Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 1007–1012. Pergamon Press. Printed in Great Britain.

STEROID CONTROL OF GONADOTROPIN RELEASE

LYNN G. NEQUIN,* JULIAN ALVAREZ† and NEENA B. SCHWARTZ†

* Southern Illinois University School of Medicine, Carbondale, Ill., U.S.A.
† Northwestern University, Dept. Biological Sciences, Evanston, Ill., U.S.A.

SUMMARY

Several studies have led to the concept that the length of the rat estrous cycle is dependent on the timing of estrogen (E) secretion. According to this hypothesis E secretion during the 5-day cycle is delayed. However, we have found LH and E levels in both 4 and 5 day cycles are identical. Therefore, even though E is critical for a cyclic gonadotropin release to occur it does not appear to be the factor, which under normal circumstances, determines cycle length. During the day of proestrus, progesterone (P) increase coincided, but did not precede the gonadotropin(s) surge in our study thus eliminating P as the normal physiological trigger of gonadotropin release. If P was the determining factor, one would expect to find 5-day P levels higher than 4-day cycle levels during the earlier stages of the cycle. Approximately 48 h following ovulation, on the day of diestrus, we found that the 5-day cycle did indeed have higher P levels. We propose that the regulation of P secretion during the early stages of the cycle controls the timing of the normal periodic gonadotropin release.

A great deal of evidence exists implicating certain steroids, specifically estrogen and progesterone, in the regulation of tonic and periodic gonadotropin release. Since Long and Evans reported that the rat estrous cycle [1] varied between 4 and 5 days rather than being a uniform 4–5 days in length, numerous interspecies, as well as intraspecies variations have been observed. Figure 1 compares the 4- and 5-day rat estrous cycle. Numerous variations between the two types of cycles are apparent. The most obvious difference, of course, is in the timing of ovulation, which occurs at either a 4- or 5-day interval, sometime during the evening of proestrus and the morning of the day of estrus. These variations have prompted various theories concerning the etiology of the length of the rat estrous cycle. By various experimental manipulations, such as the administration of exogenous estrogen or progestins or by the administration of antisera specific to a particular gonadotropin or steroid, during different stages of the cycle, various workers have obtained evidence which has lent credance to one or another of the various theories, and has created a few new theories as well. Until recently, however, no studies have involved the simultaneous measurement of all the gonadotropins and several steroids at short enough time intervals or in both types of cycles, so as to be able to definitively correlate steroid secretion patterns with gonadotropin release. During this presentation, we will review the current theories and examine the evidence linking estrogens or progestins to periodic gonadotropin release and thus to estrous cycle length.

Since we will be talking about gonadotropin

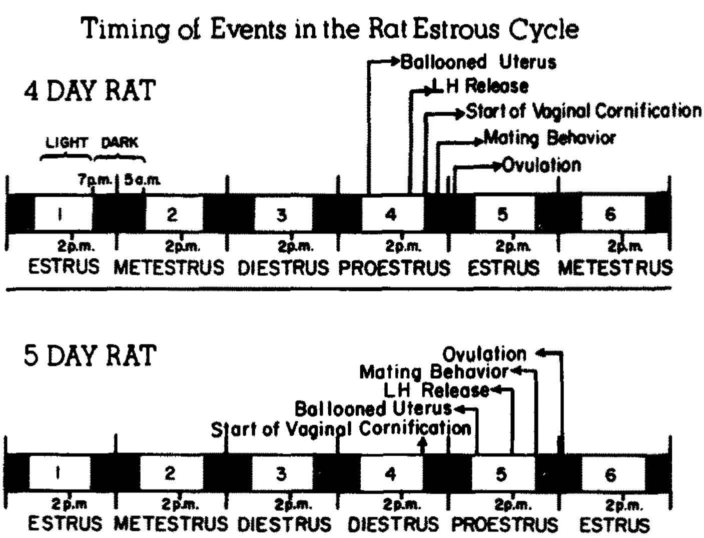

Fig. 1. Differences in timing of: ovulation, vaginal cornification, uterine swelling, uterine ballooning, time of mating, are seen during the 4- and 5-day cycle.

