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The role of PGE2 and PGF2α in follicle wall rupture and their implications in the development and treatment of luteinized unruptured follicles

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Summary: The article summarizes the results of several studies, which highlight the relevance of prostaglandins E2 (PGE2) and F2 (PGF2α) during the ovulatory process in mares and their implication in the pathogenesis of luteinized unruptured follicles. Following the preovulatory luteinizing hormone (LH) surge, the two prostaglandins are synthesized in granulosa cells. They subsequently trigger a cascade of events that lead to ovulation. The exogenous administration of intrafollicular PGE2 and PGF2α can induce follicle rupture and ovulation within 12 h of injection, even in mares in early estrus, and before the beginning of the abrupt rise of the preovulatory LH surge. The systemic administration of a prostaglandin synthesis inhibitor (flunixin-meglumine) during the periovulatory period blocks ovulation and induces the development of luteinized unruptured follicles (LUFs). LUFs share similar ultrasonographic and hormonal characteristics with hemorragic anovulatory follicles (HAFs). The administration of intrafollicular PGE2 and PGF2α in mares treated with a prostaglandin synthesis inhibitor restores normal ovulation and allows oocyte release and fertilization. However, the systemic administration of prostaglandins does not appear to have any effect on hastening ovulation.

Keywords: mare / reproduction /ovulation / LUF / PGE2 / PGF2α

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Introduction

The inducible isoform prostaglandin G/H synthase 2 (PGHS-2) begins to be expressed in granulosa cells about 10 h before ovulation in several domestic species (Sirois and Dore 1997). In the mare, this timing corresponds with 26 to 30 h after the beginning of the preovulatory luteinizing hormone (LH) surge (Sirois and Dore 1997). The enzyme PGHS-2 has a cyclooxygenase activity responsible for the production of prostaglandin H2 (PGH2), the precursor of all prostaglandins. The follicular fluid of the preovulatory dominant follicle shows increasing amounts of prostaglandins E2 (PGE2) and F2 (PGF2) beginning 33 h after hCG treatment, and reaching peak concentrations of 40 and 10 ng/mL, respectively, 36 h after human chorionic gonadotropin (hCG; Sirois and Dore 1997). The endogenous preovulatory LH surge and resultant induction of PGHS-2 expression (Sirois and Dore 1997) can be induced by exogenous treatment of hCG in the mare (Ginther et al. 2009a).

The obligatory role of PGE2 and PGF2α in the process of ovulation

Prostaglandins E2 and F2 are essential key factors during the ovulatory process. Its obligatory role in ovulation has been proven by several studies in which their synthesizing enzyme (PGHS-2) was inhibited by systemic treatment with flunixin-meglumine (FM), a non-steroidal anti-inflammatory drug (Cuervo-Arango and Domingo-Ortiz 2011; Cuervo-Arango et al. 2011; Cuervo-Arango 2011). The majority of mares treated intravenously with FM during the periovulatory period did not ovulate but developed a luteinized unruptured follicle (LUF; *Cuervo-Arango* et al. 2011). The ultrasound morphology of the FM-induced LUF is similar to that reported in mares with naturally occurring haemorrhagic anovulatory follicles (HAF; *Cuervo-Arango* and *Newcombe* 2012). The first evidence of LUF development (presence of echoic specks floating freely within the follicular antrum) is observed 44 h after hCG treatment (*Cuervo-Arango* et al. 2011).

On the other hand, the timing of FM treatment related to hCG administration appears to be important for the outcome of ovulation or LUF formation. When FM (1.7 mg/kg) is administered every 12 h beginning at the time of (Hour 0), or 24 h after hCG treatment, the incidence of LUF is 83% and 80%, respectively (*Cuervo-Arango* 2011). However, if FM is administered only once, either 24 or 30 h after hCG, 100% of mares ovulate normally. Finally, mares treated with FM twice at 28 and 36 h after hCG, have a LUF incidence of 16.7% (*Cuervo-Arango* 2011). Thus, it can be concluded that in order to block ovulation, PGHS-2 function must be inhibited during the critical time window between 24 and 36 h after hCG treatment.

Are PGE2 and PGF2α able to induce ovulation before the LH surge?

