RESEARCH REGARDING THE INFLUENCE OF CELLULOSE ON THE MORPHOLOGICAL AND STRUCTURAL DEVELOPMENT OF RABBIT OVARY

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ABSTRACT

The biologic material used in the research was represented, on one hand, by 4 batches of female animals (10 heads per batch) belonging to Oryctolagus cuniculus domesticus (OCD) breed and by a series of specific mixed fodders, on the other hand. During the period of the experiments, into the feed provided to the animals form the 4 batches, were used specific mixed fodders with the following nutritional characteristics: 2500 kcal DE/kg, 160 g CP/kg, 12-17 g Ca/kg and 7-10 g P/kg. The feed distributed to the animals from the 4 batches were different regarding the level of crude (raw) cellulose, so, the recipe R1 administrated to the control batch (LM) had 12% RC (100%); the one administrated to the experimental batch 1 (LE1) had 16% RC (+33.3% face to LM); the one administrated to the experimental batch 2 (LE2) had 20% RC (+66.7% face to LM) and the one administrated to the experimental batch 3 (LE3) had 24% RC (double face to LM). The high content in raw cellulose (16-24%) from the feed administrated to the studied animals didn't influence significant negative the structure and the functionality of the ovaries and also didn't influence the appearing and development of the puberty stage at the studied doe.

Keywords: *rabbits, raw cellulose, ovary*

INTRODUCTION

The development of the animal organism, in general, and of genital apparatus, particularly, is influenced by various factors such as: breed, race, individual, age, sex, alimentation (the most important environmental factor) (*Stan Gh. and Simeanu D., 2005*) etc.

At breed *Oryctolagus cuniculus domesticus*, at females, the development of gonads and the appearing of puberty, seems to be produced, in normal feeding and maintenance conditions, around the age of 100 days. At this age, the ovo-genesis is active, in ovarian cortical becoming mature in the same time a high number of ovarian follicles (*Bram L., 1974*; *Teuşan V., and col., 2007*).

Because the studied breed, consume an exclusive vegetal food, rich in nutrients and parietal carbohydrates, we try to study if the increase over the normal limits of raw cellulose in doe dietary ratios have any influence over the development of ovaries and on appearing of puberty stage at these animals (*Halga P., and col., 2005*).

MATERIAL AND METHOD

Biologic material used in our research was represented on one hand by 4 batches of females animals belonged to breed *Oryctolagus cuniculus domesticus* (OCD) and by a series of specific mixed fodders, on the other hand.

The animals represented the three-line hybrid "Cunirom-PF_{310}", obtained by crossing the parental lines P_{231} and P_{232}. Were divided in four batches of 10 individuals each, were grown from weaning age to the age of 120 days.

At the age of 120 days the average live weight of the animals was around 2500 grams. During the experimental period, in the food provided to the animals from the 4 batches, were used specific mixed fodders, noted with symbols R1 – R4. Those fodders were characterised by an energetic content 2500 kcal DE/kg; protean content of CP/kg and by an adequate content in Ca (12-17 g/kg) and in P (7-10 g/kg).

The food distributed to the animals from the 4 batches was different by raw cellulose level. So, recipe R1 administrated to the control batch (LM) had a content of 12% raw cellulose (RC) (100%); the one administrated to the experimental batch 1 (LE1) had a content of 16% RC (with 33.3% higher, in comparison with LM); the one administrated to the experimental batch 2 (LE2) had a content of 20% RC (with 66.7% higher, in comparison with LM) and the one administrated to the experimental batch 3 (LE3) had a content of 24% RC (double face to LM) (*table 1*).

