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INFLUENCE OF UNILATERAL OVARIECTOMY ON THE FOLLICULAR GROWTH OF THE RABBIT OVARY

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Introduction

Many studies on the effects of hemicastration on the compensatory growth of follicles exist.

It is, in fact, a model for the study of the renewal of follicles in the ovary and the understanding of mechanisms involved in the regulation of the number of ovulations.

Most authors concluded that, either, an accelerated rate of growth of the follicles or a decrease in the rate of atresia restored the number of follicles destined to ovulate in the remaining ovary (Hermreck and Greenwald 1964; Hirshfield 1982).

A direct estimation of the modifications in cell proliferation after hemicastration has, however, never been established. The aim of this study was to demonstrate that hemicastration in the adult rabbit induces an accelerated rate of growth of the follicles without changing the rate of atresia in the remaining ovary.

Material and Methods

46 rabbits were used, 22 females were hemispayed at 16 weeks of age and the second ovary was collected one month later. One or both ovaries of 24 sixteen weeks old females were collected as controls.

Ovaries of both control and hemicastrated rabbits were prepared for histology. The ovaries were included in paraplast and serially sectionned. All sections were mounted and stained with Schiff stain. The size of each follicle was measured on the section in which the nucleolus of the oocyte was present. Mitosis and pyknotic grains (< 150) were counted on the same section and on the two sections 25 µm distant, on either side, of the first one.

In this study, all follicles larger than 300 µm were considered since it is from this pool of follicles that the follicles destined to ovulate emerge (Hulot and Mariana 1985).

Follicles exhibiting any serious disorganization of the cell layers, such as indistinct theca and granulosa layers were discarded from the study. Follicles larger than 300 µm were gathered within 7 classes of size. The lower bounds of each class were 300, 337, 401, 476, 567, 674 and 800 µm respectively.

The results were analysed with STATITCF a statistical program. The tests were done using the 5 % threshold for the risk of first type error.

Results

We observed in the 4 000 follicles which were analysed, that granulosa cellular proliferation only stopped completely at the highest level of atresia ; that is, more than 150 pyknotic bodies.

We defined three types of atresia on the basis of the mean numbers of mitotic bodies associated to the mean number of pyknotic bodies in a follicle section.

Type 1: Where the average number of pyknotic bodies (p.b) was between 0 and 1.33 and we observed that the average number of mitotic bodies was not significantly different.

Type 2: Where the average number of p.b was between 1.33 and 80 and the average number of mitotic bodies slowly and significantly decreased.

and Type 3: Where there were between 80 and 150 p.b and the average number of mitotic bodies abruptly fell to zero

Consequently we classified follicles according to both size and the type of atresia.

The total number of follicles per ovary in the first 3 size classes (300-447 µm) was significantly different between controls and hemicastrates, whith it being lower in the hemicastrates.

For the 3 following size classes (447-800) the total number was either non significantly or significantly different (fig. 1). The number of follicles larger than 800 µm was twice more in hemicastrates than in the control ovaries.

Type 1 follicles less than 400 µm in diameter were significantly less numerous in hemicastrate than in control ovaries (table 1).

There were two fold more type 1 and type 2 follicles larger than 800 µm in hemicastrate than in control ovaries (table 1).

The relative proportions of the 3 types of follicles observed in each stage of growth were similar in control and

hemicastratre ovaries (fig 2). The proportions of early and late atretic (Type 2 and 3) follicles increased to the detriment of normal follicles (Type 1) up to the 4 th size class (566 µm). Where after the proportion of type 2 and 3 follicles decreased in favour of the type 1 follicles.

In the last size class, the preovulatory one, the proportion of late atretic follicles was still diminished but the proportion of early atretic follicles had increased to the detriment of normal follicles.

The homogeneity of the type 1, 2 and 3 follicles proportions between controls and hemicastrates was tested in each class. The proportion of type 1 follicles in class 3 (400-476 µm) was more important in the hemicastrate ovary than in the control whilst the proportions of type 2 and 3 follicles in the same size class were less important in hemicastrate ovaries relative to control ovaries.

There were no other differences between control and hemicastrate ovaries in the proportions of type 1, 2 and 3 follicles in the other size classes.

The follicular growth was partly ensured through the proliferation of follicular cells. We compared therefore, the proliferative activity of granulosa cells as measured by the mean number of mitotic bodies per follicle in each class in controls and hemicastrates (fig 3).

In all classes and for the 3 types of follicles, the average number of mitotic bodies was larger in hemicastrate than in control ovaries. It takes indeed 9.9 days on average for a given follicle of 300 µm in diameter to grow to 800 µm in diameter in the control ovary but only 7.7 days for a follicle of the same size in a hemicastrate ovary.