The latter series of studies showed the obligatory role of prostaglandins during the process of follicle wall rupture and ovulation. However, the prostaglandin-dependent mediators of the follicular extracellular matrix degradation required for ovulation are unknown. Studies in other species (cattle) have shown that prostaglandins activate extracellular matrix degrading

enzymes such as the MMP (matrix-metalloproteinase) family and plasmin (*Li* et al. 2006). On the other hand, it is likely that many other factors are involved in the process of ovulation, oocyte maturation, and release, all of them orchestrated by the action of gonadotropins (*Robker* et al. 2000). A recent experiment (*Martínez-Boví* et al. 2015) tested the hypothesis of whether the intrafollicular administration of PGE2 and PGF2α in a 4:1 ratio (similar to that observed during the physiological process of ovulation) would induce ovulation in mares in early estrus (before the beginning of the abrupt rise of the preovulatory LH surge). In this study, 6 mares were treated during 2 consecutive cycles with either a placebo (0.5 mL of water for injection) or a solution of 0.5 mL containing 500 μg of PGE2 (dinoprostone 10 mg/mL, PGE2-Pfizer; Pfizer España, Alcobendas, Spain) and 125 μg of PGF2α (dinoprost 5 mg/mL, Dinolytic; Pfizer España) by intrafollicular administration into the dominant follicle during early estrus (follicle diameter of 30 to 32 mm without concurrent treatment with hCG). Mares were inseminated with frozen/ thawed semen immediately before follicle puncture. No mare treated with the placebo ovulated within 24 h of follicle puncture: the mean interval from treatment to ovulation was 72 ± 10.7 h. On the other hand, 4 out of 6 mares treated with the prostaglandin solution ovulated within 12 h of treatment (mean interval to ovulation of 20 ± 8.9 h). It was concluded that the mixed solution containing PGE2 and PGF2α was able to induce follicle wall rupture and collapse despite being administered early in estrus, apparently before the beginning of the abrupt rise of the preovulatory LH surge. This finding highlights the potency of these prostaglandins to trigger ovulation on their own. Furthermore, this protocol would be potentially applicable to equine reproduction because of the rapid induction of ovulation. However, in treated mares the postovulatory rise in progesterone was delayed about 2 days compared with mares from the placebo group that ovulated spontaneously. In addition, no mare became pregnant. Whether these unsuccessful inseminations were due to a lack of oocyte maturation because of low LH concentrations, is unknown and requires further study.

Can PGE2 and PGF2 restore normal ovulation in mares treated with a prostaglandin synthesis inhibitor?

Once it is evident that an intrafollicular administration of PGE2 and PGF2α on its own can induce follicle wall rupture and ovulation, it is logical to suspect that it could be used as a potential treatment of anovulatory conditions such as HAFs. A recent study partially tested this hypothesis (*Martínez-Boví* and *Cuervo-Arango* 2014). Since HAFs are an unpredictable condition, an experimental model involving systemic administration of FM to inhibit PGHS-2 and induce LUF was used. In the reported study, mares were treated with FM every 12 h from Hour 0 (Hour of hCG administration) during two consecutive cycles to block the synthesis of follicular prostaglandins. Subsequently, mares were either treated with a placebo (0.5 mL of water for injection) or a mixed solution of 0.5 mL containing 500 μg PGE2 and 125 μg PGF2α, 32 h after hCG administration. Three mares in each group were inseminated with fresh semen 24 h after hCG. All mares in the treatment group ovulated within 12 h of PGE2 and PGF2α administration (5/5), and became pregnant (3/3). However, only one mare (1/5) ovulated in the placebo group. The rest (4/5) developed a LUF and none become pregnant (0/3).

In this experimental model, the prostaglandin treatment solution was able to overcome the anovulatory effect of FM and induce ovulation and apparent successful release of the oocyte, since all inseminated mares became pregnant. It is worth emphasizing that all mares were treated with hCG to initiate the abrupt rise of the preovulatory LH surge and therefore ovulation in mares from the treatment group took place between 36 and 48 h after hCG administration, a time window considered physiological for normal ovulation in hCG-treated mares.

The pathogenesis of naturally occurring HAF remains largely unknown. It is likely that factors other than intrafollicular prostaglandins are involved in the inhibition of ovulation in mares with HAF. However, because of the highly similar morphological and hormonal characteristics between naturally occurring HAF and FM-induced LUF (*Cuervo-Arango* and *Newcombe* 2012), as well as the ability of a combination of exogenous PGE2 and PGF2α to induce ovulation on its own, the potential usefulness of this protocol to prevent the formation of HAF should be studied further.

Systemic treatment with PGE2 and PGF2α: follicular vs. systemic effects

It seems logical to research the effect of prostaglandins on restoring ovulation administered by the systemic route. Although the intrafollicular route has been proven to be successful, it is more time consuming and requires specialized equipment (i.e. transvaginal ultrasound probe), which are not always available for the field practitioner.