Table 1. The experimental design

Batch	Nr. of animals (heads)	Recipe type	Raw cellulose	
			(g/kg)	(%)
LM	10	R1	**120**	**100.0**
LE1	10	R2	**160**	**133.3**
LE2	10	R3	**200**	**166.7**
LE3	10	R4	**240**	**200.0**

At the age of 120 days, the animals from the 4 batches were slaughtered and the ovaries were taken and after that were placed in a Bouin fixative for 72 hours. Later the histological parts gathered were processed in according with the technique of paraffin sectioning, so the samples were dehydrated in alcohol baths, clarified with amyl-alcohol, impregnated and included in paraffin. The cutting of paraffin blocks was done with a microtome realising serial sections with a width of 5 µ. These were displayed and trichromic stained with hematoxylin, eosin and methylene blue (HEA) (*Vaissaire J.P., 1972*; *Gondos B., 1970*).

After mounting and drying, the lamellas with samples were studied at a photonic binocular microscope. The apparatus have 3 types of oculars

and 4 types of lens, but the observations of microscopic fields were made with 3 associations of oculars and lens, respectively, at association OC 10 × OB 10; at association OC 10 × OB 20 and at association OC 10 × OB 40.

In the microscopic field were observed and measured different histological structures at the level of ovary cortical. So were measured: the diameters of primordial, primary, secondary, and mature ovarian follicles. The most representative microscopic aspects were photographed with an EXA-1-A apparatus type at three associations of OC and OB, respectively: OC 16.8 × OB 10 (168 zoom); OC 16.8 × OB 20 (336 zoom) and OC 16.8 × OB 40 (672 zoom).

Microscopic measurements were made using an ocular micrometer after the apparatus was previous calibrated. Calibration was made: at association OC 10 × OB 10, calculating a micrometric value of 9.0000 μ; at association OC 10 × OB 20, with a micrometric value of 4.4444 μ and at association OC 10 × OB 40, when the calculated micrometric value was of 2.3775 μ (*Vaissaire J.P., 1972*; *Gondos B., 1970*).

The food administrated to the animals was characterized regarding the recipe structure and the brut chemical content by the digestibility of organic substance and nutritive content (*table 2*).

Table 2. The structure and the nutritional parameters of the mixed fodders recipe used in experiments

Raw materials (%)	Recipe:			
	R1	R2	R3	R4
Corn	10.0	11.0	18.7	8.0
Barley	10.0	11.0	2.0	-
Oat	17.0	15.0	2.0	-
Soybean grist	9.2	2.5	9.0	7.5
Sunflower grist	-	10.0	9.0	10.0
Fodder yeast	2.0	2.0	2.0	1.0
Fish meal (flour)	1.0	1.0	1.0	2.0
Wheat bran	29.0	13.0	1.0	1.0
Alfalfa meal (flour)	13.0	31.0	39.0	51.0
Milled wheat straw	3.8	-	9.5	10.7
Feed fats	-	0.3	3.8	6.0
Calcium carbonate	1.0	1.2	1.0	-
Monocalcium phosphate	3.0	1.0	1.0	1.8
Premix (%)	1.0	1.0	1.0	1.0
TOTAL	100.0	100.0	100.0	100.0
Nutritional parameters				
Digestible energy – kcal/kg m.f.[1]	2502	2510	2495	2488
– MJ/kg n.c.	10.46	10.49	10.43	10.40
CP[2] (g/kg)	160.0	160.2	160.1	159.9
RC (g/kg)	120.0	160.0	200.0	240.0
RSO[3] (g/kg)	764.4	800.4	778.8	793.6
DOS[4] (%)	72.5	72.0	61.5	54.3
Ca (g/kg)	17	12	12	12
P (g/kg)	10	7	7	7

Note: [1] m.f. –mixed fodder; [3] RSO – raw organic substance;
[2] CP – crude protein; [4] DSO – digestibility of organic substance.

The values that characterised the dimensions of different structures from ovarian cortical were statistical processed and interpreted.

RESULTS

The analyse of the histological samples obtained through processing and section of ovaries from the animals from the four batches, put in light some very interesting aspects.

