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THE CONTRIBUTION OF THE ADRENAL GLAND TO THE TOTAL AMOUNT OF PROGESTERONE PRODUCED IN THE FEMALE RAT

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SUMMARY

1. The amount of progesterone contained in both adrenal glands of a rat was similar to or larger than the amount of progesterone in the ovaries of the same rat. This was found in unstressed rats, in stressed rats and also in pregnant rats.

2. After ether anaesthesia and exsanguination the adrenal progesterone content was increased by 75%; the ovarian progesterone content remained unchanged.

3. In contrast, prolonged operative stress resulted in a rise in the ovarian content of progesterone and 20-dihydroprogesterone whereas the adrenal progesterone content of these rats was lower than that of unstressed rats.

4. The rate at which progesterone was secreted by the adrenal glands of stressed rats was similar to the ovarian progesterone secretion rate. Rats which were kept under mild stress conditions before the experiment showed higher adrenal progesterone secretion rates.

INTRODUCTION

Progesterone is present in all steroid-producing organs. In the ovaries progesterone is the major secretion product. In addition it is an intermediate in the synthesis of other pregnane derivatives and probably of the oestrogens. In the adrenal gland progesterone is mainly regarded as a precursor steroid for the synthesis of the gluco- and mineralocorticoids. However, progesterone is also secreted by the adrenal gland (Short, 1960) at a rate which is controlled by ACTH (Heap, Holzbauer & Newport, 1966;

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Holzbauer, Newport, Birmingham & Traikov, 1969; Holzbauer & Newport, 1969). In the ovariectomized, oestrogen-primed rat and guinea-pig ACTH can stimulate the secretion of sufficient quantities of progesterone to facilitate female sexual receptivity (Feder & Ruf, 1969).

The present paper describes experiments which provide some information on the amount of progesterone contributed by the adrenal gland to the total amount of progesterone available to the female rat. For this purpose, ovarian and adrenal progesterone secretion rates were measured and also the amounts of steroids contained in these two tissues at the end of the blood collections. In addition the effect of brief and of prolonged stress on adrenal and ovarian progesterone concentrations was studied.

METHODS

Operative procedures

The experiments were carried out on virgin female rats (Wistar strain) weighing 180–220 g. They were kept either in natural light (November to February, average length of daylight 9 hr) or under artificial lighting conditions with 10 hr white light (08.00–18.00 hr) and 14 hr red light. The experiments were usually carried out after the rats had shown three or four regular oestrous cycles of 4 days in length. The animals were only handled by the persons involved in the final experiment. Ovarian venous blood was collected from rats anaesthetized with sodium pentobarbitone (30–50 mg/kg body wt., I.P.) through a cannula inserted into the left renal vein after the renal peduncle, the adrenal vein, the uterine vein and the renal vein at its entry into the vena cava had been ligated (Fajer & Barraclough, 1967). The collection periods were 15–30 min and 2–8 ml. of blood were collected. The blood lost was replaced by an infusion of arterial blood from male rats or of a 0·9 % sodium chloride solution. The body temperature was maintained between 36·8 and 38° C. Adrenal venous blood was collected in a similar manner, the ovarian vein being ligated instead of the adrenal vein (Vogt, 1955). The adrenal glands and the ovaries were dissected out immediately at the end of the blood collections and frozen. 'Unstressed' rats were killed by rapid decapitation, 'acutely stressed' rats were anaesthetized with ether and bled from a carotid artery.

Chemical procedures

The methods used for the extraction of the glands and the whole blood, the purification of the extracts, the separation of the individual steroids by paper chromatography (systems E_1 and E_2B, Eberlein & Bongiovanni, 1955) and the quantitative estimation of progesterone by gas–liquid chromatography were described previously (Holzbauer & Newport, 1967, 1969). 20α-Hydroxypregn-4-en-3-one (20-dihydroprogesterone) was separated from progesterone in the E_1 system. Its retention time on the gas chromatograph relative to that of cholestane (RRT) was 0·92. Androstenedione or pregnenolone were used as internal standards for the quantitative estimation. Eluates of the 20-dihydroprogesterone region of the E_1 chromatogram sometimes contained an impurity which produced an atypically shaped peak at about RRT 0·98 on some 3·8 % SE-30 columns. This peak was well separated from that of 20-dihydroprogesterone if the column temperature was kept below 225° C. [4-^{14}C]Progesterone (10 nCi) was added to the blood and to the gland extracts to

permit correction for losses during the chemical procedures. Corticosterone was eluted from the E_2B chromatogram and estimated by its reaction with blue tetrazolium (Vogt, 1955; Holzbauer, 1964). The values given for progesterone and 20-dihydroprogesterone were corrected for losses. The methods used were not sensitive enough to detect the steroids in 5 ml. arterial blood of the same rats.

