

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE BOVINE  
CORPUS LUTEUM AND ITS RELATIONSHIP TO PAST  
REPRODUCTIVE PERFORMANCE

by

BERT HAZEN ERICKSON

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## INTRODUCTION

The accurate and extensive records of the cow's reproductive performance, resulting from the widespread use of artificial insemination, have made both dairy scientist and husbandman acutely aware of a vast reproductive problem. Considerable research has been directed toward the detection and the elimination of venereal and other communicable diseases that affect reproductive efficiency. However, comparatively little research has been pointed toward physiological irregularities.

If efforts to detect an infection of the reproductive tract prove futile, the cause of reproductive failure is often attributed to ovarian mal-function. Treatments designed to correct ovarian mal-function are commonly used, without adequate knowledge of what constitutes normal or abnormal functioning of the bovine ovary.

It is common knowledge that a corpus luteum is formed from the cellular elements of a follicle subsequent to ovulation. With this as an established fact, histological descriptions of successive stages of regressing corpora lutea should provide basic information by which research workers could determine whether an excised ovary of a cow was functional and, if not, the approximate length of time it had been mal-functional. Such information could form the basis for determining the existence, frequency, and relative duration of abnormalities, now thought to affect the bovine ovary.

## REVIEW OF LITERATURE

## Histology of the Bovine Corpus Luteum

McNutt (14), observed that following ovulation, the follicle contracted markedly and the walls were thrown into deep folds leaving a small irregular central cavity. It was also possible for the cavity to be entirely obliterated by the folds. The follicle would shrink in size from a diameter of 16 to 19 mm. to one of 5 to 8 mm. and the corpus luteum would develop to a size that exceeded the remainder of the ovary. Proliferation of the lutein cells and vascularization of the developing corpus luteum were noted to take place together in early growth, but vascularization continued for some time after cell proliferation had ceased. He listed nine days post ovulation as the time required for the corpus luteum to attain its maximum size.

Foley and Reece (7), described the cells of the corpus luteum between 25 and 78 days of pregnancy. At 25 to 30 days of pregnancy, considerable variation in size and shape of cells was noted. The largest cells occupied an area eight to ten times that of the nucleus, and possessed globular shapes with regular outlines. The smallest cells were reported to be very irregular in shape, being characterized by long and narrow shapes, with an area two to three times that of the nucleus. All sizes and shapes were noted to occur between these extremes and the cells were freely interspersed, lacking any characteristic arrangement. The nuclei of the cells described were large and either round or oval in shape. They were slightly basophilic and had prominent nucleoli. The cytoplasm was referred to as being granular and tending to be eosinophilic.

At 78 days of pregnancy, these workers reported an increase in the size of the lutein cells with the largest occupying areas 12 times that of their



nuclei. The nucleus stained lightly and the cytoplasm tended to become more granular and less eosinophilic.

They found the general cellular arrangement of the corpus luteum at 25 days of pregnancy to be very loose. Open areas were noted between cells at this stage, but as gestation proceeded to 78 days the cell arrangement became more compact, with only occasional open areas appearing between groups of cells.

Elder (6), reported that the bovine corpus luteum undergoes a fatty degeneration. He described the corpus rubrum as containing large masses of fatty material held between large well developed fibers of connective tissue. An attempt was made to associate the presence of many corpora rubra with infertility.

McNutt (15), in a description of the regressing corpus luteum, stated that fatty degeneration begins with a general decrease in size of the lutein cells. By the fifth month of gestation or the fourteenth day of the estrous cycle, fat deposits begin to appear where luteal cells had undergone fatty degeneration. Regression was reported to continue slowly until the eighth month of gestation, or the twentieth day of the cycle, and then become more marked. At this stage, the majority of the lutein cells were noted to be replaced by connective tissue, but some cells persisted and were transformed into pigment cells; thus, forming the corpus rubrum of the bovine ovary.

#### Histochemical Considerations

Histochemistry of the Follicle. Moss et al. (17), using Gomori's (10), technic for alkaline phosphatase determination, found that during the early development of the follicle the granulosa cells contain more phosphatase than the theca cells, but in later stages of development this condition was reversed. The nucleus was the only portion of the ovum found to contain phosphatase.

They found that in tertiary follicles, only the theca contained phosphatase, with activity being highest in the region where the follicle approached the ovarian surface.

Histochemistry of the Corpus Luteum. In a study conducted to determine the cellular make-up of the corpus luteum, Corner (4), used Gomori's alkaline phosphatase technic on sows' ovaries. He found that cells of thecal origin were dispersed throughout the corpus luteum, and could easily be identified during the first seven weeks of pregnancy. After this period, the cells of granulosa origin became reactive and sites of cell origin were not easily distinguished. He stated that the nuclei of practically all cells contain alkaline phosphatase.

Moss et al. (16), reported that the amount and distribution of alkaline phosphatase in the bovine corpus luteum could be directly related to the stage of the estrous cycle. Generally, the over-all phosphatase activity of the corpus luteum increased to a maximum at mid-cycle, and then decreased. In a detailed description of phosphatase activity in the corpus luteum at diestrus, they reported that alkaline phosphatase was present in large amounts in the endothelium of capillaries and blood vessels, as well as in both the theca and granulosa lutein cells. In corpora lutea of pregnancies ranging from 30 to 66 days duration, alkaline phosphatase was prominent only in the endothelium of arterioles, with the lutein tissue showing little or no phosphatase activity.

Foley et al. (8), studied the deposition of alkaline phosphatase in the corpora lutea of pregnant heifers. The corpora lutea of heifers slaughtered between 24 and 45 days of pregnancy contained alkaline phosphatase of high concentration in the endothelium of the blood vessels, but only moderate amounts were deposited in the nuclei. The nucleoli and the cytoplasm contained no alkaline phosphatase.

## Abnormalities Affecting the Bovine Ovary

The known factors causing abnormal functioning of the bovine ovary can be grouped under four general heads: (1) Cystic follicles, (2) Cystic corpora lutea, (3) Retained corpora lutea, and (4) Functional ovarian inactivity.

Literature concerned with each of these conditions will be reviewed separately.

Cystic Follicles. This condition, according to Albrechtsen (1), Garm (9), and Wiltbank et al. (21), can influence the reproductive cycle by: (1) causing permanent heat, or intense heat at frequent intervals, (2) produce normal heat at regular intervals, (3) result in mild symptoms of heat at infrequent intervals, and (4) cause a complete absence of heat.

The features of the ovaries, in each of the groups mentioned, were found by Garm (9), to be identical. He reported that solitary or multiple cysts being much more common. A corpus luteum may or may not be present. Histologically, he observed that the granulosa cells are often completely absent from the follicular wall, both in large and small cysts. The granulosa, when present, was described as a scanty layer that was frequently partially luteinized. The vascularization of the theca interna was considerably reduced, and many cells showed evidence of degenerative changes. Numerous erythrocytes were seen throughout the theca interna and the granulosa layers. When large cysts were present, the remainder of the ovarian tissue had undergone marked atrophy.

Bone (3), listed the following methods to justify his criterion for a follicular cyst. Serial sectioning through follicles believed to be cysts failed to reveal either cumulus oophorous or ovum, the essential components of a normal follicle. Further examination did not reveal any extensive vascularization of the theca interna, loosening of the granulosa, or inward

proliferation of connective tissue cells, which are accepted characteristics of normal atresia.

