INDUCTION OF OVULATION IN MARES WITH A SINGLE INTRAVENOUS INJECTION OF LECIRELIN AT A DOSE OF 200 MG: A COMPARATIVE TRIAL WITH THE GOLD STANDARD, HCG (HUMAN CHORIONIC GONADOTROPHIN)

L. Berlande[1], L. Vavasseur[2], C. Gourtay[3], L. Briot[3], JF. Bruyas[4]

[1]ENVA, Maisons-Alfort, France; [2]Veterinary Clinic of Argentan, Argentan, France; [3]IFCE, Le Pin au Haras, France; [4]ONIRIS, Nantes, France

Abstract

The objective of this study was to test whether an intravenous injection of 200 µg of lecirelin could be a safe and effective alternative to 1,500 IU of hCG. We compared ovulation times after lecirelin and hCG injections in 42 mares over 156 cycles, with each mare serving as her own control.

This study provides encouraging results regarding the effectiveness of a single injection of 200 µg of lecirelin. However, the rate of ovulation within 48 hours post-injection is significantly different between hCG (88%, 66/75 cycles) and lecirelin (64%, 48/75). The success rate of ovulation induction within the 24-48 hour post-injection interval is also significantly different between hCG and lecirelin, with 76% (57/75) and 60% (45/75), respectively. The rate of late ovulations after 48 hours is notably higher after lecirelin injection (36%) compared to hCG (12%). The main cluster of ovulations occurs between 24 and 36 hours, with 41% after lecirelin and 61% after hCG.

There is no difference in pregnancy rates after hCG or lecirelin injections, which are 54% and 68%, respectively.

This study shows that lecirelin is less effective than the gold standard hCG, but it suggests further studies using either higher doses or subcutaneous administration for injections. Its advantages in terms of cost, adherence to cascade rules, and ease of use (single injection) make lecirelin an alternative to hCG worth further exploration.

1 | INTRODUCTION

Ovulation induction in mares is a key element in equine reproduction management. hCG is the reference molecule, but immunization after two injections per breeding season is recognized. GnRH agonists are also frequently used and have shown results comparable to hCG. However, only buserelin has equine market authorization in France, and its use in iterative intravenous injections is restrictive. Triptorelin is used in a single subcutaneous injection but with a human formulation. Lecirelin has veterinary approval

for use in cattle and pigs. A few studies, with encouraging but limited results due to experimental biases, have tested this molecule on small samples of donkeys and mares.

The primary objective of our study is to compare the frequency of ovulation within 24-48 hours after lecirelin and hCG injections, distinguishing between estrus induced by luteolytic treatment and spontaneous estrus, with mares serving as their own controls to minimize individual variability. The secondary objective is to compare pregnancy rates following lecirelin and hCG-induced ovulation.

2 | MATERIALS AND METHODS

The study was conducted from April 19 to August 8, 2022, on 42 mares from the herd at Le Pin IFCE stud farm. The mares were aged 3 to 22 years, with an average of 13 ± 5 years.

2.1 | Protocol

In our study, each mare underwent four estrus cycles: two where hCG was used for ovulation induction and two where lecirelin was used. In addition, two estrus cycles—one for each induction treatment—were "spontaneous," meaning without prior luteolytic treatment, and the other two were "induced," meaning initiated by luteolysis. During the two spontaneous estrus cycles, the mares were inseminated and underwent early pregnancy diagnosis (DG, repeated from day 12 to 14). The cycle order alternated between spontaneous and induced estrus, and the mares were randomized into two groups: half of the mares started with two hCG injections (followed by two lecirelin injections) and vice versa for the other half, to balance the injection distribution of the two molecules over the season.

The luteolytic treatment consisted of an intramuscular injection of 375 µg of Cloprostenol (Estrumate®). Ovulation induction was performed intravenously when the mare presented for the first time with a follicle diameter ≥35 mm and uterine edema ≥3 or the cervix resting on the vaginal floor. The hCG injection (Chorulon®) was administered at a dose of 1,500 IU, and the lecirelin injection (Reproreline®) was administered at a dose of 200 µg.

Throughout estrus and pregnancy diagnosis, a single experienced veterinarian performed transrectal ultrasound exams. The cervix was also examined, with daily check-ups becoming bi-daily when two induction criteria were met, continuing twice daily until ovulation. The mares were inseminated during spontaneous estrus cycles, 16-20 hours after hCG or lecirelin injections, using a mix of semen from two fertile stallions at the Le Pin stud farm.

2.2 | Statistical Analysis

Since each mare served as her own control, a Chi^2 test for paired series was used.

3 | RESULTS

A total of 156 cycles were analyzed, comprising 75 cycle pairs, including 35 spontaneous estrus cycles and 40 induced estrus cycles, with 56 inseminations and pregnancy diagnoses.

