Great Basin Naturalist

Volume 1 | Number 2 Article 5

1-28-1940

A preliminary histological study of the ovary of the kangaroo rat *Dipodomys ordii columbianus*

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A PRELIMINARY HISTOLOGICAL STUDY OF THE OVARY OF THE KANGAROO RAT DIPODOMYS ORDII COLUMBIANUS

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INTRODUCTION

The kangaroo rat, *Dipodomys ordii columbianus* Merriam, is a member of the Heteromyidae, and is well represented in the semi-arid portions of the central Utah region of the Great Basin.

The literature dealing directly with Dipodomys appears to be rather limited. Grinnell (1919, 1922, 1929) has described the geographic distribution of Dipodomys and some new species from California. Vorhies and Taylor (1922) published a paper dealing with the life history of the genus. The implantation of the blastocyst was studied by Lee (1918), and a brief analysis of the placentation and fetal membranes was published by Mossman (1937 a). Midgley (1938) described the visceral anatomy from a comparative point of view. Other papers dealing with this group may have been published, but I have seen only those mentioned. In this paper a preliminary histological study of the kangaroo rat ovary is described.

MATERIAL AND METHODS

The material consists of the ovaries of 42 animals. Thirty of the specimens were taken alive, while 12 were dead in the traps. Although a year-round collection is highly desirable for a study of this kind, only animals taken during January (19 animals), March (1 animal), April (2 animals), June (1 animal), July (3 animals), August (1 animal), and September (15 animals) have been available for study.

All the ovaries, with the exception of two sets, were fixed in Bouin's fluid. The two exceptions were fixed in Allen's B-15 modification of Bouin's fluid. Most of the ovaries were sectioned at seven micra, although some were cut at ten. Sections were mounted serially and stained with hematoxylin (both Delafield's and Ehrlich's) and eosin.

I wish to thank Dr. Vasco M. Tanner of Brigham Young University, Provo, Utah for sending me the ovaries of 18 of the animals. The animals were collected about two miles northwest of Provo, Utah.

OBSERVATIONS

GENERAL DESCRIPTION. The ovary of Dipodomys is small (1.3 x 1.3 x 1.9 mm.) and is non-lobulate. It is covered by a very thin germinal epithelium whose nuclei are similar to those of mesothelial cells. In some ovaries small groups of cells of the germinal epithelium penetrate the thin tunica albuginea, and some of their nuceli are enlarged. Here and there oocytes are to be seen with their outer surfaces flush with the surface of the germinal epithelium (fig. 1).

The peripheral region of the ovary, below the tunica albuginea is usually occupied by a varying number of oocytes and growing follicles. The oocytes may occur singly or in nests of two or more (fig. 2). Follicles in which the granulosa is made up of several layers of cells are usually found deeper in the ovary than are the smaller follicles, although both large growing follicles and Graafian follicles are to be found relatively near the surface.

Unless degeneration overtakes a follicle the mature Graafian follicle frees its ovum into the oviduct by the process of ovulation. Some or all (the origin of the corpus luteum is beyond the scope of this paper) of the remaining cellular layers of the ruptured follicle are modified into the lutein cells of the corpus luteum. If degeneration occurs in any follicle in which the thecal layers are differentiated, the cells of the theca interna metamorphose into the interstitial cells of the ovary. Between the follicles are found spindle-shaped stroma cells, interstitial cells, and fibrous connective tissue containing blood vessels.

THE GROWTH OF THE FOLLICLES. The primary oocytes usually lie in the outermost part of the cortex. They are small (13-18 micra in diameter) with a round, centrally located nucleus (10-12 micra in diameter). The oocytes are naked, as a rule, excepting for one or two small fibroblastic-like nuclei which lie against the cell membrane. Oocytes are found: (a) immediately below the germinal epithelium; (b) within the tunica albuginea; and (c) below the tunica in the cortex. Throughout this paper the follicles will be designated as types 1, 2, 3, and Graafian follicles.