Retained Corpora Lutea. A retained corpus luteum has been defined by Williams (19), as one which remains in a functional condition beyond its normal involution time.

Bone (3), reported that a cow with a retained corpus luteum will show persistent failure to come into heat, and that on rectal palpation a large soft corpus luteum will be found in one or both ovaries. He also reported that in a retained corpus luteum the fibrous connective tissue had broken the luteal cells into irregular masses, the vascularization was considerably reduced, little or no change had taken place within the luteal cells and that there was a more clear-cut demarcation of the luteal tissue from the underlying stroma of the ovary.

Roberts (18), stated that a retained corpus luteum is usually observed in association with some uterine pathology or distention, such as pyometra, fetal maceration and fetal mummification.

Cystic Corpora Lutea. In his attempt to associate this condition with infertility, Bone (3), examined the ovaries of 103 pregnant cows and found no case in which a cyst was present in the corpus luteum. Conversely, he found 12 cases of cystic corpora lutea among 55 nonpregnant cows.

Elder (6), failed to find a cystic corpus luteum in connection with pregnancy, in all but one case. The cow that constituted the one exception was six weeks pregnant with a cyst 5 mm. in diameter in the corpus luteum.

Bone (3), reported that, with the exception of the centrally located cyst, the histology of a cystic corpus luteum is similar to that of a normal corpus luteum.



Asdell et al. (2), stated that a cyst in a corpus luteum is an abnormal behavior of little consequence.

Functional Ovarian Inactivity. Laing (12), defined a functionally inactive ovary as one that had produced follicles and corpora lutea which resulted in normal estrous cycles, after which, it relapsed into an inactive state. He reported that the most acute form of this condition was evidenced by the ovaries being extremely small with no visible follicular development. The opposite extreme was one in which the follicles develop and ovulate but the usual symptoms were lacking.

#### MATERIALS AND METHODS

The ovaries studied were obtained from 72 cows which were from the Kansas State College dairy herd. The reproductive history of each of these cows was known from first breeding as a heifer to date of disposal. The Holstein, Ayrshire, Guernsey and Jersey breeds were represented, and the ages ranged from two to twelve years. With the exception of 30 cows sacrificed to obtain timed stages of embryogeny or fetal development, the cows involved were eliminated from the herd because of failure to reproduce.

The cows were slaughtered at a local abattoir and the ovaries, except those selected to be kept fresh for photographic purposes, were immediately placed in a fixative. Ten percent buffered formalin was the fixing agent for tissues used for normal histological study, and 80 percent alcohol for tissues used for alkaline phosphatase determinations.

The tissues were dehydrated in a graded series of alcohols and embedded in paraffin, using the isopropyl alcohol technic. Ten-micron sections were taken from the longitudinal medial surface of the ovary.



Tissues for histological study were stained in Harris' hematoxylin, and counterstained in a mixture of acid fuchsin and orange G. Alkaline phosphatase determinations were made by Gomori's (10) technic of 1939.

The ages of the regressing corpora lutea were determined by correlating the number, histological appearance, and size of corpora lutea present in the ovaries, with recorded periods of estrus.

Photomicrographs were taken, using "Kodachrome Professional Type "A" film at a magnification of 600X. Deviations from this will be appropriately noted.

Case histories subsequent to the last parturition before slaughter are reported for cows with abnormal ovaries to support points made in the discussion.

All gross measurements were made with vernier calipers.

#### OBSERVATIONS

On the basis of post-mortem examination of reproductive tracts and ovaries, the 72 cows included in this study were classified as follows:

Pregnant and clinically normal.	30
Not pregnant - cause of failure to conceive unknown.	16
Not pregnant - infected uterus.	11
Not pregnant - oviducts occluded.	4
Not pregnant - cystic corpora lutea.	7
Not pregnant - cystic follicles.	2
Mummified fetus.	1
Functionally inactive ovaries.	<u>1</u>
	72

The corpora lutea in the ovaries of the above 72 cows were studied and correlated with respective recent reproductive histories.

## Development and Regression of the Bovine Corpus Luteum

Only those stages of the development and regression of the corpus luteum that were characterized by distinctive changes are discussed. The corpora lutes studied were from apparently normal ovaries.

The Corpus Luteum of One Day Post Estrus. Following ovulation the theca externa of the follicle was found to have contracted and forced the theca interna and stratum granulosa into deep folds (Plate I, Fig. 3)<sup>1</sup>. The theca interna formed the core of the folds and was distinguished from the stratum granulosa by the intense eosinophilic character of its cells and the intermittent occurrence of blood vessels. The nuclei of the granulosa cells were very small and were surrounded by negligible amounts of cytoplasm (Plate IV, Fig. 1).

The corpus luteum at this stage measured 13 x 10 x 12 mm.

The Corpus Luteum of Seven Days Post Estrus. The corpus luteum was markedly larger, and measured 27 x 20 x 20 mm. The theca interna, as an organized structure, was visible only at the periphery and core of the corpus luteum, in the form of trabeculae. Other cells of the theca interna were freely dispersed among the granulosa cells. The granulosa cells had undergone a considerable increase in size, due primarily to the increase in the cytoplasm that occupied eight times the area of the cell nucleus (Plate IV, Fig. 2). Small capillaries were prominent throughout the luteal tissue. The general cellular arrangement was quite loose.

The nuclei of the theca luteal cells were highly reactive to alkaline phosphatase. The nuclear granules were the only portion of the nuclei of

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All plates in the Appendix.

granulosa luteal cells that exhibited reactivity. The cytoplasm of theca and granulosa luteal cells showed a uniform negativity to the presence of alkaline phosphatase (Plate X, Fig. 1).

The corpus Luteum of 18 Days Post Estrus. Cell spacing had become compact (Plate IV, Fig. 3) and the entire structure measured 25 x 18 x 18 mm. The cells had become intensely basophilic. All other morphological characteristics were similar to those found in the seven-day corpus luteum. At this stage cell differences were not readily distinguished by alkaline phosphatase activity.

The Corpus Luteum of 33 Days Post Estrus. The luteal body was firmly enclosed by ovarian stroma and its measurements had decreased to 13 x 14 x 12 mm. The trabeculae had again become prominent with the decrease in size. The nuclei of the granulosa lutein cells had lost their circular shape and had become definitely pyknotic. Cell membranes in most cases were indistinct and the amount of cytoplasm in granulosa and theca luteal cells was greatly reduced (Plate VI, Fig. 1).

The regressing luteal body was still distinctly bordered by the theca externa (Plate V, Fig. 1).

The Corpus Luteum of 61 Days Post Estrus. The measurement was 7 x 6 x 4 mm. and the cells of blood vessels occupied one third of the luteal area. The nuclei of the luteal cells had become progressively more pyknotic and the number of luteal cells between vessels had decreased leaving a general openness of structure (Plate VI, Fig. 1). The distinct line remained between the corpus luteum and theca externa.

The Corpus Luteum of 86 Days Post Estrus. The granulosa luteal cells were reduced to clumps of nuclei with only traces of cytoplasm, isolated between blood vessels and connective tissue-like cells (Plate VI, Fig. 3). The nuclei of all remaining cells were characterized by intense basophilia. The general

cells arrangement had again become very compact. The decrease in cell size and resultant decrease in luteal area had made the location and numbers of blood vessels highly prominent (Plate V, Fig. 2). In limited areas the line between the regressing corpus luteum and general ovarian tissue was indistinct. The regressing luteal body measured 8 x 5 x 4 mm.