So at the level of ovarian cortical could be observed numerous primordial follicles which contain ovo-gonies. Those primordial follicles have an average diameter of 45.45 ± 0.491 µ at the control batch (LM); 42.84 ± 0.520 µ at batch LE1; 38.02 ± 0.393 µ at batch LE2 and of 39.15 ± 0.437 µ at batch LE3 (*table 3*) and (*photo 1*). Ovo-gonies have a diameter of

24.35 – 28.82 µ. At the ovarian surface stands out clearly a simple cubic germinal epithelium.

Table 3. Statistical significance of the differences between the studied batches regarding some structural characteristics at ovary level

Specification	Mean of the compared batches (µ)	Differences between means (µ)	F calculated at 1; 38 GL	F_α at 1; 38 GL for			Tukey w=0.01	Statistic signif.
				p≤0.05	p≤0.01	p≤0.001		
Diameter of primordial ovarian follicles (µ)	LM=45.45	2.61	2.590	4.098	7.360	12.746	4.396	n.s.
	LE1=42.84							
	LM=45.45	7.43	33.622				3.472	***
	LE2=38.02							
	LM=45.45	6.30	20.980				3.729	***
	LE3=39.15							
	LE1=42.84	4.82	11.912				3.782	**
	LE2=38.02							
	LE1=42.84	3.69	6.194				4.214	n.s.
	LE3=39.15							
	LE2=38.02	1.13	1.047				2.980	n.s.
	LE3=39.15							
Diameter of primary ovarian follicles (µ)	LM=83.81	7.54	3.889	4.098	7.360	12.746	10.362	n.s.
	LE1=91.35							
	LM=83.81	9.00	4.759				11.184	n.s.
	LE2=92.81							
	LM=83.81	7.99	3.910				10.951	n.s.
	LE3=91.80							
	LE1=91.35	1.50	0.128				11.083	n.s.
	LE2=92.81							
	LE1=91.35	0.45	0.013				10.848	n.s.
	LE3=91.80							
	LE2=92.81	1.05	0.056				11.636	n.s.
	LE3=91.80							
Diameter of secondary ovarian follicles (µ)	LM=142.2	4.50	0.806	4.098	7.360	12.746	13.591	n.s.
	LE1=137.7							
	LM=142.2	6.50	2.095				12.221	n.s.
	LE2=135.7							
	LM=142.2	1.10	0.068				11.731	n.s.
	LE3=143.3							
	LE1=137.7	2.00	0.238				11.253	n.s.
	LE2=135.7							
	LE1=137.7	5.60	2.024				10.719	n.s.
	LE3=143.3							
	LE2=135.7	7.60	5.407				8.919	n.s.
	LE3=143.3							
Diameter of mature ovarian follicles (µ)	LM=653.85	23.18	0.494	4.098	7.360	12.746	89.359	n.s.
	LE1=630.67							
	LM=653.85	19.80	0.782				60.683	n.s.
	LE2=634.05							
	LM=653.85	19.35	0.924				54.588	n.s.
	LE3=634.50							

	LE1=630.67 LE2=634.05	3.38	0.011				86.592	n.s.
	LE1=630.67 LE3=634.50	3.83	0.016				82.436	n.s.
	LE2=634.05 LE3=634.50	0.45	0.001				49.933	n.s.
Diameter of oocytes of I order (μ)	LM=75.58 LE1=73.57	2.01	0.118	4.098	7.360	12.746	15.782	n.s.
	LM=75.58 LE2=77.51	1.93	0.117				15.338	n.s.
	LM=75.58 LE3=74.83	0.75	0.019				14.638	n.s.
	LE1=73.57 LE2=77.51	3.94	0.566				14.173	n.s.
	LE1=73.57 LE3=74.83	1.26	0.065				13.412	n.s.
	LE2=77.51 LE3=74.83	2.68	0.317				12.886	n.s.
Diameter of oocytes of II order (μ)	LM=109.01 LE1=115.31	6.30	3.799	4.098	7.360	12.746	8.766	n.s.
	LM=109.01 LE2=117.34	8.33	6.014				9.206	n.s.
	LM=109.01 LE3=117.00	7.99	6.955				8.214	n.s.
	LE1=115.31 LE2=117.34	2.03	0.310				9.857	n.s.
	LE1=115.31 LE3=117.00	1.69	0.262				8.937	n.s.
	LE2=117.34 LE3=117.00	0.34	0.009				9.369	n.s.

