned, 4 – toned). A B-mode ultrasound scanner (GE Ausonics, Universal Ultrasound, Bedford Hills, NY, USA) equipped with a 5.0 MHz linear-array transducer was used to monitor ovarian activity and to subjectively score endometrial edema from 0 (no edema, homogeneous grey) to 4 (marked edema with a distinct black and white pattern) [24]. At the beginning of the study, the reproductive status of all mares was defined as winter anestrus due to the absence of luteal tissue and follicles >15 mm in diameter on repeated examinations. Mares were examined every 2 or 3 d until a follicle $\geq$18 mm in diameter was detected and daily thereafter until 3 d post ovulation(s), and on Day 8 after ovulation (day of embryo recovery attempt).

2.2. Experimental design and treatment groups

When a follicle $\geq$25 mm in diameter was detected, mares were assigned to one of two treatment groups, using a sequential alternating treatment design. Thus, the first mare that had a follicle $\geq$25 mm in diameter was randomly assigned to a treatment group. Thereafter, mares were assigned to a treatment group by alternate sequence in order to balance the date of treatment initiation and reduce the effect of mare variability between groups.

In the eFSH group, mares ($n = 18$) were treated with eFSH (12.5 mg im; eFSH®, Bioniche Animal Health Canada Inc., Belleville, ON, Canada) twice daily until a follicle $\geq$35 mm in diameter was detected, and approximately 36 h later hCG (2000 IU im; Chorulon®, Intervet Canada Ltd., Whitby, ON, Canada) was administered. In the deslorelin group, mares ($n = 18$) were treated with deslorelin (63 μg, im; BET Pharm, Lexington, KY, USA) twice daily until a follicle

≥35 mm in diameter was detected, at which time hCG was administered.

In both treatment groups, mares were artificially inseminated 24 h after a follicle ≥35 mm in diameter was detected, and again every 48 h until ovulation (Day 0 = day of the first ovulation) with fresh semen collected from a stallion of proven fertility. A minimum dose of 5×10^8 progressively motile spermatozoa extended 1:1 with Kenney extender (EZ-Mixin, Animal Reproduction Systems, Chino, CA, USA) was used to inseminate the mares.

A treatment failure was defined as a mare that did not develop a follicle ≥35 mm in diameter within 12 d of eFSH/deslorelin treatment, or if a mare developed a follicle ≥35 mm but failed to ovulate within 72 h after hCG administration. Mares that were deemed treatment failures were followed until their first ovulation was detected.

2.3. Embryo recovery attempts

In mares that ovulated following eFSH or deslorelin treatment, embryo recovery attempts were performed 8 d after ovulation, using a routine nonsurgical transcervical technique as described elsewhere [25]. A total of 4 L of embryo flush media (ViGro Complete Flush Solution®, Bioniche Animal Health Canada Inc.) was used for each mare, and mares were administrated oxytocin (40 IU oxytocin iv; Vêtoquinol N.-A. Inc., Lavaltire, QC, Canada) before the procedure was completed to facilitate recovery. Embryos were identified using a stereomicroscope, enumerated, and rinsed three times in Holding Media (Vigro Holding Plus®, Bioniche Animal Health Canada Inc.). Embryos were scored for quality (1 – excellent, 2 – good, 3 – fair, 4 – poor) according to their morphology as described by McKinnon and Squires [26], and were subjectively assessed for age according to their developmental stage (morula/early blastocyst/expanded blastocyst) and size.

2.4. Evaluation of subsequent cyclicity

Mares received PGF2α (5 mg SC; Lutalyse®, Pharmacia Animal Health, Orangeville, ON, Canada) after an embryo recovery attempt was completed. Mares were examined every 1–3 d to evaluate whether they continued to cycle. When a follicle ≥35 mm in diameter was detected, hCG was administered to induce ovulation. The mares were not bred in this second cycle, but they were monitored as in the first cycle until 8 d after their second ovulation.

