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# Proposal of a new model for CL regression or maintenance during pregnancy on the basis of timing of regression of contralateral, accessory CL in pregnant cows

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ABSTRACT

In bovine pregnancy, regression or maintenance of the corpus luteum (CL) is mediated through local communication pathways between embryo, uterus, and ovary with Days 16 to 25 of pregnancy generally recognized as the pivotal period determining either luteolysis or prevention of luteolysis. To evaluate this concept, accessory CL was generated by treating Holstein lactating dairy cows (n = 718) with GnRH on Day 5 of the first follicular wave to produce an accessory CL on the ovary either contralateral or ipsilateral to the gravid horn. In pregnant cows, 66.2% (86/130) of contralateral CL regressed by Day 75 of pregnancy, whereas few ipsilateral accessory CL regressed (11.9%; 8/67), on the basis of similar criteria ($P < 0.0001$). As hypothesized, some contralateral CL regressions (22/86 = 25.6%) happened on Days 19 to 25 of pregnancy. However, most contralateral CL regressions (64/86 = 74.4%) happened later than expected, from Days 33 to 60 of pregnancy. Later contralateral CL regression was more common in primiparous (84.3%) than multiparous (60.0%; $P = 0.02$) cows. Early accessory contralateral CL regression (Days 19–25) may be related to lack of exposure of the contralateral horn to interferon tau from the elongating embryo because pregnant cows without early accessory CL regression had a smaller uterine volume than nonpregnant cows or pregnant cows that had early accessory CL regression ($128.4 \pm 3.9$ vs. $147.0 \pm 3.8$ vs. $143.6 \pm 10.9$ mm$^3$, respectively; $P = 0.003$). These results indicate that there is a second distinct period for CL protection during bovine pregnancy from Days 30 to 60 and implicate local and not systemic pathways in occurrence or prevention of luteolysis during both the early (≤25 days) and later (≥33 days) critical periods since accessory contralateral CL regressed whereas the accessory ipsilateral CL of pregnancy remained.



## 1. Introduction

In most mammals, including ruminants, either regression of the corpus luteum (CL) in the nonpregnant animal (luteolysis) or maintenance of the CL during pregnancy (luteolysis avoidance) involves communication between the embryo, uterus, and CL. The period between Days 16 to 20 of pregnancy is designated as maternal recognition of pregnancy or the "decisive" period determining whether CL regression or nonregression will occur in cattle [1,2]. The uterus produces the first signals for initiation of CL regression in ruminants and some other species because hysterectomy prolongs the lifespan of the CL in these species [3]. Uterine-induced regression of the CL is mediated

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by local and not systemic pathways in ruminants, as reported by the finding that unilateral hysterectomy only prolonged the lifespan of the CL when performed ipsilateral to the ovary bearing the CL and not if the uterine horn contralateral to the CL was removed [4]. The pathways for this local action involve diffusion of prostaglandin F-2α (PGF2α), the uterine-derived luteolytic substance [5], from the uterine vein into the ovarian artery that lies in close apposition to the uterine vein [6–9]. The PGF2α that does not diffuse through this countercurrent system goes into the general circulation and is rapidly metabolized in the lungs of ruminants [10,11].

Rescue of the CL during pregnancy is also generally viewed as a local process rather than mediated by systemic mechanisms in ruminants. The primary evidence for a local mechanism is based on studies involving surgical anastomosis of the ovarian artery from the gravid to the non-gravid side or vice versa [12]. Cows with isolated contralateral horns bearing CL that had the ovarian artery from the gravid side surgically connected to the opposite ovary were found to maintain the contralateral CL [12]. Nevertheless, recent studies have called into question the local mechanism since interferon tau (INFT), the primary conceptus factor involved in rescue of the CL of pregnancy [13], has been shown to exit the uterus and act on the CL causing luteal expression of interferon-stimulated genes (ISGs) [14,15]. Also, systemic administration of INFT was found to maintain the CL, supporting a systemic route for rescue of the CL [16]. In addition, some studies have implicated local diffusion of prostaglandins E1 and E2 (PGE) from the uterus in pregnancy recognition in ruminants [17]. The evidence for this idea is based on increased uterine PGE secretion during pregnancy [18,19] and prevention of CL regression using various methods of PGE treatment [20–24]. Thus, maintenance of the CL during pregnancy seems to also be primarily mediated by local mechanisms, potentially involving uterine-secreted PGE, although the importance of changes in PGF2α secretion patterns and of direct systemic actions of INFT on the CL is also being investigated [25,26].