Note: n1, n2, n3, n4 = 20.

Comparing the dates regarding the dimensions of those follicles, between the four batches, we observe that are very significant differences from statistic point of view between LM and LE2 and between LM and LE3 and distinct significant differences between batches LE1 and LE2. The rest of the differences are statistical insignificant.

In ovarian cortical (at all the four batches) could be also observed an appreciable number of primary, secondary, tertiary and mature follicles, however, the last ones being less.

Primary follicles have dimensions that varies between 83.81 ± 0.781 µ at LM and 92.81 ± 0.832 µ at LE2, they contain oocytes of I order, which at their turn are characterized by an average diameter of 73.57 ± 0.924 – 77.51 ± 0.893 µ. The nucleus of those cells were spherical or oval, were placed central or eccentric and had the dimensions of 28.1 ± 0.454 – 31.77 ± 0.460 µ. Note that oocytes of I order were measured at the level of primary ovarian follicles and also at the level of the secondary ones.

Secondary ovarian follicles are quite numerous in ovarian cortical, at all the studied animals, having dimensions (diameters) of 135.67 ± 0.746 – 143.33 ± 0.693 µ (*table 4*). They are multi-cellular ovarian buildings in which develop (grow) oocytes of I order, these ones being surrounded by two rows of follicle cells with low waist (8 - 10 µ) and by internal follicular sheath (*photo 2, 3 and 4*).

Mature ovarian follicle were quite few, but had the greatest dimensions, respectively 630.67 ± 2.517 µ (at LE1) – 653.85 ± 1.941 µ (at LM) (*table 4*). They contain oocytes of II order (haploid cells), a follicular cavity full with follicular liquid, radial granulosa crown, Slawianski membrane and at exterior two follicular sheaths. Oocytes of II order had diameters of 109.01 ± 0.684 – 117.34 ± 0.773 µ (*table 4*), eccentric nucleus with dimension of 29.77 ± 0.411 – 39.36 ± 0.387 µ, a lot of nutritive yolk and a thick yolk membrane and very visible (*photo 5 and 6*).

Internal follicular sheath from the level of mature ovarian follicles is well represented and well vascularized, presenting all the premises for a normal production of oestrogen hormones (*photo 7*). Also interstitial gland of the ovary is well developed and with an intense mitotic activity (*photo 9 and 10*). Was shown also the presence of yellow bodies, which put in light the follicular dehiscence as also the presence of mature ovaries (*photo 8*).

In some cases was observed some mature ovarian follicles which contain two oocytes, fact which we appreciate that belongs to some multiparous breeds (fox).

As regarding the differences founded between the four studied batches regarding the dimensions of the primary, secondary and mature ovarian follicles or the dimensions of oocytes I and II and of ovocitar nucleus, the concretion of statistical analyses show that those differences are insignificant. This denotes the fact that the administrated food did not influence in a negative way neither the structure nor functionality of the ovaries nor the appearing of puberty state at those animals. The literature confirms the fact that genetic factors are the ones which influence in a decisively way the evolution and functionality of females' glands. In our

opinion, it seems that this thing is confirmed by the situation founded at the level of primordial follicles (differences distinct or very significant between some batches), which are the ones with whom the animal starts his extra-uterin life and their number and size are genetically determinate. Anyway the morph-structural picture of the ovaries at the females from the four batches, present all the animals' characteristics which could be found at the end of puberty period.