2.5. Measurement of serum estradiol-17β and progesterone concentrations

During the first cycle, jugular blood samples were collected into sterile plain vacutainer tubes at the time of treatment assignment, just prior to hCG administration (hCG-Day), on the day of ovulation (Day 0), and 8 d post ovulation (Day 8) just prior to the embryo recovery attempt. For the second cycle, jugular blood samples were collected on hCG-Day, Day 0, and Day 8. The blood samples were centrifuged, and the sera were separated and stored frozen (−20 °C) until hormone assays were performed. Serum concentrations of progesterone (P4) and estradiol-17β (E2) were determined using RIA validated for use in horses. For P4, all samples were analyzed in duplicates in one assay, using the Coat-A-Count® Progesterone In-vitro Diagnostic Test Kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) [27]; the intra-assay coefficient of variation was <4.9%. For E2, samples were analyzed in nine runs, using an assay developed and validated by the Western College of Veterinary Medicine endocrinology laboratory, University of Saskatchewan [28,29]. For this assay, standards (17β-Estradiol, Sigma–Aldrich Canada Ltd., Oakville, ON, Canada) were prepared in charcoal-stripped equine serum, and ranged from 1 to 100 pg/mL. Samples from each mare were analyzed in duplicates in the same run, and the numbers of samples from the two treatment groups were balanced within a run. For E2, the intra- and inter-assay coefficients of variation were <6.1 and <11.8%, respectively.

2.6. Statistical analyses

Statistical analyses were performed with analytic computerized software (Statistix 8 Student Edition, Analytical Software, Tallahassee, FL, USA). Continuous data were evaluated for normality of distribution and for equality of variances using the Shapiro-Wilk Test and the Bartlett's Test, respectively. Accordingly, comparisons between groups were performed with either Student t-test or Kruskall–Wallis non-parametric one-way ANOVA; Student t-test was used to analyze normally distributed data that had equal variances between groups (the number of days of treatment, interval to first ovulation, interval between first and second ovulations); Kruskall–Wallis non-parametric one-way ANOVA was used to analyze data which were not normally distributed and/or had unequal variance between groups (number of preovulatory follicles, number of ovulations, number of embryos). The general effects of the treatment, the day, and the day by treatment interaction, on serum steroid

hormone concentrations were analyzed in a General Analysis of Variance test, followed by Tukey HSD All-Pairwise Comparisons test. Pearson's Correlation test was used to determine the correlation between the number of preovulatory follicles and serum E2 concentrations, and the correlation between the number of ovulations and serum P4 concentrations. Pearson Chi-square analysis was used to compare proportional data, e.g. proportion of mares that ovulated, proportion of mares with multiple ovulations, and proportion of successful embryo recoveries. Categorical data (embryo morphological grade and embryo age) were compared with Kruskall–Wallis non-parametric one-way ANOVA. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Folliculogenesis and ovarian stimulation

Folliculogenesis and ovarian stimulation data are summarized in Table 1. In the eFSH group, 18/18 (100%) mares developed at least one follicle ≥35 mm in diameter, and 14/18 (78%) ovulated and had embryo recovery attempts. In the deslorelin group, 16/18 (89%) developed at least one follicle ≥35 mm in diameter, and 14/18 (78%) ovulated and had embryo recovery attempts. Mean date (range) of the first ovulation of the season was March 30th (March 13th to April 27th) for mares treated with eFSH, and April 6th (March 14th to April 24th) for mares treated with deslorelin ($P > 0.10$). The four mares that failed to ovulate following the eFSH treatment protocol, ovulated on April 26th, April 29th, May 2nd, and May 15th. The four mares that failed to ovulate following the deslorelin treatment protocol, ovulated on April 25th, April 29th, May 6th, and May 9th.

Unless specified differently, subsequent results are presented as mean ± S.E.M. for ovulating mares in

Table 1
Ovarian response in spring transitional mares treated with twice daily eFSH or deslorelin followed by hCG administration. Results are presented as mean ± S.E.M. or percentages (%).

	eFSH ($n = 18$)	Deslorelin ($n = 18$)
Duration of treatment (d)	4.5 ± 0.4	5.3 ± 0.5
Proportion of mares that ovulated	78% (14/18)	78% (14/18)
[1]No. preovulatory follicles (>30 mm)	3.1[a] ± 0.4	1.1[b] ± 0.1
[1]No. ovulations	3.4[a] ± 0.4	1.1[b] ± 0.1

