Journal of Reproduction and Development, Vol. 68, No 2, 2022

Review

Ovarian response to prostaglandin $F_{2\alpha}$ in lactating dairy cows: A clinical update

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Abstract. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogs are used to induce luteolysis in estrus synchronization programs to terminate unwanted pregnancies or to promote ovulation in certain cow subpopulations. In the past few decades, the luteolytic dose of $PGF_{2\alpha}$ has remained unchanged. This review explores the clinical implications of increasing the standard dose for these applications in high-producing dairy cows. Ultrasonography may assist in selecting the most appropriate $PGF_{2\alpha}$ dose and improve the results. A reference has been used for $PGF_{2\alpha}$ for promoting ovulation in herds showing poor reproductive performance.
Key words: Luteolysis failure, Multiple ovulations, Terminating pregnancy

(**J. Reprod. Dev. 68:** 104–109, 2022)

Introduction

The corpus luteum (CL), named by Marcello Malpighi (1628–1694), because of its yellow appearance in the cow [1], was first described by Regnier De Graaf (1641–1673) in his study "*The Mulierum Organis Generationi Insirvientibus*" (1672). De Graaf observed that "globular bodies" appeared on the ovaries of rabbits after mating and remained there until delivery of the same number of offspring [2]. Ludwig Fraenkel, in 1901, when testing an unpublished hypothesis of his teacher, the anatomist Gustav Jacob Born, showed that pregnancy could be terminated by removing the corpora lutea from a pregnant rabbit [3]. In 1923, the first bioassay for female sex hormones was introduced [4], and in 1934, the luteal factor that maintains gestation was crystallized and named progesterone (P4) [5]. This was the beginning of early clinical research on P4 [6]. During this period, the ejaculates of both ram and man were found to induce contractility of uterine strips *in vitro*. The causative agent was named prostaglandin by von Euler in 1936 [7]. However, while P4 was already used in the 1950s to synchronize estrus in cows [8–10] and the race was on for developing the contraceptive pill for women [11–13], prostaglandins inspired little enthusiasm in the scientific community. It was not until 1965, during the Second Brook Lodge Workshop on problems in reproductive biology, Babcock wondered whether prostaglandins released from the uterus might be the luteolytic factor controlling regression of the CL (cited by Lauderdale [14]). This question prompted research in the late 1960s that led to the identification of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [15] as the uterine factor that initiates luteolysis [16–19] and its production for commercial use in domestic animals. Prostaglandin $F_{2\alpha}$ or its analogs were soon used to manipulate the estrous cycle and ovarian function in cows [20–22], mares [22], ewes [23] and gilts [24, 25]. Since then, the role of prostaglandins in reproduction has been extensively described. Apart from luteolysis, $PGF_{2\alpha}$ supports the ovulation process, favors uterine contractility and sperm transport, and induces luteinizing hormone (LH) release [26–29]. This report reviews the use of $PGF_{2\alpha}$ or its analogs in dairy cattle as the basis for developing protocols for breeding management and terminating pregnancy. Wherever possible, clinical implications are discussed. The use of $PGF_{2\alpha}$ as a therapeutic agent for reproductive disorders has not yet been addressed.

Received: October 6, 2021
Accepted: December 8, 2021
Advanced Epub: December 30, 2021

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Improvement of Fertilization with $PGF_{2\alpha}$

The importance of prostaglandins in the process of ovulation, acting mainly on the Graafian follicle, was suggested in 1972. Independent studies showed that the treatment of rats with aspirin or indomethacin could block ovulation [30–32]. Granulosa cells of the pre-ovulatory follicle produce large amounts of $PGF_{2\alpha}$, besides PGE, which is considered the main trigger of ovulation, and are responsible for the overall steroidogenic effects of LH on oocyte maturation and release [26–29]. However, the full potential of $PGF_{2\alpha}$ in promoting ovulation has not been fully exploited in breeding synchronization protocols.

In a study population of 390 cows and heifers, intravenous (IV) administration of a 50-µg dose of cloprostenol (a synthetic $PGF_{2\alpha}$ analog) (10% of the recommended luteolytic dose of 500 µg), at the time of artificial insemination (AI), was reported to significantly increase the pregnancy rate by 15.2% [33]. An IV dose of 500 µg cloprostenol at AI in 810 lactating dairy cows showed no benefit in cows with acceptable reproductive performance, promoted ovulation under heat stress conditions (70.5%–90.9%), induced double ovulation (10.5%–23.4%), and increased the pregnancy rate in primiparous repeat breeder cows (35%–66%) and in cows inseminated at spontaneous estrus for second services (36%–53%) [34]. An intramuscular (IM) $PGF_{2\alpha}$ dose at an AI of 10 mg dinoprost tromethamine (40% of the recommended luteolytic dose of 25 mg) increased the pregnancy rate (36%–45.8%; n = 451 lactating cows), but 5 mg had no such effect (n = 307 lactating cows) [35]. Body condition score and parity, factors mostly influencing reproductive parameters, did not interact with the beneficial effects on pregnancy rates of 10 mg $PGF_{2\alpha}$ observed in the latter study [35]. Conversely, no improvement in pregnancy rates was observed in 404 cows [36], and 532 cows and heifers [37], using an