The Corpus Luteum of 109 Days Post Estrus. The granulosa lutein cells could no longer be distinguished from the theca lutein cells, ( i.e. the connective tissue-like elements of the regressing corpus luteum). The corpus luteum at this stage had been reduced to a mass of blood vessels interwoven with theca luteal cells (Plate VII, Fig. 1). An eosinophilic material, relatively free of nuclei, had begun to appear at the periphery and about the larger blood vessels within the stroma. The measurement of the regressing corpus luteum was 7 x 4 x 4 mm.

The Corpus Luteum of 127 Days Post Estrus. The eosinophilic material had increased within the stroma (Plate VII, Fig. 2) and at the periphery of the corpus luteum. This was the only change noted at this stage. The measurement was 5 x 5 x 4 mm.

The Corpus Luteum of 141 Days Post Estrus. The eosinophilic material had practically obliterated the regressing corpus luteum. At the periphery, the eosinophilic mass had been infiltrated by cells of the surrounding ovarian stroma, obliterating the marginal demarcation. The cells of the small blood vessels and the cells that had been oriented about them were completely disorganized. (Plate VII, Fig. 3).

The corpus luteum at this advanced stage of regression was characterized by a small mass of intensely basophilic cells (Plate IV, Fig. 3).

Corpora Lutea Older Than 141 Days Post Estrus. Older corpora lutea were observed only in cases in which pregnancy intervened. When pregnancy intervened,

corpora lutea in the later stages of regression remained much longer before losing their identity. In one case, in which pregnancy had closely followed pregnancy, a regressing corpus luteum was aged at 443 days. The remaining cell mass was similar to that seen in the 127 day regressed corpus luteum in which regression was not interrupted by pregnancy (Plate VII, Fig. 2) and (Plate VIII, Fig. 3).

Corpora lutea of pregnancy were observed up to 240 days gestation. The cells and cellular arrangement of the 240 day corpus luteum were much like those seen in a corpus luteum of 17 days post estrus. It was particularly interesting to note the large plump condition of the granulosa lutein cells and cell nuclei (Plate III, Fig. 3) and (Plate II, Fig. 3).

At 21 days post partum the cellular details of the regressing corpus luteum of a non-pregnant cycle. In both cases the nuclei of the granulosa lutein cells were pyknotic and the cell membranes were indistinguishable (Plate VI, Fig. 1) and (Plate VIII, Fig. 2).

### Pathological Findings

Not Pregnant - Cause of Failure to Conceive Unknown. The cows in this group had been inseminated an average of six times following their last parturition, but failed to conceive. For this same period, a regressing corpus luteum was found in the ovaries for each observed heat period; indicating that the ovaries were functionally normal. The tissues of the uteri appeared normal, and showed no evidence of infection. The passage through the oviducts was not obstructed and apparently free from infection.

Not Pregnant - Infected Uterus. The length of the estrous cycle of cows in this group was highly erratic, with consecutive cycles of normal length being the exception rather than the rule. In five cases, one horn of the uterus was



infected as evidenced by a pocket of pus encircled by inflamed tissue. The tissues of two uteri were completely infected and swollen. In one instance, both horns of the uterus were occluded, one by scar tissue and the other by a cyst. The remaining two cases were characterized by a generalized uterine infection plus a gelatinous plug in the cervix.

The ovaries in all cases appeared normal.

Not Pregnant - Oviducts Occluded. The length of the estrous cycle of the cows in this group were normal, as were the uteri and ovaries.

Not Pregnant - Cystic Corpora Lutea. Seven cows were in this group and the reproductive history for each, following the parturition before slaughter is presented. Cow 21 was inseminated three times following her last parturition. The intervals between the three breedings were 20 and 39 days. She was slaughtered 35 days following her last breeding and was found not pregnant. The uterus was normal and showed no evidence of conception. A 9 mm. cyst was in the center of the most recent corpus luteum.

Cow 71 was inseminated five times following parturition, with an average cycle length of 18 days. Slaughter occurred 20 days post estrus and no evidence of pregnancy was found. A cyst 17 mm. in diameter was enclosed by a corpus luteum measuring 26 mm. at its widest point.

Cow 81 was inseminated twice following parturition. She was slaughtered 10 days post insemination and found not pregnant. Twenty-two days elapsed between insemination dates. The cyst in the corpus luteum was 11 mm. in diameter.

Cow 8 was inseminated three times and found not pregnant at slaughter, 21 days after last insemination. The cycles were 49, 47, and 41 days in length. The most recent corpus luteum contained a 10 mm. cyst and the nonfunctional ovary possessed a centrally located follicle 12 mm. in diameter with a single

layer of granulosa cells bordering the follicular cavity.

Cow 11 was inseminated during 10 consecutive estrus periods and failed to conceive. The four cycles preceeding slaughter averaged 21 days in length but prior to this, three 40 day cycles and one 90 day cycle had occurred. A 10 mm. cyst was located in the most recent corpus luteum that was 27 mm. at its widest point. Slaughter occurred 14 days post insemination.

Cow 19 was inseminated three times following her fifth parturition, she was slaughtered thirty days post insemination, and found not pregnant. Remnants of embryonic membranes were found and the caruncles showed evidence of stimulation. The three cycles were each 21 days in length. An 11 mm. cyst was found in the most recent corpus luteum (Plate VIII, Fig. 1).

Cow 54 was inseminated at four consecutive heat periods and slaughtered 19 days post insemination. The cycles were of normal length. The uterus contained embryonic membranes in addition to showing evidence of caruncle stimulation. A 9 mm. cyst was present in the corpus luteum.

In all cases observed, the cyst was inclosed in a connective tissue capsule that was approximately 1 mm. in thickness and normal luteal cells were not present for an additional 2 mm. beyond the connective tissue.

Not Pregnant - Cystic Follicles. Cow 48 was inseminated once following parturition and diagnosed pregnant 45 days post insemination. Eight months later, following slaughter, she was found not pregnant. She had been reported as being aggressive and nervous for a considerable period of time prior to slaughter.

The right ovary contained follicles of 17, 15, 12, and 9 mm. in diameter, and in all cases the granulosa layer of the follicle was absent. A blood clot was present in the most recent regressing corpus luteum.

Follicles of 25, 25, 20, 14 and 9 mm. with the granulosa absent were in the left ovary. This ovary also possessed a regressing corpus luteum containing a blood clot.

Cow 38 was inseminated once following parturition and found not pregnant at slaughter 17 days later. The right ovary contained follicles of 26 and 22 mm. in diameter, both of which were void of a granulosa layer. The left ovary contained a 21 mm. centrally located follicle that also had no granulosa layer.

Mummified Fetus. Cow 31 carried a fetus mummified at about half term for 315 days. The corpus luteum of pregnancy had remained large, and histological sections indicated that it had remained functional up to time of slaughter. No heat cycles were reported during the 315 day period and only the regressing corpora lutea were found as evidence of recorded cycles prior to conception.

Not Pregnant - Functionally Inactive Ovaries. Cow 40 had cycled four times following her last parturition and then failed to show signs of estrus for four months, at which times she was slaughtered. Only corpora lutea in advanced stages of regression were found in both ovaries. Primary and secondary follicles were absent.

