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Morphology of the pronghorn ovary

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MORPHOLOGY OF THE PRONGHORN OVARY

(ANTILOCAPRA AMERICANA ORD)

By

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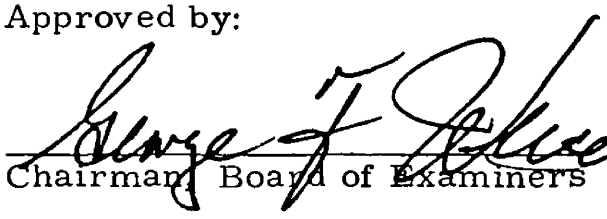

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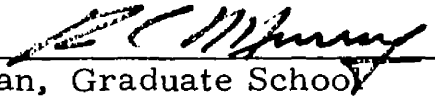

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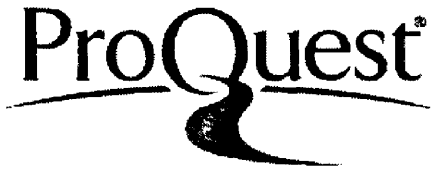

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ABSTRACT

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Morphology of the Pronghorn Ovary (Antilocapra americana Ord)

Director: George Weisel

Morphological characteristics, relative hormonal production, and ovulation incidences of ovaries of pronghorn (Antilocapra americana) were elucidated largely by microscopic examination of stained sections of ovaries from 26 does.

Numbers of vesicular follicles increased in pre-estrus and estrus, but average diameters of these follicles and their standard deviations did not differ markedly throughout the year.

In atresia, the fates of the ovum and zona pellucida were variable, and were poor indicators of the stage of atresia.

Two instances of polyovulation were found in ovaries from two different does. In each case, the follicle had two normal ova.

Luteal cells of the corpus luteum began degenerating at parturition. The structure was considered a corpus albicans after about 4 weeks, when no luteal cells remained and many of the spaces formerly occupied by these glandular cells were delineated by lacunae. The corpora albicantia could be distinguished from older corpora for 4 months and sometimes longer.

It was hypothesized that superovulation, the outstanding feature of the pronghorn ovary, was a mechanism serving to provide the species with large amounts of luteal tissue, which in turn would provide large amounts of progesterone for maintenance of the prolonged pregnancy characteristic of the pronghorn.

With one possible exception, no accessory corpora lutea were found. The exception was the occurrence of a probable silent heat in one doe.

One fawn definitely was bred at 4 months of age. Two others had old corpora albicantia as yearlings, indicating that they ovulated and probably also were bred as fawns.

ACKNOWLEDGEMENT

Most sincere gratitude is extended to those who exerted strong, albeit extremely diverse influences on this thesis--the Dick Baker family, staff and inmates of Bellairs Research Institute, Joan Bird, Dr. Raymond Corro, Anthony Kastella, Patrick Koelsch, Gary Matson, Bill Morrelles, Dr. J. R. Nursall, B. D. C. A. Punnett, Jennifer Roseland, W. S. Wolf, and especially Dr. B. W. O'Gara, Dr. George Weisel, Dr. P. L. Wright. Of most of you I am quite fond; of others . . .

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Antilocapra americana: Nothing can surpass the delicate and elegant finish of their limbs in which lightness, elasticity and strength are wonderfully combined. All the attitudes and movements of this wonderful animal are graceful and picturesque; and it is altogether a fit subject for the fanciful uses of the poet.

--Washington Irving

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CHAPTER I

INTRODUCTION

An important tool for intelligent management of game species is knowledge of their reproductive physiology. Most information on reproductive function of artiodactyls, a group which includes many important game animals, has been ascertained from domestic animals, in which selective breeding may have altered reproductive characteristics. Studies of the ovaries of wild ungulates have been mostly macroscopic. Species studied include mule deer (Odocoileus hemionus hemionus) (Sears 1955, Robinette et al. 1955), white-tailed deer (O. virginianus) (Cheatum and Severinghaus 1950, Haugen and Trauger 1962), black-tailed deer (O. hemionus columbianus) (Golley 1957), moose (Alces alces) (Markgren 1969), kob (Adenota kob) (Morrison 1971), and pronghorn (Antilocapra americana) (O'Gara 1968, 1969). Microscopic studies have been made of ovaries of impala (Aepyceros melampus) (Kayanja 1969), wapiti (Cervus canadensis) (Morrison 1960), and black-tailed deer (Thomas 1970).

This study of the microanatomy of pronghorn ovaries was undertaken to supplement knowledge of reproductive processes for possible management purposes and to add to the body of information

concerning comparative histology and morphology of the mammalian ovary. Knowledge of pronghorn reproduction was greatly expanded by Bromley (1967) and O'Gara (1968). The pronghorn is unusual in its reproductive strategy. Two to seven ova are ovulated and fertilized, but in nearly all cases, parturition results in twins. O'Gara was primarily concerned with uterine changes throughout the year and embryonic and fetal development. He studied ovaries macroscopically to determine time of follicular maturation and ovulation, number of ova produced, and development and regression of corpora lutea during and after pregnancy. Using selected ovaries of the same specimens with which O'Gara worked, the specific objectives of this investigation were to determine the:

1. morphological characteristics of the pronghorn ovary;
2. relative hormonal production of various ovarian structures as indicated by their morphology; and
3. ovulation incidence by analysis of corpora albicantia in context with other ovarian features.

CHAPTER II

METHODS

Histological preparations of ovaries from 26 pronghorns taken on the National Bison Range, Moiese, Montana, and Yellowstone National Park, were examined. Ovaries were fixed approximately 30 minutes after death in AFA and subsequently stored in 70% ethanol. Animals were identified by number, and ages and data on reproductive condition were recorded (O'Gara 1968). Sections representing nine animals (six from Yellowstone Park and three from the Bison Range) were previously prepared by O'Gara and supplemented my material. The animals varied in age classes from 0-1 year to 9+ years. In all but a few cases, both left and right ovaries were studied.

For his macroscopic examination, O'Gara (1968) made cross-sectional slices through the ovaries at about 1 mm intervals to permit measurement and counting of corpora lutea. These slices divided each ovary into five to seven pieces. Each piece was embedded in paraplast and sectioned at 8 microns. Two slides with 5 to 10 sections were made of each piece, yielding 10 to 14 slides and 50 to 140 representative sections per ovary. Sections from all ovaries were stained with hematoxylin and eosin. Selected sections were stained with fast

green, others with a trichrome stain developed by Gary Matson of Matson's Audiovisual and Microscopic in Milltown, Montana.

Ovaries from five animals were sectioned and mounted in their entirety to better visualize the three-dimensional characteristics of the structures.

When examining and measuring sections, I found it useful to set microscope slides on top of one another to determine which structures were which in consecutive slides, and which section showed the maximum size of the structure. Diameters were taken by finding the greatest diameter, adding the least diameter perpendicular to it, then dividing the sum by two, as suggested by Mossman and Duke (1973) for standardization of comparative studies. For follicles, measurement was from basement lamina; thecae or capsule were not included. A calibrated ocular micrometer was used for measuring structures.

During long-term storage of ovarian tissue in ethanol, as well as in the embedding process, shrinkage occurs. My data presented here differ from that taken in the field on the same ovaries by O'Gara (1968).

CHAPTER III

RESULTS

Surface Epithelium and Tunica Albuginea

The pronghorn ovary has a simple, single layer of surface epithelium, generally cuboidal, over a thin stroma. The tunica albuginea, as typical of artiodactyls, except the pig (Sus scrofa) and mouse deer (Tragulus javanicus) (Mossman and Duke 1973), is thick and fibrous, ranging from 0.10 to 0.35 mm in depth. The average for most ovaries was 0.16 to 0.20 mm and crypts were occasionally observed. Ovaries from two does had several of these indentations from the surface lined with flattened surface epithelium, but similar structures were rare in other specimens.