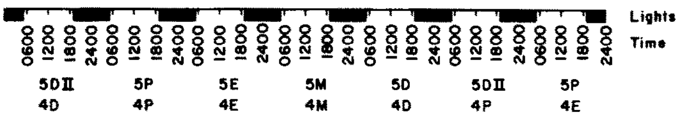

Fig. 2. Time axis used in comparing 4- and 5-day cycle: 5 = 5-day cycler; 4 = 4-day cycler; M = metestrus; D = diestrus; DII = diestrus 2—occurs only in 5-day cycle; P = proestrus—day of LH surge; E = estrus—ova in oviducts.

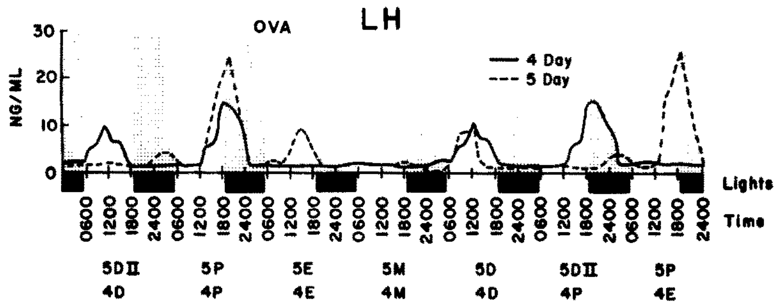

Fig. 3. See legend Fig. 2.

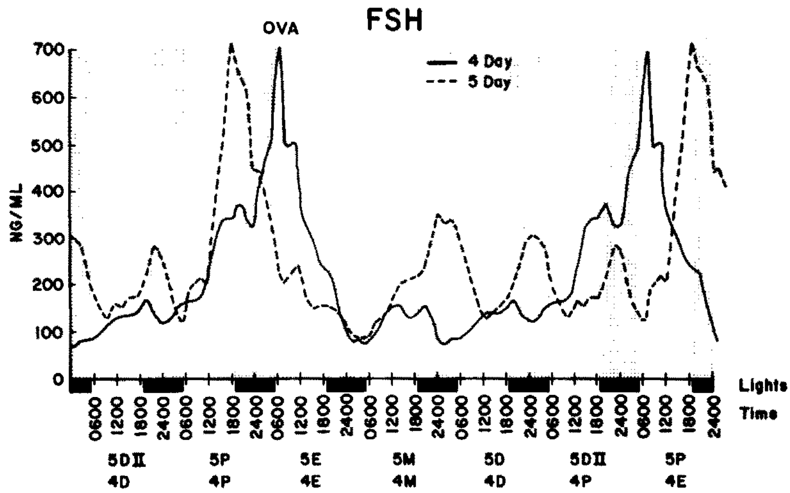

Fig. 4. See legend Fig. 2.

release throughout this presentation, let us first look at LH, FSH and prolactin throughout each cycle and note the similarities or differences. Three animals were sacrificed at 2 h intervals throughout each day of the 4- and 5-day cycle. All figures were constructed using a moving 3-point average. This procedure, which is a method of curve smoothing, is helpful in displaying underlying trends. We felt it was important to be able to visually contrast the 4- and 5-day cycle prior to, and following ovulation. Therefore in figures where the 4- and 5-day cycle are contrasted, the time axis (Fig. 2) begins with the day before proestrus (the day during which the ovulatory surge of LH is released) in both cycle types. This permits us to contrast the day before proestrus, proestrus, estrus (the day when ova are found in the oviduct), metestrus and diestrus in both the 4- and 5-day cycle. But since the time axis continues past the day of diestrus, it also enables us to contrast cycle stage with regard to the last ovulation (i.e. the 3rd day following ovulation is diestrus II (DII) in the 5-day cycle and proestrus in the 4-day cycle).

Figure 3 shows the results of LH determinations throughout each cycle. An occasional animal (two 5-day and two 4-day rats) had surge levels of LH (14–56 ng/ml) 2 days following ovulation (5D, 4D). These animals exhibiting high LH levels during diestrus, were those collected at the 1000 and 1400 h times. The proestrous LH surge coincided in both the 4- and 5-day cycle (5P, 4P) [2]. During the day of estrus, two out of six 5-day cyclers exhibited surge levels of LH at 1200 h and 1400 h. All of these rats have small uteri and ova present in their oviducts implying that the expected proestrous surge had occurred on schedule on the previous day and the LH detected on the day of estrus was a second wave of LH secretion.

