

## eFSH in clinical equine practice

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### Abstract

Equine follicle stimulating hormone (eFSH) has been used to induce follicular development in transitional mares and problem acyclic mares, as well as superovulate cycling mares. The most efficacious protocol is to administer 12.5 mg eFSH, intramuscularly, twice daily beginning 5 to 7 days after ovulation when the diameter of the largest follicle is 20 to 25 mm. Prostaglandins are to be administered on the second day of eFSH therapy. Treatment with eFSH is continued for 3 to 5 days until follicle(s) are  $\geq 35$  mm in diameter. The mare is subsequently allowed to 'coast' for 36 h, after which human chorionic gonadotropin is administered to induce ovulation.

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### 1. Introduction

Follicle stimulating hormone (FSH), produced by gonadotroph cells of the anterior pituitary, supports the emergence and the initial growth and development of ovarian follicles. Purified or partially purified equine FSH has been used clinically to stimulate follicular development or induce superovulation in mares. Initial studies utilized crude preparations of partially purified FSH, often referred to as equine pituitary extract (EPE) [1,2]. Recently, a purified equine FSH preparation (eFSH, Bioniche Animal Health Inc., Athens, GA, USA) has become available. Information gleaned from three decades of studies on EPE has been used to expedite the development of practical and successful protocols to superovulate mares with eFSH.

The goal of this paper is to review the potential uses of purified eFSH in clinical practice, including management of the transition period, induction of ovulation in problem acyclic mares, superovulation of mares in an embryo transfer program, and as an adjunct therapy for management of some types of subfertility.

### 2. Management of the transition period

The physiologic breeding season of the horse in North America occurs from April to October. However, mare owners often want to begin breeding mares by the middle of February, when most mares are not yet cycling. Exposure of mares to an artificial photoperiod (long days) is the most common and predictable technique used to induce follicular development early in the year [3]. In addition, several hormonal therapeutic regimens have been reported to advance the first ovulation of the year, including native gonadotropin releasing hormone (GnRH), GnRH agonists, FSH, progesterins, and dopamine antagonists [4–6].

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Douglas et al. [1] first reported the successful induction of ovulation in seasonally anestrous mares with exogenous hormones. An extract prepared from equine pituitaries was given to pony mares with small, inactive ovaries (follicles  $\leq 10$  mm in diameter). In that study, 87% of mares treated with EPE ovulated and 58% of those mares ovulated more than one follicle. Lapin and Ginther [2] gave EPE once daily to 11 pony mares in deep seasonal anestrus (follicles  $\leq 10$  mm in diameter). All mares treated with EPE ovulated, whereas none of the control mares (given saline) ovulated during the 14-day observation period. There was no difference in the number of ovulations for mares given only EPE ( $1.6 \pm 0.4$  ovulations) and those given EPE followed by hCG ( $1.8 \pm 0.3$  ovulations). It was subsequently reported that the efficacy of EPE in inducing ovulation in anestrous mares increased progressively along with an increase in the diameter of the largest follicle at the onset of treatment [7]. The percentage of anovulatory mares with follicles 10–15, 20–25 or  $\geq 30$  mm that ovulated within a 14-day EPE treatment period were 12.5% (one of eight), 28.6% (two of seven) and 100% (seven of seven), respectively. It was also noted that 6 of 11 treated mares (54.5%) that ovulated in response to EPE, but did not become pregnant, returned to an anovulatory condition after the cessation of treatment. Coy et al. [8] compared ovulation rates following once daily EPE administration between mares in deep winter anestrus (follicles  $< 15$  mm in diameter) and mares in transition (diameter of the largest follicle  $> 25$  mm in diameter); hCG was given when a follicle  $\geq 35$  mm was detected. A higher percentage of mares in transition (eight of nine, 89%) ovulated in response to EPE administration than mares in deep anestrus (two of nine, 22.2%). Four of the eight transitional mares had multiple ovulations. The average interval from onset of treatment to ovulation for the eight transitional mares was  $11.8 \pm 5.0$  days.