#### Histochemical Findings

Alkaline Phosphatase in the Corpus Luteum. At seven days post ovulation, the nuclei of the theca interna luteal cells were highly reactive to alkaline phosphatase. The nuclear granules were the only portion of the nuclei of granulosa luteal cells that exhibited reactivity. The cytoplasm of theca and granulosa luteal cells showed a uniform negativity to the presence of alkaline phosphatase. All blood vessels were well marked by the presence of alkaline phosphatase (Plate IX, Fig. 1) and (Plate X, Fig. 2).

The situation in a 26 day corpus luteum of pregnancy was relatively the same as that noted in a seven day corpus luteum. By 40 days of pregnancy, the nuclei of theca luteal cells had lost their intense phosphatase reactivity. The granules in the nuclei of the granulosa luteal cells remained clearly marked by phosphatase activity. The vessels of the corpus luteum remained well marked as was common in all portions of the ovary.

Alkaline Phosphatase in the Follicle. The granulosa cells of primary and secondary follicles were highly reactive to alkaline phosphatase. In tertiary follicles, the granulosa cells lost their reactivity; and the theca interna became distinctly marked by phosphatase activity (Plate IX, Figs. 2 and 3).

Phosphatase activity was not present in the theca interna of a cystic follicle (Plate X, Fig. 3).

## DISCUSSION

### Development and Regression of the Bovine Corpus Luteum

The formation of the corpus luteum as described by McNutt (15), and the description by Foley et al. (8), of cellular changes occurring during development are in agreement with observations made in this study.

McNutt (15), Elder (6), and Hammond (11), were the only investigators who have reported anything concerning the regression of the bovine corpus luteum. These investigators listed the stage of the estrous cycle and stage of pregnancy, at which regression began, but did not describe in detail the changes which occur during the regression.

The regression of the corpus luteum, as described by McNutt (15), involves a degeneration of the luteal cells and their replacement with connective tissue cells. In this study, the observations made on the regressing corpus luteum

indicate that the granulosa luteal cells are the first to be lost, and then many of the theca interna cells and cells of the smaller blood vessels. The remaining theca luteal cells or connective tissue cells and the larger blood vessels become enmeshed in the eosinophilic material and integrated with the surrounding ovarian tissue. Thus, in contradiction to the theories of McNutt (15), and Elder (6), the observations made indicate that no scar tissue remains as a lasting remnant of the corpus luteum.

Facilities were not immediately available to determine the composition of the eosinophilic material that characterizes the final regression stages of the corpus luteum.

The number and histological appearance of the regressing corpora lutea in the ovaries of the cows studied was found to be a reliable indication of the functional activity of the ovaries and the recent reproductive performance of the cow. The ovaries of a cow with a normal reproductive history that was slaughtered immediately following parturition or during gestation would contain only the corpus luteum of pregnancy and two or three corpora lutea of the cycles prior to conception in advanced stages of regression. Conversely, the ovaries of a "repeat breeder" would contain a functional corpus luteum plus a regressing corpus luteum for every non-fertile cycle. The ages of these regressing corpora lutea can be closely approximated on the basis of their size and histological appearance.

The observations made on the regression of the bovine corpus luteum are considered to be of importance not only in the evaluation of cow's past reproductive performance, but also in the determination of the frequency or existence of long periods of anestrus caused by the corpus luteum.



### Pathological Findings

Cystic Corpora Lutea. The studies of Bone (3), and Elder (6), indicate that a cystic corpus luteum is associated with infertility. McNutt (14), Hammond (11), and Asdell et al., (2) recognized the existence of these structures in functional corpora lutea, and suggested that their presence had little to do with a cows gravid or non-gravid state.

Seven cases of cystic corpora lutea were observed in this study and in every instance the affected cow was not pregnant. In two of these cases, remnants of embryonic membranes were found in the uterus. According to Bone (3), a cystic corpus luteum causes infertility by interfering with the normal functioning of the ovary, (i.e., causes long periods of anestrus). Contrary to his findings, the affected cows in this study had cycled regularly following the parturition before slaughter, and all were slaughtered within 35 days post estrus.

The histological appearance of the luteal tissue not closely associated with the cyst appeared normal for the age of the corpus luteum. The 30 and 35 day corpora lutea were in early stages of regression.

The histories of the affected cows and the histological appearance of the tissue suggests that a cystic corpus luteum interferes with reproduction not by causing long periods of anestrus, but by causing failure of embryonic attachment or early embryonic death. The cyst plus its connective tissue capsule and the cells of doubtful functional value adjacent to the connective tissue, eliminate a large portion of the functional capacity of the corpus luteum. This evidence resulted in the presentation of the theory that a cystic corpus luteum results in early embryonic death by causing a progesterone insufficiency. Further support was given to this theory by McDonald et.al., (13) who established the essentiality of the corpus luteum in the sustaining of pregnancy in the bovine.

Wiltbank et al., (20) found that 16 out of 36 (44%) repeat breeder cows injected with 50 mg. of progesterone per day beginning three days after estrus had normal embryos at 34 days. Normal embryos were found in 12 out of 36 (33%) control animals at the end of the 34 day period.

Cystic Follicles. Garm (9), presented a thorough study on cystic follicles in the bovine, and the two cases found in this study fit well in the categories he established. The ovaries of both animals were affected by multiple cysts and histological sections revealed an absence of the granulosa layer from the wall of the follicular cavity.

Retained Corpora Lutea. A study of the reproductive histories and ovaries of 72 cows revealed no instance in which a corpus luteum had repressed normal ovarian function, when all associated reproductive structures were normal. The corpus luteum that had remained functional for 315 days in association with the mummified fetus would tend to confirm the statement of Roberts (18), that a functional corpus luteum is maintained in association with pyometra, fetal maceration and fetal mummification.

#### Histochemical Findings

Alkaline Phosphatase. Histochemical studies have been made on the bovine ovary in an attempt to detect sites of hormone production, and the stage of the estrous cycle in which the greatest secretory activity occurs.

Danielli (5), reported that alkaline phosphatase is found in association with the selectively active borders of a wide variety of secretory cells, but stated that too little is known of the functions of this enzyme to justify conclusions.

The observations made in this study as to the sites of alkaline phosphatase activity in normal ovarian tissue are in full agreement with those reported by

Moss et al., (17) and Foley et al., (8).

This study revealed that the theca interna cells could be located in the developing corpus luteum of the bovine (Plate X, Fig. 1) in the same manner as that suggested by Corner (4). In his work with the ovary of the sow, Corner reasoned that since the cells of the theca interna were marked by an intense reactivity to the alkaline phosphatase stain up to the time of ovulation, these cells could be identified by this same reactivity in the developing corpus luteum.

In this work, it was interesting to note that the theca interna of a cystic follicle was completely negative to alkaline phosphatase (Plate X, Fig. 3). The test was also found useful in confirming the presence of the mass of blood vessels in a regressing corpus luteum (Plate IX, Fig. 1).

#### SUMMARY AND CONCLUSIONS

The excised ovaries and the associated reproductive histories of 72 dairy cows were studied. The Histological appearance of the ovaries was correlated with the respective reproductive history.

Descriptions of key stages of the development and regression of the bovine corpus luteum were presented.

On the basis of the studies made of the regressing corpus luteum, it was concluded that the number of regressing corpora lutea present in the ovaries would indicate the functional condition of the ovaries and the associated reproductive status and performance of the cow.

The studies of early stages of the developing corpus luteum indicate that maximum size is attained in seven to nine days.