The 5-day animals appeared to exhibit a rhythmic release of FSH with an increase at 2400 h and a low point at 1200 h (Fig. 4). This is in contrast to the 4-day cycle which does not show a daily rhyth-

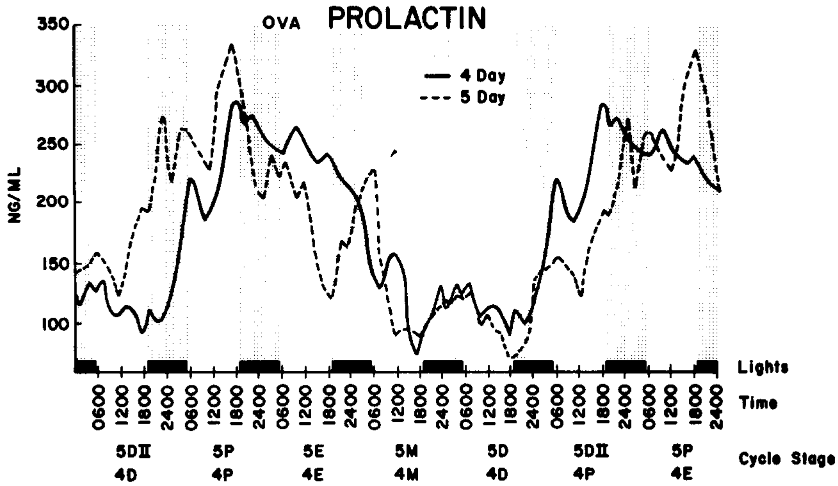

Fig. 5. See legend Fig. 2.

mic increase in FSH. Although the time course of the FSH surge seen during proestrus in each type of cycle appeared to differ, this could not be demonstrated statistically because of the large standard errors at these time points.

The 4- and 5-day patterns of prolactin secretion did not differ significantly throughout any phase of the cycle, except on the evening of estrus, Fig. 5. At 1800 h estrus, the 4-day cycler had higher prolactin levels than rats having a longer cycle length. There also appeared to be a rhythmic secretion of prolactin in the 5-day group which was quite similar to the diurnal rhythm seen for FSH, except that during the day of proestrus the prolactin levels increased prior to LH and FSH.

Studies utilizing exogenously administered estrogen [3] have demonstrated that injection of estrogen during the early stages of a 5-day cycle advances gonadotropin release and ovulation by one day. Ovariectomy or estrogen antisera given during the above period in either 4- or 5-day cycles inhibits or delays the proestrous surge of LH [4–8], FSH [7] and prolactin [4, 9]. Furthermore, the secretion of estrogen prior to the surge(s) appears to be dependent on tonic levels of gonadotropin release, as evidenced by studies in which exogenous gonadotropins 10] or gonadotropin antisera [11, 12] was given.

The above studies have led to the belief that the length of the rat estrous cycle is dependent upon the timing of estrogen secretion. This theory implies that if the threshold amount or rate of rise of estrogen was slightly delayed, the animal would exhibit a cycle of 5 days duration. If, on the other hand, the estrogen levels increased rapidly during the early stages of the cycle, periodic gonadotropin release, or surge(s), would occur at 4-day intervals. The *sine qua non* evidence in support of this theory would be, of course, the detection of the appropriate patterns of estradiol in the serum throughout the 4- and 5-day cycle. Therefore we initiated an experiment in which we measured serum estradiol, as well as LH, FSH, prolactin, progesterone, 20α-hydroxypreg-4-en-3-one (20α-hydroxyprogesterone) and uterine intraluminal fluid at 2 h intervals throughout each day of both the 4- and 5-day cycle. The results of radioimmunoassay of estradiol are shown in Fig. 6 and reveal that during both types of cycles the rise in estradiol precedes the ovulatory surge observed on the day of proestrus as predicted. However, if we contrast 4- vs 5-day estradiol patterns (Fig. 7) during the 3 days following ovulation they are observed to be virtually identical in both cycles

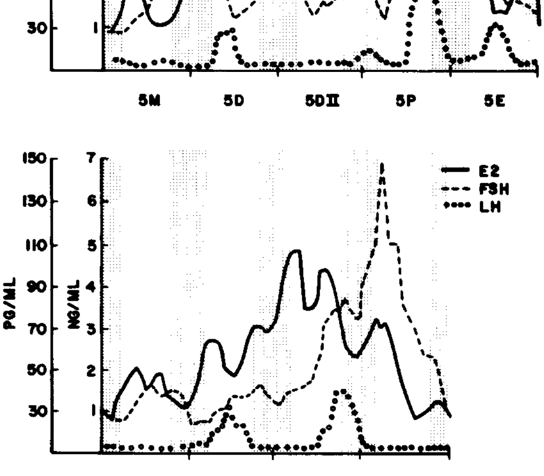

Fig. 6. Estradiol (E2) secretion in relation to LH and FSH surges: ng/ml × 10 = LH concentration; ng/ml × 100 = FSH concentration.