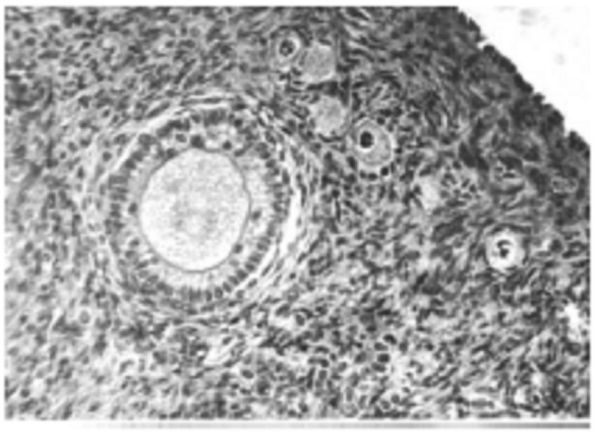

Photo 1 Ovarian cortical with primordial follicles and ovo-gonies. Secondary ovarian follicle with oocyte I. At the surface, simple cubic germinal epithelium. (16.8 x 10) (LM-LE1).

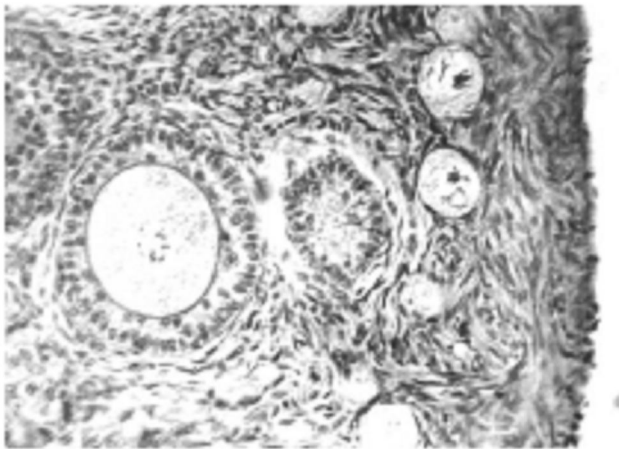

Photo 2 Ovarian cortical with primordial follicles, ovo-gonies, secondary follicle with oocyte I (16.8 x 10) (LE2-LE3).

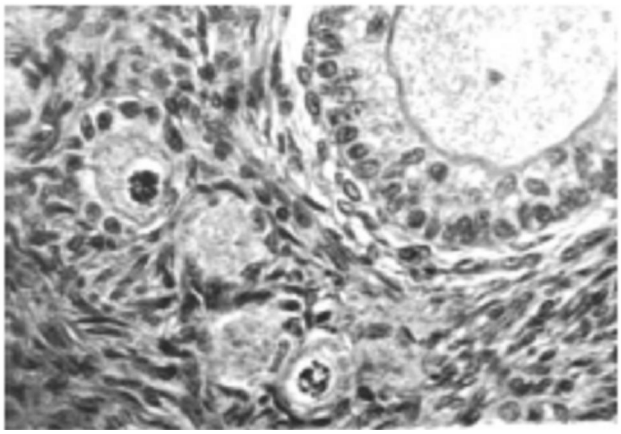

Photo 3 Ovarian cortical with primordial follicles and ovo-gonies. Tertiary follicle and oocyte I (16.8 x 20) (LM-LE3).

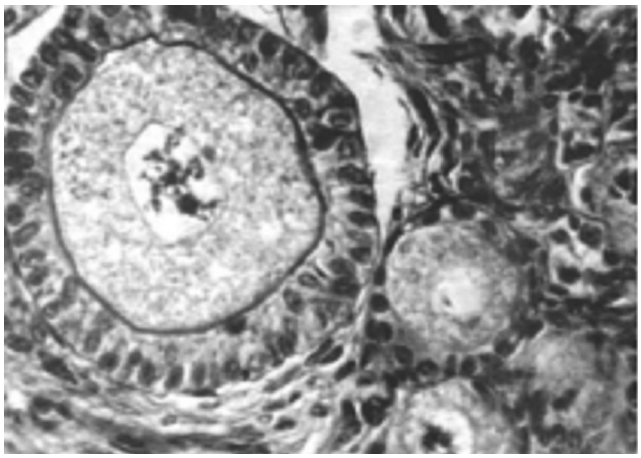

Photo 4 Ovarian cortical, primary and secondary ovarian follicles. It could be clearly observed the oocyte I with membrane, nucleus and its chromatin. (16.8 x 40) (LM-LE3).