Follicles of type 1 are as a rule found below the tunica albuginea, although in some instances they have been seen just beneath the germinal epithelium. The ova of such follicles have a diameter of 16-26 micra with a centrally located nucleus approximately 12 micra in diameter. The ova are surrounded by one complete layer of cubic epithelial cells, resulting in a follicle of 26-48 micra in diameter. Occasionally, especially in the larger follicles of this type, a rather delicate zona pellucida is seen around the ovum and also a basement membrane can be seen between the follicle cells and the stroma.

The ova and nuclei of type 2 follicles are larger than those of type 1. The follicular diameter measures 52-68 micra, the ova 26-48 micra, and the nuclei 12-18 micra in diameter. One complete layer of follicular cells is always present and the beginning of a second layer is not uncommon. The nucleus is still in a central position within the ovum. The zona pellucida and the basement membrane of the follicle cells are more distinct in this type (fig. 3) than in type 1. External to and contiguous with the basement membrane are small fibroblastic cells and delicate fibres derived from the stroma. These cells and fibres are the anlagen of the future thecal layers. Type 2 follicles are usually located beneath the tunica albuginea.

Type 3 follicles are always situated beneath the tunica albuginea and are usually rather deep in the cortical region. The ova of these follicles are much larger than those of type 2, being 52-68 micra in diameter. The nucleus is also larger, 23-37 micra in diameter, and as a rule occupies an eccentric position in the cytoplasm of the ovum. Follicles of this type possess from three to nine or more layers of follicle cells in the stratum granulosum. The increase in number of follicle cells is the result of mitosis for different stages of the division process are quite common, not only in type 3 follicles, but of types 2 and 1 as well. The zona pellucida is very distinct in most follicles of this type and both thecal layers are present. The cells of the theca interna are larger and oval, or even round, while those of the theca externa are spindle-shaped. Mitoses are common in cells of the theca interna, and tiny blood vessels vascularize the layer.

The Graafian follicles contain ova of about the same size (52-70 micra in diameter) and appearance as the preceding type. One or several fluid-filled antra of varying sizes are located in the stratum granulosum. All stages of mitosis are to be seen in the follicle cells. The theca interna of many Graafian follicles is a thickened and distinct structure in comparison to the theca externa of the same follicles. The ratio of cytoplasm to nucleus is much greater in cells of the theca interna than it is in the theca externa, and the nuclei of the former are large and oval or round while those of the latter retain their resemblance to the stroma cells, with which they imperceptibly merge. The vascularity of the theca interna is more apparent in this type than it is in type 3 follicles. A comparison of the diameters of ova and nuclei of the various follicular types is shown in fig. 7.

Follicular Atresia. Any of the above mentioned follicles may degenerate by the process of atresia. In general all the component parts of a follicle undergoing atresia ultimately atrophy. But that part of the follicle in which atresia is first recognizable is variable. Table I indicates the amount of atresia observed in each ovary.

In some follicles (types 1 and 2 as a rule) the ovum seems to be the first part of the follicle to exhibit signs of atresia. The cytoplasm of the ovum shrinks away from the granulosa cells, thus giving the ovum a shrunken, irregular appearance. The nucleus also becomes irregular in shape and may disappear, leaving a hyalinized contorted mass which stains heavily with eosin. The granulosa may remain intact (fig. 4), or it may disintegrate and some of its cells invade the degenerating ovum.

In other follicles (Graafian, and usually those of type 3) the first recognizable signs of atresia are generally seen in the follicular cells and not in the ovum. The cells of the stratum granulosum immediately surrounding the ovum pull away from the ovum, their nuclei become pycnotic, and these pycnotic cells frequently invade the follicular cavity (fig. 6).

The first maturation spindle may form in the ova of atretic follicles of type 2 and 3 and Graafian follicles. Occasionally in the latter the first polar body may be formed (figs. 5 and 6). The fate of the thecal layers of atretic follicles will be considered in the following section.

Interstitial Cells. In many ovaries, surrounding atretic follicles and scattered throughout the stroma, are masses or blocks of cells which I have called interstitial cells. The amount of this tissue within an ovary varies, being practically absent in some and very abundant in others.