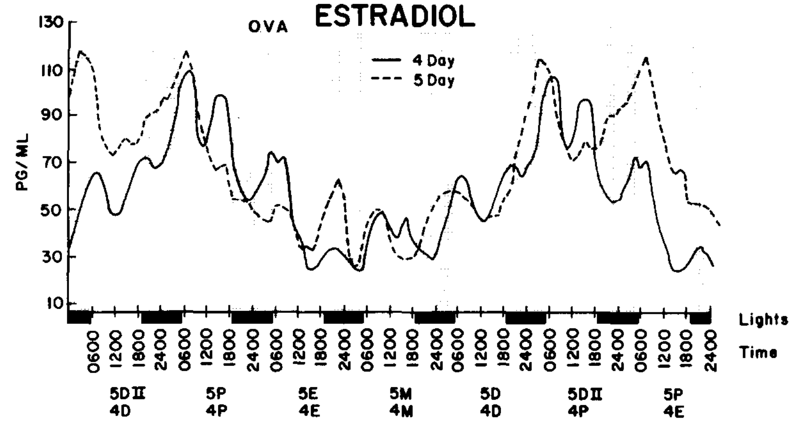

Fig. 7. Comparison of estradiol secretion patterns during 4- and 5-day cycles. See legend Fig. 2.

(5M vs 4M, 5D vs 4D, 5DII vs 4P). Estradiol secretion was prolonged approximately 24 h in the 5-day cycle (5P). These data correlate well with the vaginal changes observed in both types of cycles. The difference seen in the timing of uterine ballooning [13], however, does not correlate with estradiol secretion, since the accumulation of intraluminal fluid occurs approximately 12 h sooner in the 4-day cycle. Thus, uterine ballooning is not a reliable indicator of estrogen secretion as was believed earlier. Our current evidence does not support the proposed difference in estrogen secretion during the early stages of the 4- and 5-day cycle. Although the secretion of estrogen is necessary for a cycle to occur, it does not appear to be the primary factor, which determines cycle length in animals which have not been subjected to experimental manipulation.

During the later phases of the cycle (DII or P), after estrogen priming of the central nervous sytem has occurred, exogenously administered progesterone is capable of facilitating LH and FSH release [14–23]. Thus progesterone secreted after 0200 h proestrus, but prior to the ovulatory surges has been implicated in the control of the preovulatory LH surge [24, 25, 18, 19]. However the next figure (Fig. 8), as well as other studies [26], indicate that progesterone increases coincidentally with the rise in LH but does not precede the LH surge. Therefore, proestrous progesterone does not appear to be the primary factor, which triggers the surge during an unmanipulated cycle. Thus, it, too, is unlikely to determine cycle length.

During the early phases of the cycle (M and D) prior to the time when estrogen is released, exogenously administered progesterone is capable of delaying the LH surge 24 h, thus lengthening either a 4- or a 5-day cycle [3]. Surgical stress at comparable times will also produce the same effect [5]. Two studies have demonstrated that estrogen secretion was delayed after the administration of progesterone [27, 28]. Therefore, it occurred to us that the mechanism whereby exogenous progesterone is capable of lengthening cycle duration may be an indirect one via a depression in estrogen levels. Since the FSH and prolactin surges are also dependent on an adequate estrogen background, or priming, we would expect progesterone, given prior to estrogen priming, to also block the release of these gonadotropins. The endogenous secretion of progesterone during this early phase of the cycle is seen in Fig. 8. Progesterone levels are high during metetrus, but decrease on diestrus, prior to the LH surge. This pattern of secretion appears to be independent of tonic gonadotropin release since hypophysectomy does not cause a decrease in progesterone secretion