Discussion

One month after hemicastration, the number of follicles larger than 800 µm had doubled twice in the remaining ovary. This result concurs with the observations of Desaive (1949) and Fleming (1984) in the same species.

As Desaive we also observed one significant decrease in the number of small follicles (300 and 476 µm in diameter) in hemicastrate ovaries. The effects of hemicastration are similar to those obtained in the adult rat after stimulating the ovaries with 10 or 20 IU Ecg (Mauleon and Mariana 1977). Following gonadotropin stimulation the number of small follicles between 70 µm and 230 µm in the rat ovary decreased and the number of follicles larger than 230 µm increased.

In both cases, in the hemicastrated rabbit or in the superovulated rat, this results from the fact that the growth of follicles is not homogeneous throughout the range of follicle sizes.

The growth of follicles smaller than 300 µm in the rabbit is slower than the growth of larger follicles (Mariana et al., 1989).

After stimulation of the ovaries by Ecg follicles larger than 70 µm in the rat were propelled in the larger size classes and were not replaced by an equivalent number of smaller follicles at the same time (Mauleon and Mariana 1977). We make the hypothesis that the same phenomenon occurs in the rabbit ovary after hemicastration; follicles larger than 300 µm grow faster and accumulate in classes of size larger than 566 µm in diameter. The same phenomenon of trough is observed for the 3 types of follicles with various number of pycnotic bodies in the granulosa cell layer.

The accelerated rate of follicular growth results in part from an increase in follicular cell proliferation, as the increase in the number of mitotic bodies per section of follicle hemicastrate ovaries is significantly greater for the 5 first size classes.

The growth rate of follicles between 300 and 673 µm had accelerated and consequently these follicles grow and add the number of follicles between 673 µm and 800 µm in diameter. The rate of growth becomes slower before the last preovulatory size class where follicles accumulate and can ovulate after an LH peak.

The endocrine mechanism controling the quick renewal of preovulatory follicles which disappeared after the ablation of one ovary is known in its great lines.

During the first hours following hemicastration, basal FSH levels increase significantly and FSH ensures an accelerated rate of terminal growth of follicles toward the preovulatory stages.

Consequently, the levels of oestradiol, inhibin and FSH are restored to normal values by feedback control.

Finsheep, hemicastrated on day 14 of the cycle, ovulate on average 3 days later from the remaining ovary (Land 1973).

8 hours after hemicastrating adult rats, the FSH levels are significantly raised relative to controls at the same moment of the cycle (Welschen et al., 1974). Between 5 and 12 hours after hemicastration FSH levels are significantly higher in hemicastrated than in control Sheep (Findlay et Cumming 1977).

In the rabbit, FSH levels are elevated less than 24 hours after hemicastration and 3 days later, they are again identical to controls (Fleming et al., 1984).

The increased proliferation of granulosa cells observed in Type 1 or 2 follicles one month after hemicastration is hard to explain as the FSH levels are similar to the control as soon as 1 week after the hemicastration (Fleming 1984).

We propose the hypothesis that the rapid increase of preovulatory follicles after hemicastration in the remaining ovary induces a large local increase of the oestradiol levels.

The locally secreted oestradiol would act on the small follicles larger than 300 µm and reinforce the FSH action on stimulating the proliferation of granulosa cells.

Bradbury (1961) cleary demonstrated that a crystal of oestradiol inserted in the ovary of an immature hypohysectomized rat treated with FSH dramatically increased the number of antral follicles in the same ovary.

Goldenberg et al., (1972) also demonstrated that oestrogens interacted wiht FSH to increase granulosa cell proliferation by injecting oestrogens and FSH to hypophysectomized rats. It is also possible that FSH bioactivity had been modified, at least temporarily after hemicastration.

The transitory modification of the feedback control between the ovary and the hypothalamic system following hemicastration could induce an increase of GnRH secretion and changes in the bioactivity of secreted FSH (Huhtaniemi et al., 1988; Chappel et al., 1983).

In conclusion, one month after hemicastration, the follicular dynamics of the rabbit ovary had been significantly modified: new equilibra between the numbers of follicles in the various size classes were established and the growth had accelerated whilst pattern of atresia remained similar to controls.

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Table 1. Mean number of follicles (a) according to type and size (diameter) in control (C) and hemicastrates (H).