These patches or blocks of interstitial cells appear under low power as rather homogenous light-stained areas containing several nuclei. With high power, or better still with oil immersion, the cytoplasm appears more or less vacuolar, cell membranes are very faint and often absent, and the shape of the nuclei varies from a smooth oval or round contour to an irregular, crenated-appearing contour.

The interstitial cells originate from the theca interna of atretic follicles. In some atretic follicles the theca interna has lost the appearance characteristic of normal follicles—the cell walls have disappeared, the cytoplasm has become more vacuolar and has little or no affinity for eosin, and the nuclei have a crenated or irregular shape.

That the presence of these interstitial cells may be cyclic or periodic is suggested by the varying amounts in which they are found in the various ovaries. Table I gives the relative amount of interstitial cells in each ovary.

EXPLANATION OF TABLE I

It is realized at the outset that the survey summarized in this table is superficial and possibly inadequate. Yet it will serve as a basis for comparison of the animals making up the collection here studied.

These counts are of one section, as near the central portion of the ovary as could be determined, for each animal. The data on the oocytes and follicles and corpora lutea are more accurate than that on the interstitial cells, since the follicles, corpora, etc. could be counted, while in the case of the interstitial cells only an estimate could be made since no way has been found to make a quantitative study of them.

TABLE I

Specimen No.	Date taken	No. oocytes	No. foll.	% Foll. atretic	Corp. lut.	Inst. cells	Stage of preg.
78	1/16	13	15	47%	..	**	......
79	1/16	16	29	41	..	**	......
80	1/17	5	27	52	..	***	......
81	1/17	28	11	45	2	**	......
82	1/17	18	17	41	..	**	Bilaminar blastocyst
83	1/17	51	11	9	..	**	......
84	1/18	43	23	48	..	**	......
85	1/18	45	12	17	..	***	ovulation
86	1/18	65	16	44	..	**	......
87	1/18	104	11	36	1	*	Bilaminar blastocyst
88	1/18	31	26	50	..	**	......
89	1/22	11	22	59	..	***	......
90	1/22	42	19	21	..	**	......

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TABLE I (cont.)

Specimen No.	Date taken	No. oocytes	No. foll.	% Foll. atretic	Corp. lut.	Inst. cells	Stage of preg.
91	1/22	13	22	36	..	**	......
92	1/22	19	23	30	..	**	......
93	1/22	57	10	40	1	***	11 mm. embryos
94	1/22	15	14	71	1	**	5 mm. embryos
95	1/22	44	14	64	2	***	......
96	1/22	67	26	42	2	**	......
12	3/10	6	10	30	2	**	......
97	4/5	62	11	36	..	***	......
98	4/5	19	27	37	..	***	......
70	6/5	Tissue unsuited for study					13 mm. embryos
74	7/2	5	3	0	1	**	......
68	7/10	108	29	38	..	*	......
1	7/30	52	9	11	..	**	......
3	8/20	4	5	20	..	***	......
69	9/1	..	14	50	..	***	......
76	9/2	14	7	57	..	***	......
77	9/3	5	9	44	..	**	......
75	9/4	13	11	18	..	**	......
72	9/7	25	17	65	..	***	......
71	9/8	Tissue unsuited for study					
73	9/8	42	15	40	..	**	......
4	9/15	18	18	22	1	**	8 mm. embryos
5	9/21	4	16	69	2	***	Bilaminar blastocyst
6	9/21	88	10	30	1	***	Stage unknown

TABLE I (cont.)

Specimen No.	Date taken	No. oocytes	No. foll.	% Foll. atretic	Corp. lut.	Inst. cells	Stage of preg.
7	9/22	85	8	0	1	**	......
8	9/22	32	20	45	2	***	16 mm. embryos
9	9/22	81	21	19	..	**	15 mm. embryos
10	9/22	6	8	13	2	***	22 mm. embryos
11	9/22	41	5	60	..	***	......

