

STRESS-INDUCED RELEASE OF PROLACTIN IN CYCLING AND ANOESTROUS EWES, AND IN WETHERS

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OPSOMMING: VRYSTELLING VAN PROLAKTIEN ONDER SPANNINGSTOESTANDE BY OOIE IN VERSKILLENDE STADIUMS VAN REPRODUKSIE, EN BY HAMELS

Aangesien dit bekend is dat spanning die afskeiding van prolaktien by skape beïnvloed, is die vrystelling van hierdie hormoon gedurende die estrussiklus ge-revalueer. Bloedmonsters is twee-uurliks, vir 'n totale periode van 40 uur, verkry vanaf estrus, inter-estrus en anestrus ooie, sowel as vanaf hamels. 'n Akute afskeiding van prolaktien is by al die ooie, afgesien van hul reproductiewe status, waargeneem. Prolaktien konsentrasies in die geslagsaktiewe en anestrus ooie het by tye 1000 ng/cm³ oorskry. Relatief kleiner hoeveelhede prolaktien (25-400 ng/cm³) is in die hamels afgeskei. 'n Tipiese luteïeniserende hormoon (LH) piek is kort na die aanvang van hitte by die estrus ooie gemeet. Die resultate is bespreek in die lig van die sogenaamde bestaan van 'n prolaktien-piek wat geassosieer word met die voorkoms van estrus by ooie.

SUMMARY:

Since stress has been shown to influence prolactin release in sheep, the secretion of this hormone during the oestrous cycle of the ewe was re-evaluated. At intervals of two hours, for a total period of 40 hours, blood samples were obtained from oestrous, inter-oestrous and anoestrous ewes, and from wethers. An acute release of prolactin was elicited in all the ewes, irrespective of their reproductive state. Prolactin levels in excess of 1000 ng/cm³ were at times measured in the cycling and anoestrous ewes. Relatively smaller quantities of prolactin (25-400 ng/cm³) were secreted in the wethers. A typical pre-ovulatory luteinizing hormone (LH) peak was measured shortly after the onset of heat in the oestrous ewes. The results are discussed in relation to the reputed existence of an oestrous-related prolactin surge in ewes.

A variety of stressful stimuli result in an increased secretion of prolactin in the rat (Neill, 1970; Ajika, Kalra, Fawcett, Krulich & McCann, 1972), the cow (Raud, Kiddy & Odell, 1971), the ewe (Cumming, Brown, Goding, Bryant & Greenwood, 1972) and the goat (Hart, 1973). The magnitude of this response varies between individual animals, and consequently misleading results may be obtained when prolactin levels are monitored under conditions of stress.

According to Kann (1971) plasma prolactin levels vary from 10-40 ng/cm³ during the luteal phase of the oestrous cycle in ewes, after which a surge in the level of this hormone occurs during pro-oestrus and oestrus. An acute release of prolactin during oestrus in ewes was also detected by Cumming *et al.* (1972). However, Davis (1972) found that the stress of venipuncture profoundly influences prolactin secretion in lambs, and he subsequently suggested that the secretion of this hormone during the ovine oestrous cycle should be re-investigated. In the present study an attempt was therefore made to establish whether the occurrence of oestrus in ewes is associated with an acute release of prolactin, or whether this prolactin "surge" is in fact stress-induced. For this purpose, prolactin levels were quantitated in male and female sheep of different reproductive states which were subjected to identical stressful stimuli, including the frequent collection of blood samples and joining with vasectomized rams.

Procedure

The following 4 experimental groups, each consisting of 5 Merino sheep, were utilized in this experiment:

1. Ewes which exhibited overt oestrus during the experiment - "oestrous" ewes. The onset of oestrus occurred 1 to 16 hours before the commencement of the trial.
2. Ewes which exhibited oestrus 5 days prior to the commencement of the experiment - "inter-oestrous" ewes.
3. Anoestrous ewes, in which oestrus had not been observed during the 28 days which preceded this trial.
4. Wethers, aged 7 months.

At the commencement of the experiment the sheep were grouped, and placed in small pens within a large building. A silastic cannula was inserted into the jugular vein of each animal and a sample of blood (5,0 cm³) immediately obtained. Sampling via the cannula was repeated at intervals of two hours for the following 40 hours. Following centrifugation, the plasma was stored at -15°C.