Seven cases of cystic corpora lutea were found and all were associated with nonpregnancy. The presence of a cystic corpora lutea was associated with early

embryonic death.

Two cases of multiple follicular cysts were noted. The affected cows had experienced long periods of anestrus prior to slaughter.

One case of functionally inactive ovaries was found. This condition was associated with a four month period of anestrus.

A histological study of the ovaries and related reproductive history of 72 cows revealed no evidence of a retained corpus luteum.

Alkaline phosphatase was found in the cytoplasm of the granulosa cells of primary and secondary follicles, but was in the cytoplasm of only the theca interna cells in tertiary follicles.

The cells of theca interna and granulosa origin were clearly differentiated by alkaline phosphatase deposits in the corpus luteum until 26 days of pregnancy.

With alkaline phosphatase deposits in the early stages of development of the corpus luteum and late follicle as evidence, it was concluded that the corpus luteum is made up of the freely intermingling cells of the granulosa and theca interna.

### ACKNOWLEDGMENTS

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**APPENDIX**

#### EXPLANATION OF PLATE I

Fig. 1. A macroscopic view of bovine ovaries. Note the recent point of ovulation. (1.3X).

Fig. 2. Medial longitudinal section of the ovaries shown in figure one. 1. One day corpus luteum. 2. Regressing corpus luteum, 86 days post estrus. 3. Regressing corpus luteum, 33 days post estrus. 4. Regressing corpus luteum, 109 days post estrus. (1.3X).

Fig. 3. Photomicrograph of a histological section of the one day post estrus corpus luteum showing the folds formed by the cells of the granulosa and theca interna layers of the follicle. (5X).



## PLATE I

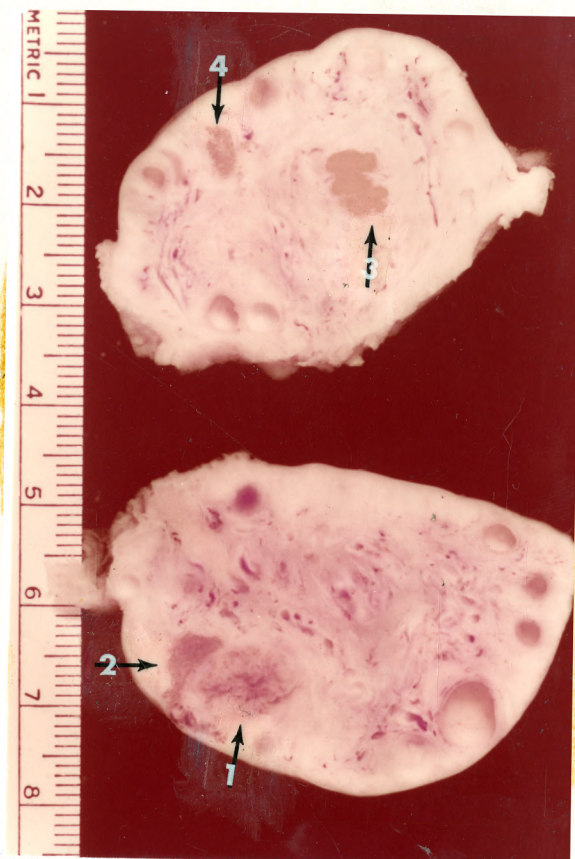


Fig. 1

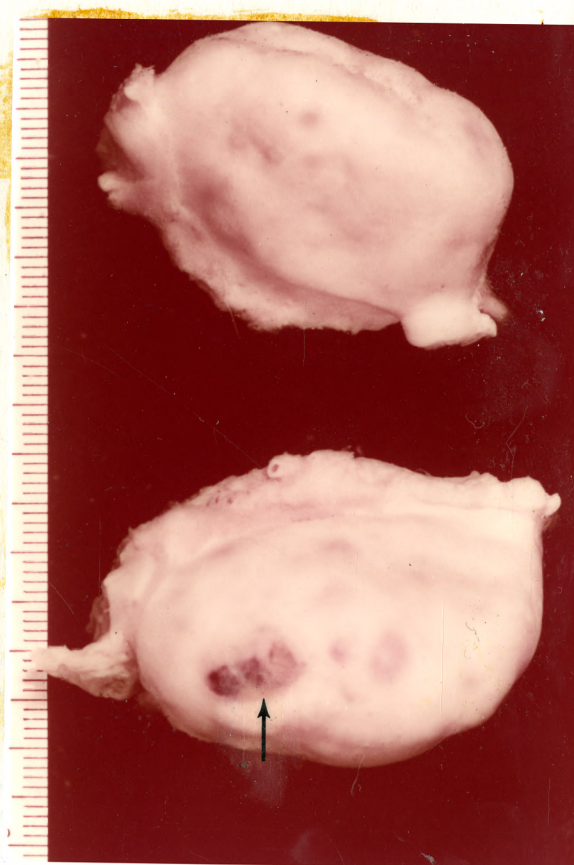


Fig. 2

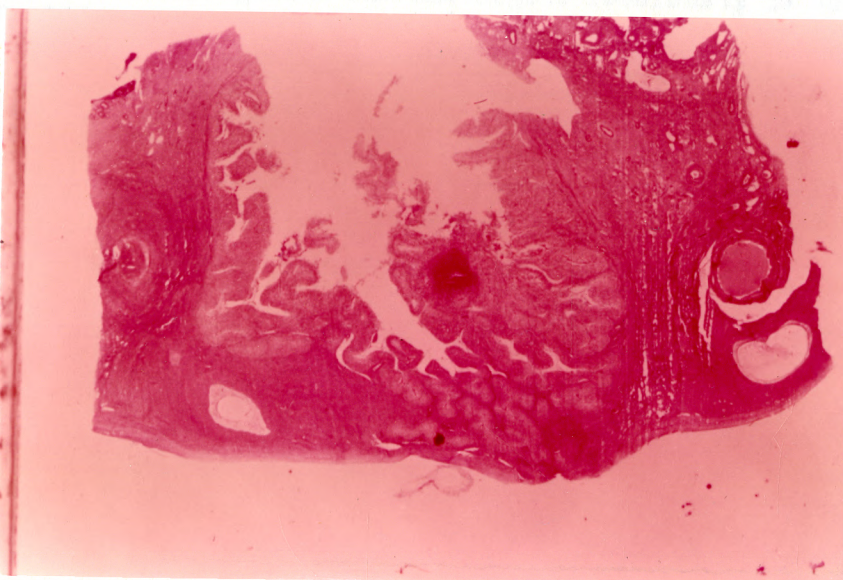


Fig. 3



#### EXPLANATION OF PLATE II

Fig. 1. A macroscopic view of bovine ovaries. The lower ovary contains a 17 day post estrus corpus luteum which is marked by the circular indentation in the tissue. (1.3X).

Fig. 2. A macroscopic view of the medial longitudinal section of the ovaries shown in figure one. The lower ovary was cut through the center of the 17 day post estrus corpus luteum. Note the tertiary follicles in the upper ovary. (1.3X).

Fig. 3. A photomicrograph of the cells of the 17 day post estrus corpus luteum. The large oval cells with the circular nuclei are considered to be of granulosa origin and the oblong cells with dark staining nuclei of thecal origin. (600 X). Compare with Plate III, Fig. 3.