DISCUSSION

It is not the purpose of this paper to discuss the literature dealing with ovarian histology and cytology, since that has been adequately done by several workers. The reader is referred to Corner's discussion and bibliography in Cowdry's Special Cytology (1932).

Post-natal and post-pubertal ovogenesis in mammals is still a field for intensive investigation. Hargitt (1930 a and b) is convinced that there is a continuous production of germ cells from the germinal epithelium of the ovary of the albino rat throughout the reproductive period. Mossman (1937 b) and Pliske (1938) have indicated that the same is true in the pocket gopher and thirteen-lined ground squirrel. Mossman's paper is more a suggestion than a verified conclusion of post-pubertal ovogenesis, since he deals with ovogenesis only incidentally to a study of the so-called thecal gland. On the other hand, Pliske's study on the ground squirrel deals directly with the problem of the follicular cycle, and he concludes that the process of ovogenesis continues in the adult. Both the type of germinal epithelium and tunica albuginea of the ovary of Dipodomys suggest this animal as a possible type for such a problem if the difficulties of determining the age of specimens and of making large enough collections can be overcome.

The term "interstitial cells" is one more of convenience than anything else. This is due (1) to the fact that the interstitial cells of the ovary constitute a point of controversy, different investigators differing in their conception of the term, and (2) the material used for this study was not fixed for cytological study.

It would be worth while to study thoroughly the cytology and fate of these cells. As has been mentioned before, they appear to be cyclic or periodic in nature. The number of these cells in any one ovary varies; their degree of vacuolation varies; in some ovaries their cell membranes can be seen while in others they cannot; and in some ovaries the nuclei of these cells are rather smooth in outline, while in others they are very irregular. It is known that some other mammals exhibit a cyclic production of interstitial cells; Rasmussen (1918) and Guthrie and Jeffers (1938) found this to be the case in the woodchuck and bat respectively. However, any similarity between the woodchuck or bat and the kangaroo rat in this respect would be limited, since the interstitial cell cycle in the woodchuck and bat is correlated with hibernation, which in turn affects the reproductive cycle. Evidence from this study indicates that the kangaroo rat does not hibernate, viz., different stages of pregnancy have been found in January, March, June and September (see Table I). Then, too, Table I seems to show no correlation between the number of interstitial cells present and the time of year the animal was collected. All this would seem to argue strongly for year-round activity, which in turn would have its effect upon the reproductive cycle.

Although the ovary of the kangaroo rat is probably quite similar to that of other rodents, yet it seems that the study of a complete cytologically fixed and well prepared collection might possibly yield some light on points that are now controversial.

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EXPLANATION OF FIGURES

Figures 1-6 were drawn with a camera lucida at the magnifications indicated.

List of abbreviations:

bm . . . . basement membrane
f ep . . . follicular epithelium
ge . . . . germinal epithelium
ms . . . . maturation spindle
nu . . . . nucleus
oc . . . . oocyte
ov . . . . ovum
p b . . . . polar body
p f ep . . pycnoctic follicular epithelium
th . . . . undifferentiated thecal layers
zp . . . . zona pellucida

PLATE I

Figure 1. Oocyte just below germinal epithelium. x 970.
Figure 2. Nest of four oocytes beneath the germinal epithelium. x 970.
Figure 3. Follicle of type 2. The zona pellucida and basement membrane are both clearly differentiated. x 970.
Figure 4. Atretic follicle of type 2. The ovum is hyalinized and shrunken. Granulosa layer (f ep) is intact. x 430.
Figure 5. Ovum of atretic Graafian follicle, showing first polar body. x 430.
Figure 6. Ovum of an atretic follicle of type 3. Pycnotic cells of follicular epithelium have become loosened and pulled away from the ovum. Maturation spindle is present. x 430.
Figure 7. Chart showing the relationship of nuclear diameter to ovum diameter in follicles of types 1, 2, 3, Graafian follicles, and oocytes.

