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Abstract
Induction of multiple ovulation in mares can be used as a means of improving embryo recovery, and thus, lowering the cost of embryo transfer. Crude equine pituitary extract (EPE) primarily has been used for superovulation, but more recent studies have reported success with a purified, commercially available equine follicle-stimulating hormone (eFSH) product. Stimulation of multiple follicles with EPE or eFSH may also be a means of obtaining multiple follicles for oocyte collection, improving fertility of mares inseminated with frozen semen, or increasing pregnancy rates of mares bred to stallions of low fertility. EPE and eFSH have also been used successfully to hasten ovulation in transitional mares.

1. Introduction
Approximately 50\% of mares provide an embryo after flushing the uterus on a given cycle. However, mares that spontaneously double ovulate or are induced to ovulate multiple follicles have higher embryo recovery. The most successful studies on superovulation have used crude equine pituitary extract (EPE) prepared in private laboratories. Rates of three to four ovulations per mare and approximately two embryos per attempt have been published [1-4]. However, the amount of equine follicle-stimulating hormone (eFSH) and luteinizing hormone (LH) injected was not always known or reported, and EPE is not commercially available. Thus, a standardized, commercial eFSH product is now available. Presented here are factors that affect the success of inducing multiple ovulation and the results of studies on inducing multiple ovulation with EPE and eFSH [a].

2. Factors Affecting the Superovulatory Response
The response of cycling mares to exogenous gonadotropins is dependent on the follicular population at the onset of treatment. Ideally, EPE or eFSH should be started at the beginning of a follicular wave, before the appearance of the dominant follicle. Response to EPE was better if initiation of treatment was on day 5 rather than day 12 after ovulation [2]. The use of ultrasound to identify mares with follicles less than 25 mm enhances the superovulatory response. Several approaches have been used to control follicular development before EPE treatment. Injections of progestins and estradiol before treatment have been used to suppress follicular development before EPE treatment [5]. In cattle, the dominant follicle is often aspirated before FSH treatment as a means of increasing the superovulatory response [6]. Apparently, this has not been attempted in mares. Two studies were conducted in our laboratory to determine whether administration of a gonadotropin releasing hormone (GnRH) agonist during the time of EPE treatment would enhance the response. Unfortunately, mares treated with GnRH agonist and EPE had similar responses to those given EPE only [2,3]. One of the most dramatic improvements in superovulation was obtained with injection of EPE q 12 h versus q 24 h [7]. Mares given EPE q 12 h had 7.1 ovulations and 3.5 embryos per mare versus 2.4 ovulations and 1.5 embryos for mares given injections q 24 h. It was determined in a subsequent study that the enhanced embryo recovery for mares given EPE q 12 h was primarily because of the frequency of administration and not the increased dose [3].

3. Studies with a Commercial eFSH Product
Recently, two studies evaluated the response of cycling mares given a purified eFSH product [a]. In the first study, conducted at Colorado State University, mares were assigned to one of four groups: group 1, untreated controls; group 2, 25 mg eFSH q 12 h; group 3, 12.5 mg eFSH q 12 h plus deslorelin acetate for induction of ovulation; and group 4, 12.5 mg
eFSH q 12 h plus human chorionic gonadotropin (hCG) for induction of ovulation. Administration of eFSH was initiated 5 or 6 days after ovulation, and prostaglandins were administered 1 day after the onset of eFSH treatment. Treatment was continued until most of the cohort of follicles reached a diameter of ≥ 35 mm. At that time, either hCG or deslorelin acetate was given. All mares were inseminated with 800 million frozen/thawed sperm from one of four stallions. Pregnancy status was determined at 14 and 16 days after the first ovulation. The number of follicles greater than 35 mm was 1.1, 6.7, 3.8, and 3.4 for groups 1, 2, 3, and 4, respectively. Number of ovulations was greater for groups 2 and 4 than controls, and number of pregnancies per mare was greater for group 4 (1.8 ± 0.8) than for controls (0.6 ± 0.1). Based on this study, the 12.5-mg dose given twice daily plus hCG was selected for a subsequent study conducted during the last breeding season in Brazil. Sixteen light-horse mares were used over two cycles. During the first cycle, mares were not treated with eFSH and were inseminated with fresh semen every other day during estrus. Embryo recovery was attempted 7 days after ovulation. After flushing, the mares were given prostaglandin F2α (PGF2α) to induce estrus, and eFSH injections were initiated. Mares were injected q 12 h until the majority of follicles were > 35 mm; hCG was then given, and mares inseminated and flushed for embryos as with the control cycles. The number of ovulations during the treatment cycle was 1.0/mare, and embryo recovery was 0.5 embryos/mare. During the treatment cycle, 15 of 16 mares ovulated, and 14 of 16 had ≥ 2 ovulations. The mean ovulation rate and embryo recovery rate was 3.9 ovulations and 1.9 embryos.

These studies were based on either 14- to 16-d pregnancy rates or embryo recovery at Day 7 after ovulation. Further studies are needed to determine foaling rates of mares given eFSH or embryo recipients receiving embryos from superovulated mares.

4. Response of Transitional Mares
Numerous methods have been used to shorten the transition period and hasten the onset of the first ovulation of the year. Coy et al., [8] compared stimulation of follicular development in deep seasonal anestrus and vernal transition mares with EPE. Twenty-seven light horse mares were used between January and June. All mares having follicles <15 mm in diameter for 3 consecutive wk were considered in deep anestrus. On January 15, mares were randomly assigned to one of three groups: group 1, controls; group 2, EPE-treated in deep anestrus; group 3, EPE-treated in transition (follicle > 25 mm at onset of treatment). All mares were given hCG when a follicle ≥ 35 mm was detected. Treatment with EPE in groups 2 and 3 continued for a maximum of 15 days. Only two of nine mares treated with EPE while in deep anestrus ovulated versus eight of nine mares treated in transition. None of the controls ovulated during the same time period. The mean interval from onset of treatment to ovulation for group 3 mares was 11.8 ± 5.0 days. Apparently, the small follicles of mares in deep anestrus have insufficient FSH receptors to respond to EPE. EPE seemed to be a viable management tool for inducing ovulation in transitional mares.

5. Summary
EPE and eFSH can be used to enhance reproductive performance of mares. Treatment results in a four-fold increase in ovulation rate and embryo recovery at a cost of approximately $400 - 500 for the eFSH. This procedure can be used to lower the cost or increase the efficiency of embryo transfer. Stimulation of multiple follicles may also enhance fertility of mares inseminated with frozen semen or mares bred with stallions of reduced fertility. In addition, eFSH treatment may be a means of stimulating multiple preovulatory follicles for aspiration and subsequent in vitro fertilization or oocyte transfer or for advancing the first ovulation of the year in transitional mares. EPE or eFSH can be used to stimulate ovulation in transitional mares.

Footnote
[a] Bioniche Animal Health USA, Athens, GA 30601.
References


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