Induction of Uterine Cyclooxygenase by Leutenizing Hormone and Estradiol is an Important Determinant of Bovine Luteolysis

Shemesh, M.,[1] Shore, L.S.,[1] Fields, M.J.[2] and Hansel, W.[3]

[1] Kimron Veterinary Institute, Bet Dagan, Israel 50250.
[2] University of Florida, Gainesville, FL 32611, United States of America.
[3] Pennington Biomedical Research Center, Louisiana State University System, 6400 Perkins Road, Baton Rouge, LA, 70808, United States of America.

* **Corresponding Author:** Prof. Mordechai Shemesh, Kimron Veterinary Institute, Bet Dagan, Israel 50250. E-mail: mordechai.shemesh@mail.huji.ac.il

ABSTRACT

Termination of the functional bovine corpus luteum is associated with a precipitous fall in peripheral plasma progesterone which is induced by a pulsatile release of prostaglandin $F_{2\alpha}$ (PGF) from the late luteal phase uterus. The production of uterine PGF from arachidonic acid is greatest in endometrial cells 2-5 and 15– 17 days post-ovulation. Similarly exposure of uterine endometrial cells to estradiol 17β or to lutenizing hormone (LH) results in a significant increase in LH receptors, cyclooxygenase-2 (COX-2) and PGF at 2-5 and 15-17 days post-ovulation. It is therefore suggested that hormonal regulation of uterine COX- 2 by pulses of estradiol and LH may play a role in bovine luteolysis.

Keywords: Lutelysis; COX-2; Lutenizing Hormone; Prostaglandin F2α

REVIEW

Events associated with regression of the bovine corpus luteum (CL)

This review presents an overview of research carried out in our laboratory over some forty years associated with the subject of the regression of the bovine CL.

Peripheral plasma of progesterone

Peripheral plasma concentrations of progesterone are a valuable indication of the concentration of hormone reaching target organs and control centers governing secretion of gonadotropins at various phases of the reproductive cycle. Alterations in circulating progesterone also supplies information regarding the functional state of the corpus luteum (CL) or of alternative sources of the hormone such as the adrenal or placenta (1-3).

In our early work (4-6), we demonstrated that in the absence of an early embryo in the uterus, there is a precipitous fall in luteal progesterone secretion toward day 18-19 of the cycle. This divergence of blood concentrations of progesterone in pregnant and non-pregnant cows constitutes the earliest means of pregnancy diagnosis in the cow as well as in other ruminants. It is likely that the anti-luteolytic action of the conceptus is in fact initiated several days earlier (4).

Arachidonic acid as a luteolytic agent

The mechanism of the anti-luteolytic action of the fetus is not known. It is presumably related to metabolism of arachidonic acid, the precursor to prostaglandin $F_{2\alpha}$ PGF (PGF). Hansel *et al.* (7) isolated arachidonic acid from the bovine endometrium and demonstrated its luteolytic effect when injected into the ovarian bursa of pseudo-pregnant hysterectomized hamsters. Similarly Hoffman (8) reported that intra-peritoneal injections of arachidonic acid were luteolytic in the pseudo-pregnant rabbit. Based on these observations it was hypothesized that bovine endometrial

tissues might be the source of arachidonic acid, which is then subsequently converted into prostaglandins by the uterus and corpus luteum (9).

PGF in uterine vein and endometrial tissue during the estrous cycle

PGF was measured in plasma obtained from the uterine vein as well as endometrial tissues at various times during the bovine estrous cycle (9). Low concentrations of PGF were measured in the endometrium and uterine venous blood on day 1-14 of the cycle. Higher values were found at day 15 until the day of estrus, the time that the corpus lutein begins to regress and plasma progesterone fall. However by the time of the first signs of estrous behavior, plasma PGF had already begun to decline. This rise and fall in plasma PGF before the onset of estrus corresponds with the elevation and decline in peripheral plasma estradiol (10) which occurs during the 3 days preceding estrus (Figure 1.)

