hCG is more effective than the GnRH agonist buserelin for inducing the first ovulation of the breeding season in mares

Diana Fanelli1 | Matteo Tesi1 | Alessandra Rota1 | Massimiliano Beltramo2 | Giuseppe Conte3 | Mario Giorgi1 | Giovanni Barsotti1 | Francesco Camillo1 | Duccio Panzani1

1Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa, Italy
2UMR Physiologie de la Reproduction et des Comportements (INRA, UMR85, CNRS, UMR7247, Université de Tours, IFCE), Nouzilly, France
3Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Correspondence
Diana Fanelli, Dipartimento di Scienze Veterinarie, Università di Pisa, Via Livornese, 56124, San Piero a Grado, Pisa, Italy.
Email: dianafanelli@alice.it

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Abstract
Background: Human Chorionic Gonadotropin (hCG) and Gonadotropin Releasing Hormone agonists (GnRHa) are routinely used to induce ovulation in mares. However, GnRHa efficacy in transitional mares has been suggested to be low.

Objectives: The aims of this study were as follows: (a) to compare the efficacy of hCG and GnRHa in inducing the first ovulation of the breeding season and (b) to evaluate the correlation between ovulatory response, uterine oedema and teasing score at the time of treatment during the early or late transitional phase.

Study design: Randomised controlled superiority trial.

Methods: Mares in winter anoestrus were treated with sulpiride when at least two follicles reached a diameter of 25 mm. The day after the follicle reached 35 mm in diameter, mares in oestrus were treated with GnRHa buserelin (N = 29) or hCG (N = 33) and checked daily for ovulation.

Results: More mares (30/33, 90.1%) ovulated when the first ovulation after winter anoestrus was induced with hCG, than with GnRHa, (11/29, 38.0%) (P = .0001). Ovulation rate was lower in mares that did not show uterine oedema and full acceptance of the teaser stallion for at least three days before the treatment (32/41, 78% vs 9/21, 42.9%) P = .01.

Main limitations: Plasma LH and oestrogen concentrations were not performed.

Conclusions: These results demonstrate that hCG was more effective than GnRHa for inducing ovulation in the first cycle after winter anoestrus. Uterine oedema and behavioural signs of oestrus, for at least three days before the treatment, were predictors for a positive response to ovulation induction.

Keywords
horse, induction of ovulation, mares, spring transition
Mares are long-day seasonally polyoestrous animals in which the reproductive period is followed by a phase of non-cyclicity known as winter anoestrus. In between, mares enter into spring and autumn transition. Spring transition marks the changeover from the non-breeding season to the breeding season, and in most breeds does not coincide with the beginning of the commercial breeding season. Early breeding of mares is desirable in order to obtain mature yearlings at auction time and better trained young horses entering the competition. However, during spring transition, signs of oestrus are not always followed by ovulation, thus leading to low reproductive efficiency. The spring transition phase is characterised by the development and regression of steroidogenically incompetent follicles (anovulatory follicular waves), until the emergence of a steroidogenically active follicle that ovulates.

The onset of spring transition is dependent on day length: as the hours of daylight increase, melatonin production decreases and GnRH production increases. As a result, FSH release increases, inducing the recruitment of a new follicular wave, however, LH remains at a basal level for several days, probably due to the inhibition of the gene involved in the synthesis of the LH subunit. After waves of follicular development and regression, inhibition of LH subsides leading to an increase in oestrogen concentrations, enabling the synthesis of LH to be fully resumed and drive the final maturation of the dominant follicle and the first ovulation of the year.

Management with an artificial photoperiod is still the best method to hasten the first ovulation after winter anoestrus, and the use of floodlights or blue light masks can hasten the first ovulation of the breeding season. One of the limitations of these techniques is that their use for large herds that are housed outdoors can be difficult and expensive. Late-transitional mares can be treated with progestogen or, excluding EU countries, with oestradiol and progesterone to hasten the first ovulation of the breeding season.

Dopamine exerts a tonic inhibition on reproductive activity during the anovulatory season, in mares. Treatments with the dopamine-2 (D2) antagonists sulpiride, domperidone and pimozide hasten the onset of the oestrous season in mares in some studies, but not in others. This is probably due to the differences in the drugs employed, timing of treatments, routes, doses and frequency of administration, body condition scores or pre-treatments (eg, artificial photoperiod) of the mares in the various studies.

In previous work, performed under the same conditions as this study, sulpiride treatment hastened the first ovulation of the year without reducing fertility, compared to a control group.