(b) standard deviation of the mean

* significantly different at $P < 0.05$

classes (µm) / Type of follicle		1 300 – 336	2 337 – 400	3 401 – 476	4 477 – 566	5 567 – 673	6 674 – 800	7 800 et plus
		(a) (b)						
Type 1	T	6.58 (4.97)	6.80 (4.57) *	3.58 (3.38)	2.20 (3.02)	0.98 (1.41)	1.07 (1.58)	1.83 (1.72) *
(0 – 1.33 pycnosis)	H	4.50 (2.92)	4.81 (3.37)	3.27 (2.62)	1.95 (1.91)	1.32 (2.00)	1.45 (1.60)	3.59 (2.09)
Type 2	T	0.59 (1.36)	2.46 (3.83)	4.04 (3.65) *	2.72 (2.62)	0.85 (1.67)	0.37 (0.74)	2.08 (1.48) *
(1,33 – 80 pycnosis)	H	0.32 (0.78)	1.72 (2.81)	2.09 (2.41)	2.27 (1.96)	1.27 (1.55)	0.45 (0.96)	5.31 (2.60)
Type 3	T	0.13 (0.45) *	0.91 (1.11)	3.02 (2.97) *	3.50 (3.21)	0.89 (1.22)	0.35 (0.90)	0.58 (0.88)
(80 pycnosis 150)	H	0 ( )	0.55 (0.80)	1.59 (2.04)	2.55 (3.11)	0.77 (1.02)	0.32 (0.57)	0.86 (1.16)

Table 1

Fig. 1. Mean number of follicles per ovary according to diameter in control and hemicastrates.

( ) standard deviation of each mean is given in brackets.

* significantly different at $P < 0.05$.

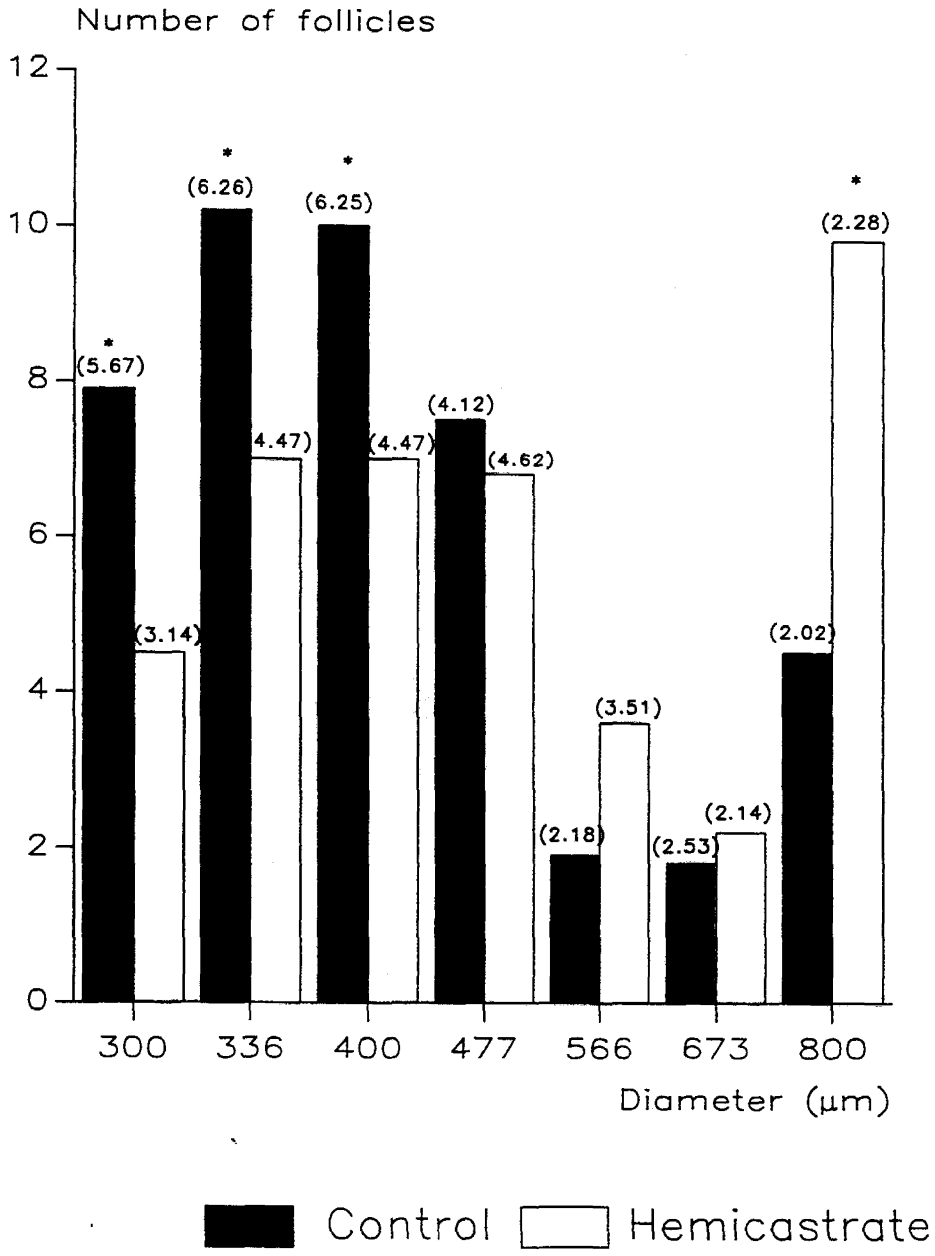

Fig. 2. The relative proportions of the 3 follicles.