## PLATE II

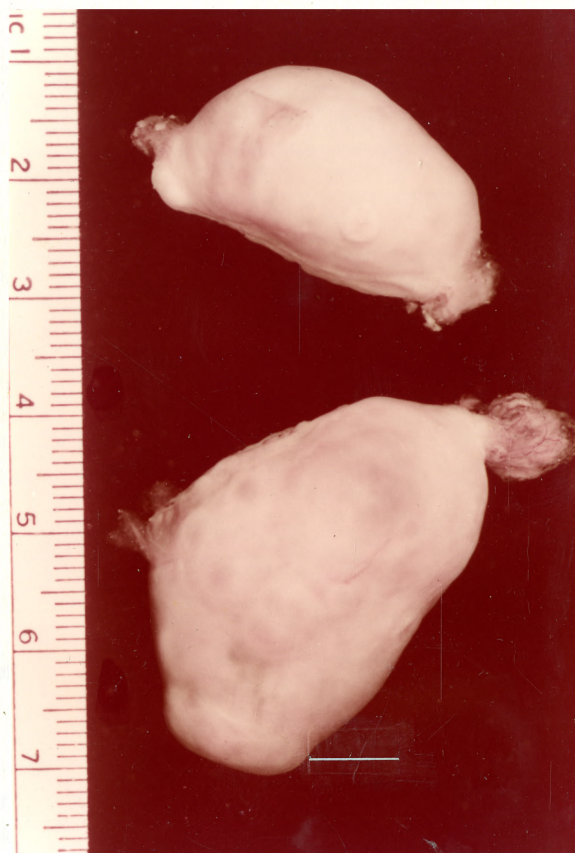


Fig. 1

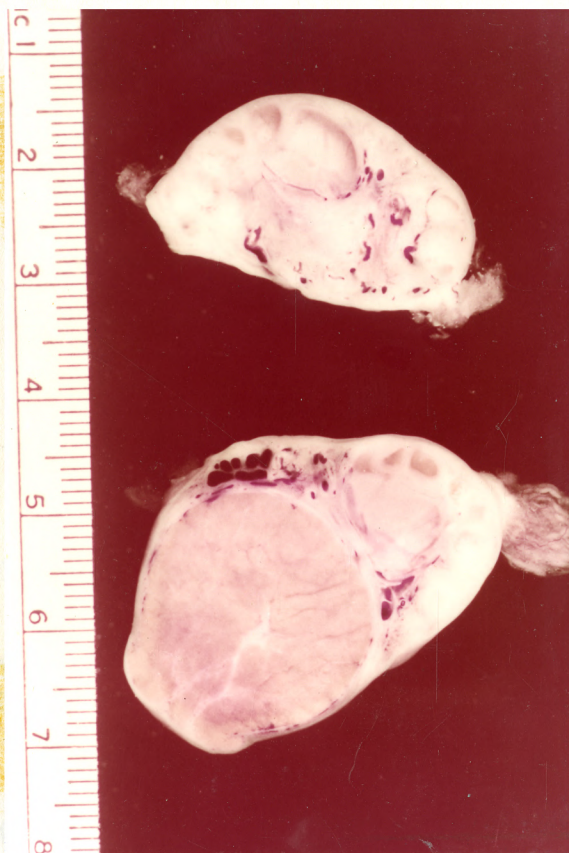


Fig. 2

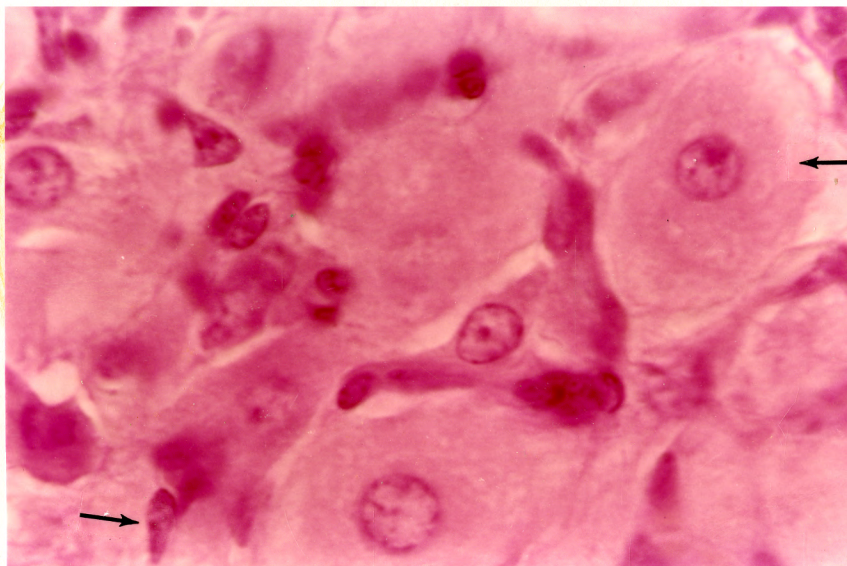


Fig. 3



### EXPLANATION OF PLATE III

Fig. 1. Macroscopic view of an ovary containing two corpora lutea associated with a 240 day gestation. The arrows mark the protrusions of the corpora lutea. (1.3X).

Fig. 2. Longitudinal section of the ovary in figure one showing the two functional corpora lutea. These corpora lutea were found in association with one fetus. (1.3X).

Fig. 3. Photomicrograph of the cells of a 240 day corpus luteum of pregnancy. Note the large plump condition of the granulosa luteal cells. (600X). Compare with Plate II, Fig. 3.