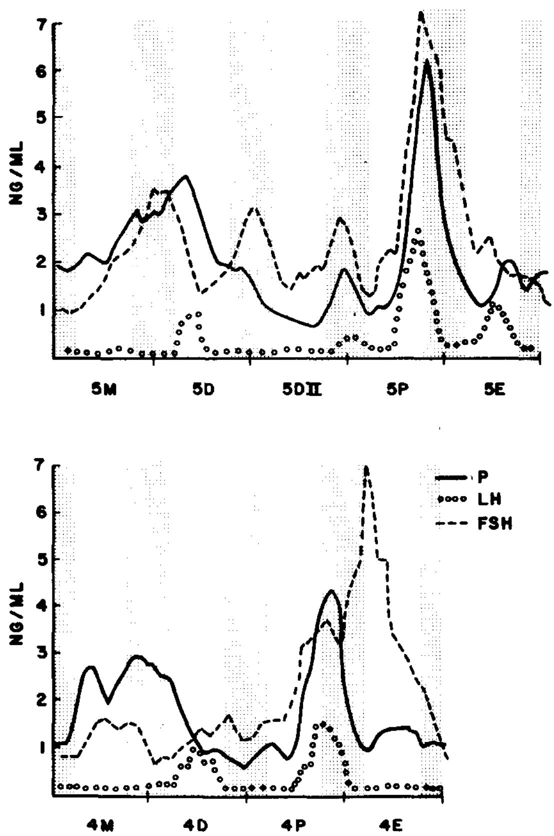

Fig. 8. Progesterone (P) secretion in relation to the LH and FSH surges: ng/ml × 10 = P; see legend Figs. 2 and 6.

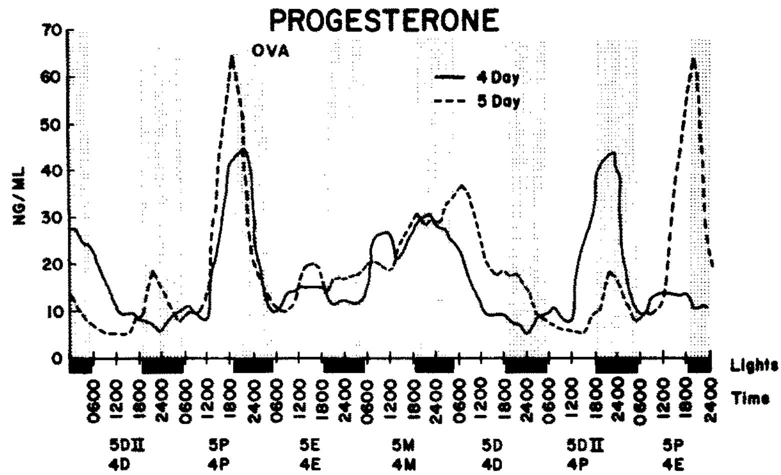

Fig. 9. Comparison of progesterone secretion patterns during 4- and 5-day cycles. See legend Fig. 2.

at this time [29]. This early autonomous secretion of progesterone could possibly be related to adrenal function [24, 30, 31] or to the functional life span of the corpora lutea [28].

The above studies imply that if the secretion of progesterone, prior to estradiol priming, was prolonged, the animal would exhibit a 5-day cycle. On the other hand, if the endogenous secretion of progesterone decreased at a more rapid rate, this would result in a cycle of 4 days length. If this theory is correct, it should be possible to detect higher progesterone levels in 5-day cyclers during some period following ovulation. The next figure (Fig. 9) compares the patterns of progesterone secretion following ovulation in both cycle types. During the days of proestrus, estrus, and metestrus, progesterone levels do not vary between cycle types. However, during the morning of diestrus, three days after the surges, progesterone levels remain elevated in the 5-day cycler, while they begin to decline in the 4-day rat. This corresponds to the times when uterine intraluminal fluid accumulation begins in each cycle [13]. Thus the beginning of uterine ballooning coincides with the decline of progesterone levels, rather than the increase in estrogen as previously assumed.