The challenge of the systemic route approach is to determine the minimum effective dose of PGE2 and PGF2α capable of reaching the granulosa and theca layers to induce ovulation with the minimal adverse side effects. Prostaglandin E2 is used in human reproductive medicine for its ecbolic and cervical relaxation properties. The main indication of PGE2 is induction of labor administered as a vaginal pessary (*Mozurkewich* et al. 2011). The use of PGE2 by the intravenous route to induce labor was attempted in the past but now it has been discontinued in favor of the vaginal route because of increased adverse side effects such as gastrointestinal symptoms and fever (reviewed by *Mozurkewich* et al. 2011). The indications stated in the data sheet of the human product (PGE2-Pfizer 10 mg/mL intravenous infusion; Pfizer España) used for the previous experiments in mares, are molar pregnancy and induction of late term abortion. The manufacturer recommends the use of diluted PGE2 (5 μg/mL) by intravenous infusion at a starting rate of 2.5 μg/min and no more than 10 μg/min. Possible adverse side effects such as nausea, vomiting, diarrhea, dizziness, trembling, hypertension, and heart attack, etc., are described.

Data on the use of PGE2 in mares are scarce and have been limited to local therapy in the follicle (*Martínez-Boví* and *Cuervo-Arango* 2014), oviduct (*Weber* et al. 1991, *Allen* et al. 2006), uterus (*Weber* et al. 1991), and cervix (*Rigby* et al. 1998). To the author's best knowledge, only one published study has reported the intramuscular treatment of 10 mg of PGE2 in six mares as a single bolus (*Weber* et al. 1991). However, no mention of side effects following the systemic administration of PGE2 was made. The objective of the latter

study was to accelerate oviductal embryo descent. Only the intraoviductal infusion of PGE2, but not the intramuscular treatment caused a positive effect and hastened the embryo's oviductal transit in some mares.

The use of systemic (intramuscular or subcutaneous) treatment of PGF2 (dinoprost) or one of its synthetic analogues is common practice in equine reproductive medicine. Following intramuscular administration of the manufacturer's recommended dose of PGF2α (5 mg), slight adverse side effects can be observed in some mares (about 10%), such as sweating, diarrhea, or colic (*Irvine* 1993). However, when cloprostenol was administered intravenously (250μg, manufacturer's recommended dose), severe adverse side effects such as ataxia and profuse diarrhea as well as sweating were noted in 100% of treated mares (*Cuervo-Arango* 2011).

In a preliminary study, (originally reported herein), two mares were treated with 10 mg of PGE2 and 2.5 mg of PGF2α either as a single intravenous or intramuscular bolus when they were in estrus with a follicle of 35 mm in diameter. The mares were scanned every 12 h until ovulation. Within 2 minutes of treatment, the intravenously treated mare showed profuse sweating and drooling, ataxia, weakness of the hind limbs, abdominal discomfort, and increased intestinal peristaltism. Signs of colic lasted for 12 h. The interval to ovulation in this mare was 84 h. The mare treated intramuscularly showed no adverse side effects other than a slight rise in heart rate. The interval to ovulation was 72 h. It seems that 10 mg of PGE2 administered as a single systemic bolus did not affect the hastening of ovulation.

In a subsequent series of experiments (originally reported herein), the same amount of PGE2 (10 mg) and PGF2α (2.5 mg) were diluted in 250 mL of saline and administered to mares in estrus with a follicle of 32–35 mm in diameter, 24 h after hCG treatment, by constant intravenous infusion either throughout 2 h (n = 5) or 4 h (n = 4). In addition, mares were treated with FM at 24 and 36 h after hCG, to ensure that ovulation would be blocked. Overall, 7/9 mares failed to ovulate but developed a LUF. One mare from each group ovulated between 60 and 72 h after hCG. The adverse side effects were mild: no mare showed sweating, diarrhea, or ataxia. Most of the mares showed some sort of abdominal discomfort and adopted a urinating position with straining every 10 to 15 min throughout the duration of the infusion.

These preliminary results were disappointing. Although the protocol chosen for systemic administration of prostaglandins appeared to be bearable for the mares, it did not have a positive effect upon follicle rupture and ovulation. A similar approach, but designed to induce full luteolysis, achieved a positive luteolytic result following intravenous administration of a low dose of PGF2α (0.1 mg) diluted in saline and infused at a constant rate over a period of 2 h (*Ginther* et al. 2009b). Needless to say, perfusion of the CL and the follicle and the resultant arrival of prostaglandin at its target receptors within the ovary may be completely different in these two ovarian structures.

Conflict of interest statement

The authors have no conflict of interest to declare.

Animal Welfare statement

All animal procedures reported in this manuscript have been approved by the local welfare committee of the Universidad CEU Cardenal Herrera (ref: PRCEU-UCH 13/14).

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