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# Efficacy of induction of luteolysis in superovulated cows is dependent on time of prostaglandin F2alpha analog treatment: effects on plasma progesterone and luteinizing hormone profiles

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ABSTRACT

The objectives were to (1) evaluate the effectiveness of induction of luteolysis in superovulated (SOV) cows at two distinct time points after embryo flushing; and (2) compare the pattern of LH release after treatment with PGF in cows with single vs. multiple ovulations. In the first experiment, Holstein cows were SOV with 400 IU of FSH following standard procedures. Uterine flushing for embryo recovery was performed 7 days after artificial insemination (Day 0), and cows were randomly allocated into two groups to receive PGF (0.5-mg sodium cloprostenol, intramascular) either immediately after flushing (Day 7 group, N = 19) or 4 days later (Day 11 group, N = 20). Time of luteolysis was determined on the basis of plasma progesterone (P4) concentrations. There was no difference ($P > 0.05$) in plasma P4 before treatment between Day 7 and Day 11 groups. A decline in plasma P4 was observed 48 hours after PGF treatment in both the groups ($P < 0.0001$). In Day 11 cows, P4 continued to decrease thereafter, whereas Day 7 animals had no further reduction in plasma P4. Luteolysis (P4 < 1 ng/mL) occurred in all Day 11 cows. In the Day 7 group, however, luteolysis failure was observed for 11 of 19 cows (57.9%). In cows without luteolysis, plasma P4 increased after the initial PGF–induced decline. The second experiment compared luteolysis in (SOV, N = 6) vs. non-SOV (control, N = 8) cows. Both groups received a single PGF treatment on Day 11 after estrus, and luteolysis was monitored daily by ovarian ultrasonography and plasma P4 measurements. In addition, plasma LH was measured in blood samples taken every 20 minutes for 1 hour during five consecutive days after treatment. A similar percentage of reduction in P4 was observed in both groups 24 hours after treatment; however, SOV cows only reached plasma P4 values similar ($P > 0.05$) to controls 96 hours after treatment. There was no difference in initial LH values between SOV and controls ($P > 0.05$). The slower decrease in plasma P4 in the SOV group prevented an increase in LH for up to 96 hours after luteolysis induction, whereas LH values increased ($P < 0.05$) in controls 24 hours after treatment. In conclusion, (1) luteolysis may fail or be incomplete when PGF treatment is given on the day of uterine flushing (Day 7) in SOV cows; (2) induction of luteolysis 4 days later (Day 11) is effective, but the initial high-plasma P4 concentrations result in a slower slope of P4 decline to basal levels, and consequently, delayed increase in LH pulses.


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## 1. Introduction

The induction of multiple ovulations is a key procedure for *in vivo* embryo production in cattle. As a consequence, many corpora lutea (CLs) develop on ovaries of superovulated (SOV) donors. Progesterone (P4) production by the CL is directly related to the mass of luteal tissue [1] and thus, greater number of CL leads to abnormally high-plasma P4 concentrations in cows used as embryo donors [2]. The nonphysiological P4 concentration has been implicated in alterations in the microenvironment of the reproductive tract [3], embryo quality [4], and pregnancy rates after embryo transfer (ET) to recipients [5]. Although it is important for the ET industry to reduce the interval between ET sessions to collect embryos from a given donor, less attention has been given to the consequences of multiple CL on the subsequent reestablishment of ovarian cyclicity and fertility of the donor cow. Superovulation protocols induce a transient endocrine imbalance, that is, concentrations of estradiol before ovulation and P4 afterward are much higher than typically experienced by cows [6]. In most cases, the endocrine disturbance is limited to the interval from the beginning of the superovulation treatment until uterine flushing for embryo recovery, usually performed on Day 7 after estrus/artificial insemination (AI). It is noteworthy, however, that failure to reestablish normal P4 and LH secretory patterns after this period is considered a risk factor for the development of cystic ovarian disease in donor cows [7].