[ab]Within a row, values without a common superscript differed ($P < 0.05$).
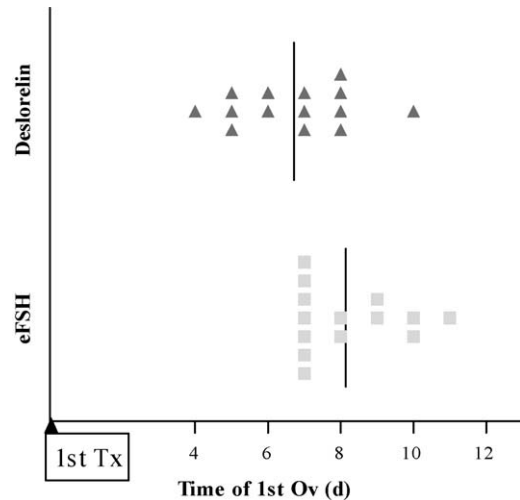[1]Values were calculated for ovulating mares only.

Fig. 1. Time of first ovulation of the breeding season in mares treated with twice daily eFSH or deslorelin, followed by hCG. Each dot represents time of ovulation of individual mare, in relation to the day of treatment initiation (1st Tx). Vertical lines represent the mean time of ovulation of the group.

each group. Duration of treatment was not significantly different between the eFSH group (4.5 ± 0.4 d) and the deslorelin group (5.3 ± 0.5 d). Mares treated with eFSH had more ($P < 0.05$) follicles ≥30 mm in diameter at the time of hCG administration (eFSH: 3.1 ± 0.4 vs. deslorelin: 1.1 ± 0.1 preovulatory follicles) and higher ($P < 0.05$) number of ovulations (3.4 ± 0.4 vs. 1.1 ± 0.1 ovulations). Higher ($P < 0.05$) proportions of mares had multiple ovulations in the eFSH group (13/14, 93%) as compared to the deslorelin group (1/14, 7%). The mean (95% CI) interval from treatment initiation to ovulation was 8.2 (7.3, 8.9) d in mares treated with eFSH, and 7.2 (6.2, 8.1) d in mares treated with deslorelin ($P > 0.10$). Data regarding the time of the first ovulation of individual mares, in relation to the day of treatment initiation, are presented in Fig. 1.

3.2. Embryo production

Embryo production data are summarized in Table 2. A total of 37 embryos were recovered from 14 ovulating eFSH-treated mares, and a total of six embryos were recovered from 14 ovulating deslorelin-treated mares. Therefore, mean embryo recovery was higher ($P < 0.05$) in the eFSH group (2.6 ± 0.5 embryos/recovery attempt) as compared to the deslorelin group (0.4 ± 0.2 embryos/ recovery attempt). Embryo recovery attempts were successful (≥1 embryo recovered) in 13/14 (93%) eFSH-treated mares, and in 5/14 (36%) deslorelin-treated mares ($P < 0.05$). Mean embryo morphology grades

Table 2
Embryo production of spring transitional mares ovulating subsequent to treatment with twice daily eFSH or deslorelin, followed by hCG administration; estrous mares were artificially inseminated with fresh semen, and embryo recovery attempts were performed 8 d after ovulation.

	eFSH (n = 14)	Deslorelin (n = 14)
[1]Embryo number	2.6[a] ± 0.5	0.4[b] ± 0.2
Successful embryo recovery attempts	93%[a] (13/14)	36%[b] (5/14)
Embryo per ovulation rate	77%[a]	38%[b]
[2]Embryo morphology grade (1 – excellent; 4 – poor)	2 (1, 3.5)	1 (1, 4)
[2]Embryo assessed age (d)	8 (7, 8)	8 (6, 8)

[ab]Within a row, values without a common superscript differed (P < 0.05).
[1]Values are presented as mean ± S.E.M.
[2]Values are presented as median (1st and 3rd quartiles).

were not significantly different between groups, and a morphology grade of good or excellent was given to 25/37 (68%) embryos in the eFSH group, and to 4/6 (67%) embryos in the deslorelin group.