## PLATE III

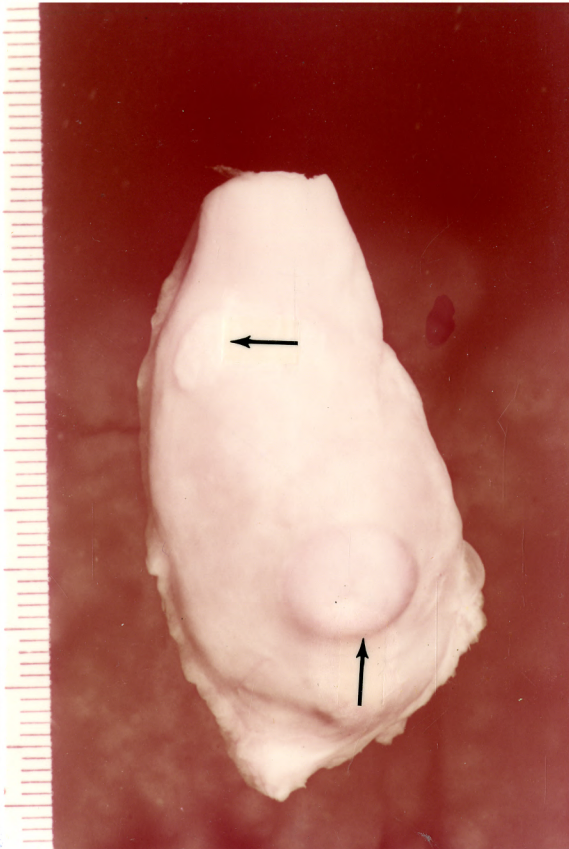


Fig. 1

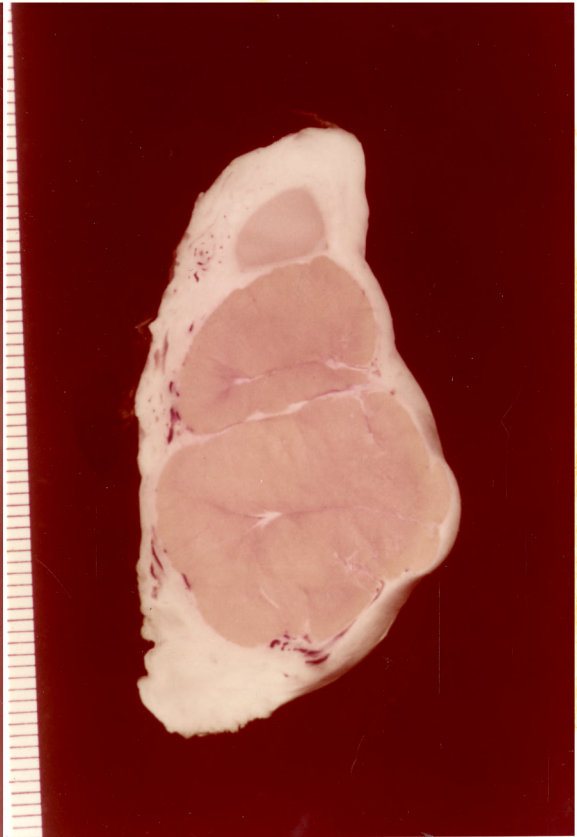


Fig. 2

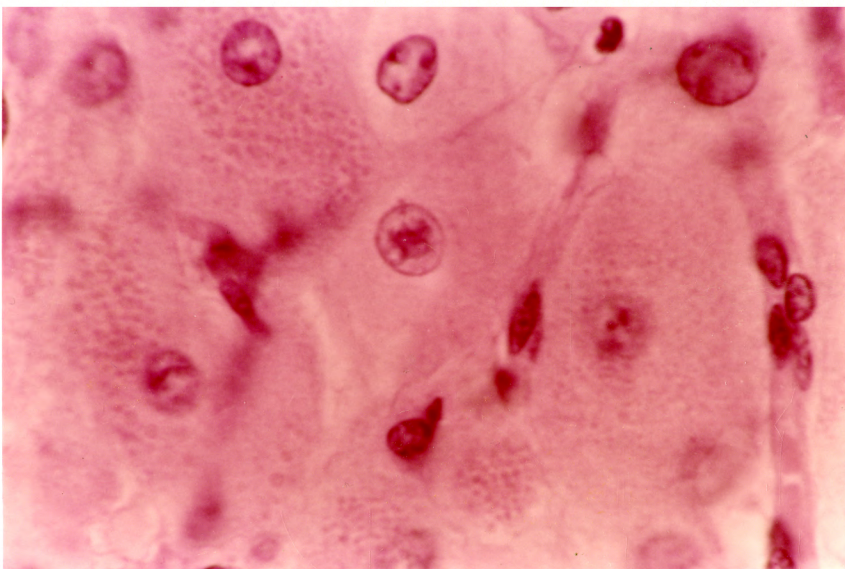


Fig. 3



#### EXPLANATION OF PLATE IV

Fig. 1. Photomicrograph of the cells of a one day post estrus corpus luteum. The nuclei are small and surrounded by a minute quantity of cytoplasm. (600X).

Fig. 2. Photomicrograph of the cells of a seven day post estrus corpus luteum. Note the general increase in size of nuclei, increase in cytoplasm about the nuclei of the granulosa lutein cells (marked by arrow) and the looseness of cellular arrangement. (600 X).

Fig. 3. Photomicrograph of the cells of an 18 day post estrus corpus luteum. The granulosa lutein cells have attained their maximum size and general cell arrangement is compact. (600X).