After uterine flushing, luteolysis is induced in embryo donors as a routine procedure in an attempt to hasten the return of ovarian cyclic activity, avoid the establishment of pregnancies from potential nonrecovered embryos, and to avoid uterine infections caused by possible contamination during the flushing procedure. The exogenous induction of luteolysis with PGF analogs is largely used in animals bearing a single or few CL undergoing estrus/ovulation synchronization protocols, as well as in other clinical approaches in which a reduction in P4 levels is important, such as induction of parturition and treatment of uterine infections (reviewed by the study done by Weems CW et al. [8]). The early CL (before Day 5 of the estrous cycle) is refractory to PGF–mediated luteolysis [9] by mechanisms not yet fully understood [10]. As the estrous cycle progresses the CL becomes progressively more sensitive to luteolytic actions of PGF [11]. Embryo flushing in SOV cattle is performed on Day 6 or 7 after AI, a moment in the estrous cycle when the CL has not reached optimal sensitivity to exogenous PGF. Thus, it is likely that CL regression in SOV donors treated with PGF is only partial.

The hypothesis tested here was that delaying PGF administration in SOV donors would increase effectiveness of induction of luteolysis so that complete regression would occur in a greater percentage of animals compared with PGF treatment on the day of flushing (Day 7). However, it is not known whether a later PGF treatment would cause regression of multiple CL in a pattern similar to physiological single-CL regression. Therefore, the aims of this study were to (1) evaluate the effectiveness of induction of luteolysis in SOV cows treated at two distinct time points after embryo flushing; and (2) compare the pattern of LH release after luteolysis in cows with single vs. multiple ovulations.

## 2. Materials and methods

### 2.1. Animals and superovulation protocol

Multiparous lactating Holstein cows (second to fifth lactation; N = 54), greater than 45 days in milk, were enrolled in this study. Cattle were maintained in a free-stall system at the Dairy Cattle Research Center of the Brazilian Agricultural Research Corporation (Embrapa), located in Coronel Pacheco, MG; Brazil. Animals were fed corn silage and grain ration to meet maintenance and production requirements, with *ad libitum* access to water, salt, and mineral mixture. Cows were cyclic, with normal ovarian activity, and had no reproductive abnormalities on the basis of transrectal ultrasound examinations with an 8.0-MHz linear probe (Aquila Vet, Esaote Genova, Italy). Likewise, they remained healthy and in good body condition (between 2.5 and 3.5, scale 1–5) [12] for the duration of the two experiments.

A standard superovulation protocol was used to induce ovulation of multiple follicles growing in the second follicular wave of an estrous cycle in 46 cows. Briefly, 10 days after standing estrus, 400 IU FSH (Pluset, Hertape Calier Saúde Animal S.A., Juatuba, MG, Brazil) were given in decreasing concentrations daily twice for four consecutive days. At the third day of the superovulation protocol, donor cows received PGF (0.5-mg sodium cloprostenol [Ciosin], MSD Saude Animal, Sao Paulo, SP, Brazil, intramuscular [i.m.]) and were observed for estrus twice a day. Artificial insemination was performed at 12 and 24 hours after standing estrus with commercial semen from bulls of proven fertility. Seven days after the first AI, embryos were collected nonsurgically by flushing the uterus with Dulbecco's phosphate buffered saline (Nutricell, Campinas; SP, Brazil). For experimental purposes, the day of the first AI after the superovulation protocol was designated as Day 0. Seven cows had poor response to superovulation (<three ova per flushing) and were excluded from the experiment. The remaining 39 cows were allocated in experiment 1. A second round of superovulation with the same protocol was then performed in a subset of cows which were used in experiment 2 (N = 6), along with non-SOV cows (controls, N = 8).

### 2.2. Experimental design

#### 2.2.1. Experiment 1

The experiment was designed to evaluate the decrease in plasma P4 concentration (an indicator of functional luteolysis) when CL regression was induced in SOV cows at embryo collection (Day 7) or 4 days later (Day 11). On the day of embryo collection, donor cows were randomly assigned to receive PGF (0.5-mg i.m. sodium cloprostenol, Ciosin) either immediately after uterine flushing (Day 7; Day 7 group, N = 19) or 4 days later (Day 11; Day 11 group, N = 20). Blood samples for P4 assays were taken immediately before and 48 hours, 96 hours, and 144 hours after the PGF treatment (a total of four samples per cow).