RESULTS

Comparison between secretion rates of ovarian and adrenal progesterone

In Fig. 1 the results of experiments are summarized in which the secretion rates of progesterone and of 20-dihydroprogesterone by the ovary were measured. Ovarian blood was collected on the day of pro-oestrus between 11.00 and 15.00 hr (early pro-oestrus) and between 18.00 and 21.00 hr (late pro-oestrus); on the day of oestrus and the two days of metoestrus blood was collected between 11.00 and 16.00 hr. The results confirm earlier observations by Fajer & Holzbauer (1968) in which the progesterone secretion was found to be very slow in early pro-oestrus and fast in metoestrus. The peak in the progesterone secretion in the evening of the day of pro-oestrus which was first demonstrated by Hashimoto, Henricks, Anderson & Melampy (1968) was also present in this strain of rats.

Fig. 2 shows the results of experiments in which the adrenal secretion rates of progesterone and of corticosterone were measured in female and male rats. One group of female rats (group 1) was unstressed until the day of the adrenal blood collection. A second group of female rats (group 2) and a group of male rats (group 3) were under conditions of mild chronic stress before the experiment; in group 2 this stress consisted in replacement of the drinking water by a 0·9% sodium chloride solution for 1 month and in group 3 of a sham adrenalectomy 2 days before the experiment.

Progesterone was secreted by the adrenal glands of all rats studied at rates which were similar to the ovarian secretion rates of progesterone under similar conditions of surgical stress. The adrenal progesterone and corticosterone secretion was lowest in group 1, the female rats which were not disturbed before the experiment. The female rats which received saline for one month (group 2) secreted four times more progesterone and one and a half times more corticosterone. The sham-operated male rats (group 3) secreted even more progesterone and corticosterone than group 2, but the differences were not statistically significant.

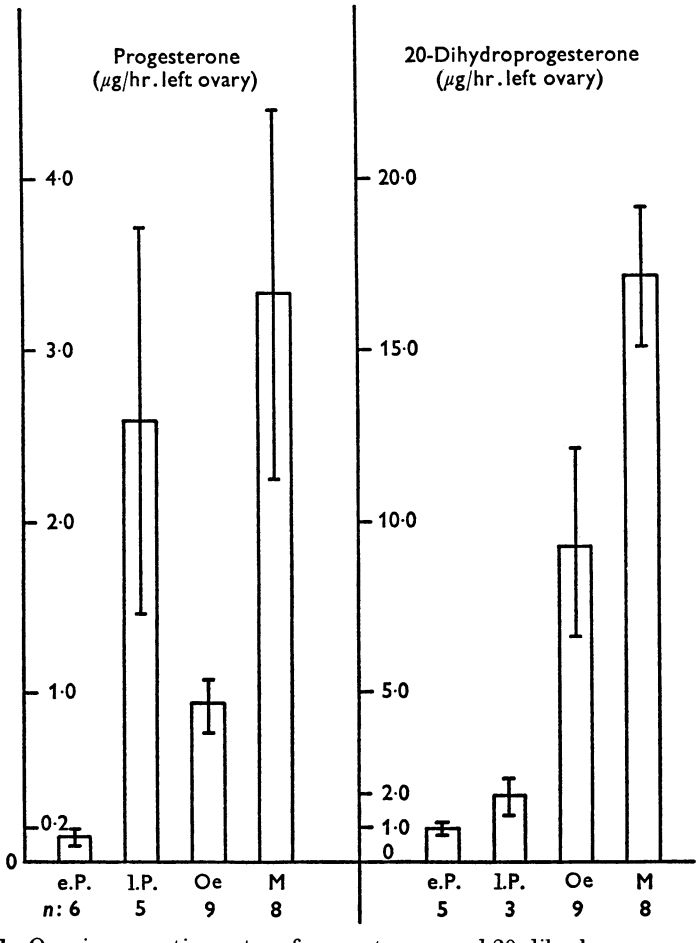

Fig. 1. Ovarian secretion rates of progesterone and 20-dihydroprogesterone during different phases of the oestrous cycle of the rat (mean values ± S.E. of mean). e.P.: early pro-oestrous; l.P.: late pro-oestrus. Oe = oestrus; M = metoestrus. n = no. of rats.