IM dose of 25 mg of $PGF_{2\alpha}$ at AI and in 220 [38] and 413 [39] cows receiving an IM dose of 500 µg and 150 µg of cloprostenol (40% of the recommended dose), respectively, or using 10 mg of $PGF_{2\alpha}$ in 1828 lactating dairy cows [40]. In the latter two studies, treated cows produced more twins than control cows using cloprostenol (6.9%–15.6%) [39] or $PGF_{2\alpha}$ in one herd (3.2%–11.7%) but not in an additional herd [40].

As stated above, $PGF_{2\alpha}$ has multiple effects on the female reproductive system in mammals [26–29]. Thus, $PGF_{2\alpha}$ treatment concurrent with AI could increase pregnancy rates not only by supporting the ovulation process, but also by inducing LH release [26, 27]. In effect, irrespective of effects on fertility, this treatment has been linked to increased double ovulation or twin pregnancy rates in only three studies providing these data [34, 39, 40]. Double ovulation is a beneficial factor in subsequent fertility [41, 42]. Further, according to the results of three of the included studies [33–35], it seems reasonable to suggest that the positive effects of cloprostenol or $PGF_{2\alpha}$ on reproductive performance were mediated by the improved ovulation rate. The influence of follicular dynamics and development at the time of $PGF_{2\alpha}$ treatment on double ovulation rate should be considered in future studies to assess this assertion. The question is why is there such a discrepancy among the results? Is it because the percentage of cows sensitive to treatment varies remarkably between studies? Alternatively, could there be marked differences among the hormones used for timed AI. For example, the average ovulation risk was greater than 90%, and in 289 cows, there were no effects of treatment [40]. This high ovulation rate precluded the possibility of demonstrating the efficacy of $PGF_{2\alpha}$ treatment, contrasting sharply with its ovulation-promoting effect noted above under heat stress conditions (70.5%–90.9%) [34]. In the latter study, cloprostenol treatment had no influence on the ovulation rate during the cool period of the year in 273 cows and 89.7% of controls versus 89.8% of treated cows [34].

Insights into the effects of $PGF_{2\alpha}$ on ovulation have been recently reported. Intrafollicular injection of $PGF_{2\alpha}$ into pre-ovulatory follicles did not influence the time of ovulation, indicating that $PGF_{2\alpha}$ alone is not able to induce ovulation locally [43]. These results suggest that inducing LH release via a luteolysis-independent mechanism might be the main mechanism of $PGF_{2\alpha}$ in the ovulation process [44, 45]. Anovular cows, up to 40% of cows at the end of the waiting period [46, 47], are a valuable study population to assess the possible effects of $PGF_{2\alpha}$ on the physiology of the pre-ovulatory follicle. An IM dose of 25 mg of $PGF_{2\alpha}$ (n = 437 lactating anovular cows) administered 2 days before timed AI was found to increase the pregnancy rate (23.1%–43.7%) in normothermic cows (rectal temperature at AI ≤ 39ºC) but not in hyperthermic cows (> 39°C) [48]. These results could be an example of a reduction in supra-basal P4 levels. In a second experiment (n = 56 anovular cows), the LH surge was longer for treated cows, and treatment increased the diameter and volume of the pre-ovulatory follicle and concentration of estradiol [48]. Prepubertal heifers have also proven to be a useful model for examining the influential role of $PGF_{2\alpha}$ in the ovulation process [49]. Fourteen prepubertal heifers were treated with an IM dose of 500 µg of cloprostenol 5 days after the emergence of a spontaneous (non-induced) follicular wave (PG group); in a further 12 heifers, a follicular wave was induced and cloprostenol was given on day 5 of the induced follicular wave (PPG group); and 14 heifers received no treatment (control group). The rates of heifers ovulating within 10 days after wave emergence were higher in PPG (10/12, 83.3%) and PG (11/14; 78.5%) than in the control group (1/14; 7.1%; $P < 0.0001$) [49].