20α-Hydroxyprogesterone has been shown to be capable of releasing both LH and FSH in the adult castrate rat [21] and may play a similar role in the intact adult. This steroid was increasing during the morning of proestrus prior to the LH and FSH surges in both types of cycles (Fig. 10), and may account for the timing of the surges on proestrus. However, if 20α-hydroxyprogesterone was the trigger for the surge, we would expect to find levels of this hormone to be low on DII in the 5-day cycle since the proestrous surge of gonadotropins is delayed until the following day in this group. Our results (Fig. 11) tend to suggest that this is the case, but the difference between the 4- and 5-day animals could not be confirmed statistically. One variable which we were able to correlate with our measurements of 20α-hydroxyprogesterone was the FSH secretion pattern seen in the 5-day but not the 4-day cycle (Fig. 10).

What events lead to a cycle of 4 or 5 days duration? Why do some strains of rats show predominately one cycle length [32], and why does a shorter light period increase the percentage of 4-day animals [33]? We feel that the answer lies in the regulation of progesterone secretion during the early stages of the cycle (M, D). Two organ systems contribute progesterone to the steroid pool, the ovary and the adrenal.

The amount and timing of progesterone secretion from these organs may account for both interspecies and environmentally induced variations in cycle

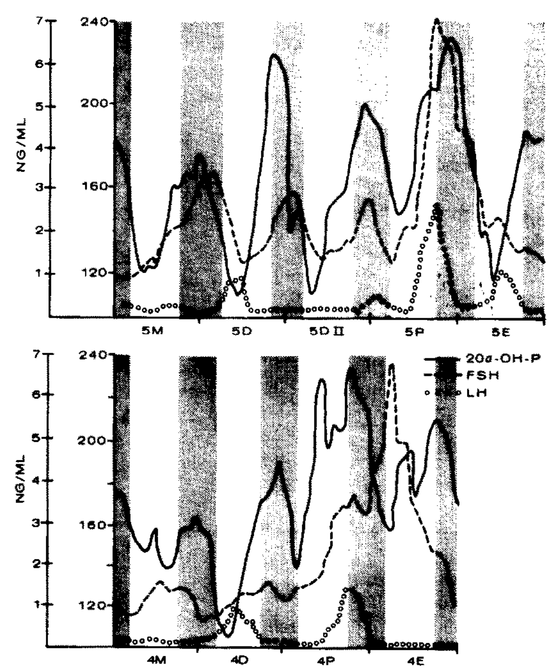

Fig. 10. 20α-Hydroxyprogesterone (20α-OH-P) secretion in relation to the LH and FSH surges. See legend Fig. 6 for ng/ml; see legend Fig. 2.

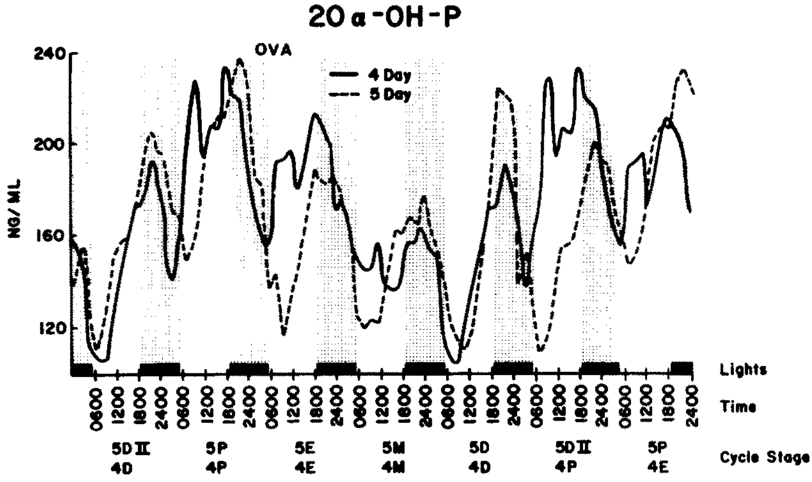

Fig. 11. Comparison of 20α-hydroxyprogesterone (20α-OH-P) secretion patterns during 4- and 5-day cycles. See legend Fig. 2.

length. A shorter light period could shift the adrenal rhythms to an earlier time of day, thereby shortening the cycle length. The hereditary makeup of the animal could determine both its endogenous ovarian and adrenal secretion and their sensitivity to environmental entrainment.

We believe that further studies will confirm the relationship between progestin patterns and cycle length, although it must be kept in mind that the interrelationships among the various hormones may be more complex than we presently expect.

