1 Thanks to the technical staff of the Clinical Chemistry Laboratory at Laurentian Hospital.
2 M.A. Persinger (ed.), ELF and VLF Electromagnetic Field Effects. Plenum Press, New York 1974.
3 J.G. Llaurado, A. Sances and J.H. Battocletti (ed.), Biologic and Clinical Effects of Low-Frequency Magnetic and Electric Fields. C.C. Thomas, Springfield 1974.
4 J.H. Battocletti, Electromagnetism Man and the Environment. Westview Press, Boulder 1976.
5 M.A. Persinger, H.W. Ludwig and K.-P. Ossenkopp, Percept. Mot. Skills *36*, 1131 (1973).
6 M.A. Persinger (ed.), in: ELF and VLF Electromagnetic Field Effects, p. 275. Plenum Press, New York 1974.
7 M.A. Persinger, Devl Psychobiol. *2*, 168 (1969).
8 K.-P. Ossenkopp, Psychol. Rep. *30*, 371 (1972).
9 K.-P. Ossenkopp, W.T. Koltek and M.A. Persinger. Devl Psychobiol. *5*, 275 (1972).
10 M.A. Persinger, G.F. Lafrenière and N.J. Carrey, Int. J. Biomet. *22*, 67 (1978).
11 M.A. Persinger, N.J. Carrey, G.F. Lafrenière and A. Mazzuchin, Int. J. Biomet. *22*, in press (1978).

Effects of postnatal progesterone treatment on ovarian function in adult rats

A. Bukovský, J. Presl and Z. Krabec

Institute for the Care of Mother and Child, nábř. K. Marxe 157, CS-147 10 Praha 4-Podoli (Czechoslovakia), 31 October 1977

Summary. Long-lasting postnatal progesterone administration in female rats induced an early or delayed ovulatory failure with persistent vaginal estrus. Short-term treatment was ineffective. The beginning and incidence of ovulatory failure appeared to depend on the beginning and duration of progesterone treatment. The necessary duration of progesterone administration exceeds the critical postnatal steroid sensitive period of sexual differentiation of the hypothalamus. Moreover, long-lasting progesterone treatment results in ovulatory failure even if started after termination of this period.

In laboratory rodents, the presence or absence of testicular testosterone during the critical steroid sensitive period of sexual differentiation of the hypothalamus (1 week of life in the rat) organizes the male or female pattern of hypothalamic control of gonadotropin secretion[1]. Unlike exogenous testosterone or estradiol both of which masculinize the hypothalamus in newborn female rats, properly timed progesterone treatment protects the developing female brain from the «damaging» effects of early postnatal testosterone or estradiol administration[2–4]. A similar protective role is suggested for placental progesterone in primate fetuses of both female and male sex, thus explaining the potentially operative positive feedback effect of estrogens in male primates[5].

In fact, high doses of progesterone injected during the early postnatal critical period of hypothalamic sexual differentiation do not impair the cyclic ovulatory release of gonadotropins in adult female rats[6–8]. It has been reported, however, that postnatal long-lasting small-dose progesterone treatment impairs the late ovulatory function in rats[9–12].

The present experiment was designed to assess the effective period of postnatal progesterone treatment required to induce an impairment of the ovulatory function in adult female rats.

Material and methods. Immature female Wistar rats of our laboratory colony were injected s.c. with different progesterone doses during different periods of time. The amount of oil solvent was 0.05 ml in ages up to 19 days and 0.1 ml from the age of 20 days. The control animals were injected with oil only. Vaginal smears were taken daily for 4 weeks after finishing the 2nd and the 5th month of life. Only then were the animals decapitated and the ovaries evaluated histologically, using standard technique. All experimental groups are summarized in the table.

