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Luteolytic efficiency of reduced doses of cloprostenol in the ewe. Effect of progesterone concentrations at the time of treatment

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ABSTRACT

Seventy six ewes were treated with 7.5, 12.5, 25 or 50 μg of cloprostenol on day 6 or 9 post-estrus to compare the luteolytic efficiency of the PGF2α analogue at each stage and to evaluate if progesterone concentrations at the time of treatment affect such efficiency. Blood samples were obtained before cloprostenol administration and 12, 24, 48, and 72 h thereafter. There was an effect of dose ($p < 0.05$) but not of day post-estrus on the proportion of animals completing luteolysis. As the dose increased, the proportion of ewes completing luteolysis also increased. Also, as the dose increased from 7.5 to 25 μg, more ewes showed a transient progesterone decline instead of an absence of response, indicating that in some ewes reduced doses initiated luteolysis but were not able to finish the process. Since the dose of 25 μg resulted in close to 50% luteolytic efficacy, this group was used to study the effects of progesterone concentrations at the time of treatment on the response to cloprostenol. Pre-treatment progesterone concentrations were higher ($p < 0.01$) in ewes experiencing luteolytic failure than in those that completed luteolysis. There was a negative correlation between initial progesterone concentrations and their reduction by 12 h post-treatment. It is concluded that high progesterone concentrations are associated with a reduction in sensitivity to small doses of cloprostenol. Possible mechanisms and implications of this luteoprotective effect are discussed.

1. Introduction

Prostaglandin F2α (PGF2α) has been extensively used for estrus synchronization in the ewe (for reviews see Abecia et al., 2011; Fierro et al., 2013). Intramuscular administration of an appropriate dose of either natural PGF2α (Hackett and Robertson, 1980) or some of its synthetic analogues (Acritopeoulou et al., 1977; Baird and Scaramuzzi, 1975) is an effective and practical method to induce luteolysis.

Considerable variation in luteolytic efficiency and estrous response has been observed both with natural PGF2α and with its synthetic analogues (Abecia et al., 2011; Fierro et al., 2013). It is known that the luteolytic efficiency is lower when the treatment is administered before day 3 post-ovulation (Hackett and Robertson, 1980; Silva et al., 2000), but there also appear to be periods of relative insensitivity at later stages: Herrera et al. (1990) reported that luteolysis failed to occur in 33% of ewes treated with PGF2α between days 7 and 10 post-ovulation, and Hernández-Cerón et al. (2001) reported an even higher failure rate on day 5 or 6 post-

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ovulation. A finding shared by those two studies was that the ewes that failed to complete luteolysis had higher progesterone concentrations before the treatment than those that responded with complete luteolysis, thus suggesting a luteoprotective effect of progesterone. A direct luteoprotective role of this hormone has been postulated in the rat (Goyeneche et al., 2003), the cow (Okuda et al., 2004), the ewe (Davis et al., 2010) and the sow (Diaz et al., 2011). Alternatively, non-genomic effects of progesterone on the oxytocin-signalling elements of the endometrial cells could interfere with the endogenous endometrial release of PGF2α that often occurs after exogenous administration of the hormone (Bishop and Stormshak, 2006; Bogacki et al., 2002; Grazzini et al., 1998) and that may help to complete luteolysis when reduced doses of exogenous PGF2α are used (Wade and Lewis, 1996). However, to our knowledge there are no studies designed to compare the effects of pre-treatment progesterone concentrations on the luteolytic efficiency of reduced doses of PGF2α in the ewe.

Cloprostenol, is currently the most widely used PGFα analogue for estrus synchronization in the ewe (Abecia et al., 2011; Amiridis and Cseh, 2012; Fierro et al., 2013). The use of different reduced doses of cloprostenol can be useful to identify differences in luteal sensitivity due either to the stage of the luteal phase or to the initial progesterone concentrations. It has been known for some time that both in ewes and in cows the administration of a reduced, sub-luteolytic dose of either PGF2α or cloprostenol can result in one of three general types of response (Juengel et al., 2000; Colazo et al., 2002; Trevisol et al., 2015). 1- In some animals there is not a significant alteration in progesterone concentrations after administration of the sub-luteolytic dose, indicating very low sensitivity. 2- Animals with intermediate sensitivity may show a significant but transient decline in progesterone concentrations, which return to pre-treatment levels within 24–48 h after treatment. 3- Animals with higher than average sensitivity experience rapid, complete and irreversible luteolysis after administration of a reduced dose that would not be luteolytic for most ewes.