Steroid contents of ovaries and adrenal glands after ovarian venous blood collection

The amounts of progesterone found in the ovaries and in the adrenal glands of the rats from which ovarian venous blood had been collected (see Fig. 1) are listed in Table 1. The adrenal glands of these rats contained equal amounts of, or more, progesterone than the ovaries. There was little difference between the weights of the adrenals and the ovaries. Under conditions of surgical stress no difference in the adrenal progesterone content during the different phases of the oestrous cycle was apparent. The glands contained about 15 times more corticosterone than progesterone.

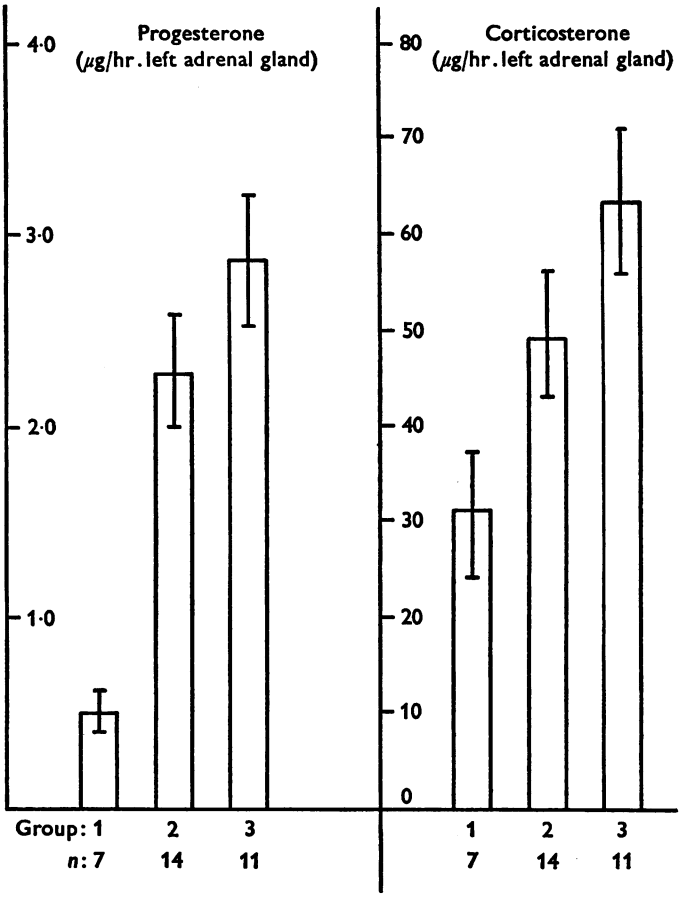

Fig. 2. Adrenal secretion rates of progesterone and corticosterone in three different groups of rats (mean values ± S.E. of mean). Group 1: ♀ rats, unstressed before blood collection. Group 2: ♀ rats, 0·9 % NaCl instead of drinking water during 4–6 weeks before blood collection. Group 3: ♂ rats, 'sham adrenalectomized' 1–3 days before blood collection. n = no. of rats.

The ovarian progesterone content showed large individual variations. There were small, but not significant, differences during the different phases of the cycle. The mean values were higher during metoestrus than during oestrus, as found for the secretion rates. In contrast, the mean value for the rats in late pro-oestrus was lower than in early pro-oestrus. The amounts of 20-dihydroprogesterone present in the ovaries was about 2 to 5 times larger than the amount of progesterone.

TABLE 1. Steroids contained in the ovary and the adrenal gland of rats after ovarian blood collection.

