Correlation of plasma LH and prolactin levels with the fate of the corpus luteum in the vole, *Microtus agrestis*

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Ovulation in the vole (*Microtus agrestis*) occurs about 9–12 hr after mating (Austin, 1957; Breed & Clarke, 1970). Following ovulation, the CL may be 'short-lived' and start to degenerate within 48 hr of their formation, or become fully functional when the animal becomes pregnant or pseudo-pregnant (Milligan, 1974, 1975a). The fate of the CL is determined by whether mating activates a reflex mechanism separate from that controlling ovulation; limited amounts of mating, e.g. a single intromission, often give rise to only short-lived CL and greater amounts of mating stimulate luteal function more consistently (Milligan, 1975a). The hormonal basis of such observations was investigated in the present study by relating the plasma levels of LH and prolactin after mating with the fate of the resulting CL.

Laboratory-bred voles (see Breed, 1969) were used; females were mature virgins and males were breeding adults. All matings took place between 08.30 and 12.00 hours. The male was introduced into a cage 5 min before the female; if mating had not been initiated within 5 min of introduction of the female, the pair were again separated. Females that mated were separated from the male immediately after a single intromission or a single series of intromissions culminating in ejaculation (see Milligan, 1975b for description of mating behaviour). The females were anaesthetized with ether and blood samples (about 0.4 ml) were taken from the jugular vein at 10 min, 30 min, 1 hr, 8 hr, or 24 hr after the start of mating. All the females were killed 4 days after mating and blood samples were again collected from some of them. The plasma was removed and kept frozen at -20°C until assay. The fresh ovaries were examined for large opaque vascular CL (i.e. functional CL), or much smaller white degenerating CL (i.e. short-lived CL). Twenty-five control (unmated) females were also bled. Six other unmated females were subjected to cervical stimulation by a glass rod attached to an electric toothbrush; the regimen was designed to simulate four ejaculatory series of mating (see Milligan, 1975a). These females were bled 30 min after the start (i.e. about 10 min after the completion) of stimulation.

Double-antibody radioimmunoassays (Niswender, Midgley, Monroe & Reichert, 1968) were used to measure LH and prolactin in each blood sample. LH was measured in an ovine–ovine system with the NIH-LH-S13 standard, and prolactin in a rat–rat system with NIAMDD-RP1 standard. Vole LH and prolactin show good cross-reactions in these systems, with dilution curves of plasma and pituitary hormones parallel to the standard curves (unpublished observations). The intra- and inter-assay coefficients of variance were 10 and 23 % respectively for both assays. The lower limits of sensitivity were 0.25 ng/ml and 1.95 ng/ml respectively.

The results were analysed by Student's *t* test and are summarized in Table 1.

A marked elevation in plasma LH levels occurred after mating, irrespective of the nature of the CL formed. This surge of LH probably constitutes the ovulatory stimulus and confirms the observations of Charlton, Naftolin, Sood & Worth (1975). In most cases, the levels at 10, 30 and 60 min exceeded the upper limit of the assay (>32 ng/ml), although at 60 min 3/7 females with short-lived CL had LH concentrations of 9.0, 6.4 and 1.3 ng/ml. At later times, LH levels were significantly lower in all the females and, although there were some differences between females with short-lived CL and those with functional CL, these were relatively small. Mechanical stimulation of the vagina and cervix

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Table 1. Mean ($\pm$ S.E.M.) plasma LH and prolactin levels in female voles (nos in parentheses) developing short-lived or functional corpora lutea as a result of mating

	LH (ng NIH-S13 equiv./ml)		Prolactin (ng NIAMDD-RP1 equiv./ml)	
	Short-lived CL	Functional CL	Short-lived CL	Functional CL
Mated (time after)	>32 (7)	>32 (14)	8.07 $\pm$ 0.83 (7)	**28.1 $\pm$ 5.2 (12)
30 min	>32 (7)	>32 (9)	9.1 $\pm$ 2.3 (7)	14.7 $\pm$ 3.6 (9)
60 min	>32 (4)	>32 (16)	9.2 $\pm$ 3.1 (7)	8.2 $\pm$ 1.4 (12)
8 hr	1.39 $\pm$ 0.08 (7)	**2.27 $\pm$ 0.29 (18)	4.1 $\pm$ 0.48 (7)	6.42 $\pm$ 1.27 (17)
24 hr	3.22 $\pm$ 0.78 (6)	2.79 $\pm$ 1.0 (6)	7.7 $\pm$ 1.1 (6)	12.0 $\pm$ 2.4 (7)
4 days	1.34 $\pm$ 0.17 (16)	*0.76 $\pm$ 0.11 (16)	9.5 $\pm$ 0.8 (15)	**15.1 $\pm$ 1.7 (16)
Unmated		1.6 $\pm$ 0.17 (21)		9.95 $\pm$ 0.88 (22)
Cervical stimulation†		1.32 $\pm$ 0.44 (6)		8.6 $\pm$ 1.45 (5)

† With glass rod (see text).