Cortex and Medulla

Pronghorn ovaries have a very distinct fibrous outer zone of the cortex contrasted with the inner zone and medulla. As in nearly all adult mammalian ovaries, the border between medulla and cortex is indistinct and is used only for general topographical reference. Vesicular follicles and corpora lutea frequently extended into the medulla, and large blood and lymph vessels are sometimes present

in the regions of the cortex. The stroma of the cortex consists of spindle-shaped fibroblasts in a dense matrix of collagenic and reticular fibers. The stroma of the medulla is irregular, dense connective tissue and is continuous with the hilus.

Interstitial Tissue

Mossman and Duke (1973) described interstitial gland tissue as any endocrine cells that occur in or closely with the mammalian ovary and that are not part of a thecal gland or a luteal gland. They further specified that unlike thecal and luteal gland cells, interstitial gland cells ordinarily do not degenerate after a functional period; instead, they dedifferentiate, either to remain as indifferent stromal cells or to redifferentiate later into the same or some other cell type. Of the several types of interstitial gland tissue described in the mammalian ovary, only a very small amount of one type, thecal, was observed in the pronghorn ovaries. Thecal-type interstitial gland tissue develops from the theca interna and thecal gland of atretic secondary and vesicular follicles. In most cases, the thecal gland consisted of small, cytoplasm-poor cells arranged about degenerating follicles. Thecal glands were best developed during estrus and early pregnancy--that is, at the time of greatest follicular atresia.

Rete

The rete ovarii in the pronghorn is a network of fine spaces

and tubules lined with an irregular epithelium located at the ovarian hilus and often extending far into the ovarian medulla. The rete is conspicuous. In tubules with diameters of 20 microns or less, a single layer of cuboidal epithelium was the rule. The largest tubules, those with diameters of 30-50 microns, had pseudostratified epithelium.

Ova

In the pronghorn, oogonia are indistinguishable from cells of the germinal epithelium on the surface. When oogonia attain a diameter of about 20 microns, they are at the primary oocyte stage and readily recognized. This compares closely to 19 microns in the impala (Kayanja 1969). Although technically germ cells remain oocytes until after ovulation, it is convenient to refer to all female germ cells in the ovary as ova (Mossman and Duke 1973). Ova grow as follicles develop. In the primordial stage, the ovum averages 22 microns, in the primary follicle 31 microns, in the secondary follicle 83 microns, in the vesicular follicle 92 microns. Ova grow very little, if at all, in the vesicular follicle. The nucleus of the ovum is 12 microns in primordial follicles, increasing to a maximum of 28 microns in vesicular follicles. In the latter, the average nucleolus is 6 microns. In histological sections, normal oocyte cytoplasm appears uniformly granular.

A zone pellucida is first present in secondary follicles. In some primary follicles, it forms in segments between adjacent follicular cells, as opposed to formation in a uniform layer surrounding the ovum. Thickness is about 4 microns. Adjacent to the zona pellucida is the corona radiata, a layer of slightly differentiated granulosa cells that are somewhat columnar.

Table 1 summarizes data on ova and their follicles.

Follicles

Four follicular stages are recognized (Fig. 1). Primordial follicles consist of an oocyte surrounded by simple squamous epithelium. Primary follicles, with low columnar epithelium around the oocyte, average a diameter of 0.05 mm. Secondary follicles have a double or stratified cuboidal epithelium of granulosa with diameters averaging 0.17 mm. Vesicular (Graafian) follicles, with fluid-filled spaces that eventually coalesce or have already coalesced to form an antrum, exhibit a tremendous amount of growth, attaining a maximum of 3.58 mm. The antrum usually forms when the follicular diameter is about 0.40 mm, which compares closely with antrum formation in the 0.40 mm follicle of the impala (Kayanja 1969) and 0.50 mm in the cow (Marion et al. 1968).

The number of vesicular follicles per ovary varies considerably, apparently a function of age, time of year, and individual

Table 1. Follicular and ovular growth in nine ovaries.

	Follicular stage			
	Primordial	Primary	Secondary	Vesicular
Mean follicular diameter (mm)	--	0.05	0.17	0.98
Standard deviation	--	0.01	0.07	0.62
Range	--	0.04-0.06	0.08-0.29	0.19-3.58
Mean antral diameter (mm)	--	--	--	0.70
Standard deviation	--	--	--	0.55
Range	--	--	--	0.05-2.14
Mean cumular diameter (mm)	--	--	--	0.17
Standard deviation	--	--	--	0.14
Range	--	--	--	0.10-0.24
Mean ovular diameter (microns)	22.0	31.3	83.4	92.3
Standard deviation	2.83	7.57	23.3	15.4
Range	20.0-24.0	26.0-40.0	44.0-108	64.0-120
Mean nuclear diameter (microns)	13.0	17.0	24.0	25.0
Standard deviation	1.41	4.36	0.00	2.00
Range	12.0-14.0	14.0-22.0	All 24.0	24.0-28.0
Mean zona pellucida diameter (microns)	--	--	3.60	3.67
Standard deviation	--	--	0.90	0.91
Range	--	--	2.00-4.00	2.00-6.00

Fig. 1. Follicular stages and atresia.

A. Primordial follicle--no epithelium around oocyte. 400x.

B. Primary follicle. 400x.

C. Secondary follicle. 400x.

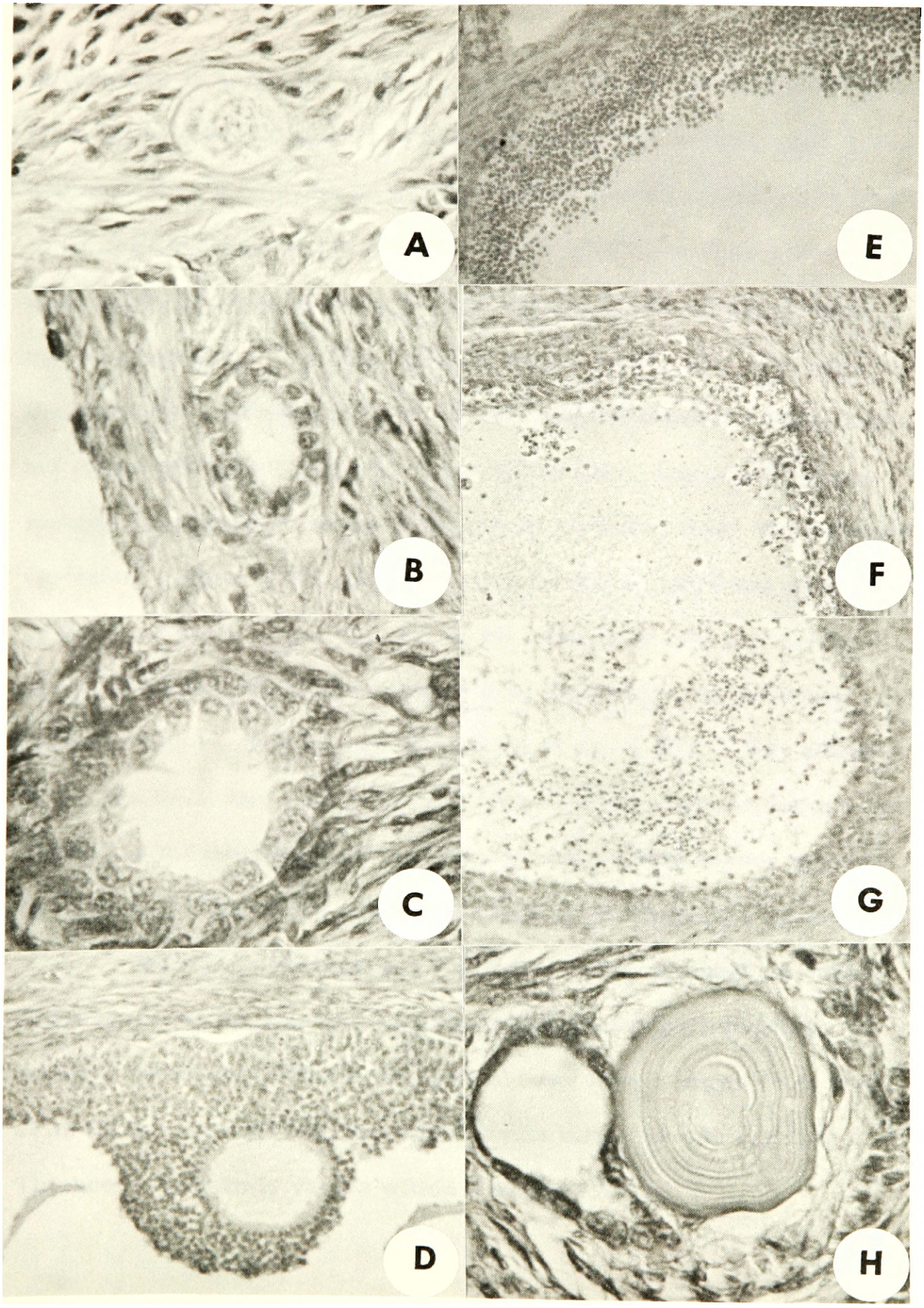
D. Growing vesicular follicle with oocyte. Mitosis is active in granulosa; theca interna is well developed. 100x.