No. of rats	Phase of cycle	Steroid contents (μg/pair)				Organ weights (mg/pair)	
		Progesterone		20-Dihydro-progesterone (ovary)	Cortico-sterone (adrenal)		
		Ovary	Adrenal			Ovary	Adrenal
6	Early pro-oestrus	0·49 ± 0·16	0·73 ± 0·08	1·90 ± 0·54	13·4 ± 2·19	70·4 ± 4·5	67·2 ± 3·50
5	Late pro-oestrus	0·35 ± 0·11	0·75 ± 0·29 (n = 2)	1·81 ± 0·23	—	80·7 ± 7·3	82·9 ± 9·60 (n = 2)
8	Oestrus	0·62 ± 0·14	0·79 ± 0·11	1·58 ± 0·24	12·0 ± 2·03	72·8 ± 3·5	79·0 ± 5·5
8	Metoestrus	0·81 ± 0·25	0·75 ± 0·09	1·52 ± 0·31	11·5 ± 2·10	73·9 ± 4·3	69·8 ± 5·5

(Figures are mean values and S.E. of mean.)

Comparison of tissue steroid contents with secretion rates

The amount of progesterone contained in the left ovary of a rat in early pro-oestrus or in oestrus was approximately equal to the quantity secreted in a period of 60 min. During late pro-oestrus and during metoestrus, when the secretion was faster, the amounts of progesterone found in the glands would have only sufficed for a 5–10 min secretion period. Similar results were obtained for 20-dihydroprogesterone. The adrenal content of progesterone after operative stress was equal to the amount of progesterone secreted by the adrenal gland in approximately 30 min.

The effect of stress on the steroid content of the ovary and the adrenal gland

The following experiments were carried out in order to investigate whether acute stress affected the ovarian steroid content in a similar manner to that of the adrenal gland. One group of thirty-eight rats was killed by rapid decapitation (unstressed rats), a second group of nineteen rats was anaesthetized with ether and exsanguinated from a carotid artery (acutely stressed rats). In each group an equal percentage of rats were in metoestrus or in oestrus. All rats were killed between 11.00 and 13.00 hr. One to three (stressed rats) and three to five (unstressed rats) pairs of ovaries or adrenal glands were pooled for the steroid estimations. The pooled organs were from rats in the same phase of the oestrous cycle.

The mean ovarian and adrenal steroid contents of these rats are shown in Fig. 3. The amounts of progesterone and 20-dihydroprogesterone found in the ovaries of unstressed rats (open columns) was not significantly different from the amounts found in the ovaries of the acutely stressed rats (horizontally hatched columns). In contrast acute stress caused a 70% rise in the adrenal progesterone content and a 90% rise in the adrenal corticosterone content. The adrenal glands of the unstressed rats contained twice as much progesterone as the ovaries of the same rats. Fig. 3 includes also the amounts of steroids found in the tissues of the rats from which ovarian venous blood had been collected (vertically hatched columns). In order to make a true comparison only the results from the rats in oestrus and metoestrus were used to calculate the mean values shown in Fig. 3. The ovarian progesterone content of these operated rats was about 60% higher than that of the unstressed rats ($P = 0{\cdot}1$–$0{\cdot}2$) and double that of the acutely stressed rats ($P = 0{\cdot}02$–$0{\cdot}05$). The ovarian content of 20-dihydroprogesterone in the operated rats was also 60% higher than in unstressed rats ($P = 0{\cdot}05$).

A comparison between the amounts of progesterone contained in the

adrenal glands of unstressed or briefly stressed rats with those found in the adrenal glands after ovarian blood collection gave an interesting result. Although this prolonged surgical stress caused a further rise in the adrenal corticosterone content, the adrenal progesterone content was not increased. On the contrary, there was even less progesterone in the adrenals of these rats than in the adrenals of the unstressed rats.