At intervals of two hours during the blood sampling period vasectomized rams were joined with all the animals, irrespective of their sex and reproductive state. This treatment was applied in order to subject all animals to this stimulus. The animals were fed and watered once daily, and at night overhead lights were used briefly during sample collection.

Prolactin was measured in the plasma by the double-anti body radioimmunoassay of Davis, Reichert & Niswender (1971). Fresh standard solutions of ovine prolactin (NIH-P-S10) were used in the assay, since these are known to deteriorate with time (Butler, Malven, Willet & Bolt, 1972).

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Plasma luteinizing hormone (LH) levels in the oestrous ewes were measured by the double-antibody radioimmunoassay of Niswender, Reichert, Midgley & Nalbandov (1969), using anti-sera raised in this laboratory (Lishman, Stielau, Dreosti & Stewart, 1973).

Results

Plasma prolactin levels in the experimental animals are diagrammatically illustrated in Fig. 1. After the completion of the trial it became evident that two ewes (70/75 and 70/147) which were initially judged to be in an anoestrous condition, had in fact resumed cyclic activity about the time that blood sampling was commenced. An acute release of prolactin was elicited in all the ewes and this release was not notably influenced by the reproductive state of the ewes (Fig. 1). Prolactin levels of 1 000 ng/cm³ were at times exceeded. Especially noteworthy was the finding that prolactin was as readily secreted in the inter-oestrous (Fig. 1B) as in the oestrous (Fig. 1A) ewes. However, prolactin was less readily secreted in the wethers, in which the level of this hormone seldom exceeded 300 ng/cm³ (Fig. 1D). The results presented in Fig. 1 further illustrate that the cannulation process (at the commencement of the trial) resulted in markedly elevated levels of prolactin in the majority of the experimental animals. No consistent changes in prolactin secretion were observed immediately after the ewes had received food and water.

Evidence for the occurrence of a typical pre-ovulatory surge of LH was noted in four of the five oestrous ewes (Fig. 2). Due to the timing of the blood sampling operation, the entire LH surge was measured in only two of the ewes. The LH peak was probably missed in ewe 66/132, in which blood samples were obtained for only 20 hours.

Discussion

The finding that the female sheep secreted similar quantities of prolactin when subjected to the identical blood sampling technique (Fig. 1) suggests that the acute release of prolactin, reputed to occur during oestrus in ewes, is not necessarily associated with, or dependant on, the occurrence of oestrus. Oestrogen can induce an acute release of both LH (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969; Radford, Wallace & Wheatly, 1970) and prolactin (Fell, Beck, Brown, Catt, Cumming & Goding, 1972) in anoestrous ewes. Furthermore, it is generally accepted that the increased secretion of oestrogen

prior to oestrus in ewes (Moor, Barret, Brown, Schindler, Smith & Smyth, 1969; Scaramuzzi, Caldwell & Moor, 1970; Obst, Seamark & Brown, 1971) results in the pre-ovulatory release of LH (Cumming, Brown, Blockey & Goding, 1971). It thereby appears likely that oestrogen induces the release of LH, as well as prolactin, during oestrus in ewes. However, prolactin can be secreted in the absence of a surge of oestrogen, such as during the milking process (McNeilly, 1972) and under conditions of stress (Cumming *et al.* 1972). *In vitro* studies conducted with rat pituitary tissue indicated that prolactin is synthesized and released at a greater rate during pro-oestrus and oestrus than during any other stage of the oestrous cycle (Ieiri, Akikusa & Yamamoto, 1971; Ieiri, Nobunaga & Yamamoto, 1972). It is thus possible that, as a result of the techniques employed in this experiment, the "oestrous-related" prolactin surge was masked by a stress-induced release of this hormone in the oestrous ewes.

Davis *et al.* (1971) and Cicmanec & Niswender (1973) detected markedly elevated levels of prolactin during the luteal phase of the oestrous cycle in ewes and suggested that stressful conditions may have induced this response. On the other hand, Kann (1971) measured prolactin levels of only 10–40 ng/cm³ during the inter-oestrous period in ewes. These levels were probably obtained under conditions less stressful to sheep than those employed in the present study.

In the event of large physiological differences, it would appear likely that treatment effects can be established when studying prolactin secretion in sheep. Thus, the wethers secreted less prolactin than any of the female sheep (Fig. 1), despite the fact that all the animals were subjected to the same amount of stress.