E. Portion of vesicular follicle in early atresia. Granulosa is pyknotic and cells are loosening. 40x.

F. Portion of vesicular follicle in mid-atresia. Granulosa is dispersing. 40x.

G. Portion of vesicular follicle in late atresia. Few granulosum cells remain on the follicular wall; theca interna is dedifferentiating. 40x.

H. Atretic oocyte showing lamellar rings. 400x.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differences in hormonal levels. Ovaries with the most follicles were from young adult animals taken near the peak of breeding season; one had ovulated, another had not. Fewest vesicular follicles were found in an old doe taken in December. Numbers of these follicles often were quite different in left and right ovaries from the same animal, but the average sizes of the follicles tended to be close in ovaries of the same pair. Average diameters and their standard deviations did not differ markedly (Table 2). The differences probably reflect an inverse relationship with the numbers of follicles rather than seasonal variation. Sears (1955) noted that there was no significant difference in seasonal sizes of the follicles in the mule deer ovary in any age class. This information compares very favorably with seasonal follicle sizes encountered by Kammlade et al. (1952). They stated that if the follicular size is the criterion used to evaluate the ovarian activity of the domestic sheep, then the nonbreeding season ovaries are just as active as the ovaries at the peak of breeding season.

Mitosis is obvious in granulosum cells, although on superficial examination pyknosis could be mistaken for cell division. In growing vesicular follicles, the granulosum layer ranges from five cells thick, about 40 microns, to 20 cells thick, or 160 microns. Thickness frequently varies within a single follicle.

Table 2. Numbers and mean diameters of vesicular follicles.

Coll. #	Coll. date	Age class	# Ves. Foll.			Mean diameter (mm)						Max L/Max R (mm)
			L	R	L+R	L	St. dev.	R	St. dev.	L+R	St. dev.	
20	8 Sep	2-3	38	59	97	1.1	0.6	0.9	0.6	1.0	0.6	2.4/2.4
21	8 Sep	4-5	72	66	138	0.8	0.4	0.7	0.4	0.8	0.4	1.9/1.6
23Y	18 Sep	2-3	25	26	51	1.4	1.0	1.7	0.9	1.6	0.9	3.2/3.1
38Y*	30 Sep	8-9	38	--	--	0.9	0.5	--	--	--	--	2.0/ --
46Y*	7 Oct	7-8	--	--	--	--	--	--	--	--	--	-- / --
129	11 Oct	6-7	25	23	48	1.1	0.4	0.9	0.4	1.0	0.4	2.2/2.3
49	15 Oct	1-2	42	50	92	0.9	0.5	1.0	0.6	1.0	0.5	2.6/3.2
134	20 Oct	2-3	33	29	62	0.8	0.4	0.9	0.4	0.8	0.4	1.9/1.6
59	4 Nov	3-4	31	16	47	1.2	0.7	1.2	0.5	1.2	0.6	2.8/2.1
74	2 Dec	4-5	14	24	38	0.8	0.5	1.3	0.9	1.1	0.8	2.2/3.5
75	2 Dec	9+	6	8	14	1.5	0.6	1.4	0.6	1.4	0.6	2.4/2.4
85	10 Dec	6-7	13	12	24	1.3	0.6	1.2	0.6	1.3	0.6	2.4/2.4
94	27 Jan	3-4	11	20	31	1.5	0.6	1.5	0.7	1.5	0.7	2.7/3.0
95 *	27 Jan	1-2	20	--	--	1.0	0.6	--	--	--	--	2.0/ --
99 *	24 Feb	5-6	--	--	--	--	--	--	--	--	--	-- / --
100 *	24 Feb	0-1	--	5	--	--	--	0.7	0.4	--	--	-- /1.1
110	24 Mar	1-2	21	10	31	1.4	0.7	1.7	0.7	1.5	0.7	2.4/3.0
112	21 Apr	5-6	18	2	20	1.7	0.5	1.7	0.8	1.7	0.5	2.6/2.2

Table 2. (continued)

Coll. #	Coll. date	Age class	# Ves. Foll.			Mean diameter (mm)						Max L/Max R (mm)
			L	R	L+R	L	St. dev.	R	St. dev.	L+R	St. dev.	
113	21 Apr	2-3	26	36	62	0.9	0.5	1.2	0.7	1.1	0.6	2.6/2.7
116	19 May	3-4	23	12	35	1.6	1.0	1.0	0.7	1.2	0.8	2.7/2.9
117	19 May	5-6	16	23	39	1.3	0.7	1.7	0.8	1.5	0.8	2.9/3.6
118Y*	3 Jun	2-3	--	--	--	--	--	--	--	--	--	-- / --
120Y*	3 Jun	3-4	--	--	--	--	--	--	--	--	--	-- / --
121	23 Jun	3-4	14	32	46	1.5	0.6	0.8	0.6	1.0	0.7	2.8/2.9
123Y*	9 Jul	6-7	--	--	--	--	--	--	--	--	--	-- / --
125	19 Jul	2-3	23	19	42	1.4	0.6	1.3	0.6	1.4	0.6	2.6/2.9

Y indicates animals from Yellowstone herd. All other animals are from the National Bison Range.

* indicates limited study material, i.e., incomplete ovaries.

Thecae

Adjacent to the basement lamina, the theca interna, a layer rich with capillaries for nourishment of follicle and egg, first appears in secondary or early vesicular follicles. Cells tend to be rounded and have many mitotic figures. In the pre-ovulatory or ripe follicle, the theca interna develops into a thecal gland (Mossman and Duke 1973), a highly vascularized zone of endocrine gland cells that degenerated soon after ovulation. Mossman and Duke generalize that the thecal gland is usually only a few cells thick, is often interrupted in the family Cervidae, and is of intermediate thickness in the family Bovidae. The gland was 20 microns thick in elk and 50 microns in bovine follicles (Marion et al. 1968). The pronghorn thecal gland, however, is much thicker--atypical of artiodactyls except the goat (Capra hircus) (Harrison 1948). In three pre-ovulatory follicles in specimen #21, thickness of the thecal glands is 40-120 microns, 40-72 microns, and 50-120 microns. In three growing vesicular follicles prior to the pre-ovulatory stage, average thickness of the thecal glands is 40, 64, and 56 microns. Three follicles in late atresia with granulosum cells shedding into the antrum have thecal glands of 40 microns each. Other follicles in the same stage of atresia, however, have thecal glands of 0-20 microns. If the thecal gland and theca interna of the pronghorn often disappears in atresia, it would explain why very little thecal interstitial gland tissue is found in this species.

In early vesicular follicles, a thin theca externa of elongated cells forms around the theca interna. Upon ovulation, the theca externa thickens, probably because of contractile properties of the cells of this zone (O'Shea 1971), but the cells soon disperse and this zone is no longer apparent.

Numerous blood and lymphatic vessels in the thecal layers do not penetrate the granulosum. Morris and Sass (1966) reported similar observations in the ovary of the ewe (Ovis aries).

