

# Control of the Corpus Luteum: A Model System for Toxicology Research

by Marc E. Freeman\*

The rodent corpus luteum is an ephemeral structure releasing progesterone for only a few hours rather than several days. However, in response to the mating stimulus, sufficient prolactin is released from the pituitary gland in a unique pattern to activate the corpus luteum and maintain it in a secretory state for several days. Progesterone, secreted from the corpus luteum, prolongs the secretion of prolactin, while the uterus may secrete a substance which terminates the functional life of the corpus luteum directly and also acts upon the prolactin secretory apparatus within the hypothalamopituitary axis to terminate prolactin secretion.

In order to gain insight into the loci at which a toxic agent might intervene, one must have an appreciation for the normal function of the organism. When studying effects on female reproductive processes, one can focus on the primary organ of reproduction, the ovary, or the endocrine organ which provides support for the ovary, the pituitary gland. This gland controls two aspects of ovarian function: release of the egg from the ovarian follicle or ovulation and the initiation and maintenance of corpus luteum function. The corpus luteum forms on the ovary immediately after release of the egg and begins to secrete progesterone. In most non-pregnant mammals, the corpus luteum persists for 12-14 days. If the recently shed ovum is fertilized, pregnancy ensues and progesterone secretion is maintained due to the hormonal stimuli provided by the newly formed placenta. In the absence of a fertile mating the corpus luteum wanes after the 12-14 day "luteal phase," progesterone secretion ceases and a new estrous or menstrual cycle begins. The corpus luteum of the nonfertile cycle is maintained in most mammals by the basal levels of luteinizing hormone (LH) secreted from the pituitary gland after ovulation. On the other hand, rats, mice, and hamsters are unique in that their estrous cycles are short, usually 4-5 days, and are characterized by a "luteal phase" lasting only a day or

two. However, prolactin, secreted from the pituitary gland is capable of initiating a functionally prolonged luteal phase. The following sections will describe the initiation and maintenance of luteal progesterone secretion by pituitary prolactin and show that one function of luteal progesterone in turn, is to modify pituitary prolactin secretion. The possible sites at which toxic agents may intervene to modify these processes will be summarized.

## Activation of the Corpus Luteum in the Rat

Ovulation occurs on the morning of estrus in response to the release of LH on the previous day. Over the next two days, the newly formed corpus luteum begins to function as reflected by the gradually rising blood levels of progesterone (1). In the absence of mating, the corpus luteum begins to fail within two days of its formation and progesterone secretion wanes (1). During the ensuing days a new crop of ovarian follicles grow which rupture on the morning of estrus and a new set of corpora lutea are formed. In rodents the corpus luteum can be induced to function for a prolonged interval by the mating stimulus. That is, a fertile mating results in a pregnancy characterized by heightened secretion of progesterone for 20-22 days (2). On the other hand, a sterile mating or artificial mechanical or electrical stimulation of the uterine cervix on proestrus results in a "pseudopregnancy" characterized

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by enhanced secretion of progesterone and a distinctive persistent leukocytic vaginal smear pattern lasting 10-13 days (2, 3). Thus, in response to the mating stimulus the corpus luteum of the estrous cycle is "rescued" and transformed into a functional state during which it secretes progesterone for a prolonged interval.

## Control of the Corpus Luteum of Pseudopregnancy and Pregnancy

In order to evaluate the control of this period of enhanced and extended secretion of progesterone brought on by mating, one can compare the circulating levels of the pituitary hormone follicle stimulating hormone (FSH), LH, and prolactin as well as the ovarian hormone estradiol during pseudopregnancy and the estrous cycles (1). Of the hormones known to play a role in reproductive function, blood levels of FSH, LH and estradiol do not differ in mated and unmated animals (1). However, mating institutes a unique pattern of pituitary prolactin secretion in rodents (1, 4-6). Though the unmated animal presents low unchanging blood levels of prolactin for all but a brief period on the afternoon of proestrus, the mated animal begins to secrete two daily "surges" of prolactin. The early morning surge begins by 1 AM, peaks by 5 AM and returns to basal levels by noon. This has been called the nocturnal surge (5, 6). The other surge, called diurnal (6), begins by 3PM, reaches peak levels by 7PM and returns to basal levels by midnight. These surges occur daily through the tenth day after cervical stimulation in both pregnant and pseudopregnant rats (6, 7). During pregnancy, the last surge, a nocturnal surge, is secreted on day 10. Basal levels of prolactin are secreted thereafter (7). Since implantation of the blastocyst within the uterus is complete by this time, it has been suggested that substances secreted by the placenta may be responsible for termination of the surges (8, 9). On the other hand, during the luteal phase induced by a sterile mating, a nocturnal and diurnal surge are found on day 10. The last nocturnal surge is secreted on day 11 with no diurnal surge on this day. During the afternoon of day 12 a proestrous-like surge of prolactin occurs, the corpus luteum fails, the pseudopregnancy is terminated and the animal returns to short estrous cycles (7).