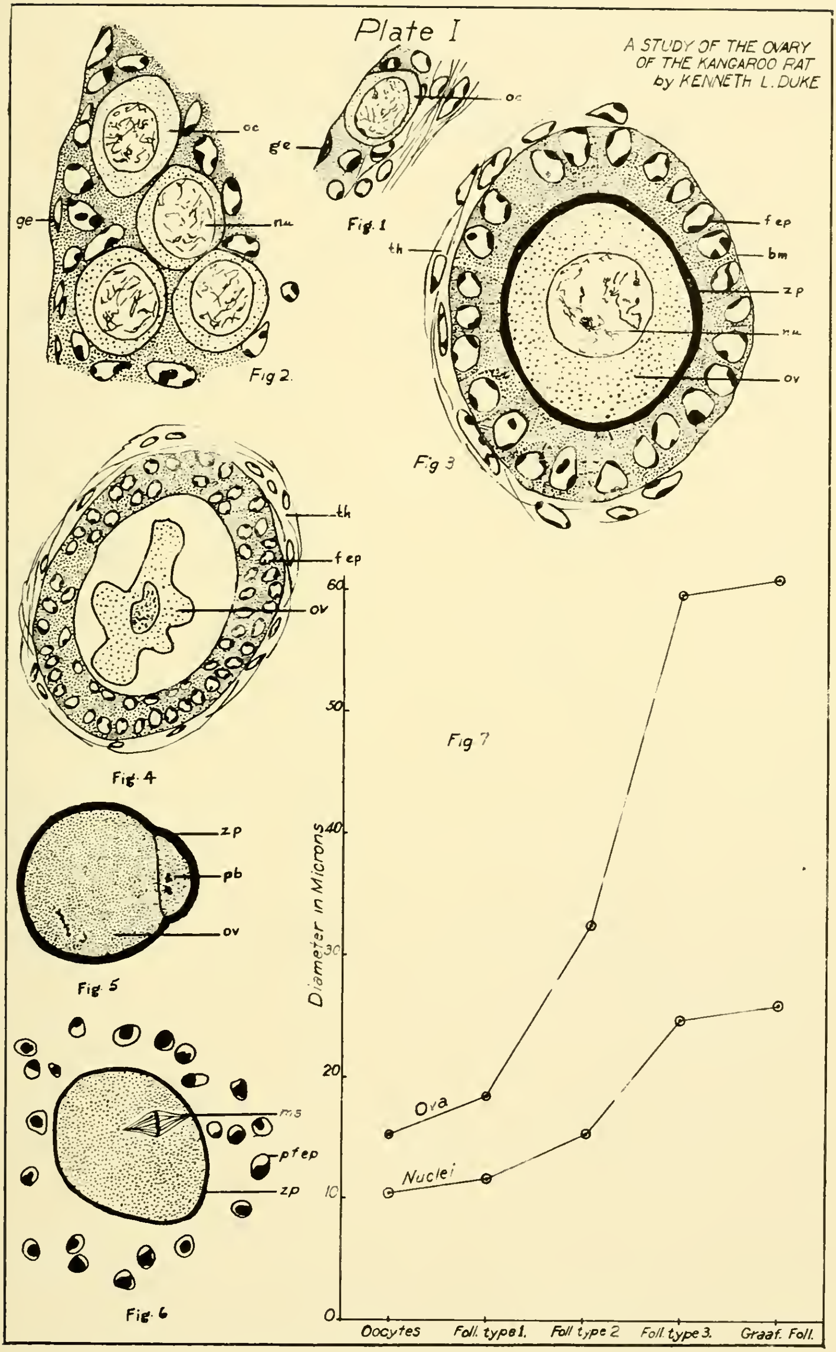
Plate I
A STUDY OF THE OVARY
OF THE KANGAROO RAT
by KENNETH L. DUKE
oc
ge
nu
Fig 2.
oc
ge
Fig. 1
fep
bm
zp
nu
ov
th
Fig 3
th
fep
ov
Fig. 4
zp
pb
ov
Fig. 5
ms
pfep
zp
Fig. 6
Fig 7
Diameter in Microns
60
50
40
30
20
10
0
Ova
Nuclei
Oocytes
Foll. type 1.
Foll type 2
Foll. type 3.
Graaf. Foll.