3.3. Continuation of cyclicity

In the second cycle, each mare ovulated a single follicle. The mean interval from the first to second
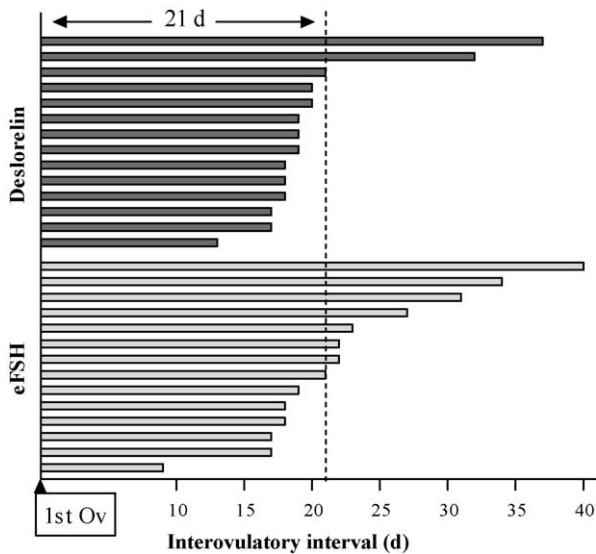

Fig. 2. Inter-ovulatory interval between the 1st and 2nd ovulation of the breeding season in mares. First ovulation was following eFSH or deslorelin treatment of spring transitional mares; $PGF_{2\alpha}$ was given 8 d after the 1st ovulation, and hCG was given when a follicle ≥35 mm in diameter was detected to induce the 2nd ovulation. Each bar represents the inter-ovulatory interval of one mare.

ovulation was not significantly different between mares in the eFSH group (22.7 ± 2.1 d) and the mares in the deslorelin group (20.6 ± 1.7 d). However, the proportion of mares which ovulated within a 21 d interval tended (P = 0.10) to be lower in the eFSH group (7/14, 50%) as compared to the deslorelin group (12/14, 86%). The inter-ovulatory intervals of individual mares are shown (Fig. 2).

3.4. Serum progesterone and estradiol-17β concentrations

Serum P4 and E2 concentrations data are shown (Fig. 3). The number of preovulatory follicles was positively correlated (P < 0.05) with the serum E2 concentrations on the hCG-Day (r = 0.9), and on Day 0 (r = 0.6). The number of ovulations was positively correlated (P < 0.05) with the serum P4 concentrations on Day 0 (r = 0.8), and on Day 8 (r = 0.6). The treatment, day, and treatment by day interaction significantly affected both serum P4 and E2 concentrations. At the
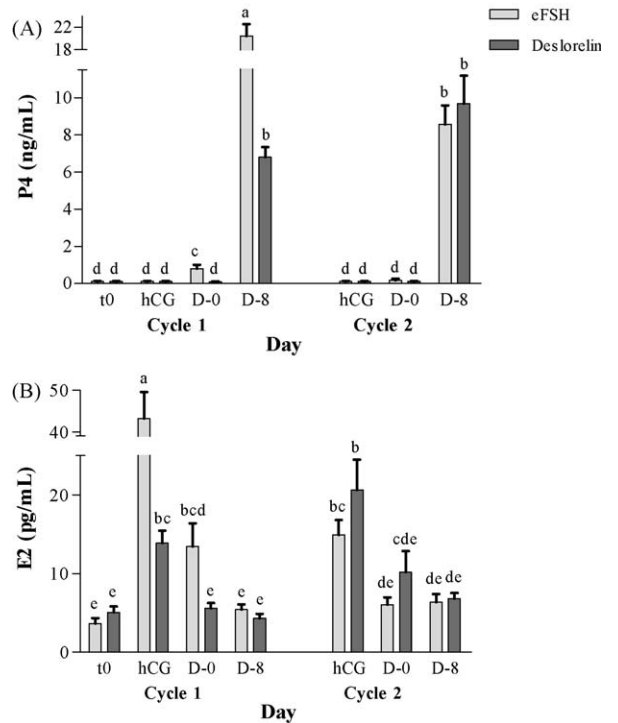

Fig. 3. Serum P4 (A) and E2 (B) concentrations in spring transitional mares treated with eFSH or deslorelin. For the eFSH or deslorelin treatment cycle (Cycle 1), values are presented for the day of treatment assignment (t0), day of hCG administration (hCG), day of ovulation (D-0), and 8 d post ovulation (D-8). For the subsequent cycle, values are presented for hCG, D-0 and D-8. Error bars represent S.E.M. Different letters above bars represent significant differences (P < 0.05).