In cyclic mares, ovulation is routinely induced with hCG or a GnRHa, but transitional mares have been reported to fail to respond to induction with GnRHa more frequently than cyclic mares.

The aims of this study were as follows:

(i) To compare the efficacy of hCG and GnRHa for the induction of the first ovulation of the breeding season in mares pre-treated with sulpiride;

(ii) To evaluate whether factors such as time period (early or late) in the transitional phase, uterine oedema and teasing score at the time of the treatment are correlated with the ovulatory response.
leovulsulpiride (Levopraid 50 mg®, Teofarma) IM, once a day until ovulation, or for a maximum of 21 days.17 After the beginning of the treatment, ovarian activity and oestrous behaviour were checked daily until ovulation.

The mares were considered to be in full heat when both grade 2 or 3 uterine oedema24,26 and full oestrous posture25 in the presence of the teaser stallion were observed for at least three consecutive days. If only one of the two parameters was observed for at least three consecutive days, the mares were not considered to be in full heat but were still suitable for ovulation induction (classified as incomplete heat). In other cases, the mares were considered not to be in heat and were not submitted to induction of ovulation, irrespective of follicular diameter.

When a growing follicle with a diameter of ≥35 mm was detected, mares were assigned to one of the two groups (hCG or GnRHa) in order to have a similar distribution of animals of the same age, breed and BCS in each group. The following day (D0) ovulation was induced with 2500 IU of hCG (Corulon®, Intervet Italia Srl) IV (for the hCG group, N = 33) or 1 mg of GnRHa buserelin (Suprefact®, Sanofi Spa) SC (for the GnRHa group, N = 29).

2.4 | Data analyses

IBM® SPSS® Version 26 for MacOsX was used for all statistical analyses. The Shapiro-Wilk normality test was used in order to determine the normality of distributions within mare groups (GnRHa vs hCG and ovulating or not) for age, breed, BCS and sulpiride treatment (days). An independent sample t test or a Mann-Whitney U test was applied as appropriate.

To identify variables associated with ovulation rates, univariable analysis of the data was performed with Fisher’s exact test with Bonferroni correction to compare ovulation rates between agents (GnRHa vs hCG), phases of the year (early transitional period vs late transitional period) and heat expression (full heat vs incomplete heat), while Chi square test with Bonferroni correction was used to assess the influence of breed (Standardbred, Thoroughbred and other breeds) and year of the study.

Mares ovulating between 24 and 48 hours after the treatment were considered to have responded to the induction treatment. P < .05 was considered to indicate statistical significance. For data showing significant differences in univariable analysis, a forward stepwise multivariable logistic regression model based on the Wald statistics criterion of a P value smaller than 0.10 was used to establish significant predictors for ovulation occurring between 24 and 48 hours after the treatment.27,28

3 | RESULTS

Data within groups (GnRH vs hCG and ovulating vs not ovulating) were not normally distributed in terms of age, breed, BCS and sulpiride treatment (days). Mann-Whitney U Tests were thus used to compare these groups. No differences were found between GnRH- and hCG-treated mares expressed in median (interquartile range) for age: 7 (6) vs 7 (5) years (P = .2); breed; BCS: 3 (0.25) vs 3 (0.25) (P > .9) and length of sulpiride treatment: 7 (8) vs 7 (6) days (P = .8). Age 7 (7) and 7 (4) years (P > .9), BCS 3 (0.25) and 3 (0.25) (P > .9), length of sulpiride treatment 6 (4) and 8 (4) days (P = .2) and breed 25/38 (65.8%), 15/20 (75%), 1/4 (25%). Standardbred, Thoroughbred and other breeds, respectively, (P = .2), did not differ between mares that ovulated or did not ovulate after the induction. Overall ovulation rate was similar for the first, second and third years of the study: 10/18 (55.6%), 11/16 (68.8%) and 20/28 (71.4%) (P = .5). Data were thus pooled for further analysis.

Table 1 presents the ovulation rates according to the factors analysed. More mares ovulated after induction of the first ≥35 mm follicle of the breeding season with hCG, 30/33 (90.9%), than with GnRHa, 11/29 (37.9%), (P < .001) (Table 1, agent). In addition, more mares ovulated after treatment in the late than the early transitional period, 24/30 (80.0%) and 17/32 (53.1%), respectively, (P = .03), irrespective of the agent employed (Table 1, transition phase). Finally more mares in full heat ovulated than mares not in full heat, 32/41 (78%) and 9/21 (42.9%), respectively, (P = .01), irrespective of the agent employed and of the stage in the transitional period (Table 1, heat expression).

