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Induction of estrus and ovulation: Why some mares respond and others do not

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Abstract

The two most common procedures for breeding management of mares involve induction of luteolysis and induction of ovulation. Although both of these events are usually achieved, physiologic conditions affect the timing of the response. In a diestrus mare treated with prostaglandin $F_{2\alpha}$ (PGF), or a PGF analogue, it is well documented that, on average, the interval from treatment to the onset of estrus is 3–4 days, whereas ovulation occurs 8–10 days after treatment. However, the diameter of the ovulatory follicle, as well as its status at the time of PGF treatment, determines the intervals from treatment to onset of estrus and to ovulation; these intervals can range from 48 h to 12 days. Ovulation is routinely induced with human chorionic gonadotropin (hCG), recombinant LH (rLH), or the GnRH analogue Deslorelin. On average, ovulation occurs approximately 36 h after treatment, but the effectiveness of any of these treatments can be affected by the stage of the estrus cycle, follicle size and maturity.

Keywords: Estrus induction; Prostaglandin; Ovulation induction; Mare

1. Introduction

The use of ovulation-inducing agents is a key tool in the breeding management of mares. In order to maximize the efficiency of ovulation-inducing hormones, it is imperative to have a clear understanding of the physiology and the dynamics of follicular growth during the mare's estrus cycle. Estrus is characterized by receptivity to a stallion, cervical relaxation, presence of a large (dominant) follicle, and endometrial edema. The duration of behavioral estrus averages 5–7 days, but can vary widely among mares. Diestrus is characterized by lack of stallion receptivity, presence of a CL, a tight cervix, and lack of endometrial edema (blood progesterone concentrations >2 ng/mL). Although diestrus lasts approximately 15 days on average, mares can (and often do) develop large preovulatory-size follicles during diestrus.

2. Monitoring the estrus cycle

Ultrasonographic examination of the reproductive tract of the mare has become a standard procedure in breeding management. Monitoring and determining the stage of the estrus cycle, early pregnancy diagnosis, diagnosis of pathological reproductive conditions, and monitoring responses to uterine therapies, are some of the uses of ultrasonography in equine reproduction.

In order to implement therapies to hasten (and synchronize) equine ovulation, the presence, detection, and interpretation of endometrial edema is critical. Normal mares under the influence of estrogen will have edematous hyperplasia of the endometrial folds. Endometrial edema increases progressively during the

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first few days of estrus, but decreases as the mare approaches ovulation [1]. Alterations of this normal pattern, such as the presence of endometrial edema after ovulation or during diestrus, could be an indication of endometrial inflammation or an underlying problem.

To maximize pregnancy rates, breeding should be done at the following times relative to ovulation: natural mating, within 48 h before ovulation; AI with cooled shipped semen, 12–24 h before ovulation; and AI with frozen–thawed semen, <12 h before ovulation to <6 h after ovulation. In order to breed mares at these ''ideal'' times, there should be good estrus detection, and in particular, accurate prediction of ovulation.

3. Induction of estrus

In the normal cycling mare, estrus is induced by terminating the luteal phase with an injection of PGF. The author gives 5 mg of prostaglandin PGF or 250 μg of cloprostenol IM, as early as 5 days postovulation. On average, onset of estrus and ovulation occur 3–4 days, and 8–10 days, respectively, after treatment. However, elevated blood progesterone concentrations do not suppress increases in blood FSH concentrations; therefore, follicular development continues during diestrus. Consequently, there are often follicles of various sizes and stages of growth during the luteal phase. When there is a large, growing follicle present at the time of PGF treatment, ovulation is possible as early as 72 h after treatment, without overt signs of estrus. Conversely, if the follicle has reached its maximal diameter during the progesterone-dominated luteal phase, this follicle will inevitably regress, and a new cohort of follicles will have to be recruited; therefore, estrus and ovulation will be delayed [2]. The degree of endometrial edema can be used to predict the response to treatment. In that regard, mares with a large, growing follicle will have substantial endometrial edema within 24 h after treatment. However, in mares with large atretic follicles, increases in endometrial edema are not detected until 3–5 days after treatment.

4. Induction of ovulation

The main advantages of having ovulation at a predictable time include: (1) one service per cycle for high-demand stallions; (2) scheduled breedings for mares transported to stallions; (3) fewer breedings and reduced uterine contamination (particularly in mares with delayed uterine clearance or increased susceptibility to uterine infections); (4) inseminations close to ovulation for cooled-transported or frozen–thawed semen, or for stallions with low fertility; (5) ensuring adequate intervals between natural service for specific stallions; (6) synchronization between donor and recipient mares in embryo transfer programs; and (7) reduced labor and veterinary costs.