Fell, Findlay, Cumming & Goding (1973) have suggested that prolactin is less readily secreted in anoestrous than in cycling ewes. The results presented in Fig. 1C do not support this suggestion, since prolactin levels in the three anoestrous ewes varied from 100–1 600 ng/cm³ during the 40 hours in which blood samples were obtained. However, it is possible, that the ewes studied by Fell *et al.* (1973) were in a deeper stage of anoestrus than those used in the present study.

The results obtained in this experiment indicate that prolactin secretion is profoundly influenced by stress in sheep. Furthermore, it is evident that the natural pattern of prolactin secretion during the oestrous cycle of the ewe requires clarification. For this purpose, practical means of defining, and eliminating stressful conditions in sheep should be established.

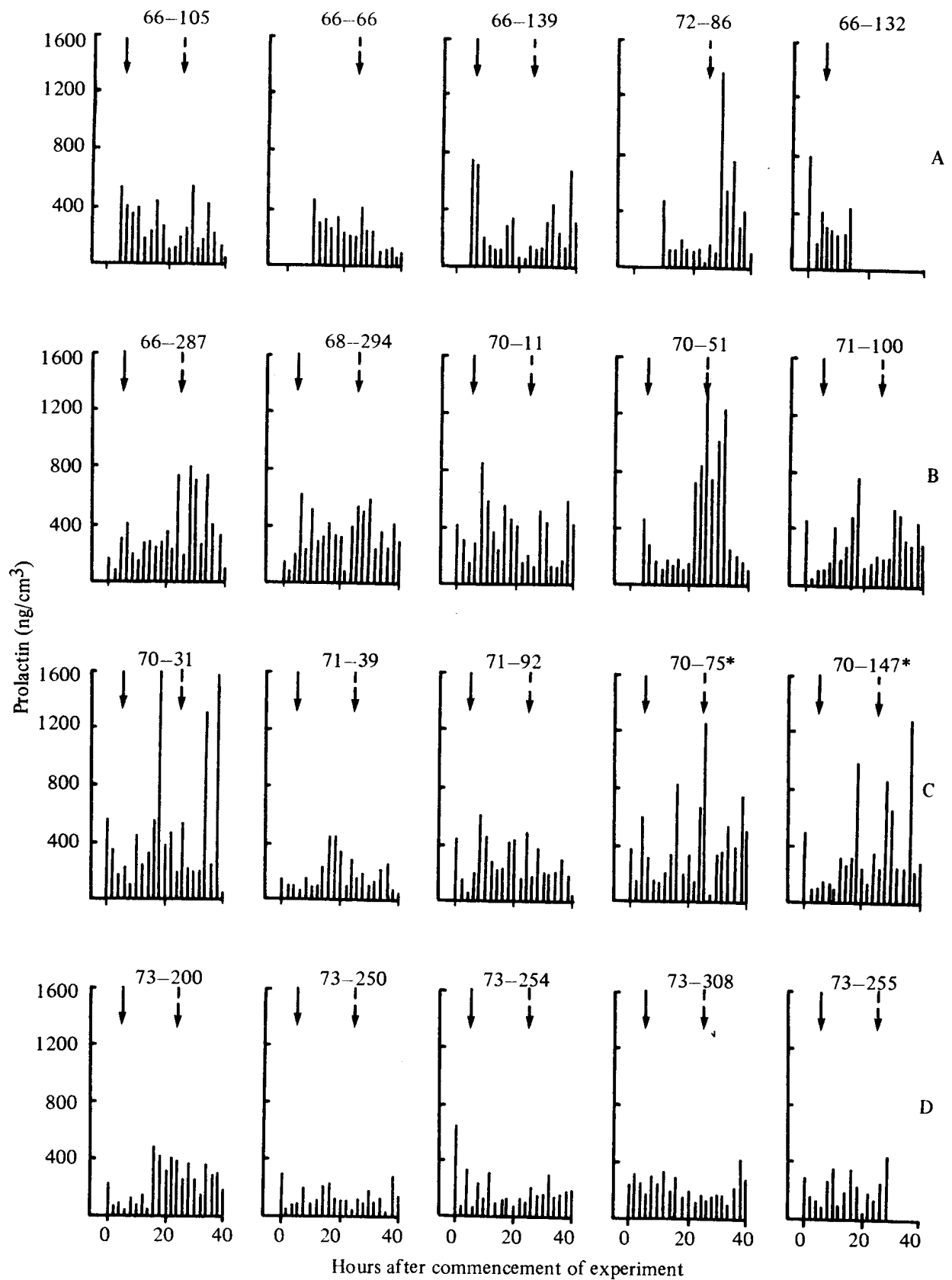


Fig. 1 Plasma prolactin levels in oestrous (A), inter-oestrous (B) and anoestrous (C) ewes; and in wethers (D). ↓;⇓ indicates supply of feed and water, respectively.