Atretic Follicles

I observed the following stages in pronghorn follicles (Fig. 1):

Growing--mitosis very active;
Transitional--some mitosis, granulosum cells loosening;
Early atresia--mitosis very slow or ceases, granulosum cells loosened, pyknotic nuclei;
Mid-atresia--no mitosis, granulosa dispersing;
Late atresia--one or no layers of granulosum cells remain on antrum periphery, granulosum cells floating in antrum, thecal gland dedifferentiating in some;
Very late atresia--no definite border, fine web of connective tissue with few cells fills former antral space;
Very, very late atresia--no definite border, dense connective tissue with few nuclei fills former antral space.

In all ovaries there are many more atretic follicles than growing ones. Sometimes, especially in proestrus, estrus, and early pregnancy, all vesicular follicles are atretic. For example, in #20R there are 59 vesicular follicles, 55 of which are atretic.

In #23L, all vesicular follicles are atretic. Kayanja (1969) noted the same phenomenon in impala.

Some features of atretic follicles are quite variable. Degeneration of the ovum can begin at any stage and is a poor indicator of the level of atresia, as also noted by Marion et al. (1968) in the bovine ovary. Some atretic ova appear to consist of lamellar rings. There are particularly good examples of such ova in #85. The fate of the zona pellucida in atresia shows much variation. The zona is extremely thin, or indistinct, or non-existent in all stages of atresia. In some instances, however, the zona is persistent after the ovum has degenerated and forms a ring around an empty space. The zona is not a reliable indicator of the degree of atresia. The corona radiata, closest of the granulosum cells to the ovum, shows the last evidence of mitosis in atretic follicles. As previously stated, atresia of secondary and vesicular follicles sometimes results in development of interstitial gland tissue from theca interna or thecal gland. Luteal-type cells are present in late atretic follicles in two ovaries. No other instances of luteinization of atretic follicles to form possible accessory corpora lutea, and subsequently corpora albicantia, were found.

Polyovular Follicles

Two does each had one follicle with two ova. One was in early atresia and ova occurred opposite each other, each with its own

cumulus appeared normal in all respects. The other follicle was in mid-atresia with one ovum floating in the antral space and the other still tenuously associated with its dispersing cumulus. Polyovular follicles are found in many mammalian species. Among artiodactyls, such follicles have been reported in black-tailed deer (Thomas 1970) and impala (Kayanja 1969).

Luteal Glands

The corpus luteum of the pronghorn is a solid spheroidal mass of large polyhedral gland cells and smaller fusiform or stellate cells. Because of the glandular nature of the corpus luteum, it may be more aptly termed a luteal gland, as suggested by Mossman and Duke (1973) (Fig. 2).

Prior to ovulation, the granulosum cells of the cumulus loosen and free the ovum, which along with its corona cells floats in the antrum and is ovulated. At that time, the follicular liquid is also discharged, and as a result of the change in internal pressure, the walls of the follicle collapse and the granulosum layers become folded. In #21, collected 8 September, at the ovulation point many erythrocytes are scattered in with the granulosum and thecal cells and even into surrounding stromal cells. No luteal cells are present. A specimen taken approximately 2 weeks after ovulation, #38, shows a forming corpus luteum with luteal cells. Although there is rampant

Fig. 2. Corpora lutea and early corpus albicans.

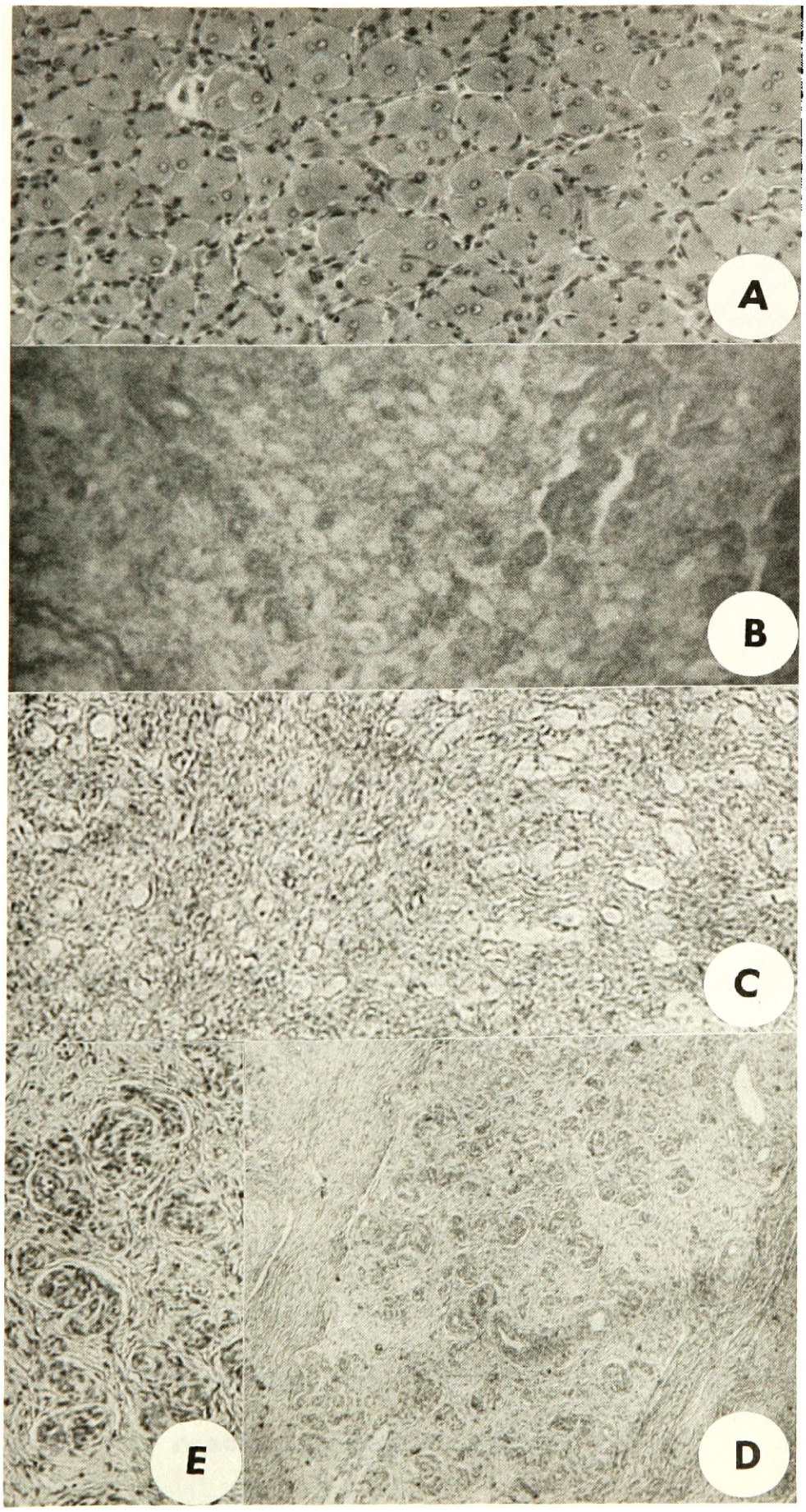
A. Section of corpus luteum 3 weeks prior to parturition. Note two types of cells. 100x.

B. Corpus luteum immediately postpartum. Large polyhedral cells are degenerating and structure is shrinking. 100x.

C. Corpus luteum 3 weeks postpartum. Few luteal cells remain; nuclei of some luteal cells can be seen in lacunae. 100x.

D. New corpus albicans, approximately 5 weeks postpartum. No luteal cells remain, a few lacunae remain. 40x.

E. Detail of D. 100x.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confusion in the literature concerning the origin of luteal cells, those of the pronghorn seem to originate only from the granulosa. Warbritton (1934) studying the domestic ewe and Corner (1919) studying swine, concur in this, but state that other elements of the corpus luteum do come from the theca interna. Corner considers smaller cells of the corpus luteum to be modified epithelial cells of the theca interna. Thomas (1970) states that in the black-tailed deer smaller cells of the corpora lutea are derived from less differentiated cells of the thecal region.