The efficacy of eFSH in stimulation of follicular development and advancement of the first ovulation of the year was initially reported in 2004 [9]. Ten mares in spring transition with follicles  $> 25$  mm in diameter were given 12.5 mg of eFSH i.m. twice daily. Ten additional transitional mares served as untreated controls. Mares were given hCG (2500 IU, i.v.) when one or more follicles reached 35 mm in diameter. Administration of eFSH followed by hCG resulted in ovulation in 80% of mares treated; the mean ovulation rate was  $2.5 \pm 1.7$ . Multiple ovulations were induced in 40% of transitional mares treated with eFSH. The interval from onset of treatment to ovulation was  $7.6 \pm 2.4$  days, whereas control mares ovulated an

average of  $39.5 \pm 17.2$  days after the onset of the observation period. None of the control mares ovulated multiple follicles at the first spontaneous ovulation of the year.

Treatment with eFSH was less effective for induction of follicular development and ovulation in transitional mares when given only once daily when either the standard dose or a reduced dose of eFSH was used (McCue, unpublished data). Thirty transitional mares (follicle diameter  $> 20$  mm) ( $n = 10$  per group) were given either saline placebo, 6.25 mg eFSH or 12.5 mg eFSH once daily for a maximum of 10 days; hCG (2500 IU) was given when a follicle reached 35 mm in diameter. Ovulation rates were 20, 50, and 60% following saline, low-dose eFSH, and high-dose FSH treatment, respectively. It was concluded that twice daily treatment with eFSH, as used the previous season, was more advantageous in stimulating follicular development in transitional mares than a once-daily protocol.

A combination of an artificial photoperiod and eFSH therapy may be used to advance the date of the first ovulation of the year. Administration of eFSH (12.5 mg, twice daily) would be initiated once the mare has become transitional (i.e. follicles  $\geq 25$  mm in diameter). An ovulation inducing agent should be given when one or more follicles  $\geq 35$  mm in diameter are present.

### 3. Management of postpartum acyclic mares

Postpartum mares usually develop follicles and ovulate early in the postpartum period (foal heat) and continue to cycle if they do not become pregnant at a foal heat breeding. In some mares, a foal heat ovulation may be followed by a variable period of anestrus or anovulation until the mare resumes normal cyclic activity. A few mares may lack substantial follicular development or may exhibit moderate to substantial follicular development without ovulation during the postpartum period. Mares in the latter groups may remain anestrus or anovulatory for weeks or months before cyclic ovarian activity is initiated.

Most mares with postpartum acyclicity are mares that foal early in the year. Consequently, it may be difficult to distinguish between postpartum anestrus due to a short ambient photoperiod versus anestrus due to the effects of lactation. In general, failure to ovulate postpartum is more likely to be attributed to seasonal affects than lactation affects [10,11]. However, some mares do not develop follicles in the postpartum period or become anestrus following a foal heat ovulation and will exhibit rapid follicular development and estrus as

soon as the foal is weaned [12,13]. The incidence of lactation-associated anestrus in mares has been reported to be 21–74% [11]. In contrast, other investigators have reported that suckling had no effect on postpartum ovarian activity [14].

Poor body condition in late gestation and the early postpartum period may also contribute to poor reproductive performance. The effects of inadequate nutrition and poor body condition may be manifested in delayed return to reproductive cyclicity postpartum, reduced pregnancy rates, and increased embryo loss rates [15]. The phenomenon of ‘lactational anestrus’ may represent the combined effects of season, body condition and lactation [12]. Maintenance of late-term pregnant mares due to foal between January and March (Northern Hemisphere) under a stimulatory artificial photoperiod for the last 2–3 months of pregnancy may be beneficial. Pregnant mares housed under lights have been reported to foal approximately 10 days earlier than mares not maintained under lights [16], are more likely to have a foal heat ovulation, have ongoing estrous cycles [11], and ovulate earlier in the postpartum period [17].

LeBlanc (unpublished data) gave eFSH to five Thoroughbred mares with a history of postpartum anestrus. All mares foaled between 21 January and 20 March 2006, and were 40–60 days postpartum at the onset of hormone therapy. Mares were given 6.25 mg eFSH twice daily for 5–10 days and 2500 IU of hCG when a 32–35 mm follicle was identified. All mares developed follicles in response to eFSH and all ovulated after administration of hCG. Three mares that were bred on the induced ovulation all conceived.

There are no published reports on the efficacy of eFSH when used to stimulate follicular development in other types of noncyclic mares, such as mares with equine cushing’s disease, ovarian senescence, or mares with a single inactive ovary following removal of a granulosa cell tumor.

