Histochemistry of Ovarian 20α-Hydroxysteroid Dehydrogenase in the Rat During the Estrus Cycle

The ovarian tissue of the rat catalyses the reduction of progesterone to 4-pregnen-20α-ol-3-one[1] by means of the 20α-hydroxysteroid dehydrogenase (20α-HSD), a NADP-linked enzyme.

Since the progestational activity of the 4-pregnen-20α-ol-3-one is low compared to that of progesterone[2-5], the variations of the enzymatic activity in the gland and of the secretion of 4-pregnen-20α-ol-3-one during the estrus cycle and pseudopregnancy strongly suggest a control mechanism of the ovarian progestational activity[6].

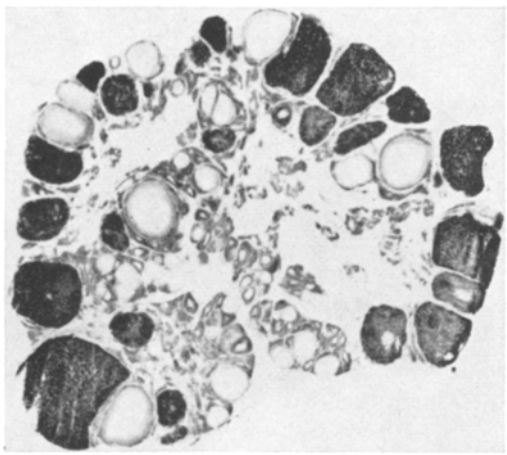

Fig. 1. Proestrus. Marked 3β-HSD activity in corpora lutea, in thecal cells of follicles, and in interstitial cells. × 25.

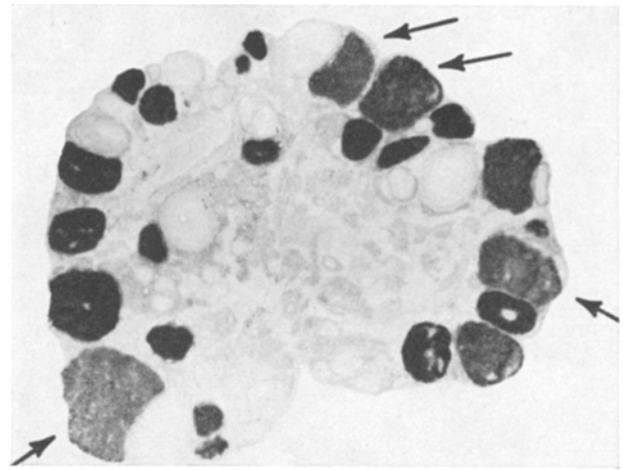

Fig. 2. Proestrus. Section contiguous to that of Figure 1. All the corpora lutea show 20α-HSD activity. Those with only weak activity are the most recently formed (arrows). × 25.

The histochemical visualization of the 20α-HSD activity[7] allows the study of its topographic localization in ovarian structures during the estrus cycle, and the comparison of the morphologic findings with the biochemical data.

Contiguous cryostatic sections of the ovaries of 40 Sprague-Dawley female rats (10 animals for each phase of the cycle determined by vaginal smears) were cut at various levels, i.e. in the 1st, 2nd, and 3rd quarter respectively. The sections, each of 12 μ, were then prepared for the demonstration of the 3β-hydroxysteroid dehydrogenase (3β-HSD)[8] and of the 20α-HSD[7]. The number of the corpora lutea (CL) 3β-HSD and 20α-HSD positive was recorded and the percentage of CL 20α-HSD positive was evaluated in comparison with the total number of CL in the different phases of the estrus cycle. A statistical analysis using the formula of BRANDT and SNEDECOR[9] was performed.

In each section intense 3β-HSD activity occurred in every CL, in the follicular thecal cells, in the interstitial tissue, and in the granulosa cells of atretic follicles.

20α-HSD activity was demonstrated in the CL cells exclusively. CL that were 20α-HSD positive and 3β-HSD negative have never been observed. The ratio between CL 3β-HSD positive and CL 20α-HSD positive appeared modified in the course of the cycle as follows. In *proestrus* and *estrus* all the CL present were 3β-HSD and 20α-HSD positive (Figures 1 and 2). The involuting CL represented by small cellular masses, exhibited a more marked 20α-HSD positivity (Figure 2). In *metestrus* and *diestrus* the 3β-HSD positive newly-formed CL, identified by their large size, totally lack 20α-HSD activity, this latter enzyme being demonstrable only in the CL of the previous cycles (Figures 3 and 4). It is possible occasionally to observe a weak 20α-HSD activity during the diestrus in the newly-formed CL.

The results are summarized in the Table. Statistical analysis demonstrated a high significance in the differences between proestrus and diestrus ($P < 0.001$) and those between estrus and metestrus ($P < 0.05$).

The percentage of CL 20α-HSD positive, as compared with the total number of CL identifiable by means of the 3β-HSD visualization, gets modified with the different

Corpora lutea (CL) 3β- and 20α-HSD positive scored in ovary of normal rats during the estrus cycle

Phase of cycle	No. of rats	Average number of active CL per ovary 3β-HSD	20α-HSD	Ratio $\frac{\text{20α-HSD}}{\text{3β-HSD}} \cdot 100$
Proestrus	10	38.2 ± 3.2[a]	38.2 ± 3.1	100
Estrus	10	35.8 ± 3.9	35.7 ± 3.6	100
Metestrus	10	36.3 ± 4.1	31.0 ± 4.6	85.4
Diestrus	10	33.6 ± 4.1	26.2 ± 3.2	77.9

[a] Standard error.