The proportion of animals with each type of response to reduced doses of cloprostenol can be useful to identify differences in CL sensitivity between stages of the luteal phase, and may also help to explain differences in the luteolytic efficacy of a full dose. Also, a dose resulting in close to 50% effective luteolysis and 50% luteolytic failure could allow for the identification of differences between the animals that respond or not with complete luteolysis to such a dose. Therefore, the objectives of this study were to compare the luteolytic response to different reduced doses of cloprostenol administered on day 6 or day 9 post-estrus, and to evaluate the effect of initial progesterone concentration on the luteolytic efficacy of the treatment.

2. Materials and methods

2.1. Ethics and preparation of the animals

The experiment was conducted at an experimental farm in the Central Plateau of Mexico. The protocol was approved by the Institutional Committee for the Care and Use of Experimental Animals of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, according to Mexican Official Norm NOM-062-ZOO-1999 (SAGARPA, 2001). Eighty adult, mixed-breed ewes were initially used. The ewes were feed on pasture and supplemented with concentrate according to requirements. Before the onset of the experiment they were synchronized with an intravaginal sponge impregnated with 20 mg of fluorogestone acetate (Chronogest, MSD Animal Health, Mexico) that remained in place for 7–9 days. At the time of sponge removal the ewes were treated with 250 IU of eCG (Folligon, MSD Animal Health, Mexico) and 125 μg of cloprostenol (Celosil, MSD Animal Health, Mexico).

2.2. Experimental design

After withdrawal of the sponges, estrus was detected using intact rams fitted with an apron to prevent mating. Each ewe was assigned at the time of sponge removal to one of eight cloprostenol-treatment groups. In four groups the ewes that showed estrus were treated on day 6 post-estrus with either 7.5, 12.5, 25 or 50 μg of cloprostenol/ewe, and in the other four groups the same treatments were administered on day 9 post-estrus. Initial assignment to the groups was random with the exception that fewer animals were assigned to the groups treated with 7.5 μg ($n = 7$ instead of $n = 11$), since a very high rate of luteolytic failure was expected to occur in those groups. The animals that were not detected in estrus during the first 5 days after sponge removal were not treated with cloprostenol, and they were eliminated from the study.

Jugular blood samples were obtained in heparinized tubes immediately before cloprostenol was administered (time 0), and at 12, 24, 48 and 72 h after treatment. The samples were centrifuged for plasma separation and stored at −20 °C until assayed for progesterone using a solid-phase radioimmunoassay kit (Coat-A-Count P4; Siemens Medical Solutions, Los Angeles, CA), that has been validated for ewes (Padmanabhan et al., 1995). The sensitivity of the assay was 0.02 ng/mL, the intra and inter- assay coefficients of variation were 7.4 and 4.9% respectively.

2.3. Definitions

Complete luteolysis in response to cloprostenol was considered to have occurred if progesterone concentrations decreased to less than 1 ng/mL within 24 h after treatment and remained at basal levels until 72 h post-treatment (Herrera et al., 1990, Hernández-Cerón et al., 2001). Any other progesterone profile was classified as luteolytic failure. The failures were further classified as minimal response, transient progesterone decline or delayed response: Minimal response to cloprostenol was assumed when progesterone concentrations never fell below 1 ng/mL during the first 72 h after treatment, even if some fluctuation was observed during that period. Transient progesterone decline was defined as a decrease in progesterone concentrations below 1 ng/mL within the first 12–24 h after treatment, followed by recovery to more than 1 ng/mL at 24, 48 or 72 h, a response called “partial luteolysis” by other