* Ewes 70-75 and 70-147 were not anoestrus during the experiment

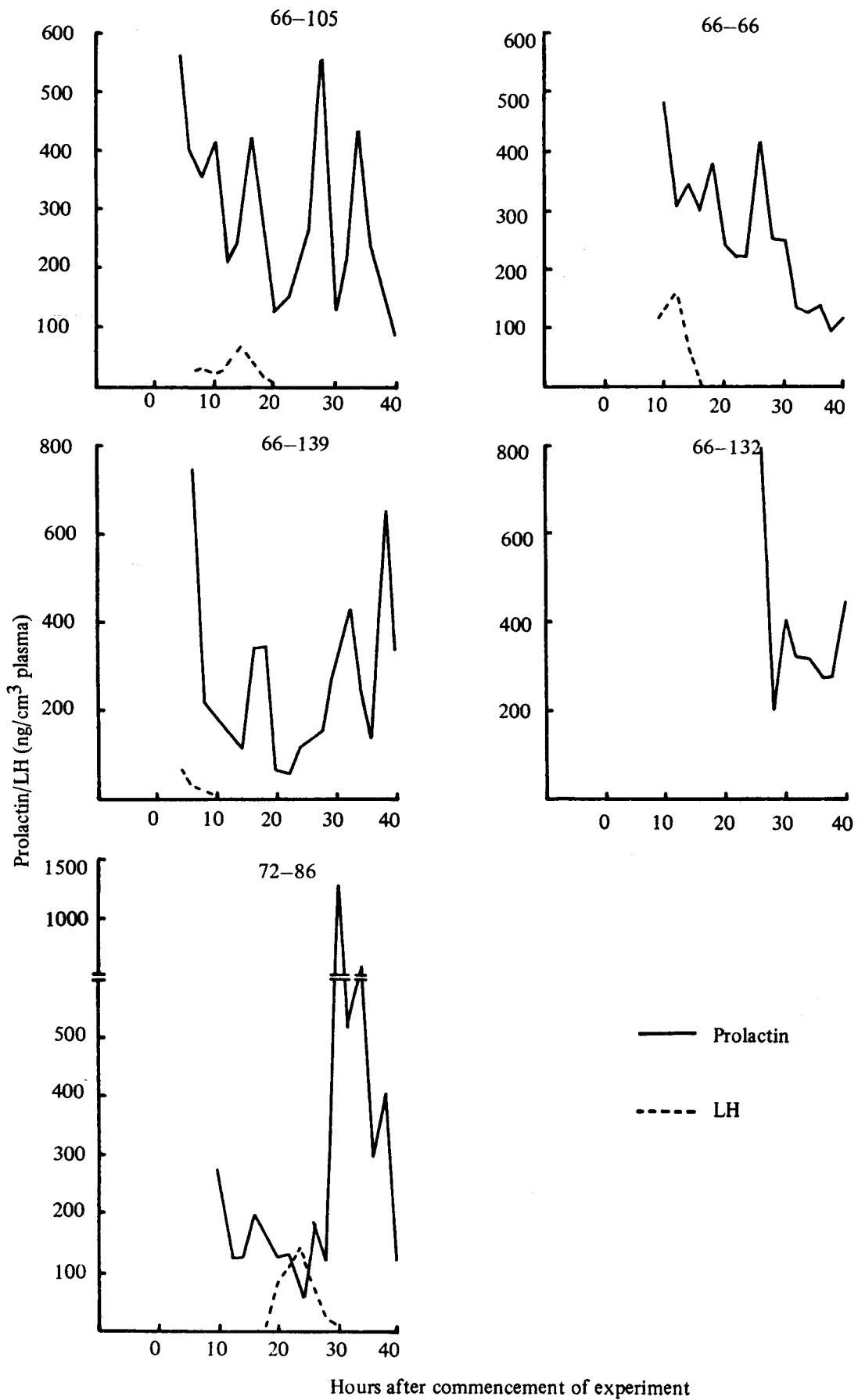


Fig. 2 Plasma prolactin and LH levels in the oestrous ewes. Blood samples were obtained at two-hour intervals

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