Types per ovary according to size in controls and hemicastrates.

Type 1 + Type 2 + Type 3 = 100 %

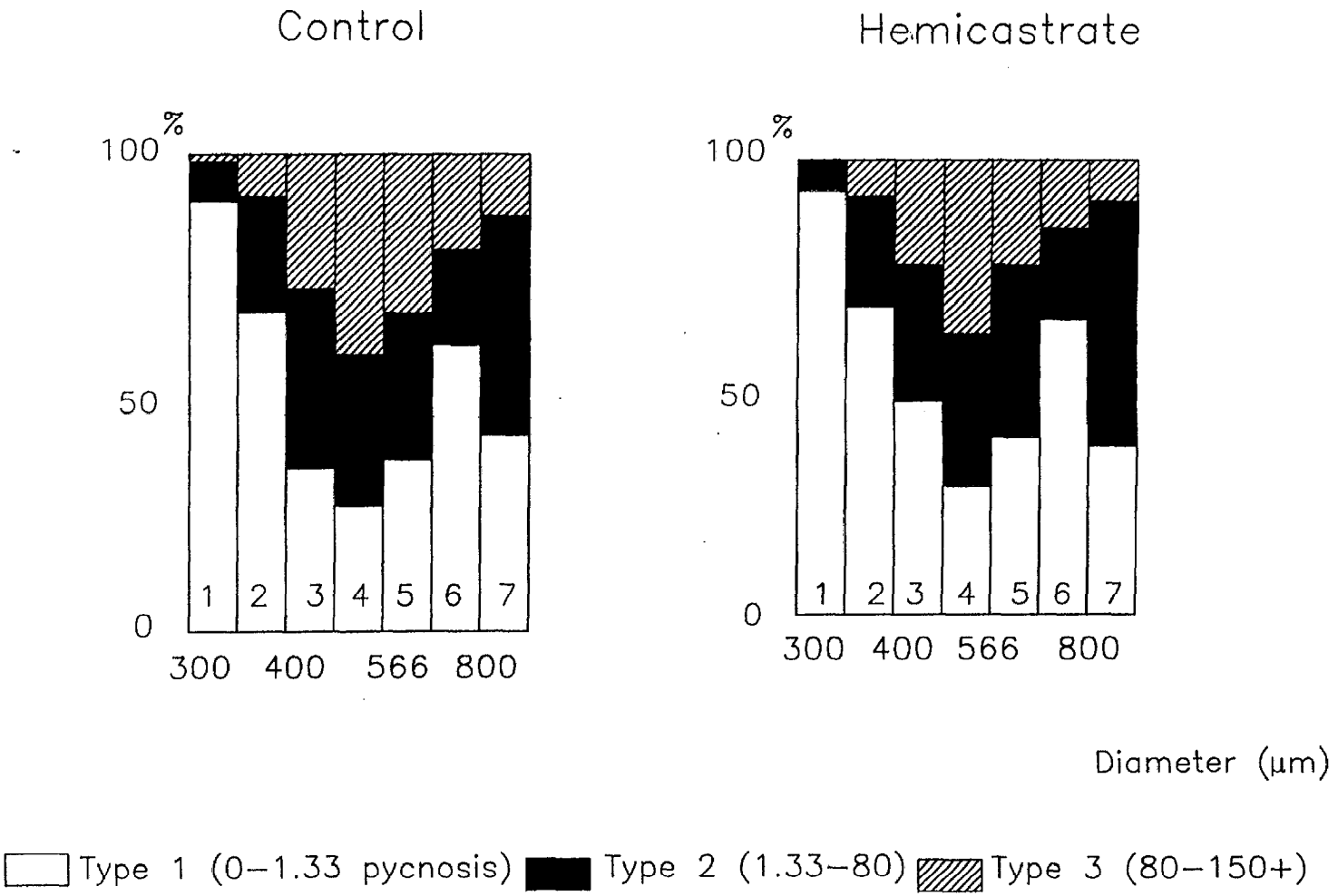

Fig. 3. Mean number of mitotic cells according to diameter, follicles type (1, 2 and 3) (see text) and treatment in control (C) and hemicastrates (H).

Fig 3