time of treatment assignment all mares had serum P4 concentration <0.1 ng/mL. Serum P4 concentrations were higher ($P < 0.05$) on Day 0 and on Day 8 of the first cycle in mares treated with eFSH, as compared to mares treated with deslorelin. In all mares that ovulated (both groups, both cycles) serum P4 concentrations on Day 8 were >3.5 ng/mL. Serum E2 concentrations were higher ($P < 0.05$), in the first cycle, on the hCG-day and Day 0 in mares treated with eFSH as compared to mares treated with deslorelin. In the second cycle, there were no significant differences between groups in serum P4 and E2 concentrations when analyzed by day, but there was a significant day effect.

4. Discussion

This is the first study that has critically compared the efficacy of eFSH and deslorelin treatment protocols in early transitional mares. The intention of both treatment protocols is to promote the development of preovulatory follicle(s), responsive to ovulation-induction. The deslorelin treatment protocol works through stimulation of gonadotropin secretion from the pituitary, whereas the eFSH treatment protocol bypasses the hypothalamic–pituitary axis and stimulates the ovary directly. The dose and frequency of the eFSH treatment used in the study were selected according to the manufacturer's recommended protocol, and as previously reported [30]. The dose and frequency of the deslorelin treatment were selected according to preliminary results and information provided to us by BET Pharm, the compounding pharmacy supplying the deslorelin. Administration of hCG was included in both treatment protocols in order to increase the likelihood of successful ovulation, regardless of the ability of the hypothalamic–pituitary–ovarian axis to generate an endogenous LH surge. In the eFSH group, hCG was given after a 36-h delay, following the last eFSH treatment, a period that is termed "coasting" and has been reported to be beneficial when cycling mares were treated with eFSH [31]; the rationale for the coasting period is to allow the high FSH concentration to decrease during the final maturation phase of the follicles. A similar coasting period was not utilized in the deslorelin group, as we believed that the endogenous FSH concentrations were not expected to be as high, and since a coasting period was not a part of the protocol recommended by BET Pharm, or in other previously reported GnRH-analogue treatment regimes.

Treatment assignment was performed in a sequential alternating design to balance the date of treatment initiation and reduce the effect of mare variability between the groups. We followed the mares from winter anestrus to the transitional phase in order to initiate the treatment as early as possible before the breeding season, and as mares become more likely to be responsive to exogenous hormonal stimulation. Based on serial examinations of ovarian activity, and hormonal analyses, we concluded that mares were indeed in the early transitional period at the time of treatment initiation [5,32].

The University of Saskatchewan is located in the Canadian Northwest, which has a naturally long and cold winter. In our experience, the majority of mares under ambient light at our location commonly have their first spontaneous ovulation of the breeding season in May. In the current study, both eFSH and deslorelin treatment protocols were effective and stimulated the first ovulation of the season in 78% of mares after only a few days of treatment. Mean date of the first ovulation of the season was March 30th for mares treated with eFSH, and April 6th for mares treated with deslorelin. We did not have a group of untreated mares to evaluate the extent in which the treatments advanced the breeding season, as this was not one of the objectives of this study. However, our results may be consistent with such an effect, based on our clinical data, and in concurrence with previous reports [1,22,23,33,34].

Equal proportions of mares successfully ovulated in both groups; however, treatment with eFSH resulted in a significantly greater degree of ovarian stimulation, which was apparent in more preovulatory sized follicles and more ovulations. This was also reflected by the differences between groups in serum concentrations of E2 and P4 concentrations; on the hCG-Day and on Day 0, serum concentrations of E2 were correlated with the number of preovulatory follicles, and were significantly higher in mares treated with eFSH; consistent with the greater number of ovulations in eFSH-treated mares, serum P4 concentrations increased faster and reached higher concentrations, on Day 0 and Day 8, respectively. These differences in the magnitude of ovarian response between the two groups may be related to the physiologic differences in the routes of ovarian stimulation, and to the subsequent amount of gonadotropins that were available to stimulate the ovary. Bioavailability, potency, and the doses of eFSH or deslorelin used in the study, may have also led to such differences.