The effects of $PGF_{2\alpha}$ or cloprostenol used to promote ovulation appear to be influenced by the health status of the cow, timing of treatment, the administered dose, and herd. Since the IV administration of $PGF_{2\alpha}$ is not practical for routine use in a herd and is off-label in some countries, 10 mg of $PGF_{2\alpha}$ IM at AI could be used as a reference to promote ovulation in herds with poor reproductive performance [35]. A normal IM dose of 25 mg of $PGF_{2\alpha}$ two days before AI appears to be effective for anovular cows [48]. More dose-response studies are needed for each cow subpopulation, particularly using cloprostenol. The first issue that needs to be explored is the incidence of twins after promoting ovulation with $PGF_{2\alpha}$ or its analogs. The positive effects of prostaglandin-induced ovulation may be compromised by a greater risk in twin pregnancies.

Breeding Synchronization Protocols

Luteolytic treatment with $PGF_{2\alpha}$ or its analogs is only effective when a functional CL exists from days 5 to 16 of a normal estrous cycle [50]. The fertility of induced estrus was already noted in the early 1970s to be similar [14, 51] or greater than [52] that of naturally occurring estrus. In fact, the luteolytic doses needed to synchronize estrus were also established in the 1970s as $PGF_{2\alpha}$, 25 mg; cloprostenol, 500 µg; and fenprostalene, 1 mg [14, 51]. These luteolytic doses have not been modified throughout the development of different estrus synchronization protocols over the past 50 years in dairy cattle. However, the clinical implications of increasing the luteolytic dose of $PGF_{2\alpha}$ in dairy cattle has been recently addressed [53]. The basis for this proposal was the presence of a young CL or multiple CLs in a fixed-time AI (FTAI) protocol, or the presence of multiple CLs in pregnant cows for therapeutic abortion [53].

Because of today's large herd sizes and intensive milking and feeding rhythms, individual animal monitoring poses a problem, and breeding synchronization protocols for FTAI have become routine components of the reproductive management of dairy herds. An example is the $PGF_{2\alpha}$-based ovulation synchronization protocol denoted “OvSynch” which is extensively used for the FTAI of lactating dairy cows [54, 55]. The OvSynch method consists of a gonadotropin-releasing hormone (GnRH) treatment administered at random stages of the estrous cycle to synchronize a follicular wave; $PGF_{2\alpha}$ administered 7 days later to lysate a CL; a second dose of GnRH administered 36 h after the $PGF_{2\alpha}$ treatment to synchronize ovulation; and finally, FTAI 16 to 20 h later [54, 55]. However, around 60% of cows ovulate after the first GnRH treatment, forming a new, very young CL (5–6 days) at the time of $PGF_{2\alpha}$ treatment [56]. As a result, 20% of cows subjected to the OvSynch protocol underwent delayed or incomplete CL regression [57, 58]. Incomplete luteal regression decreased fertility. In a recent meta-analysis that included data derived from seven randomized controlled experiments in a final study population of 5356 cows, additional $PGF_{2\alpha}$ treatment 24 h after the first dose during the OvSynch protocol was found to offer improvements in luteal regression of 11.6% and in pregnancy per insemination of 4.6% [59]. The need to increase the $PGF_{2\alpha}$ dose twice [60] or the cloprostenol dose 1.5- [61] or 2-fold [28] to promote luteolysis in cows with a 3.5-day-old [60] or 5- to 6-day-old [61, 62] CL reinforces these results. Furthermore, it was recently shown that the presence of two or more corpora lutea influenced the luteolytic response to prostaglandin $F_{2\alpha}$ in a study population of 2436 lactating dairy cows: 1683 cows with a single CL (control cows) and 753 cows with two or more CLs [63]. Using a single $PGF_{2\alpha}$ dose (25 mg of $PGF_{2\alpha}$), the presence of multiple CLs reduced the estrous response compared to that observed in control cows (74–15.6%), and milk

production was inversely associated with this response. Importantly, an increased $PGF_{2\alpha}$ dose (37.5 mg of $PGF_{2\alpha}$) improved the estrous response in cows with two or more CLs (82.9%) [63].