The polyhedral gland cells grow from a mean diameter of 16 microns in September to a maximum of 36 microns in May. Vacuolations are present throughout this period. The nuclei are 8 microns throughout pregnancy. Nucleoli are clearly visible, and nuclear cytoplasm appears granular. The smaller fusiform cells do not grow during pregnancy. The mean length is 7 microns; the width is usually about the same as the diameter of the nucleus, which is 4 microns. These nuclei are usually elongate, but sometimes rounded, like those in connective tissue of the thecal gland.

O'Gara (1968) described the growth of luteal glands in these specimens. Growth was rapid during the first month, slowed or ceased in winter months, and increased again in early spring until a maximum was reached at parturition, the end of May or beginning of June. As with vesicular follicles, there is a tendency toward decrease

of mean diameters of luteal glands as their number within an ovary increases (Table 3).

Vessels of the thecal gland grow into the luteal gland, forming a complex network ranging from minute capillaries to vessels of a millimeter or more in diameter.

In some instances, a fibrous capsule surrounds the luteal gland. Most often incomplete, the capsule has few cells and a mean thickness of 20 microns. This capsule does not develop from the theca externa.

Mean numbers of luteal glands increase from 2.0 in fawns to 5.7 in 5-6 year olds and thereafter decline to 5.3 in does of 6-7 years and older (Table 4).

Accessory (or secondary) corpora lutea are defined as luteal tissue occurring in structures that did not contribute viable ova, and are distinguished by their smaller size. In the ovaries studied, no accessory corpora lutea were found. This does not mean that they do not exist in the pronghorn, but if they do, they are uncommon.

Corpora Albicantia

The luteal gland begins to decline at parturition. Degenerated luteal cells immediately result in reduced size and loss of the regular spheroid shape of the luteal gland. After about a month, no luteal cells remain; the former mass of glandular tissue shrinks around the

Table 3. Numbers and mean diameters of corpora lutea (CL) and corpora albicantia (CA).

Coll. #	Coll. date	Age class	# CL	Mean CL diameter (mm)		# CA	Mean CA diameter (mm)		Comments
				CL	St. dev.		CA	St. dev.	
20	8 Sep	2-3	0	--	--	7	1.22	0.36	pre-ovulation
21	8 Sep	4-5	6	1.95	0.20	12	1.34	0.36	fol./CL; ov. sites
23Y	18 Sep	2-3	0	--	--	5	2.14	0.26	pre-ovulation
38Y*	30 Sep	8-9	4	3.70	0.09	24	1.89	0.17	fol./CL stage
46Y*	7 Oct	7-8	6	3.80	1.10	--	--	--	
129	11 Oct	6-7	4	4.67	0.17	4	1.48	0.39	
49	15 Oct	1-2	5	4.54	0.36	2	1.63	0.19	
134	20 Oct	2-3	3	4.91	0.14	2	1.08	0.10	
59	4 Nov	3-4	6	4.78	1.00	7	1.28	0.22	
74	2 Dec	4-5	5	5.15	0.28	14	0.87	0.34	
75	2 Dec	9+	6	4.55	0.74	8	1.13	0.17	
85	10 Dec	6-7	7	3.84	0.95	12	0.75	0.20	
94	27 Jan	3-4	5	5.66	0.63	8	1.76	0.36	
95*	27 Jan	1-2	5	4.63	0.63	0	--	--	
99*	24 Feb	5-6	6	4.80	0.58	--	--	--	
100*	24 Feb	0-1	2	5.05	0.07	0	--	--	
110	24 Mar	1-2	4	4.92	0.17	0	--	--	
112	21 Apr	5-6	5	4.50	0.54	20	0.93	0.35	

Table 3. (continued)

Coll. #	Coll. date	Age class	# CL	Mean CL diameter (mm)		# CA	Mean CA diameter (mm)		Comments
				CL	St. dev.		CA	St. dev.	
113	21 Apr	2-3	4	5.47	0.46	6	1.43	0.59	
116	19 May	3-4	5	6.23	0.38	4	0.88	0.40	
117	19 May	5-6	6	4.74	0.57	5	0.88	0.32	
118Y*	3 Jun	2-3	3	7.30	2.12	--	--	--	prepartum
120Y	3 Jun	3-4	4	3.70	0.35	--	--	--	CL degenerating
121	23 Jun	3-4	3	2.20	0.34	--	--	--	CL 3 weeks post-partum
123Y*	9 Jul	6-7	5	--	--	5	2.10	0.17	
125	19 Jul	2-3	2	--	--	2	1.49	0.24	CA both $\frac{1}{2}$ mo. old

Y indicates animals from Yellowstone herd.

* indicates limited study material, i.e., incomplete ovaries.

Table 4. Mean numbers of corpora lutea (CL) and corpora albicantia (CA) by age class.

Age class	# in sample	Mean # CL	St. dev.	Mean # CA	St. dev.
0-1	1	2.0	0.0	0	0
1-2	3	4.7	0.6	0.7	1.1
2-3	5	2.0	1.9*	4.4	2.3
3-4	5	4.6	1.1	5.2	2.1
4-5	2	5.5	0.7	14.0	0.0
5-6	2	5.7	0.6	12.0	11.3
6-7	3	5.3	1.5	7.0	4.3
7-8	0	--	--	--	--
8-9	1**	4.0*		18+	
9+	1	6.0	0.0*	8.0	0.0

*Biased sample responsible for misleading mean values. O'Gara's larger sample gives 4.3 for 2-3 year olds, 5.3 for 8-9 year olds and 9+ years.

**Data from one ovary only.

numerous blood vessels. These vessels, after persisting actively for about 10 months (first in the theca interna and thecal gland nourishing follicle and ovum, then in the luteal gland as nourishment and transport systems during pregnancy), degenerate slowly. This remnant, the corpus albicans, dated from about 1 July when luteal cells are no longer present (Fig. 2). In animals taken in the September breeding season two types of corpora albicantia can be distinguished. The 2-3 month-old scars are relatively large (Fig. 3). They often have lacunae delineating the spaces once occupied by the luteal gland cells and are generally less compact than the older type which were smaller and had no lacunae. Because there is little evidence of accessory corpora lutea, these are probably remnants of corpora lutea of previous pregnancies. None were found in animals that could not have had fawns, and few were found in the 1-2 year age class. These scars probably persist for at least 2 years and quite possibly longer. Morrison (1960) and Thomas (1970) believe that this is also the case in elk and black-tailed deer. (See Table 3 for numbers and mean diameters of corpora albicantia and Table 4 for mean numbers of corpora albicantia by age class.)

Cystic Ovaries

Two ovaries (#46L and #134R) were found to be cystic. Within each was a cystic vesicle lined with a single layer of cuboidal cells very like granulosum cells in a vesicular follicle; the underlying

Fig. 3. Corpora albicantia.