The corpora lutea that developed following the eFSH or deslorelin treatments appeared ultrasonographically normal, and resulted in Day 8 serum P4 concentrations capable of supporting embryonic development and pregnancy. Embryo recovery results were superior in mares treated with eFSH due to the higher number of

ovulations, and higher embryo per ovulation rate. Embryos were recovered from mares in both groups, and most of them were graded as good to excellent in quality; this may indicate normal release of cumulus–oocyte-complexes, fertilization, and early embryo development following at least some of the eFSH- and deslorelin-induced ovulations in transitional mares. However, some of the recovered embryos were of lower quality (Grades 3 or 4). This was in accordance with previous studies which suggested that in many species superovulation treatments for ovarian stimulation may impact the viability of a proportion of embryos recovered; nevertheless, this is a controversial issue in the mare [35–42].

Treatment of transitional mares with eFSH have been reported previously following studies conducted in Colorado, USA (latitude 40°585′N) [22], and Sao Paulo, Brazil (latitude 22°52′S) [22,23]. The eFSH treatment protocol reported here is different as mares were maintained under ambient light before and during the study, and an hCG coasting period was utilized. The duration of eFSH treatment, the proportion of mares that successfully ovulated, and the number of ovulations obtained following the eFSH treatment in the current study were comparable with those reported in the studies conducted in Colorado and Sao Paulo. Using this eFSH treatment protocol, we obtained multiple ovulations in most ovulating mares, and a high embryo recovery rate. Embryo per ovulation rate in cycling superstimulated mares was reported to be equal or lower than expected in cycling control mares [31,41,43–47]; interestingly, the embryo per ovulation rate in the current study was notably high. Perhaps the eFSH treatment is more valuable for transitional mares than for cycling mares, however, this has not been critically examined to date.

Several studies utilized GnRH, or GnRH analogues, in a combination of doses, routes, and patterns of administration, for induction of ovulation in seasonally anovulatory mares, which makes it difficult to compare them with this study [1,2,9–13,33,34,48–61]. Nevertheless, the current study utilized a practical treatment protocol that combined injectable deslorelin and hCG, which are commercially available. Ovulation rate (78% of mares) seemed superior to most GnRH treatment regimes previously reported, and mean ovulation number (1.1 ± 0.1) was similar to that accepted from cycling mares spontaneously ovulating [5]. Higher incidence of multiple ovulations following GnRH or GnRH analogs regimes have been reported by few investigators [10,55]; others, like us, have failed to obtained similar results [11,34,56,62]. The embryo

recovery rate obtained in the current study (0.4 ± 0.2 embryos/recovery attempt) was lower than what is expected in single-ovulating mares (0.5 embryos/recovery attempt), but still acceptable. This was anticipated, considering the normal pregnancy rate previously reported following ovulations induced by GnRH in seasonally anestrous mares [10,56,57,59,61].

The mean inter-ovulatory interval from the first, eFSH- or deslorelin-induced ovulation, to the second ovulation was longer than the expected interval during the breeding season, particularly when PGF2α and hCG are used to shorten the cycle and hasten ovulation [5,63]. This may reflect lack of full seasonal maturity of the hypothalamic–pituitary–ovarian axis in a few of the treated mares, even after they successfully ovulated and produced embryos. Anovulatory mares induced to ovulate with GnRH have been reported to have prolonged intervals to their next ovulation, particularly when ovulation was induced early before the onset of the natural breeding season [10,34,64]. Prolonged inter-ovulatory interval was also anecdotally reported following eFSH treatment [23,64]. We found a tendency for more eFSH-treated mares than deslorelin-treated mares to have a delayed return to normal estrus in a reasonable interval; these findings may be due to chance, the date of ovulation of each individual mare, the potential effect of each hormone on the hypothalamic–pituitary–ovarian axis, or the possible suppression of the high levels of P4 and E2 found in eFSH-treated mares.

In summary, twice-daily administration of eFSH or deslorelin for a short interval, followed by hCG, were equally effective in inducing ovulation in transitional mares. However, treatment with eFSH resulted in a significantly higher number of preovulatory size follicles, a greater number of ovulations, and a higher embryo recovery rate than obtained following the deslorelin treatment. Corpora lutea developed following both treatment protocols produced adequate serum P4 concentrations. Prolonged intervals between the first and second ovulation of the season tended to be more common in mares treated with eFSH than in mares treated with deslorelin.

Acknowledgements

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