Results and discussion. An early ovulatory failure, which is characterized by persistent vaginal estrus observed already after the 2nd month of life, was induced with daily progesterone administration up to the 40th day of life (experiment 1). Shortening this period to 26 days with the dosage of 200 µg progesterone daily was similarly effective (experiment 2). Lowering the daily dose of progesterone to 100 µg on days 1–19 and using the daily dosage of 200 µg on days 20–26 did not change the incidence of the early ovulatory failure (experiment 3), whereas further lowering of the daily progesterone dose to 100 µg on days 1–26

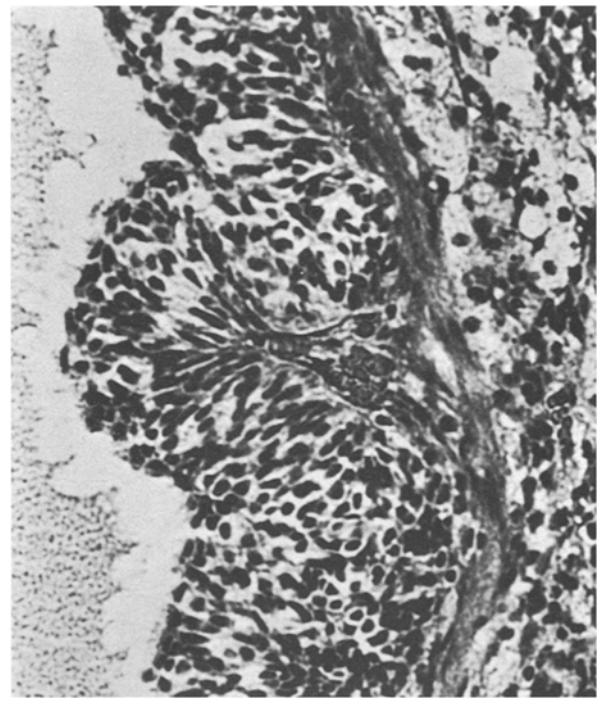
Experiment No.	Number of rats	Day of treatment Dose of progesterone daily			Total dose of progesterone (µg)	Persistently estrous type of smear*	
		1200 µg	100 µg	200 µg		3rd month	6th month
1	6	–	1–19	20–40	6100	6/6	6/6
2	12	–		1–26	5200	12/12	12/12
3	9	–	1–19	20–26	3300	9/9	9/9
4	23	–	1–26		2600	10/23	20/23
5	9	–	10–19	20–26	2400	0/9	9/9
6**	8	–	1–19	–	1900	0/8	8/8
7	5	–	1–10	–	1000	0/5	2/5
8	16	–	10–19	–	1000	0/16	3/16
9	18	5	–	20–26	3600	0/18	0/18
10	15	–	–	20–26	1400	0/15	0/15
11	11	5	–	–	1200	0/11	0/11
12***	14	–	–	–	–	0/14	0/14

* Number of animals with persistent estrus/total number. ** Experiment published previously[12]. *** Control group treated with oil solvent from days 1 to 26.

resulted in the early ovulatory failure only in some animals (experiment 4). Additional shortening of the progesterone treatment on days 10–26 with the daily dose of 100 µg during days 10–19 and 200 µg during days 20–26 did not induce an early ovulatory failure but resulted in a delayed failure (persistent vaginal estrus observed only after the 5th month of life) in all animals (experiment 5). Progesterone administration of 100 µg daily from days 1–19 was similarly effective (experiment 6). Shortening of this dosage only to the days 1–10 decreased the incidence of delayed ovulatory failure (experiment 7). Even less effective was a progesterone treatment during the days 10–19 with the daily dose of 100 µg (experiment 8). A single injection of 1200 µg progesterone on the 5th day of life followed up during days 20–26 with 200 µg daily, was completely ineffective (experiment 9). Equally ineffective was a progesterone treatment on days 20–26 with the daily dose of 200 µg (experiment 10) or a single dose of 1200 µg at the age of 5 days (experiment 11). A 26-day administration of the oil solvent only, beginning from the 1st day of life, was likewise without effect (experiment 12).

In all animals showing persistent vaginal estrus at decapitation, ovaries lacking corpora lutea and with the frequent presence of thecal hyperplasia were observed, the latter protruding into the large cavitated follicles with proliferating granulosa (figure).