A. Corpus albicans 2-3 months old. 40x.

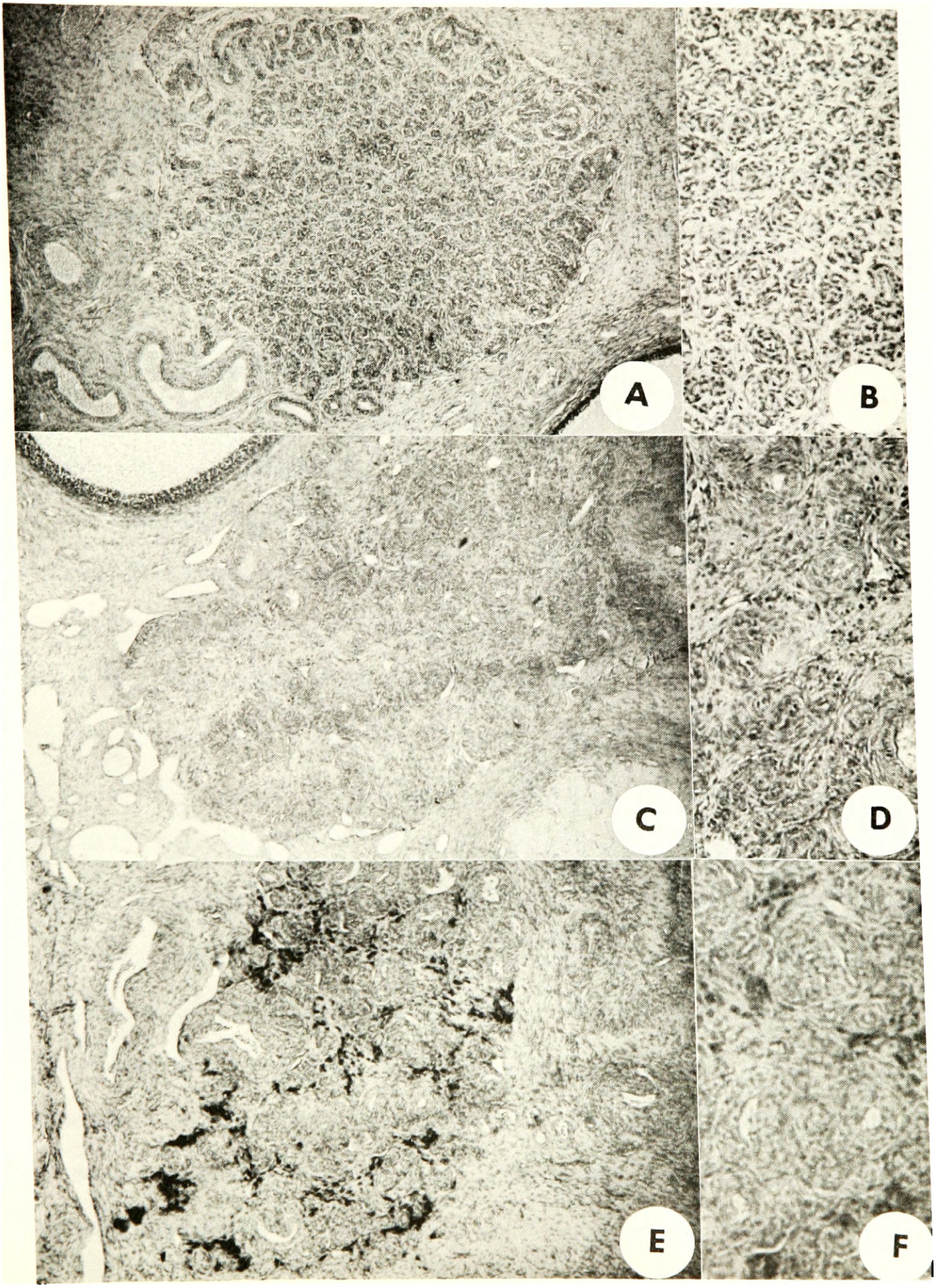
B. Detail of A. 100x.

C. Corpus albicans 4 months old. This scar is more compact than the corpus albicans in A and B. 40x.

D. Detail of C. 100x.

E. Corpus albicans of 8 months, heavily pigmented. 40x.

F. Detail of E. 100x.


A
B
C
D
E
F

layers were also similar. O'Gara (1968) reported that when fresh these cysts contained brownish fluid. Apparently they did not interfere with reproductive function--one had two full-sized corpora lutea and the other had one large corpora lutea and embryos in the uterine horns.

Summary of Annual Ovarian Cycle

Annual ovarian events in the Bison Range pronghorn can be summarized as follows:

In September estrogen production by the thecal gland reaches a peak as the pre-ovulatory follicle nears the surface of the ovary. This high estrogen level, indicated by well-developed thecal gland cells has three effects (Deane 1952, Ham 1974). It produces mating behavior in the doe. Virtually all does, except fawns, mate successfully (O'Gara 1968). Secondly, by positive feedback, high levels of blood estrogen cause increased production of LH (luteinizing hormone), controlled by the hypothalamus, which causes ovulation of two to seven ova at approximately the same time as mating. Dilation of lymph vessels in the thecae at this time indicate that perhaps increased fluid pressure has a mechanical influence on expulsion of the ovum. The theca externa thickens, probably because of contractile properties of some smooth muscle-like cells of this zone, and may also contribute to follicular rupture. Soon after, the theca externa degenerates. At

ovulation, blood vessels of the thecae rupture to release the RBC's observed in the antrum. These RBC's and other blood plasma components provide necessary materials for the stigmatic clot. Thirdly, the increased production of LH causes luteinization of the granulosum cells which fill the former antral space. These become the luteal gland cells whose function is steroid synthesis (Priedkalns and Weber 1968), most certainly progesterone. Diameter of the newly differentiated, polyhedral cells is 16 microns. There is no evidence of cellular division. Less differentiated cells of the thecae appear in the lumen after the granulosum cells begin luteinization and differentiate to become the smaller cells of the corpus luteum, whose probable function is lipid storage (Priedkalns and Weber 1968) of progesterone precursors (Blanchette 1966). The size of these cells is a little less than half that of the new, large, glandular luteal cells. Fully differentiated cells of the thecal gland degenerate. Vessels of the thecae begin invasion of the forming corpus and form a complex vascular plexus which nourishes the new structure and serves as a transport system for the hormone produced therein. The ovum is fertilized and begins cleavage.

In October, luteinization of the granulosum cells is complete and they grow to a diameter of 18-20 microns. The corpora of the previous pregnancy are about $3\frac{1}{2}$ months old and have taken on the dense, compact appearance of old corpora albicantia. Three to four

weeks after conception, intrauterine death via thread stage knotting occurs (O'Gara 1969). The cells deeper within the corpus luteum are hypertrophied. Cavazos et al. (1969) found that there was a close correlation in swine between morphological changes during the cycle and the level of progesterone in luteal tissue, and that larger cells secreted more progesterone. Breed and Clark (1970) also found that there was a quantitive correlation between healthy corpora lutea and progesterone secretion.

In late October/early November, about 30 days after breeding, the embryos implant (O'Gara 1969). During November the large luteal gland cells grow to a diameter of 24 microns.

Five to six weeks into gestation, in late November/early December, the second mechanism for intrauterine mortality of embryos is affected. All but two siblings (one in each uterine horn) proximal to the cervix are eliminated when necrotic tips grow from them and disrupt the membranes of any embryos not eliminated at the thread stage (O'Gara 1969). Glandular luteal gland cells grow to a diameter of 28 microns and some show vacuolation in December.

By January, many of the glandular luteal cells are vacuolated and the mean diameter is 30 microns. For about $2\frac{1}{2}$ months following this stage, growth of the luteal gland slows or ceases. In April, cell growth and therefore luteal gland size increases. The cells attain a diameter of 32 microns.

Glandular luteal cells attain a maximum diameter of 36 microns in May. At this stage, the corpus luteum is about twice the diameter of the follicle from which it developed. After a gestation of about 255 days (Hepworth and Blunt 1966), which is exceeded in North American wild ungulates only by elk and bison, parturition occurs at the end of May or in early June. There is little or no blood in the vessels of the luteal gland. Luteal cells cease hormone production and the luteal gland begins its decline right after parturition. The size of the luteal gland decreases immediately and looses its solid, spheroidal appearance, taking on a less regular shape.

During June, the luteal cells continue to degenerate and the luteal gland shrinks further.

By the first of July, no luteal cells are left, although lacunae often delineate their former positions. This stage is considered the beginning of the corpora albicantia. They continue to shrink through August. Toward the end of August, under the influence of FSH, there is an increase in the number of developing follicles, including the follicles that will produce viable ova for the next pregnancy. Markgren (1969) notes that FSH secretion is lower in young ungulates, and that the number of ova shed is therefore less. This agrees with generally lower corpora lutea and corpora albicantia counts in younger animals. The theca interna, then the theca externa differentiate from the stromal cells of the cortex. Granulosum cells, the probable function

of which is protein synthesis (Priedkalns and Weber 1968), proliferate rapidly.

In the pre-ovulatory follicle of early September, the thecal gland has developed from the theca interna. Relatively small quantities of LH synergize with FSH to stimulate the secretion of estrogen by the thecal gland cells. The estrogen level builds to a peak in preparation for estrus and ovulation for the breeding season.