Due to the advantages of having ovulations at a predictable time, as well as the increase in pregnancy rates when mares are bred close to ovulation, many veterinarians are relying on pharmacological agents to induce ovulation (Table 1). These agents are maximally effective when given to mares with obvious endometrial edema, a relaxed cervix, and a follicle ≥35 mm in diameter. There are three products commercially available to induce ovulation in mares: human chorionic gonadotrophin (hCG); the GnRH analogue, Deslorelin; and recombinant equine LH (rLH).

4.1. hCG

This is a large protein hormone with LH-activity. The recommended dose of hCG varies among practitioners, but ranges between 1500 and 3300 international units (IU) given IV or IM. In a recent report [3], 75% of hCG-induced ovulations occurred between 24 and 48 h after treatment. However, several mares ovulated within 12 h of treatment, whereas others ovulated >48 h post-treatment. In the author's experience, the efficacy of hCG is reduced when it is given repeatedly to a mare during a breeding season. The author's recommended dose is 2500 IU given IV, 12–24 h prior to AI. In natural breeding situations, mares can be treated at the time of breeding.

Table 1
Ovulation-inducing agents commonly used in brood mare practice

Product	Dosage and route	Interval from treatment to ovulation (h)	Conditions
Human chorionic gonadotropin (hCG)	1500–3300 IU, IM or IV	36 (range, 12–48)	≥35 mm follicle and maximal uterine edema
Recombinant LH	800 μg, IV	36 (range, 24–48)	Same as above
GnRH (Cystorelin™)	10–40 μg, IM or SQ bid or tid	48–72	30 mm follicle
Deslorelin (Ovuplant™)	2.2 mg, SQ implant	38 (range, 36–42)	≥35 mm follicle uterine edema
Deslorelin injectable	1.5 mg, IM	40–46	≥35 mm follicle

4.2. Deslorelin (Ovuplant™)

This product is a biodegradable, short-term implant containing 2.2 mg of the GnRH analogue Deslorelin [4]. The implant comes as a preloaded syringe (with needle) to facilitate SQ placement. Based on recent studies, Ovuplant™ induced ovulation predictably between 38 and 42 h after treatment [5]. However, in some mares that failed to achieve pregnancy, there was a delayed return to the next natural estrus [6]. In that regard, a small percentage of mares induced to ovulate with Ovuplant™ had a delay of several days or weeks in returning to estrus, seemingly exacerbated when prostaglandin was used on days 5–7 [6]. Unfortunately, there are no reliable criteria to predict which mares will experience this delay, but it apparently may be avoided by removing the implant shortly after ovulation. Easy access for Ovuplant™ removal is facilitated by submucosal placement in the vulva instead of the neck, which is the manufacturer's recommended site. The removal of the pellet results in no deleterious effect on the subsequent cycle when compared to control mares or those treated with hCG [6].

Compounded Deslorelin is also available (from several sources) in a liquid form. The injection of 1.5 mg IM reliably induced ovulation 40–46 h after treatment. Furthermore, there was no evidence that this product delayed returns to estrus.

Although hCG and Ovuplant™ are the only approved products for induction of ovulation in horses, there is anecdotal evidence that gonadotropin releasing hormone (GnRH) is also effective. To mimic natural pulsatile secretion from the hypothalamus, 20–25 μg of GnRH was given IM or SQ twice or thrice daily for 2–3 days. Although this regime is GnRH impractical for routine induction of ovulation, GnRH can be used to induce estrus and ovulation in mares with unexplained ovarian inactivity during the physiologic ovulatory season. In most cases, twice daily injections for 2–3 weeks were required to stimulate ovarian follicular development and ovulation.

4.3. Recombinant LH (Ovi-Stim™)

Recombinant equine LH is a single chain gonadotropin that is of lower molecular weight than the two-chain hCG and therefore much less antigenic. Quarter Horse breeding stallions treated with reLH had plasma testosterone concentrations that were fourfold higher compared to saline-treated controls [7]. When mares were given 0.75 or 0.9 mg of reLH, ovulation rates were 90 and 80%, respectively, which were similar to hCG treatment (85.7%) [8]. Therefore, it was concluded that reLH was a reliable and effective ovulatory agent that did not significantly alter endogenous hormone profiles or affect interovulatory intervals [8].

In preliminary studies, PGF analogues were used to induce ovulation. Since further studies were unable to reproduce these results, PGF and its analogues are not recommended for induction of ovulation. However, veterinarians must be aware that there could be a short interval from PGF treatment to ovulation in mares with a large diestrus follicle.

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