#### 4. Superovulation of cycling mares

Substantial progress has been made over the past three decades in regards to induction of multiple ovulations in mares [1,19]. In the original eFSH study in cycling mares, horses were given either 12 or 25 mg eFSH twice daily to stimulate follicular development and then either hCG or a deslorelin implant to induce ovulation [9]. The highest ovulation rate ( $3.4 \pm 0.7$ ) was obtained using 12 mg eFSH twice daily, beginning 5–6 days after ovulation, followed by 2500 IU hCG to induce ovulation, once a majority of follicles reached 35 mm in diameter. Mares received 250  $\mu$ g cloprostenol on the second day of eFSH

treatment. The average duration of treatment with the 12 mg dose of eFSH was  $7.5 \pm 0.5$  days.

Welsh et al. [20] introduced the concept of incorporating a ‘coast period’ into the eFSH treatment protocol. Mares were given 12.5 mg eFSH twice daily, beginning 7 days after ovulation. Cloprostenol (250  $\mu$ g, i.m.) was given on the second day of eFSH treatment. Treatment with eFSH continued until approximately 50% of the cohort of developing follicles was  $>35$  mm in diameter. Administration of eFSH was then discontinued and hCG was given approximately 54 h after the last eFSH dose. Mares were treated with eFSH for an average of  $5.9 \pm 0.4$  days. Ovulation rate and embryo recovery rate per flush were  $3.8 \pm 1.2$  ovulations and  $1.7 \pm 1.4$  embryos, respectively.

Timing of the onset of eFSH therapy was evaluated by McCue et al. [21]. Treatment with eFSH was initiated in early diestrus when the diameter of the largest follicle was 20 to 25 mm. Mares received cloprostenol on the second day of eFSH therapy. Administration of eFSH (12.5 mg, twice daily) was limited to a fixed 3-day interval, after which mares were allowed to ‘coast’ until follicle(s) reached 35 mm in diameter or greater; hCG was subsequently given to induce ovulation. The mean ovulation rate for mares treated with eFSH for the fixed 3-day interval was  $3.8 \pm 2.6$  ovulations, which was significantly higher than that of control mares ( $1.2 \pm 0.4$  ovulations).

The concept of the treatment regimen described above was to allow endogenous FSH to initiate development of a follicular wave and stimulate continued follicle development throughout the ‘common growth phase’. Deviation and selection of the dominant follicle occur at approximately 22–25 mm in diameter [22]. The onset of treatment with exogenous eFSH was intended to be timed to begin when endogenous FSH levels were starting to decline. The goal of exogenous eFSH administration was to ‘rescue’ subordinate follicles in the follicular wave and promote their continued development.

Additional lessons learned from eFSH studies in the past 2 years include:

- Starting eFSH therapy when a single follicle  $\geq 30$  mm is present will only result in development and ovulation of that follicle and not superovulation.
- Twice daily eFSH treatment results in higher ovulation rates than administration of the same total amount of eFSH once daily [20].
- Treatment with eFSH over consecutive cycles may still result in superovulation, but the duration of therapy will be significantly longer after the first cycle [21].

- Administration of a lower dose of eFSH (6.25 mg, twice daily) may still result in multiple ovulations, but the ovulation rate will be lower than if the standard dose (12.5 mg, twice daily) is used [23].
- hCG, deslorelin, and recombinant equine luteinizing hormone (reLH) may all be used to induce ovulation in mares previously treated with eFSH. Ovulation rates may be highest if hCG or reLH are used in the presence of a large number of preovulatory follicles [23].
- There is no advantage of pre-treating mares with progesterone and estradiol prior to the onset of eFSH therapy [24,25].

Currently, the most efficient protocol for using eFSH to superovulate mares and enhance embryo recovery is as follows [19,26]:

1. Determine the day of ovulation.
2. Monitor follicular development in early diestrus.
3. Initiate eFSH therapy (12.5 mg, i.m., twice daily) when the largest follicle is first 22–25 mm in diameter; anticipate a 3–5 day treatment period.
4. Administer cloprostenol (250 µg, i.m.) on the second day of eFSH therapy.
5. Discontinue eFSH therapy when follicle(s) are  $\geq 32$ –35 mm in diameter.
6. Allow mare to ‘coast’ for approximately 36 h or until follicles are  $\geq 35$  mm.
7. Administer hCG (2500 IU, i.v.) to induce ovulation.
8. Breed or inseminate according to standard procedure.
9. Perform embryo recovery attempt 7 or 8 days after ovulation(s) are detected.