CHAPTER IV

DISCUSSION

Features of the Artiodactyl Ovary

Several characteristics of the pronghorn ovary are indicative of this species' phylogenetic relationships with other artiodactyls.

The instance of two classes of luteal gland cells is apparent in all artiodactyls. It was first mentioned by Corner (1919) in swine. Mossman and Duke (1973) also report two cell types in the corpora lutea of perissodactyls and cetaceans, and state that this reinforces evidences of relationships between these groups, but it is remarkable that such a minor feature should persist for so long. Provost (1962) also described two types of cells in the corpora lutea of the beaver, but they are not so strikingly different in morphology and size in that species as in the whales and hoofed mammals.

Only one type of interstitial gland tissue has been described in artiodactyls--thecal--and there is very little of that. The function of these cells is steroid synthesis, but it is not yet known just which steroids they are responsible for. They may produce estrogen in small amounts (Deane 1952). Also, in the pronghorn it is possible that the relatively large amounts of estrogen produced by the theca

interna and thecal gland preclude the necessity for differentiation of interstitial gland tissue to produce that hormone.

Other features of the pronghorn ovary that are common to all or most artiodactyls reported in the literature are a thick tunica albuginea and a relatively large antrum (Table 1 includes follicular and antral diameters).

Accessory Corpora Lutea

Except for a few luteal-type cells in two atretic follicles, no accessory luteal tissue was observed in the pronghorn. Neither have accessory corpora been reported in ovaries of swine, bison (Bison bison), cow (Bos taurus), goat, sheep, kob (Mossman and Duke 1973). Accessory corpora are reportedly rare in whitetails (Haugen and Trauger 1962), mule deer (Sears 1955), and moose (Markgren 1969). High incidences of accessory corpora are given by Thomas (1970) for black-tailed deer (47%), Halazon and Buechner (1956) and Morrison (1960) for wapiti (66 and 60%, respectively), and Douglas (1966) for red deer (Cervus elaphus) (37%). This indicates that in artiodactyls, accessory corpora lutea separate the cervids from the bovids. It is also possible that confusion over the term "accessory corpora lutea" and the lack of microscopic examination of such structures in some species have prevented more accessory corpora from being reported in the literature. If there is any evolutionary advantage to this

seemingly excess luteal tissue, perhaps it is provided in the pronghorn by the comparatively large amount of luteal tissue that results from superovulation.

Superovulation

The outstanding feature of pronghorn reproduction is superovulation--the ovulation of many more eggs than can be successfully gestated. The pronghorn shares this characteristic with the South African long-eared elephant shrew (Elephantulus myurus) (Horst and Gillman 1941) and the plains viscacha (Lagostomas maximus) (Weir 1971), two other mammals exhibiting even more remarkable superovulation than Antilocapra americana.

O'Gara (1968) states:

> The evolutionary significance of losing a large number of conceptuses seems rather obscure. Possibly the pronghorn once had greater numbers of young, but in comparatively recent times predation or other pressures have made two precocious offspring of greater survival value than a larger number of smaller and less developed young.

Addressing the situation from a different angle, Mossman and Duke (1973) suggest:

> Conceivably the five to eight eggs ovulated and fertilized by the pronghorn antelope . . . are insurance that at least one or two will successfully implant . . . The more reasonable interpretation would seem to be that . . . it is related to something other than litter size.

Two other characteristics of pronghorn reproduction bear examination. First, the species has an extremely long gestation for an animal of its

size, only bison and wapiti in North America exceed it. This probably necessarily evolved along with the social behavior described by Bromley (1967) because of habitat requirements, and could demand a large, continuous supply of progesterone for maintenance of prolonged pregnancy. Second, the corpus luteum does not degenerate until parturition and in fact reaches its maximum size just prior to that event, indicating a necessity for increasingly large amounts of progesterone right up to the birth of the fawns. If for some reason a species requires a considerable amount of progesterone, superovulation and subsequent formation of luteal glands is a way to get it. Possibly, other features of pronghorn reproductive physiology may be directly or indirectly related to ovarian structures--the atypically thick thecal gland, lack of interstitial gland tissue, or lack of accessory corpora lutea, as mentioned previously.

The two explanations for superovulation are not mutually exclusive. Ancestors of our present-day pronghorns could have had the capacity to gestate large numbers of young when environmental pressures changed to make such reproductive strategy unrewarding. Nature conservatively utilized the excess luteal tissue provided by superovulation to best advantage under dynamic conditions.

Analysis of Reproductive Performance

Ovarian analysis as discussed by Cheatum (1949), Golley

(1957), Haugen and Trauger (1962), Thomas (1970), and others is meant to be a useful tool for investigation of reproductive performance. Ovulation incidence, or the number of eggs each doe ovulates during pregnancy-producing estrus, is determined by counting the corpora albicantia resulting from corpora lutea of the previous pregnancy. Two assumptions are necessary for success of the technique--the corpora albicantia in question must persist until hunting season, when specimens are likely to be obtained, they must be distinguishable from any other corpora albicantia that are not the result of corpora lutea of pregnancy, and from corpora of earlier years.

In the pronghorn, both of these criteria are satisfied, but we know before we start that nearly all incidences of parturition produce twins, and that ovulation incidence will be much higher than the two fawns that result. Because of these differences between pronghorns and other game animals, examination of ovaries from pronghorn does killed during hunting seasons would not serve as the management tools that Cheatum's (1949) methods provided for other species. However, results of this morphological study of the ovaries coupled with Cheatum's basic method of examining corpora albicantia can be utilized to determine ovulation incidences of the does which were used in this study.

To aid in this analysis, all corpora for each pair of ovaries

are graphed according to their diameters (Fig. 4). Because of the lack of evidence for accessory corpora lutea and subsequent corpora albicantia, it is hypothesized that the resulting clumps represent previous breeding seasons (Morrison 1960, Thomas 1970). Several factors must be kept in mind, however:

1. Corpora albicantia sizes and shapes can be distorted by growing follicles, causing overlap of groupings.

2. Fawns can be expected to breed later than yearlings and adults.

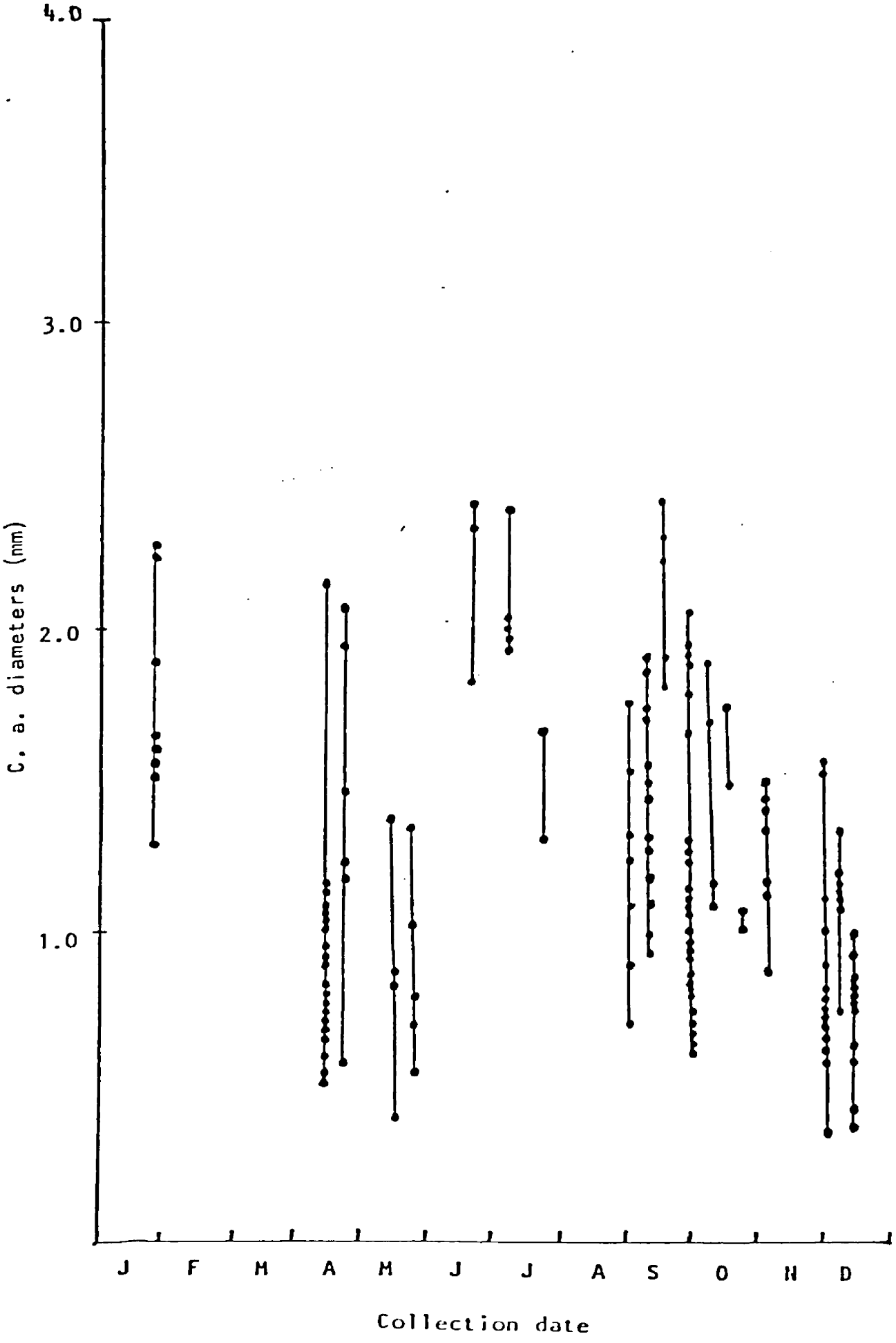
3. Many corpora lutea in a single ovary tend to decrease the mean diameter and thus the mean diameter of the resulting corpora albicantia.

4. Silent heat and recurrent estrus have not been ruled out in the pronghorn (O'Gara 1968).

Because of these factors, estimations of reproductive performance beyond one breeding season, except in a few cases, move progressively farther into the realm of guesswork. Animals #20, 21, 23Y, and 38Y were collected during or near the breeding season. Explanation of the analysis of their ovulation incidences requires more extensive comment than other specimens because ovulating follicles, new corpora lutea, and old and new corpora albicantia may all be found in the ovaries.

Doe #20, collected 8 September, was in the 2-3 year age class. Pre-ovulatory follicles and lack of forming corpora lutea indicate that she had not ovulated. Corpora albicantia totaled seven.

Fig. 4. Diameters of corpora albicantia.


4.0
3.0
2.0
1.0
C. a. diameters (mm)
J
F
M
A
M
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Collection date

Table 5. Ovulation incidences.§

Coll. #	Age class									
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9+
20	5	2								
21	(0	5	3)	4	6					
23Y	0	5								
38Y*	(-	-	-	5	4	4	4)	3	6,4	
46Y*	-	-	-	-	-	-	-	6		
129	(-	-	-	-	2)	2	4			
49	2	5								
134	0	2	3							
59	(1	2)	4	6						
74	(1	8	3)	2	5					
75	(-	-	-	-	-	-	-	1	6)	6
85	(-	-	2	2	5	2)	7			
94	(1	5	2)	5						
95*	-	5								
99*	-	-	-	-	-	6				
100*	2									
110	0	4								
112	(0	3	7	3	7)	5				
113	0	5	4							
116	(1	3)	5							
117	(-	-	3	2)	6					
118Y*	-	3								
120Y*	-	-	4							
121	-	-	3							
123Y*	-	-	-	-	-	5				
125	0	2								

§Table 5 summarizes ovulation data. Parentheses enclose possible explanations for corpora albicantia of inexplicit age; groupings of diameters for the corpora albicantia in Fig. 4 are the basis for these explanations.

Y indicates animal from Yellowstone Park; all other animals from the Bison Range.

* indicates limited material, i.e., incomplete ovaries.

Two were relatively new, indicating that she ovulated two ova as a 1-2 year old. The remaining five were of the older type, and represented that number of ova ovulated when she was a fawn.

Doe #21, taken 8 September, was in the 4-5 year age class. Six forming corpora lutea signify that she had ovulated, somewhat earlier than usual, that same number of ova. Corpora albicantia totaled 12. All of these are rather dense, but four show signs of more recent origin than the others, indicating that this animal produced four viable ova as a 3-4 year old. Their appearance is older than one might expect for a 2-month old corpus albicans, probably the result of early parturition and distortion by pre-ovulatory follicles. The remaining scars are not well clumped on the graph, but their arrangement suggests that this doe might have ovulated three ova as a 2-3 year old and five ova as a 1-2 year old, or four as a 1-2 year old and one as a fawn.

Doe #23Y was taken 18 September and was in the 2-3 year age class. Absence of corpora lutea and pre-ovulatory follicles means that she was in pre-estrus. Five corpora albicantia, all about 2 or 3 months old, indicate that she ovulated that many ova as a 1-2 year old and probably did not breed as a fawn.

Doe #38Y was collected on 30 September and was 8-9 years old. Only one ovary from this animal was available for microscopic study. One forming corpora lutea and field data indicate recent

ovulation of four ova. Six degenerating corpora lutea with luteal cells still present are evident. These indicate parturition or silent heat within the last month. Twenty-four corpora albicantia were counted in this ovary. Of these, three appear to be 3-4 months old, so later parturition in September is doubtful. Neat groupings of corpora albicantia diameters on the graph point temptingly to past ovulation history, but cannot be considered reliable.

The remainder of the specimens were collected from 11 October to 19 July and present clear corpora lutea of pregnancy or young corpora albicantia to signify ovulation incidence of the most recent breeding season. Other, older corpora albicantia in these ovaries are difficult to separate into 1) corpora albicantia which resulted from the ovulation of 13 months ago, and 2) those which resulted from earlier breeding seasons.

CHAPTER V

SUMMARY

Ovaries of 26 pronghorns from the National Bison Range at Moiese, Montana, and Yellowstone National Park were prepared histologically and studied microscopically. These ovaries were from does used in a study by O'Gara (1968), who recorded data on their ages and reproductive status, as well as other information.

The following is a list of important findings:

1. Numbers of vesicular follicles increased in pre-estrus and estrus, but average diameters of these follicles and their standard deviations did not differ markedly throughout the year.

2. While a number of characteristics were used to determine in which stage follicles were (growing, transitional, early atresia, mid-atresia, late atresia, very late atresia, very very late atresia), the fates of the ovum and zona pellucida were variable in atresia and were therefore poor indicators of the degree of atresia.

3. Two instances of polyovulation were observed. They were in ovaries from different animals and both follicles involved had two normal ova.

4. Luteal cells of the corpus luteum began degenerating at parturition and within a month no luteal cells could be seen, marking the scar as a corpus albicans. Lacunae were seen in new corpora albicantia delineating the spaces formerly occupied by the luteal cells.

5. Corpora albicantia of 4 months and sometimes more were distinguishable from corpora albicantia of previous years.

6. Two classes of luteal gland cells, thecal interstitial gland tissue, relatively large antrum, and thick tunica albuginea are indicative of the species' phylogenetic relationship to other artiodactyls.

7. Superovulation, the outstanding feature of pronghorn reproduction, can perhaps be accounted for as a) a remnant of a previous tendency to produce large numbers of young at one time, or b) a mechanism to produce large amounts of luteal tissue, which would in turn produce large amounts of progesterone for maintenance of pregnancy throughout the long gestation period. These two hypotheses need not be mutually exclusive.

8. An analysis of ovulation incidence revealed that silent heat probably did occur in one doe. If so, the resulting corpora lutea were the only accessory corpora found in the study.

9. Of seven does whose ovulation incidences as fawns could be determined, one definitely bred at 4 months of age. Two others ovulated and probably bred.

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