Significantly different from females forming short-lived CL: * $P < 0.05$; ** $P < 0.01$.

failed to cause an elevation in LH levels, consistent with observations that such mechanical stimulation is unable to induce ovulation (Clarke & Clulow, 1973; Charlton *et al.*, 1975; Milligan, 1975a).

In females which subsequently developed functional CL, prolactin levels increased markedly 10 min after mating. This did not occur in females which developed short-lived CL. The increase was not, however, maintained: although 2 females with functional CL had levels of 22 and 41 ng/ml at 30 min, the other 7 showed a mean level ($\pm$ S.E.M.) of 9.9 ± 0.91 ng/ml which was not significantly different from that of the control group. At 4 days after mating the prolactin levels were again significantly higher in females with functional CL than in the control females and the females with short-lived CL ($P < 0.01$ for both). At this time, the levels in the 16 females with functional CL were rather variable, with four in excess of 20 ng/ml; such high levels were only otherwise observed within 30 min of mating. Prolactin levels in females that developed short-lived CL did not increase above control values at any time and those in females that had been cervically stimulated with a glass rod did not change significantly by 30 min from the start of the stimulation.

These observations suggest that the fate of the CL following mating can be correlated most obviously with whether or not prolactin is secreted. Mating induces ovulation by causing a surge of LH, but if it fails to stimulate prolactin secretion as well only short-lived CL are formed. Results from studies involving hormone administration support this idea; the injection of LH or LH-RF causes ovulation but results in short-lived CL (Milligan, 1975a), while the daily administration of prolactin, but not LH, given after an ovulating dose of LH or LH-RF induces the formation of functional CL (Milligan, Charlton & Versi, 1976). Additional evidence that prolactin is involved in maintaining the CL of the vole is provided by the use of ergocryptine (CB154), which suppresses pituitary secretion of prolactin (Floss, Cassady & Robbers, 1973) and causes a failure of luteal function in the vole (Milligan *et al.*, 1976). The present observations cannot exclude the possibility, however, that the fate of the CL may be correlated with differences in the ovulating LH surge occurring immediately after mating; most of the readings at this time were above the limit of the assay and insufficient plasma was available for dilutions to be made for more accurate determinations. The failure of LH or LH-RF injections to produce functional CL, however, suggests that the characteristics of the ovulating LH surge are not the determining feature.

The present results indicate that the vole resembles spontaneously ovulating animals such as the rat, mouse and hamster in some of its neuroendocrine mechanisms. All these species possess a mating-activated reflex mechanism controlling luteal function which involves prolactin secretion (Everett, 1961; Milligan, 1975a). In the rat, daily prolactin surges occur during pregnancy and pseudopregnancy (Butcher, Fugo & Collins, 1972; Freeman, Smith, Nazian & Neill, 1974), and the large variation in prolactin levels 4 days after mating in voles with functional CL may indicate that surges of prolactin secretion also occur in these animals. In addition, the neuroendocrine control of the CL of the vole appears to resemble that of the rat in that vaginal stimulation, if given several days before an ovulating stimulus, can result in a 'delayed' pseudopregnancy following the ovulation (S. R.

Milligan, unpublished observations). This may reflect the activation of a mnemonic system in the luteal control mechanism (Freeman *et al.*, 1974; Beach, Tyrey & Everett, 1975). There are also similarities between the vole and the rat in the effect of mating on LH secretion; even in the normally spontaneously ovulating rat, mating can stimulate LH secretion (Spies & Niswender, 1971) and can, in some circumstances, induce ovulation (Brown-Grant, Davidson & Greig, 1973). These similarities again emphasize that the distinction between induced and spontaneous ovulators may be "one more of degree than of kind" (Eckstein & Zuckerman, 1955).

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