## 5. Management of subfertility

The embryo recovery rate is higher for mares that spontaneously double ovulate than for mares that have a single ovulation [27]. Therefore, induction of multiple ovulations in normal mares may increase the number of oocytes available for fertilization and consequently may increase the probability of establishing a pregnancy in mares intended to carry their own pregnancy to term. A study was subsequently conducted using EPE to test the hypothesis that increasing the ovulation rate would increase pregnancy rates under conditions of reduced fertility [18]. Nine mares were treated during alternating estrous cycles with either a saline placebo or EPE. All mares were inseminated with frozen-thawed semen containing  $300 \times 10^6$  progressively motile spermatozoa. Ovulation rates for placebo and EPE treatments were  $1.1 \pm 0.3$  and  $2.6 \pm 0.9$ , respectively. Pregnancy

rates for mares ovulating single and multiple follicles were 33 and 67%, respectively.

Niswender et al. [9] were the first to determine pregnancy rates in mares ( $n = 20$  total) carrying their own pregnancy that were superovulated with eFSH. One group of 10 mares was given 25 mg of eFSH twice daily. A second group of 10 mares was allocated to receive either 12 or 25 mg eFSH twice daily and induced to ovulate with either 2500 IU hCG ( $n = 5$ ) or an implant containing 2.1 mg deslorelin acetate ( $n = 5$ ). Mares were bred with frozen semen containing  $800 \times 10^6$  spermatozoa with a minimum of 30% progressive motility. Pregnancy examinations were performed 14 and 16 days after ovulation. Ovulation and pregnancies per mare were highest in mares receiving 12 mg eFSH and induced to ovulate with hCG ( $3.4 \pm 0.7$  ovulations and  $1.8 \pm 0.8$  pregnancies, respectively) and lowest in control mares ( $1.1 \pm 0.1$  ovulations and  $0.6 \pm 0.1$  pregnancies, respectively). Pregnancy rates per ovulation were not significantly different between eFSH-treated and control mares (52.9 and 54.5%, respectively).

The effect of eFSH on pregnancy rate in mares bred with a minimum of  $100 \times 10^6$  normal, motile spermatozoa was evaluated by Raz et al. [28]. Mares ( $n = 16$  cycles) were given 12.5 mg eFSH i.m. twice daily for an average of  $8.9 \pm 2.5$  days. Treatment with eFSH was discontinued when a follicle  $\geq 35$  mm in diameter was detected and hCG (2000 IU) was given 36 h later. Mares were inseminated with  $>100 \times 10^6$  normal motile spermatozoa every 48 h until ovulation occurred. Pregnancy examinations were performed 11–16 days after ovulation. Ovulation rate was greater during eFSH-treated cycles as compared to 26 untreated control cycles ( $2.2 \pm 1.9$  ovulations versus  $1.1 \pm 0.3$  ovulations, respectively). However, overall pregnancy rate was not enhanced in eFSH-treated mares versus control mares (8/16, 50% and 16/26, 62%, respectively).

Perhaps eFSH is more effective in increasing embryo recovery rates in individual mares and less effective in increasing overall pregnancy rates of a group of mares intended to carry their own pregnancies. Squires et al. [29] noted that there was considerable variation in ovulation rate among mares treated with eFSH. In addition, it was reported that approximately 25% of mares treated with eFSH fail to yield even a single embryo when flushed. Cause(s) of the variation in ovulation and embryo recovery are not yet known. Additional work is needed to determine if eFSH can increase pregnancy rate in mares with inherent low fertility. It also remains to be determined if it is possible

to increase the pregnancy rate of normal fertile mares bred to a stallion with oligospermia.

## 6. Summary

Purified eFSH was most effective in stimulating ovarian follicular development in transitional mares and cycling mares if given at a dose of 12.5 mg i.m., twice daily. Treatment should continue until follicle(s) are 32–35 mm in diameter. A ‘coast period’ of 36 h may be incorporated before administration of an ovulation inducing agent. If eFSH therapy is initiated at the end of the common growth phase of follicular wave development, the duration of treatment may be minimized to 3–5 days, but may lower the ovulation rate.

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