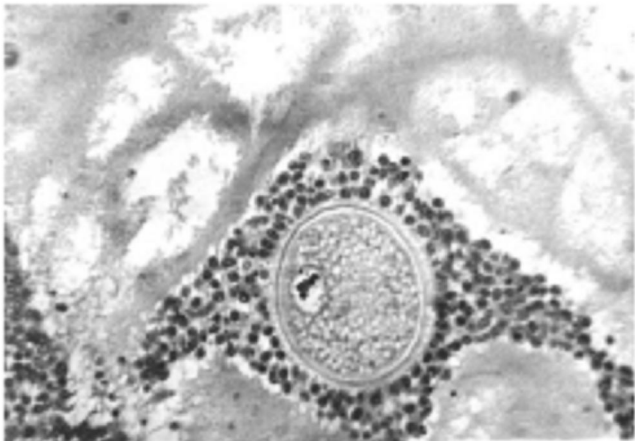

Photo 5 Oocyte II in mature ovarian follicle. It could be observed: radial crown, proliger discs and a fragment of granulosa. The nucleus of oocyte II is eccentric placed, and the nutritive yolk is well presented. (16.8 x 20) (LM-LE3).

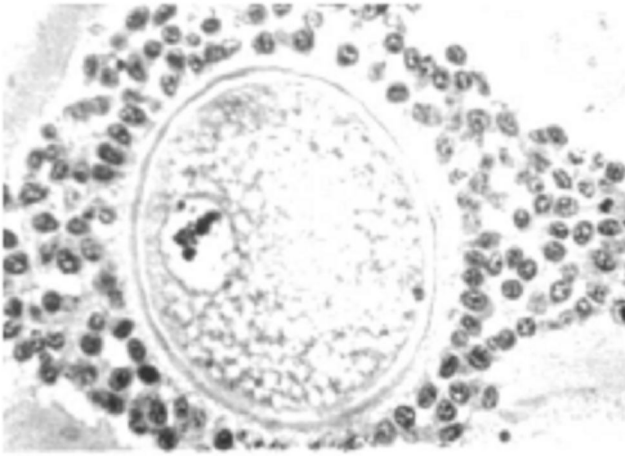

Photo 6 Oocyte II. It could be observed the yolk membrane and pellucida area, eccentric nucleus, nucleolus and chromatin in nucleus. (16.8 x 40) (LM-LE3).

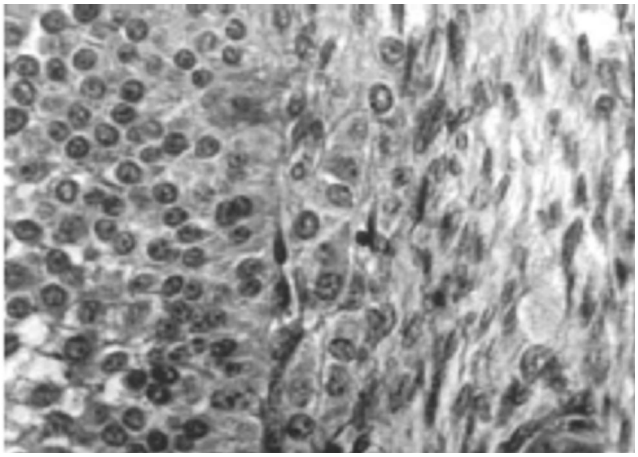

Photo 7 A part of a mature ovarian follicle. It could be observed: granulosa, Slawianski membrane and internal follicular sheath with theca cells that produce oestrogen hormones. (16.8 x 400) (LM-LE3).