Since the only pituitary hormone which is secreted in this unique pattern in response to mating is prolactin, this suggests that prolactin is the so-called luteotrophic hormone which prevents the corpus luteum from waning on day 2. This concept can be tested with the use of an ergot alkaloid,

2-Br- $\alpha$ -ergocryptine or CB-154, known to block only prolactin secretion by acting at the specific cell within the pituitary gland (10). Indeed, administration of 1 mg of this drug at 11 AM and 11 PM on the first day of pseudopregnancy blocks both the diurnal surge of prolactin on this day as well as the nocturnal surge of prolactin the following morning (10). As a result of this, the "rescue of the corpus luteum" which would have occurred on day 2 is prevented as reflected by the waning levels of progesterone in ergocryptine-treated rats (10). That the death of the corpus luteum is attributed to inhibition of prolactin secretion by CB-154 is suggested by the fact that prolactin is the only hormone whose administration will prevent luteal death when administered simultaneously with the drug (10). Moreover, neutralization of circulating levels of LH by administration of an antiserum to the hormone failed to prevent "rescue" of the corpus luteum in cervically stimulated rats. These data, together with the unique pattern of its secretion in response to the mating stimulus, suggests that only prolactin secretion initiated by this stimulus serves as the initial luteotrophic hormone activating a prolonged luteal phase. However, it is now well established that corpora lutea become dependent upon LH for maintenance of progesterone secretion during midpregnancy (11). Though prolactin satisfies the luteotrophic requirement during the early part of pregnancy which corresponds to pseudopregnancy, beyond this time the corpus luteum requires LH and ultimately placental lactogen for functional maintenance (11). Prolactin additionally induces luteal cell LH receptors in preparation for the changing luteotrophic requirements of the cell (12).

## Control of Prolactin Secretion by the Ovaries

Given that the gonads regulate their own trophic support with positive and negative controls over the secretion of the pituitary gonadotrophins FSH and LH, it is not surprising that the prolactin surges induced by cervical stimulation may be regulated by ovarian steroids as well (Fig. 1A). However, the presence of ovarian steroids is not obligatory for surges to be secreted. Indeed, if one stimulates the cervix of animals which had been without their ovaries for at least two weeks, both the nocturnal and diurnal surges of prolactin occur at the usual time of day. But, both surges occur in an attenuated form in ovariectomized rats (13). Ovarian steroids have a profound influence on the magnitude and recurrence of prolactin surges initi-

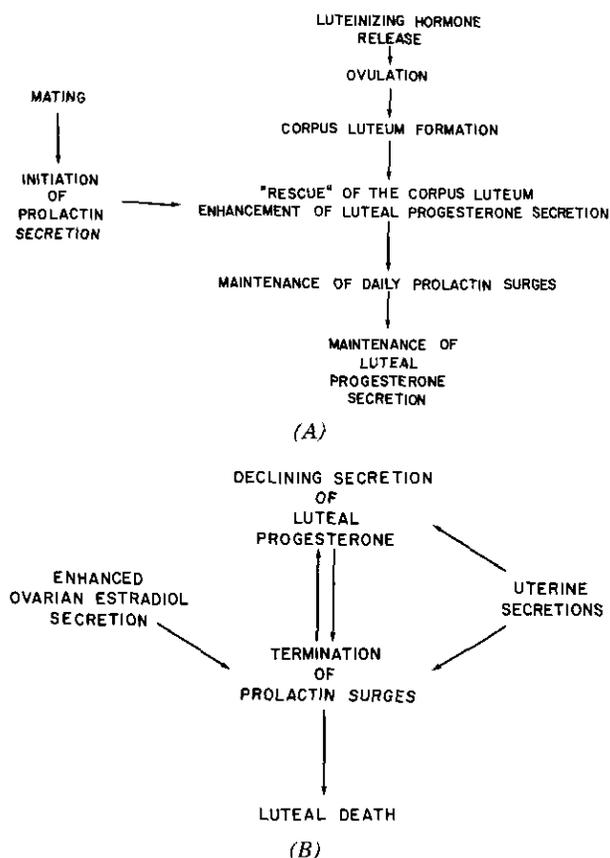


FIGURE 1. The major steps resulting in the initiation: (A) maintenance and (B) failure of progesterone secretion by the corpus luteum.