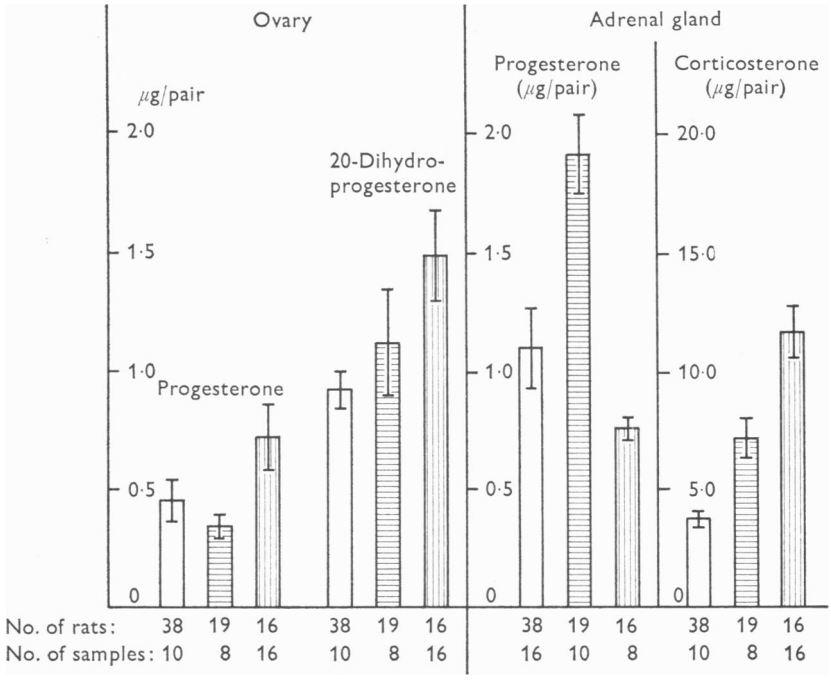

Fig. 3. Steroids contained in the ovary and the adrenal glands. Open columns: unstressed rats; horizontally hatched columns: acutely stressed rats (ether anaesthesia, exsanguination from carotid artery); vertically hatched column: after ovarian blood collection under sodium pentobarbitone anaesthesia (mean ± S.E. of mean).

In order to exclude interference with the blood circulation of the left adrenal glands of these rats as the cause for the lower progesterone content, experiments were carried out on six rats in which progesterone and corticosterone were estimated separately in right and left adrenal glands. The veins of the left adrenal glands were ligated for 20 min and ovarian venous blood was collected during this time. In each experiment two left and two right glands were analysed. The progesterone content of the left adrenal glands was 1·17, 0·82 and 0·52 (μg/two adrenals), of the right adrenal glands it was 0·94, 1·04 and 0·62. For corticosterone the corre-

sponding values were 3·3, 3·5 and 9·0, and 3·5, 2·0 and 7·1. These differences in the steroid content of the left and the right glands were not large enough to explain the relatively small amounts of progesterone found in the adrenals after ovarian blood collection.

Steroid content of the ovaries of unstressed rats in early and late pro-oestrus

The increased ovarian content of progesterone and of 20-dihydroprogesterone in operated rats in contrast to unstressed or acutely stressed rats indicate some abnormality in the 'storage mechanisms' of steroids. Although the differences in the progesterone secretion rates during oestrus

TABLE 2. Steroids in the ovaries of unstressed rats during early and late pro-oestrus

Phase of oestrous cycle	No. of rats	Progesterone (μg/pair)	20-Dihydro-progesterone (μg/pair)	Ovarian weight (mg/pair)
Early pro-oestrus	16	0·10 ± 0·03	1·15 ± 0·11	70·5 ± 3·2
Late pro-oestrus	17	0·24 ± 0·04	1·80 ± 0·20	78·0 ± 3·7

(Figures are mean values and S.E. of mean.)

and metoestrus were paralleled by the differences in the ovarian progesterone contents of the same rats, this was not the case during early and late pro-oestrus (see Fig. 1 and Table 1). In unstressed rats, however, Lindner & Zmigrod (1967) observed higher ovarian progesterone concentrations during the evening of pro-oestrus. In order to investigate whether the difference between our operated rats and the unstressed rats of Lindner & Zmigrod was due to the manipulations involved in the ovarian blood collection and not to a difference in strain or the conditions under which the rats were kept prior to the ovarian blood collection the following experiment was carried out on unstressed rats. On the day of pro-oestrus sixteen rats were killed by rapid decapitation between 14.00 and 15.00 hr (early pro-oestrus) and seventeen rats between 21.00 and 22.00 hr (late pro-oestrus). The steroids were estimated in pairs of ovaries from individual rats. The results are listed in Table 2. They confirm the observations of Lindner & Zmigrod (1967). The ovaries contained significantly more progesterone in the evening of pro-oestrus than in the morning. The amount of progesterone found in the ovaries of unstressed rats in early pro-oestrus was less than a quarter of that found in the operated rats during the same phase of the oestrous cycle.