Hormonal regulation of cyclooxygenase-2 (COX-2) in the bovine endometrium

Expression of cyclooxygenase-2 (COX-2) in the bovine endometrium was demonstrated with plated cells at various stages of the estrous cycle (11, 12). It was observed that COX-2 was not consistently expressed throughout the estrous cycle, i.e., the signal for COX-2 was strongest prior to luteolysis (15-17 days post- ovulation), weak around estrus and non-existent in endometrium of ovulation. We next determined the endocrine factors that regulate COX-2 and timing of PGF secretion from the bovine uterus (11). Endometrial cells were incubated with LH or estradiol. Using western blot analysis it was found that endometrial COX-2 was increased 2-3 fold by both LH and estradiol at 2-5 days post-ovulation and at mid-cycle. In contrast when endometrial cells at ovulation were used, LH or estradiol resulted in enzyme suppression rather than stimulation. To demonstrate the correlation between COX-2 induction and PGF synthesis, endometrial cells were incubated with arachidonic acid. A significant increase in PGF secretion was observed mid-cycle, prior to luteolysis and at estrus. However, only a non-statistically significant PGF secretion was observed at ovulation (11).

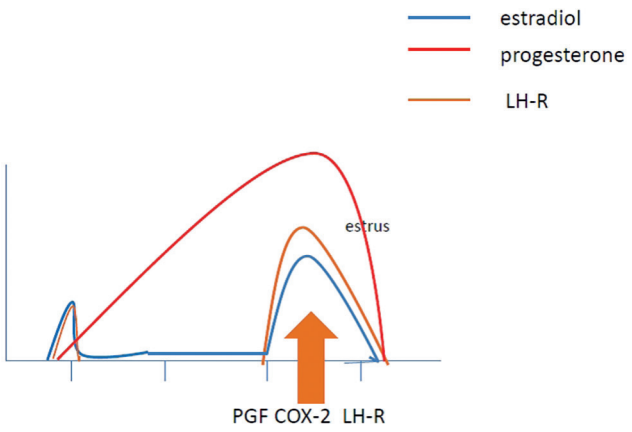

Figure 1: The fall in peripheral plasma progesterone and increase in peripheral plasma estrogen is associated with increased level of LHR, COX-2 and PGF in the endometrium is associated with increased level of LHR, PGF2α and COX-2 in the uterine vein.

Hormonal regulation of uterine vein cyclooxygenase

Two hormones were found to control COX-2, LH and estrogen (11-14). Incubation of endothelial minces of uterine vein with LH resulted in 2-fold increase in COX-2 as determined by western blot analysis. The increase in cyclooxygenase-2 was maximal in cows in proestrus/estrus compared with post-ovulatory and luteal phase cows. Activation of the PGF pathway with activation of PGE2 production by LH was seen only in the uterine vein. Both PGE2 and PGF production by vascular tissues increased linearly over 15 hours of incubation in culture media. Similarly, in a few preparations of uterine veins where a stimulatory effect of estradiol on PGF was detected, PGE was not affected.

Pro-estrus rise in estradiol

The abrupt fall in peripheral plasma progesterone during the bovine estrous cycle (4, 6) is associated with the induction of endometrial and uterine vein PGF secretion (9). In addition to progesterone, there is a pro-estrus rise in plasma estradiol, whose onset coincides with the precipitous fall in plasma progesterone. It seems likely that the opposed changes in the plasma concentrations of these two hormones is associated with the rise of endometrial and uterine vein PGF, and induction of luteolysis. Since the onset of behavioral estrus occurred only after plasma estrogen had passed its peak, it appears that this neural mediated response of estrogens has a prolonged latency, or more likely that additional hormone such as LH, plays a role in its full manifestation (4, 15).

A minor rise in peripheral blood estrogen was observed on day 4 of the cycle, and a more sustained increase on day 10 to 15 (Shemesh *et al.*, 1972). It is interesting to note that LH

receptors in the endometrium are more numerous on days 2-5, 15-17 and pro-estrus than on other days of the estrous cycle. The ability of estradiol to induce uterine LH receptors was shown (Ziecik *et al.*, 1992). The induction of LH receptors by estradiol is of interest as estradiol can induce COX -2 in the bovine endometrium (Freidman *et al.*, 1995, Shemesh *et al.*, 1996). These observations suggest that COX-2 is carefully regulated during the estrous cycle and this regulation of COX-2 is involved in PGF production by endometrial and uterine vein cells and induction of luteolysis (Shemesh *et al.*, 1997). Furthermore, the stimulatory effect of mellitin on PGF secretion at the follicular stages but not on the estrous cycle suggests that hormonal regulation of uterine COX-2 by estradiol and LH plays a major role in bovine luteolysis.