In herds subjected to a good estrus detection and FTAI protocol, $PGF_{2\alpha}$ treatment is commonly used to provoke and synchronize estrus in those with a mature CL [54]. Single, double, or triple $PGF_{2\alpha}$ treatment 11 to 14 days apart, followed by AI at the subsequently detected estrus, resulted in pregnancy rates similar to those of FTAI protocols in a meta-analysis based on the results of 71 trials consisting of control and treatment comparisons [54]. Neither pregnancy rates differed with respect to controls in response to two $PGF_{2\alpha}$ doses given 12 to 14 days apart, a GnRH dose 24 to 48 h after the last $PGF_{2\alpha}$ dose, and AI 16–20 h later [54]. Unfortunately, estrus is not precisely synchronized following a single $PGF_{2\alpha}$ regimen. Although most cows show estrus between 3 and 5 days after treatment, estruses are generally detected between 2 and 7 days [64–66]. The stage of the follicular wave [66–68] or the presence of multiple corpora lutea [63] at treatment are factors that determine the time of estrus onset. Simultaneous treatment with $PGF_{2\alpha}$, equine chorionic gonadotropin (eCG), and GnRH 48 h later followed by FTAI in cows with silent ovulation (cows with a mature CL and no signs of estrus detected in the preceding 21 days; n = 1266 cows) led to improved fertility over spontaneous estrus (n = 4615 cows) [69]. It should be noted here that eCG treatment influences both FSH and LH secretion, and simultaneous administration of eCG and $PGF_{2\alpha}$ has been successfully used in FTAI protocols [70]. However, simultaneous treatment with cloprostenol and GnRH [71, 72] or FSH [73] disrupts follicular dynamics, promoting premature ovulation or ovulation failure. However, when $PGF_{2\alpha}$ treatment is administered to a group of cows with no further hormone treatment, subsequent estrous behavior and detection are dramatically improved as many cows simultaneously enter estrus, promoting tighter synchrony [74]. A cow will show estrous behavior as a result of sexual stimulation by other cows in estrus [75]. In effect, each additional cow in estrus simultaneously has been associated with a 6.1% increase in walking activity [76] and with an augmented intensity and duration of estrus [77].

In single $PGF_{2\alpha}$ protocols, a possible subsequent luteal deficiency should also be considered. It has been suggested that pharmacological manipulation of the estrous cycle may cause lower conception rates and impaired fertility [52, 78]. Estrus synchronization by $PGF_{2\alpha}$ or its analogs may decrease P4 concentration and thus modulate steroidogenesis in the subsequent CL [79–81]. The consequence of disturbances in the morphology and functionality of the newly formed CL potentially decreases the sensitivity of luteotropic factors, such as LH and PGE2 [80], and may reduce the ultimate overall weight of the CL [82]. Hansen *et al*. [79] showed that luteal cells derived from CLs following $PGF_{2\alpha}$-induced estrus were less responsive to LH. Thus, pharmacological manipulation of the estrous cycle could impair the function of granulosa and theca cells responsible for the production of healthy oocytes, proper follicle growth and ovulation, and CL formation and function, such as disorders in P4 production by the subsequent CL. Lower P4 production and reduction of CL sensitivity to luteotropic factors may be the reason for luteal deficiency, may influence the fertility of inseminated cows, and may also decrease the pregnancy rate after embryo transfer in recipient cows.

Given the reduced effects of a single $PGF_{2\alpha}$ dose on a young CL, of particular importance because of its high incidence rate in FTAI protocols, the use of two $PGF_{2\alpha}$ treatments administered 24 h apart in such protocols is recommended. In single $PGF_{2\alpha}$ protocols, ultrasonography procedures are commonly used to identify luteal structures and may therefore help to determine the best $PGF_{2\alpha}$ dose to improve the estrus response. A young CL can be identified as a small luteal structure with a high pixel intensity of the gland, in contrast to a mature CL [83, 84]. A double $PGF_{2\alpha}$ dose should be applied in the presence of a young CL, whereas a cow with multiple young CLs should be introduced in the FTAI protocol. In the remaining cows with mature CL, a single or 1.5 $PGF_{2\alpha}$ dose is recommended in the presence of a single or multiple CL, respectively.

Terminating a Pregnancy

Although it is prohibited in some countries to slaughter pregnant animals, accidental breeding of a very young heifer or a valuable cow may require termination of an established pregnancy. Termination of pregnancy may also be a suitable option when there is a diagnosis of twins, as this is a costly problem for dairy herd economy [85]. It is generally accepted that pregnancy is maintained by the CL until approximately 165 days of gestation [86] and that $PGF_{2\alpha}$-induced abortions are rapid and generally without complications up to 150 days of gestation, even when using lower than standard $PGF_{2\alpha}$ doses in heifers [87, 88]. Cloprostenol is considered a safe and effective abortifacient in heifers at a dose of 250 µg (50% of the recommended luteolytic dose) until day 120 of gestation, and at a dose of 500 µg from day 121 to day 150 [87]. However, in cows with a single CL, a double $PGF_{2\alpha}$ dose between days 40 and 120 of gestation led to abortion in all treated cows, as opposed to a single or lower dose, which were either less effective or totally ineffective [89]. These results suggest that for terminating pregnancy, a double $PGF_{2\alpha}$ dose is better in cows and a single dose in heifers. However, the impact of multiple CLs on the response to $PGF_{2\alpha}$ remains unclear.