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phases of the cycle: in the proestrus and estrus every observed CL gives a positive reaction for both enzymes; in the metestrus and diestrus, on the contrary, the newly-formed CL totally lack 20α-HSD activity. In these phases the enzyme is demonstrable only in the CL of previous cycles. Therefore the newly-formed CL exhibit evident 20α-HSD activity in the period between diestrus and proestrus. The complete involution of the CL was characterized by the lack of both 20α-HSD and 3β-HSD activity.

Bearing in mind that (1) the complete involution of the CL together with the disappearance of 20α-HSD occurs in 16–20 days[10], (2) in every cycle about 10 CL are formed[11], and (3) in the proestrus 20α-HSD positive CL substitute those that have completed their involution, it can be expected that in the proestrus about 40 CL are thoroughly present. The fluctuation of total number of CL in the ovaries and of their enzymatic activities throughout the cycle is reported in Figure 5, where the difference between the two curves in metestrus and diestrus is due to the number of fresh CL. As can be seen, there is a good correlation between the experimental and the theoretical curve.

Finally, the histochemical data, which show a maximum of 20α-HSD activity in proestrus phase, are in agreement with the biochemical assay of the enzymatic activity and with the secretion of 4-pregnen-20α-ol-3-one biochemically assessed[6,12].

We can conclude that the described fluctuations of the enzyme throughout the estrus cycle correlate to the onset of 20α-HSD in the CL in the period between diestrus and proestrus. Moreover the percentage of the CL 20α-HSD positive in the rat ovary may be regarded as an index closely related to the different phases of the cycle[13].

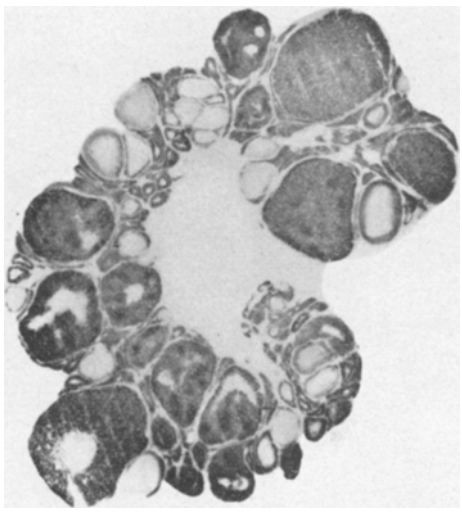

Fig. 3. Diestrus. Marked 3β-HSD activity as in Figure 1. × 25.

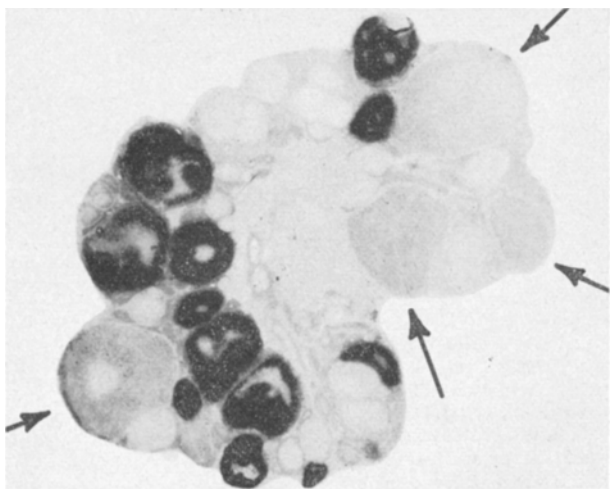

Fig. 4. Diestrus. Section contiguous to that of Figure 3. The 20α-HSD activity is lacking in the newly-formed corpora lutea (arrows). × 25.

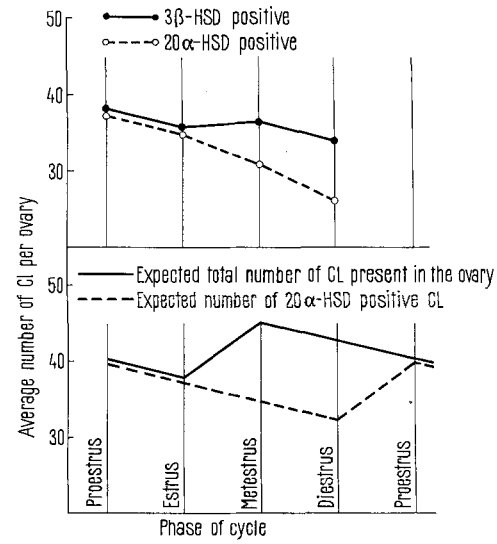

Fig. 5. Number of corpora lutea (CL) counted on the sections

Riassunto. In ovaie di ratta, durante le fasi del ciclo estrale, fu determinata istochimicamente l'attività 3β-HSD e 20α-HSD. L'attività 20α-HSD risulta localizzata esclusivamente nei corpi lutei. Essa compare nei giovani corpi lutei durante il proestro e si mantiene fino alla completa involuzione dell'organulo.

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