3.1 | Lot Homogeneity

The compared groups were perfectly homogeneous regarding age, breed, and body condition score since they consisted of the same mares (cross-over study). The dates of induction treatment, follicle diameter, and uterine edema at the time of induction were also comparable.

3.2 | Ovulation Timing

All results are presented in Table 1.

When the success rate was calculated over the 0-48 hour interval, hCG was significantly ($p<0.05$) more effective, with an 88% ovulation rate compared to 64%. In the 24-48 hour interval, hCG was also significantly ($p<0.05$) more effective, with 76% versus 60%. However, for the 36-48 hour interval, there was no significant difference between the two treatments, with 13% for hCG versus 19% for lecirelin.

The study shows a significantly ($p<0.05$) higher early ovulation rate (within 36 hours post-injection) for hCG (73%) than for lecirelin (34%). These early spontaneous ovulations were primarily concentrated between 24 and 36 hours, significantly ($p<0.05$) higher for hCG than lecirelin (61% vs 41%). Early ovulations occurring within 24 hours post-injection were rare, with no significant difference between the two treatments (12% vs 4%).

Conversely, the failure rate due to dysovulation or late ovulation occurring more than 48 hours after injection was significantly ($p<0.05$) higher for lecirelin (36%) compared to hCG (12%).

3.3 | Early Pregnancy Rates

Analysis of pregnancy rates did not reveal a significant difference between the two treatments, with 54% for hCG and 68% for lecirelin.

4 | DISCUSSION

4.1 | Discussion of Results

The success rate of ovulation induction within 24-48 hours obtained in our study for hCG is highly favorable (69% and 83% depending on whether cycles were spontaneous or induced) and is similar to previous studies on this molecule, which is considered the gold standard for which the action time is between 30 and 48 hours post-injection.

Lecirelin's advantage lies in its lack of immunization and veterinary availability, which could make it a very interesting alternative to hCG, especially in mares with many cycles per season, such as subfertile mares and embryo donor mares.

4.2 | Success Rate of Ovulation Induction Treatment

The desired ovulation interval varies depending on the reproduction technique used. When using fresh semen (natural mating or artificial insemination with fresh semen), the 0-48 hour or 24-48 hour ovulation intervals are important. In this case, and in our study, hCG appears significantly more effective ($p < 0.05$). However, when the success rate is calculated over the 36-48 hour interval, which is recognized as the interval during which ovulations typically occur after induction injections, there is no significant difference between the two treatments. This 36-48 hour interval is particularly useful when the number of frozen semen doses available is limited, and to ensure only one artificial insemination per estrus, it is performed within 6-8 hours post-ovulation. However, our study observed a low ovulation rate during this 36-48 hour interval with both molecules. Simultaneously, we observed a significant clustering of ovulations within the 24-36 hour interval.

Several explanations may account for these observations. First, the minimum follicle diameter required to induce ovulation was set at 35 mm in our protocol. However, the average diameter at the time of induction ranged from 37 ± 4 to 45 ± 4 mm depending on the groups, due to the need for another induction criterion. The larger the follicle diameter, the higher the risk of spontaneous early ovulation. Second, due to practical reasons, gynecological exams were performed at most twice daily. Ovulation timing was then determined as the average of the two exams between which ovulation occurred, introducing some relative but real imprecision that may have led to classification errors.

In conclusion, the clustering of ovulations within the 36-48 hour interval is not necessarily detrimental, as it has little practical influence. A 24-48 hour cluster, which may be preferred, could likely be achieved by adjusting the protocol based on the points discussed above.

4.3 | Late Ovulations

The rate of late ovulations is significantly different between the two treatments, with higher percentages observed for lecirelin. These results are due to actual treatment failures, which could be reduced by adjusting the dose or administration route. The chosen dose of 200 µg is double that indicated in the product's leaflet for cattle, as many studies on GnRH agonists have shown that the effective dose for mares is at least double that for bovines. The intravenous route was used in our study, but it may be worth testing lecirelin via subcutaneous administration, as this appears to be the most effective route for GnRH agonists in recent studies using a single injection of a high dose of agonists (buserelin or triptorelin).

5 | CONCLUSION

Our interventional, randomized study with a large sample size offers strong scientific evidence. It presents lecirelin as a promising ovulation induction molecule for mares, offering an alternative to hCG and other GnRH agonists, given its advantages in terms of use. Success rates for induction could be improved in future studies testing subcutaneous administration and refining the protocol.

CONFLICT OF INTERESTS

There are no conflicts of interest associated with this publication, and there was no significant financial support for this work that could have influenced its outcome (the project was primarily supported by IFCE through the provision of mares and the facilities at the Le Pin stud farm).

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