ated by cervical stimulation (13). Progesterone enhances the nocturnal surge but does not influence the magnitude of the diurnal surge of prolactin initiated by cervical stimulation (13). Estradiol abolishes the nocturnal surge and enhances the diurnal surge of prolactin (13). Though both nocturnal and diurnal surges of prolactin will occur in the presence of progesterone, estradiol will insure the recurrence of diurnal surges only (13, 14). Indeed, these data suggest that maintenance of both surges of prolactin requires luteal progesterone secretion and that termination of the prolactin surges of pseudopregnancy is the result of the waning secretion of luteal progesterone and the enhanced secretion of follicular estradiol (14).

## Control of Prolactin Secretion by the Uterus

In addition to direct support provided by the pituitary gland, the corpus luteum in most mam-

mals is influenced by the uterus (15). Indeed, removal of the uterus, hysterectomy, leads to prolonged secretion of progesterone by the persisting corpora lutea of pseudopregnancy (2, 16, 17). These data suggest that uterine input, which is lytic in nature, hastens the death of the corpus luteum. This can obviously be achieved by several means. The most time-honored concept is that the uterus secretes a substance into the blood which acts directly at the corpus luteum to terminate its function (15). The most widely accepted candidates for such "luteolytic" agents are members of the family of compounds known as prostaglandins (18, 19). The most potent luteolytic member of this family is prostaglandin  $F_{2\alpha}$  (19). A distinct correlation has been established between prostaglandin  $F_{2\alpha}$  levels in the uterus and death of the corpus luteum in many species (19). A second mechanism by which the uterus might terminate corpus luteum function is through direct inhibition of the secretion of prolactin from the pituitary gland. Indeed, inhibition of the secretion of this luteotrophic hormone should lead to the death of the corpus luteum. Such a mechanism is suggested by the fact that hysterectomy leads to prolongation of both surges of prolactin beyond their normal day of termination at the end of pseudopregnancy (20). Substantiation of this mechanism (Fig. 1B) is found in the fact that nocturnal prolactin surges are prolonged in long-term ovariectomized cervically stimulated rats in the absence of the uterus but not when the uterus is in place (20). Since there is no luteal tissue maintained in these animals, the continuation of prolactin surges can not be due to the prolonged secretion of progesterone (13), but must be due to removal of direct restraint provided by the uterus. The nature of this restraint, though probably humoral, is not known at this time.

## Overview

This prolactin secretory response to cervical stimulation is a thoroughly unique neuroendocrine reflex. The usual neuroendocrine reflex, exemplified by the prolactin-release response to the suckling stimulus, is closely tied to the application of the stimulus. That is, suckling induces almost immediate release of prolactin which ceases immediately upon withdrawal of the stimulus and does not reappear until the stimulus is reapplied. The prolactin-secretory response to briefly applied cervical stimulation, on the other hand, leads to 12-14 days of two daily surges of the hormone. This pattern seems to be independent of the ovaries for its initiation and yet dependent upon the ovaries and uterus for its termination.

# A Model System for Toxicology Research

Progesterone is the hormone responsible for maintenance of pregnancy in all mammals. The intervention of toxic agents in reproductive processes, can, in many cases, compromise the secretion of progesterone by the corpus luteum. The toxicologist can examine several loci to describe the nature of such effects on luteal function. Based upon the information provided in this brief review one can propose multiple approaches to these studies. For example, one can describe: the way toxic agents intervene in the progesterone synthetic pathway, the action of toxic agents at the site of the interaction of prolactin with its receptor on the luteal cell membrane, the influence of toxic agents on the release of the luteotrophic hormone, prolactin from the pituitary gland and the mechanism through which toxic agents might influence the ovarian steroid and possible uterine effects on the control of prolactin release. These areas of research serve as appropriate models for the determination of the influence of toxic agents on reproductive processes.

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