The induction of COX-2 by LH and estradiol was shown to be time-dependent. COX-2 increased linearly during 6 hours of culture and induction of the enzyme occurred within 3-6 hours of culture. Using endometrial cells obtained at various times of the cycle it was shown that LH and estradiol can induce COX-2 at 2-5 and 15-17 days post-ovulation, but inhibit the enzyme in post estrous endometrial cells (11). A possible explanation is that both LH and estradiol activate an endometrial protease at ovulation. Furthermore, LH receptors are more numerous on day 2-5 and 15-17, than on other days of the estrous cycle (11, 12). These actions of LH, apparently unrelated to its ovulatory peak, indicate that novel intracellular factors such as COX -2 are involved in the regulation of prostaglandins production by the uterus.

Role of LH in initiation of luteolysis

There is a direct temporal relationship between the induction of LH receptors, induction of COX-2 and PGF production (19, 20). This indicates a major role for LH in the initiation of luteolysis. LH binding to the uterine receptors on days 2-4 and 15-17 could affect many systems through LH dependent cAMP (21) and phosphatidyl inositol (PLC) pathways (22). The induction of COX -2 by LH and estradiol may therefore just represent one of several enzymes induced by LH and estradiol to regulate the estrous cycle and pregnancy.

There is a dichotomy on the concept that LH is involved in uterine PGF synthesis since traditionally LH is well known to drive progesterone synthesis by the CL. Our concept is reasonable considering a decline in plasma progesterone and rise in circulating LH accompanies a regressing CL. Importantly Rahe *et al.* (23) showed there are pulses of LH during this time frame. However, the temporal increased expression of the LH receptor in the uterus may be more significant than a rise in plasma LH. Although the role of LH in luteal regression has not yet been determined, these preliminary data shows that LH has a profound effect on PGF synthesis by the uterus and this effect appears to be limited to a specific time frame, i.e., middle to late luteal phase of the cycle. It may well be that rather than an initial role LH plays in reinforcing luteolysis (Figure 2).

The objective of the present review is to provide an overview of the physiological role of Lutenizing Hormone Receptors (LHR) in the regression of the CL. We also provided an insight to the relationship between blood progesterone, estradiol, LH and uterine cyclooxygenase and PGF during the regression of the CL. Prior to our work, extragonadal LHRs were considered as non-functional receptors. However, we demonstrated that LHR in the uterus regulates the bovine uterine cyclooxygenase and uterine PGF which is known to be associated with induction of bovine (14) and porcine luteolysis (24, 25). Cervical LH and FSH receptors can also regulate cervical cyclooxygenase to produce PGE2 in the bovine (25, 26), porcine (25) and ovine (28) cervices.

The up regulation of uterine PGs by gonadotropins may have use in clinical practice of veterinary medicine as the gonadotrophins can used for cervical relaxation (29-31) and, in conjunction with embryonic factors, may lead to improve reproductive efficiency in domestic species (32).

In future studies, we intend to use a novel Si RNA we developed for inhibiting COX-2 (33). This Si RNA will be used in vitro and in vivo to inhibit the expression of uterine LHR and determine the effects on the levels of COX-2 and PGs throughout the estrous cycle.

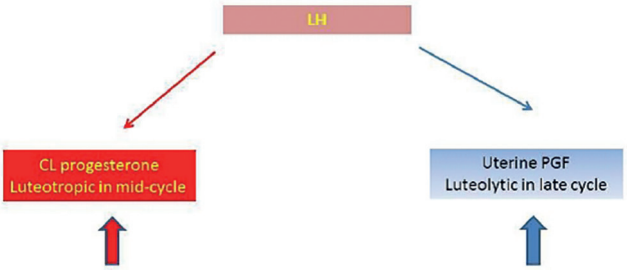

Figure 2: LH acts to increase progesterone synthesis to prolong the lifespan of the CL but can also be involved in regression of the CL by increasing PGF production in the uterine vein.

CONFLICT OF INTERESTS

This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector. There is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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