During the period of protective action of progesterone, a significant uptake of ^{3}H-progesterone by the female rat hypothalamus could not be observed[13], nor could a decrease in ^{3}H-estradiol accumulation in the hypothalamus result from progesterone pretreatment in female rats during the first 10 days of life[14]. Therefore, a hypothesis has been expressed on the extracerebral mechanism of the protective effect of progesterone[14]. Present results demonstrating a delayed ovulatory failure induced by progesterone after the critical postnatal steroid sensitive period of sexual differentiation of the hypothalamus (experiment 5) are thought to be a further contribution to the above-mentioned hypothesis. An attempt to interpret the extracerebral mechanism of protective and/or noxious progesterone action is only speculative in the meantime; presumably, progesterone does impair the normal development of the relationship between the differentiating immune system and the ovary[15].



Theca interna growing into the antrum of the preovulatory follicle bedded with protruding granulosa. 6-month-old rat after long-lasting postnatal progesterone treatment. Papanicolaou stain, ×400.

1 R.A. Gorski, Endocrinology *82*, 1001 (1968).
2 N. Takasugi, J. Fac. Sci. Univ. Tokyo, Sect. IV Zool. *7*, 299 (1954).
3 R.I. Dorfman and F.A. Kincl, IV Reunión Anual Soc. Mex. Nut. Endocr. Ixtapan de la Sal, México, 6–7 December 1963, p. 171.
4 E.G. Shipley and R.K. Meyer, in: Hormonal Steroids, vol. 2, p. 293. Ed. L. Martini and A. Pecile. Academic Press, New York 1965.
5 J. Presl and A. Bukovský, Čslka Gynek. *41*, 449 (1976).
6 G. Shipley and K. Meyer, Int. Congr. Hormonal Steroids, Milan, Italy, 14–19 May, 1962, p. 86.
7 F.A. Kincl, A. Folch Pi and L. Herrera Lasso, Ciencia, Méx. *22*, 135 (1963).
8 J. Presl, J. Jirásek, I. Mikuláš and J. Horský, Čslka Gynek. *32*, 658 (1967).
9 R.R. Greene and M.W. Burrill, Am. J. Physiol. *133*, P302 (1941).
10 N. Takasugi, Annotnes zool. jap. *26*, 52 (1953).
11 N. Takasugi, Annotnes zool. jap. *26*, 91 (1953).
12 A. Bukovský, J. Presl and Z. Krabec, Čslka Gynek. *41*, 281 (1976).
13 J. Presl, V. Figarová, J. Herzmann and S. Röhling, Endocr. exp. *9*, 51 (1975).
14 J. Presl, J. Herzmann and S. Röhling, Čslka Gynek. *40*, 751 (1975).
15 A. Bukovský and J. Presl, Med. Hypotheses *4*, in press (1978).

Sertoli cells of adult rats in vitro. II. Effect of different steroid precursors on estradiol 17β-synthesis[1]

M.L. Marcante, A. Floridi, P.G. Natali, G. Citro, V. Cioli and C. De Martino[2]

Regina Elena Institute for Cancer Research, Rome (Italy) and F. Angelini Research Institute, Rome (Italy), 9 June 1978

Summary. The estradiol 17β-synthesis by Δ^4 pathway has been studied in homogenous cultures of Sertoli cells isolated from adult rat testes. The data reported clearly demonstrate that progesterone, androstenedione, testosterone and estrone induce an increase of the estradiol 17β-production.

Previous studies[3–5] have demonstrated that the Sertoli cells of adult rats in vitro synthetize large amounts of cholesterol, estrone (E_1) and estradiol 17β (E_2), without the addition of a steroid precursor and/or FSH and cAMP to the culture medium. These findings seem to indicate that the Sertoli cells have the capacity to produce E_2 by the Δ^4 and/or Δ^5 metabolic pathways, which recognize progesterone (P) and pregnenolone, respectively, as the leading