Multiple CLs may occur in over 50% of the older cows. In a study of 2173 pregnant cows in their third lactation or more, the presence of two or more CLs was recorded in 51.5% (1119/2173), of which 37.7% (422/1119) carried singletons [90]. Pregnant cows with multiple CLs probably show a reduced luteolytic response to $PGF_{2\alpha}$, particularly those carrying singletons. The presence of additional CL (number of CL exceeding the number of embryos/fetuses) has proven to be a very strong factor favoring pregnancy maintenance [91]. In a recent study, the $PGF_{2\alpha}$ dose-dependent abortion response was examined in cows with two dead twins at pregnancy diagnosis 28–34 days post-AI (late embryonic period [LE]) or at confirmation of pregnancy 49–55 days post-AI (early fetal period [EF]) [92]. The study population consisted of 615 cows, 415 receiving a single dose of $PGF_{2\alpha}$ (PG1 group) and 200 receiving a 1 × 1.5 $PGF_{2\alpha}$ dose (PG1.5 group). The induced abortion rate was significantly lower ($P < 0.0001$) in the EF cows (34.6%) than in the LE cows (88%) and was also reduced ($P = 0.001$) in the EF PG1 group (28%) than in the EF PG1.5 group (48.1%). After treatment, the estrus response occurred significantly ($P < 0.0001$) earlier in LE cows (2.8 ± 0.9 days) than EF cows (5.6 ± 0.9 days). Based on the odds ratio, the only factor influencing the induced abortion rate in LE cows was milk production, with an odds ratio of 0.2 ($P < 0.0001$) for high producer cows (≥ 45 kg), whereas the odds ratio for induced abortion in the EF PG1.5 group was 2.3 ($P = 0.005$) compared to the EF PG1 group [92]. The gradual dissolution of conceptuses during the late embryonic period could explain the rapid response to $PGF_{2\alpha}$ treatment irrespective of dose, whereas the longer interval to estrus or luteolysis failure after treatment during the early fetal period may be explained by the survival of trophoblastic cells. Following cloprostenol-induced abortion, the decline in pregnancy-associated glycoproteins and placental antigens expressed in the trophectoderm cells [93, 94] is delayed as gestation advances [95]. Additionally,

intrauterine infusion of embryonic homogenates [96] or trophoblast proteins [97, 98] promotes luteotropic signals and extends luteal function. Based on these findings, I recommend a double or even greater dose of $PGF_{2\alpha}$ for cows carrying dead twins during the early fetal period.

Differences detected in the best way to terminate a pregnancy in heifers [87, 88] and cows [89] could be explained by the different weights of animals and stage of gestation. A single $PGF_{2\alpha}$ dose is effective in heifers [87, 88, 95]. However, a double dose of $PGF_{2\alpha}$ should be administered to cows with a single CL carrying singletons [89] and to cows carrying dead twins during the early fetal period [92]. The dose-response to $PGF_{2\alpha}$ remains to be established in pregnant cows with multiple CL carrying live conceptuses, both in single and multiple pregnancies, and in high-producing pregnant cows.

Concluding Remarks

Over the past decades, the luteolytic doses of $PGF_{2\alpha}$ and its analogs used in dairy cattle have not been modified. While there is a large body of literature supporting the recommended dose of $PGF_{2\alpha}$, a reduced response to this agent has been described in non-pregnant [63, 99] and pregnant [92] high-producing cows. These recent findings suggest the benefits of increasing the $PGF_{2\alpha}$ dose in some circumstances.

Given the high incidence of cows with a young CL and the presence of cows with multiple CLs in FTAI protocols, two $PGF_{2\alpha}$ treatments should be administered 24 h apart in such programs. In single $PGF_{2\alpha}$ protocols and based on ultrasonography findings, a double $PGF_{2\alpha}$ dose is recommended in the presence of a young CL and a 1.5 $PGF_{2\alpha}$ dose in the presence of multiple CLs. To terminate a pregnancy, a single $PGF_{2\alpha}$ dose is sufficient in heifers, whereas a double dose should be administered to cows. Finally, a dose of 10 mg of $PGF_{2\alpha}$ at AI is a good option to promote ovulation in herds with poor reproductive performance. After promoting ovulation with $PGF_{2\alpha}$ or its analogs, the increased incidence of twins should always be monitored.

Conflict of interests: The authors declare no conflict of interest.

Acknowledgements

The authors thank Ana Burton for editing the manuscript.

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