Tables 2 and 3 show differences in ovulation induction response during the early and late transitional periods (phase1 vs phase 2) and between mares showing full or incomplete heat, according to the agent used respectively.

The forward conditional logistic regression model including as factors: agent used for the induction of ovulation, phase of the year, heat expression and all of their interactions were statistically significant, \( \chi^2 (7) = 42.211 \) (P < .001). The model explained 68.4% (Nagelkerke \( R^2 \)) of the variance in ovarulations occurring between 24 and 48 hours after induction, and correctly classified 83.9% of cases. The agents used for the induction of ovulation, phase of the year and oestrus expression predictor variables, but not their interactions, were statistically significant (Table 4).
**TABLE 2** Ovulation rate in mares given either GnRHa or hCG in early (phase 1) and late (phase 2) transition period

|          | Phase 1 | Phase 2 | P value |
|----------|---------|---------|---------|
| GnRHa    | 3/15 (20.0%) | 8/14 (57.1%) | .06     |
| hCG      | 14/17 (82.3%) | 16/16 (100.0%) | .2      |
| P value  | .001 | .005    |         |

**TABLE 3** Ovulation rate in mares displaying full heat or incomplete heat given either GnRHa or hCG

|                | Full heat | Incomplete heat | P value |
|----------------|-----------|-----------------|---------|
| GnRHa          | 9/18 (50.0%) | 2/11 (18.2%) | .1      |
| hCG            | 23/23 (100.0%) | 7/10 (70.0%) | .02     |
| P value        | P = .0001 | P = .03        |         |

**TABLE 4** Multivariable analysis of factors significantly influencing the occurrence of ovulation between 24 and 48 h after treatment with hCG or GnRHa in early (phase 1) and late (phase 2) transition period and showing full heat or incomplete heat

|                | P value | Odds ratio | 95% confidence intervals for odds ratio |
|----------------|---------|------------|----------------------------------------|
|                |         | Lower      | Upper                                  |
| hCG vs GnRHa   | .0001   | 69.9       | 7.4 - 663.5                             |
| Phase 2 vs phase 1 | .006 | 14.7       | 2.1 - 101.7                            |
| Full vs incomplete heat | .005 | 17.0       | 2.4 - 120.7                            |
| Constant       | .001    | 0.022      |                                        |

4 | DISCUSSION

Ovulation is induced routinely to manage a mare's reproductive cycles. For cyclic oestrous, mares with a dominant ≥35 mm diameter follicle, ovulation generally occurs between 24 and 48 hours after treatment with either hCG or GnRHa.\(^{21}\) Given that the ovulatory response to 0.5 mg of buserelin was significantly lower in mares treated during the spring transition than in mares treated after the first ovulation of the year,\(^{22}\) we tested differences in the efficacy of hCG vs GnRHa in terms of inducing ovulation in spring transition mares.

In this study, hCG was found to be much more effective than GnRHa in inducing the first ovulation of the breeding season in the transitional mares. This could be explained by the different activities of the two agents: hCG has an LH-like activity and induces the final follicular maturation and ovulation,\(^ {29}\) acting directly on follicular receptors, whereas GnRHa (and exogenous GnRHa) stimulates LH release from the adenohypophysis which, in turn, stimulates final follicular maturation and ovulation.\(^ {30}\)

The pituitary gland in transitional mares is known to have a lower LH content\(^ {29-34}\) and, therefore, GnRHa may be less effective in inducing LH release and ovulation in these mares, especially compared with cyclic mares,\(^ {30}\) or with late transitional mares, in which the pituitary gland LH content is higher. This is in accordance with our findings, because in this study, although lacking statistical significance, the percentage of mares ovulating after GnRHa induction during the first early transitional period was lower than in mares in which ovulation was induced during the late transitional period (20% vs 57%, respectively, \(P = .06\)). This difference in ovulation response between the two phases was not observed for the hCG-induced group (82.3% vs 100%, respectively, \(P = .2\)). This might be due to the fact that hCG is able to bypass both the LH-release step and the low pituitary LH content typical of early transitional mares.\(^ {31}\)

April 8 was chosen to distinguish between the early and late transitional phases as it was the first day of the year when day length reached 13 hours, at the latitude of the study. It is well documented that 14.5-16 hours of artificial light per day are stimulatory factors for the early resumption of ovarian activity of the mare after winter anoestrus.\(^ {32,33}\) In addition, the second half of April represents the expected time of the spontaneous first ovulation of the breeding season in warmblood mares at latitudes similar to that of this study.\(^ {1}\) Therefore, it is not surprising that induction of ovulation performed near to the time of the physiological resumption of ovarian activity (phase 2 or late transitional period), was more successful.