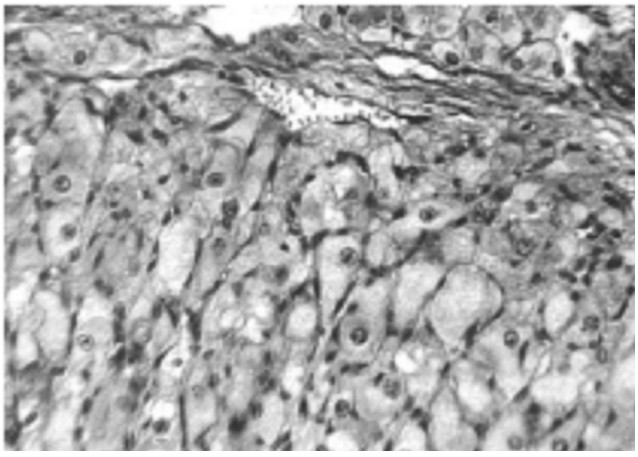

Photo 8 Yellow body (fragment). It could be observed LH cells, of an oval or even triangular shape, with their nucleus. Also could be observed sanguine vessels. (16.8 x 20) (LM-LE3).

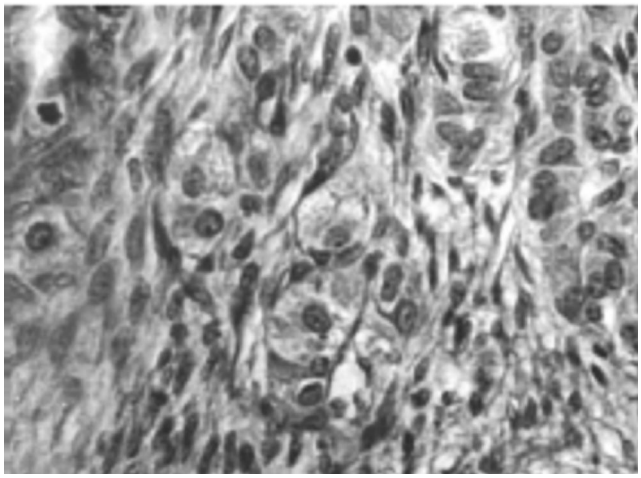

Photo 9 Ovarian interstitial gland at rabbits (spherical or oval cells with oval nucleus). (16.8 x 20) (LM-LE3)

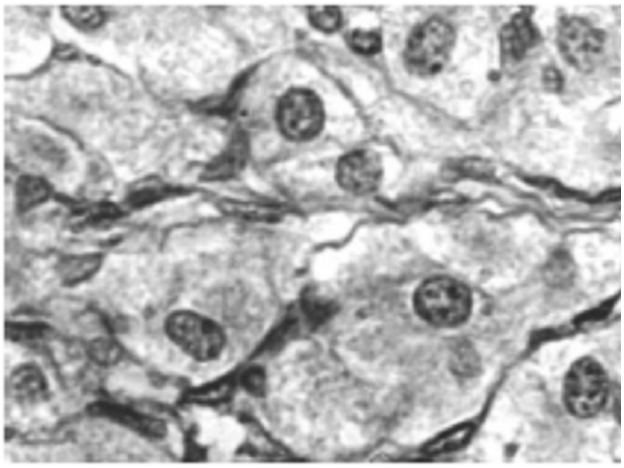

Photo 10 Ovarian interstitial gland at doe. (16.8 x 40) (LM-LE3).

CONCLUSIONS

1. High content of raw cellulose (16-24%) from the administrated food of the studied doe didn't influence in a negative way the structure and functionality of ovary.
2. As regarding the appearance and evolution of puberty stage at the studied doe we didn't observe a significant negative modification in the case in which the food had a high content in raw cellulose (16-24%).

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