Pregnant rats

Ovarian progesterone secretion rates and tissue contents were also measured in six pregnant rats at different days of pregnancy. The total quantity of progesterone contained in the left ovary at the end of the ovarian blood collection was equal to the amount secreted by the same ovary in 1·5–5·5 min. This indicates that the synthesis of progesterone during pregnancy is as fast as its secretion rate. The ovaries contained 1·21 ($\pm$ 0·15, S.E. of mean) μg progesterone/pair, which was 70 % more than the ovaries of operated, non-pregnant rats. The progesterone content of the adrenal glands of three pregnant rats at the end of the ovarian blood collection was 0·73, 3·61 and 2·28 μg/pair.

DISCUSSION

Tissue content

The amount of progesterone contained in the adrenal glands of a rat was either similar to or larger than that present in its ovaries. This was observed in unstressed rats, in rats bled under ether anaesthesia (acute stress), in rats which were under operative stress for 30–50 min and in pregnant rats. The acute stress caused a rise in the adrenal progesterone and corticosterone content, as already seen on a previous occasion (Holzbauer & Newport, 1967). In the same rats there was no elevation in the ovarian progesterone content. This is in accordance with the observation of Guiliani, Martini, Pecile & Fochi (1961) that the plasma concentration of luteinizing hormone (LH) did not increase in rats which were anaesthetized with ether and exposed to a short operative stress.

The adrenals of rats which had been exposed to the prolonged surgical stress of ovarian blood collection contained less progesterone than those of unstressed rats, although their corticosterone content was three times that of unstressed rats and nearly double that of rats exposed to the short stress. In these adrenals the synthesis of progesterone could probably not keep pace with its fast conversion into C_{21}-hydroxylated steroids over a longer period of time.

The ovaries of the rats from which adrenal venous blood had been collected contained more progesterone and 20-dihydroprogesterone than the ovaries of acutely stressed rats. A comparable increase in ovarian progesterone secretion was observed when rat ovarian venous blood was collected for as long as 2 hr (Yoshinaga, Grieves & Short, 1967). This rise in the ovarian production of progesterone and 20-dihydroprogesterone during prolonged operative stress could be caused by an increase in the release of gonadotrophins. Strong stimuli which cause a maximum release

of the ACTH-releasing factor may simultaneously have an unspecific effect on the various gonadotrophin-releasing factors. LH release could also have been caused by an increased secretion of adrenaline during the operation (Guiliani *et al.* 1961). On the other hand it cannot be ruled out that the prolonged exposure of the ovaries to very high blood ACTH concentrations leads to a small increase in ovarian steroid production.

Steroid secretion rates

The rates at which progesterone was secreted by the ovary were smallest during early pro-oestrus and largest in metoestrus with a peak in the evening of pro-oestrus which is regarded as a response to the release of LH in the afternoon of the day of pro-oestrus. These findings confirm earlier observations of Hashimoto *et al.* (1968) and Fajer & Holzbauer (1968).

The adrenal secretion rates of progesterone in operated rats were dependent on the conditions under which the rats were kept before the adrenal blood collection. In previously undisturbed rats it was about one-fifth of the ovarian progesterone secretion during metoestrus; in rats which had been exposed to a mild stress for several days or weeks it was as high as the ovarian progesterone secretion during metoestrus. The ratio between progesterone and corticosterone in the adrenal venous blood of previously undisturbed rats was 1:60, in the adrenal tissue of these rats it was 1:15. The adrenal tissue of unoperated and unstressed rats contained only three times more corticosterone than progesterone. That progesterone is also secreted by the adrenal gland of unstressed rats was observed by Feder, Resko & Goy (1968).

There was good agreement between the changes in progesterone secretion rates during the oestrous cycle as seen in the present experiments and the changes in the progesterone concentrations in peripheral blood as reported by Feder, Brown-Grant, Corker & Exley (1969). Whether the quantities of progesterone secreted by the operated rats are of the same order of magnitude as those secreted by an unoperated, normal rat remains as yet unknown. The low ovarian progesterone contents of unstressed rats in early pro-oestrus and the high contents in late pro-oestrus are also in good agreement with the low progesterone secretion found in the operated rats in early pro-oestrus and the high secretion rates in late pro-oestrus. The magnitude of the differences was however larger for the secretion rates than for the steroid contents.

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