Cuervo-Arango et al\(^ {34}\) reported that progesterone-primed transitional follicles had a higher (93.1%) response to hCG induction of ovulation than the non-primed ones (58.7%, \(P < .01\), in transitional mares.\(^ {34}\) It is thus important to underline that the mares included in this study were primed with the dopamine antagonist, sulpiride, regardless of the induction agent used. Consequently, we do not know whether the same results, in terms of responsiveness to induction of ovulation with hCG, would have been achieved without this pretreatment. The various studies reported in this paper fail to agree on the effectiveness of dopamine antagonist treatments to hasten the first ovulation of the breeding season in mares.\(^ {11-19}\) Nevertheless, the great variability between the different conditions of the already published studies, including the breeding seasons, hemispheres, pretreatments, photostimulations and follicular diameter at the time of the first treatment of the mares\(^ {13,35-38}\) complicates any comparison between studies. In our study, the mean number of days of sulpiride treatment before the development of a ≥35 mm follicle was very similar to findings reported in a recent study performed under the same conditions.\(^ {17}\) This previous study reported that after the development of at least one 25 mm follicle, sulpiride treatment hastens the first ovulation of the breeding season.

We found that mares were more likely to fail to respond if they did not show either grade 2 or 3 uterine oedema,\(^ {24,25}\) or oestrous behaviour in the presence of the teasing stallion\(^ {25}\) for at least three consecutive days before ovulation induction. In the absence of progesterone, uterine oedema and oestrous behaviour depend on the number of circulating oestrogens. In the early transition phase, erratic oestrous behaviour or low-grade uterine oedema are related
to steroidogenic incompetence of the follicles,\textsuperscript{4} while clear uterine oedema and stallion acceptance are signs of oestrus and reflect the readiness of mares to ovulate.\textsuperscript{1,4,6}

In our opinion, mares treated in the absence of clear uterine oedema and full oestrous behaviour for at least three days (considered as not in full heat but suitable for ovulation induction) were probably in their early transition period and therefore less responsive to the induction of ovulation. Tables 2 and 3 show that the ovulatory responses to both agents of the mares in phase 1, corresponding to the early transitional period, were very similar to the ovulatory response of mares not in full heat (20\% vs 18.2\%, for GnRHa and 82.3\% vs 70\%, for hCG respectively). Since circulating LH and oestrogen concentrations were not determined, it is impossible to confirm the abovementioned hypotheses, which represents a major limitation of this study.

In conclusion, this study confirms the difficulties in inducing ovulation of the first preovulatory follicle after winter anoestrous, particularly if this occurs in the early part of the transitional phase. In this period, the use of hCG is preferable. The results of this study were obtained in transitional sulphiride-primed mares. It is possible that not-primed transitional mares might respond differently. Selecting mares that show both uterine oedema and clear behavioural signs of oestrus can reduce the likelihood of unsuccessful treatment, and consequently a teaser stallion is useful when managing reproduction in transitional mares.

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CONFLICT OF INTERESTS

No competing interests have been declared.

AUTHOR CONTRIBUTIONS

The corresponding author confirms that all the authors had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed equally to the research outcome, provided their consent and approved the final version of the manuscript.

ETHICAL ANIMAL RESEARCH

The study was approved by the Animal Welfare committee of Pisa University (protocol number 20/2017).

INFORMED CONSENT

Representatives of the company which owned the mares gave consent for the use of animals in this study.

DATA ACCESSIBILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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ORCID

Diana Fanelli https://orcid.org/0000-0003-0863-5898
Matteo Tesi https://orcid.org/0000-0001-8773-395X
Alessandra Rota https://orcid.org/0000-0002-3379-2003
Massimiliano Beltramo https://orcid.org/0000-0003-3472-2477
Giuseppe Conte https://orcid.org/0000-0002-7257-4762
Mario Giorgi https://orcid.org/0000-0003-3657-4703
Giovanni Barsotti https://orcid.org/0000-0002-6529-3348
Duccio Panzani https://orcid.org/0000-0002-3433-7918
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