Acknowledgements—The authors wish to thank Wm. Talley and Brigette Mann for their excellent technical assistance; Dr. John Marbarger, Director of the Research Resources Laboratory (Univ. Illinois College of Medicine) for providing controlled animal housing; the Endocrine Study Section and Dr. A. F. Parlow for the NIAMD kits used for the FSH and prolactin assay; Drs. G. D. Niswender, A. R. Midgley and L. Reichert for the materials used in the LH assay and Dr. B. Caldwell for the 17β-estradiol antibody. This work was supported in part by PHS grant HD 0440, Contract PH NIH 70-2307 and a grant to Dr. O. Hechter from the Rockefeller Foundation.

REFERENCES

1. Long J. A. and Evans H. M.: *Mem. Univ. Calif.* **6** (1922) 1–148.
2. Nequin L. G., Talley W. L., Mann B. G. and Schwartz N. B.: *Neuroendocrinology* **14** (1974) 65–71.
3. Everett J. W.: *Endocrinology* **43** (1948) 389–405.
4. Neill J. D., Freeman M. E. and Tillson S. A.: *Endocrinology* **89** (1971) 1448–1453.
5. Schwartz N. B.: *Am. J. Physiol.* **207** (1964) 1251–1259.
6. Shirley B., Wolinsky J. and Schwartz N. B.: *Endocrinology* **82** (1968) 959–968.
7. McClintoch J. A. and Schwartz N. B.: *Endocrinology* **83** (1968) 433–441.
8. Ferin M., Tempone A., Zimmering P. E. and Vande Wiele R. L.: *Endocrinology* **85** (1969) 1070–1078.
9. Freeman M. E., Reichert Jr. L. E. and Neill J. D.: *Endocrinology* **90** (1972) 232–238.
10. Lostroh A. J. and Johnson R. E.: *Endocrinology* **79** (1966) 991–996.
11. Ely C. A. and Schwartz N. B.: *Endocrinology* **89** (1971) 1103–1108.
12. Schwartz N. B. and Ely C. A.: *Endocrinology* **86** (1970) 1420–1435.
13. Nequin L. and Schwartz N. B.: *Biol. Reprod.* **9** (1973) 75–76.
14. Kalra P. S., Fawcett C. P., Krulich L. and McCann S. M.: *Endocrinology* **92** (1973) 1256–1268.
15. Uchida K., Kadowaki M. and Miyake T.: *Endocr. Japon.* **17** (1970) 99–106.
16. Brown-Grant K.: *J. Endocr.* **43** (1969) 553–562.
17. Nallar R., Antunes-Rodrigues J. and McCann S. M.: *Endocrinology* **79** (1966) 907–911.
18. Mann D. R. and Barraclough C. A.: *Endocrinology* **93** (1973) 694–699.
19. Brown-Grant K. and Naftolin F.: *J. Endocr.* **53** (1972) 37–46.
20. Nequin L. G. and Schwartz N. B.: *Endocrinology* **88** (1971) 325–331.
21. Swerdloff R. S., Jacobs H. S. and Odell W. D.: *Endrocrinology* **90** (1972) 1529–1536.
22. Caligaris L., Astrada J. J. and Taleisnik S.: *Endocrinology* **89** (1971) 331–337.
23. Ying S. and Meyer R. K.: *Endocrinology* **84** (1969) 1466–1474.
24. Barraclough C. A., Collu R., Massa R. and Martini L.: *Endocrinology* **88** (1971) 1437–1447.
25. Feder H. H., Brown-Grant K. and Corker C. S.: *J. Endocr.* **50** (1971) 29–39.
26. Piacsek B. E., Schneider T. C. and Gay V. L.: *Endocrinology* **89** (1971) 39–45.
27. Kalra S. P. and Kalra P. S.: *Endocrinology* **92** (1973) 130.
28. Brown-Grant K., Corker C. S. and Naftolin F.: *J. Endocr.* **53** (1972) 31–35.
29. Uchida K., Kadowaki M. and Miyake T.: *Endocr. Japon.* **16** (1969) 227–237.
30. Mann D. R. and Barraclough C. A.: *Proc. Soc. exp. Biol. Med.* **142** (1973) 1226–1229.
31. Feder H. H., Resko J. A. and Goy R. W.: *J. Endocr.* **41** (1968) 563–569.
32. Everett J. W.: *Physiol. Rev.* **44** (1964) 373–431.
33. Hoffmann J. C.: *Biol. Reprod.* **8** (1973) 473–480.