At all stages of pregnancy, circulating progesterone (P4) is essential for maintenance of pregnancy and many studies have utilized various strategies to increase circulating P4 in an attempt to increase fertility in cattle [27–29]. One strategy to increase circulating P4 concentrations is to induce an accessory CL by ovulation of a dominant follicle in cows that already have a CL. This strategy has generally been done by treating cows with human chorionic gonadotropin (hCG) or GnRH at 5 to 7 days after breeding in order to ovulate the dominant follicle of the first follicular wave [30,31]. Studies have generally observed a high percentage of cows with ovulation of the dominant follicle of the first follicular wave and consistent increases in circulating P4 concentrations [32,33]; however, fertility responses have been variable [34–36]. There is a greater fertility response to induction of an accessory CL in first lactation cows (primiparous) than in multiparous cows [37,38]. Currently, there are no defined physiological mechanisms to explain the observed variability between parities or between studies in fertility responses to treatment with hCG or GnRH during the first follicular wave in lactating dairy cows.

Treatment with GnRH or hCG on Days 5 to 7 after breeding can produce an accessory CL on the same side as the previous ovulation (ipsilateral) or can produce an accessory CL on the opposite ovary (contralateral). During previous studies, our group has observed accessory CL regression in pregnant lactating dairy cows with contralateral but not ipsilateral CL (JN Guenther and MC Wiltbank, unpublished results); however, the timing of CL regression during pregnancy or any differences between parities in occurrence of luteolysis of accessory CL has not been previously determined. A previous study [33] found that a new accessory CL was formed in pregnant cows (Days 26–71 of pregnancy) after treatment with hCG (50% of cows formed an accessory CL) or GnRH (26% of cows formed new CL) and this new accessory CL subsequently regressed in 36.2% of pregnant cows during the next 4 weeks. Regression of the CL was more likely if the accessory CL was contralateral to the gravid horn rather than ipsilateral [33]. A potentially related recent finding from our laboratory [39] reported that primiparous cows have a smaller uterus compared with older cows, and also, for any given parity, larger uterine size was associated with decreased fertility. It is therefore possible that the contralateral accessory CL may undergo luteolysis in cows that have a large uterine size, possibly due to inefficient delivery of INFT to the contralateral horn, whereas the accessory contralateral CL may be maintained in cows with a smaller uterus. This may help explain the parity differences in fertility responses to induction of an accessory CL because contralateral CL regression may occur in older cows with larger uterine size thereby muting the fertility responses to induction of an accessory CL.

Thus, embryonic signals maintain the CL that is ipsilateral to the pregnancy but may or may not maintain an accessory CL that is contralateral to the gravid horn. The objective of this research was to determine the percentage of pregnant lactating dairy cows that undergo regression of an accessory CL that has been produced by ovulation of a dominant follicle on the contralateral ovary. We hypothesized that a greater percentage of multiparous cows with a larger uterus would undergo contralateral, accessory CL regression compared to primiparous cows. Thus, we designed an experiment to evaluate the timing of contralateral, accessory CL regression. We hypothesized that contralateral, accessory CL regression in pregnant cows would primarily occur during the time of CL regression that has been typically described, from Days 19 to 25 after AI, probably due to insufficient signals from the embryo reaching the contralateral horn in lactating cows with a large uterus. Thus, we speculated that maternal recognition of pregnancy and rescue of the CL would occur normally in the uterine horn and ovary ipsilateral to the pregnancy, but in the contralateral horn, luteolytic mechanisms would develop in some pregnant multiparous lactating dairy cows causing contralateral, accessory CL regression. It should be emphasized that this study was not designed to evaluate the effect of GnRH treatment or an accessory CL on fertility, as has been performed in previous studies [34–38]. Therefore, we treated all cows with GnRH on Day 5 after breeding in order to maximize the number of pregnant cows with a contralateral CL, which represented the key