## PLATE IV

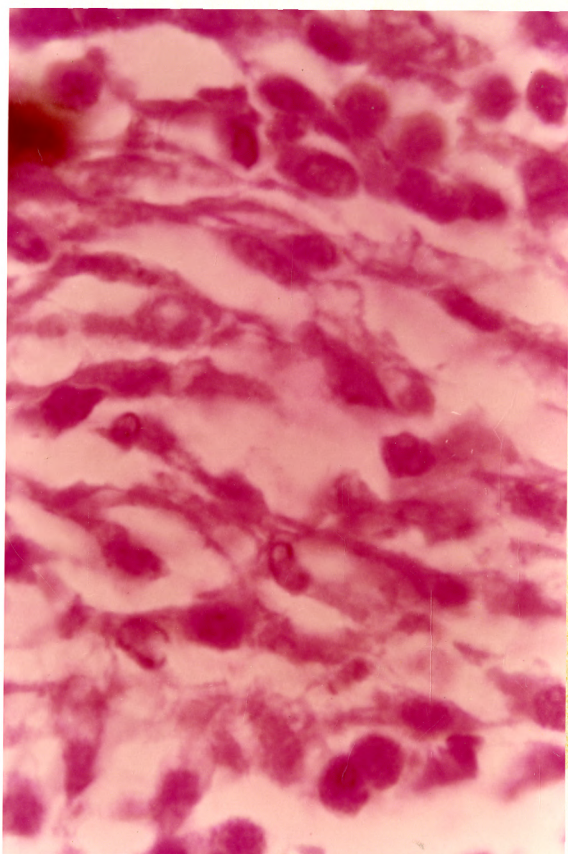


Fig. 1

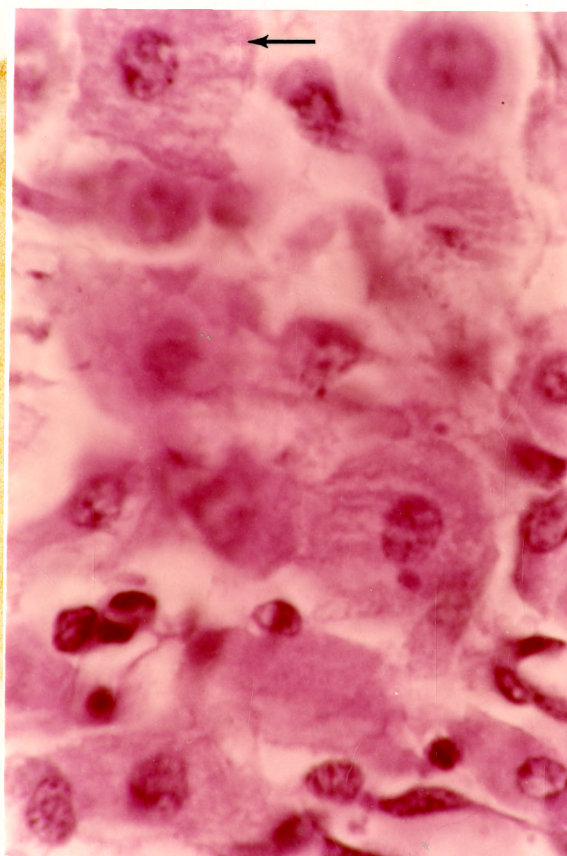


Fig. 2

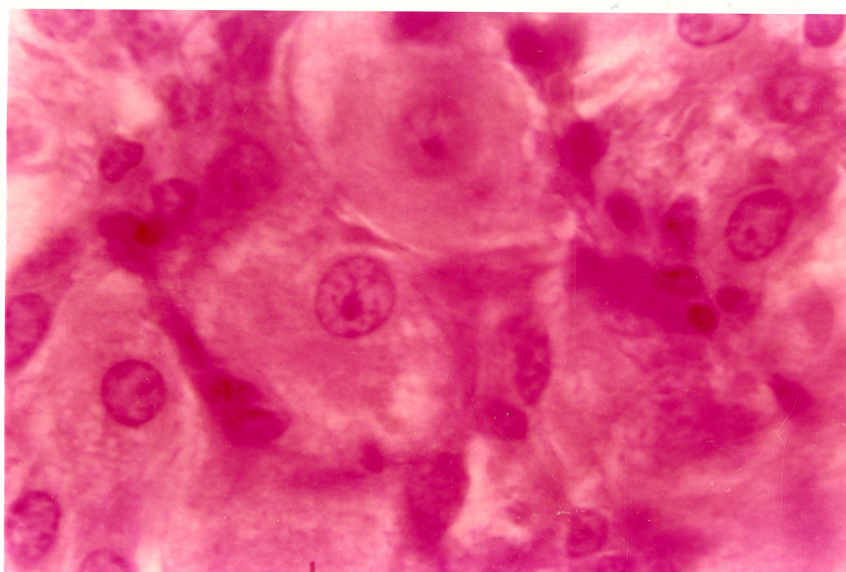


Fig. 3



#### EXPLANATION OF PLATE V

Fig. 1. Low magnification photo of a medial section of a regressing corpus luteum, 33 days post estrus. The regressing luteal body is still distinctly bordered by the theca externa. (5X).

Fig. 2. Low magnification photo of a medial section of a regressing corpus luteum, 86 days post estrus. The blood vessels have become prominent due to the loss of interstitial luteal cells. The theca externa border is no longer distinct. (5X).

Fig. 3. Low magnification photo of a medial section of a regressing corpus luteum, 141 days post estrus. Note the intense basophilia of the remaining cells. (5X).



## PLATE V

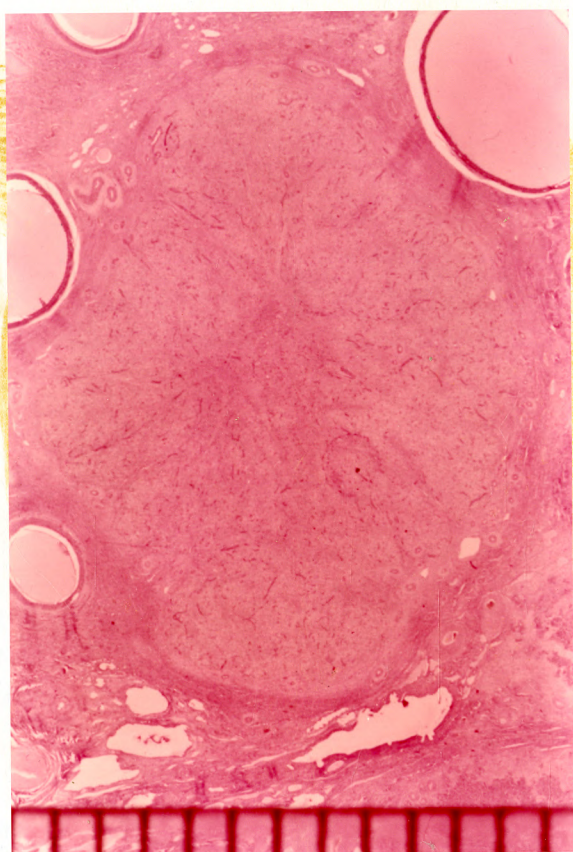


Fig. 1

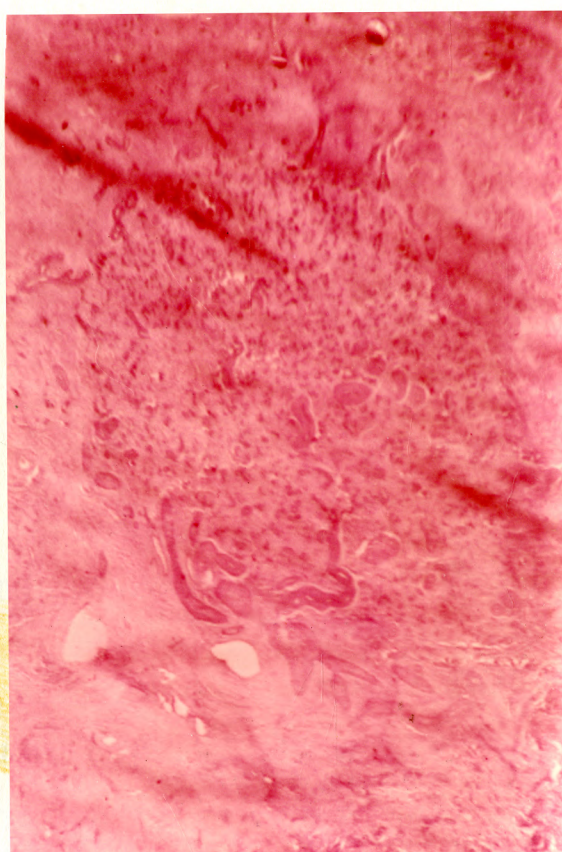


Fig. 2

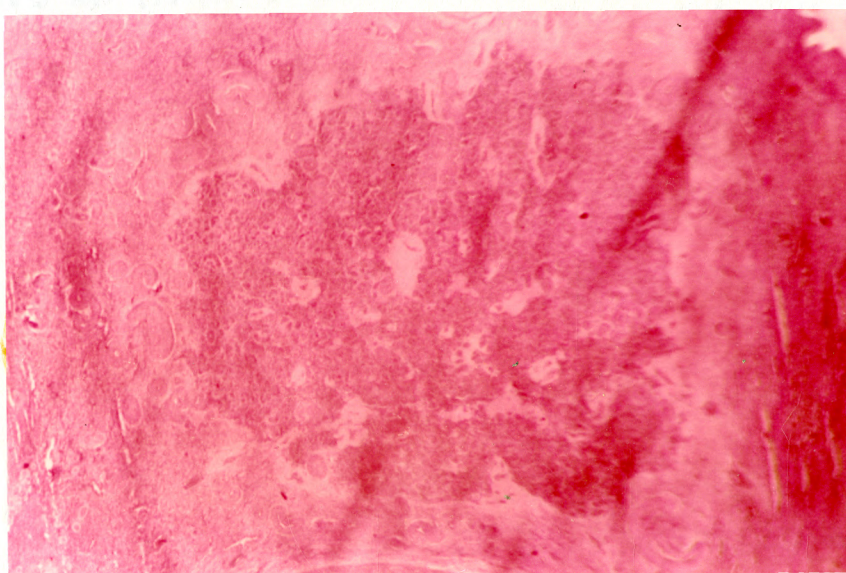


Fig. 3



#### EXPLANATION OF PLATE VI

Fig. 1. Photomicrograph of the cells of a regressing corpus luteum, 33 days post estrus. The nuclei of the granulosa luteal cells are pyknotic and cell membranes are indistinct. (600X).

Fig. 2. Photo micrograph of the cells of a regressing corpus luteum, 61 days post estrus. Note the intense basophilia of the cells surrounding the blood vessel in the lower right corner and the comparative lack of nuclei in the remaining area of the figure. (600X).

Fig. 3. Photomicrograph of the cells of a regressing corpus luteum, 86 days post estrus. Note the concentration of nuclei, the trabecula composed of theca luteal cells through the center, and remnants of granulosa luteal cells on either side. (600X).

## PLATE VI

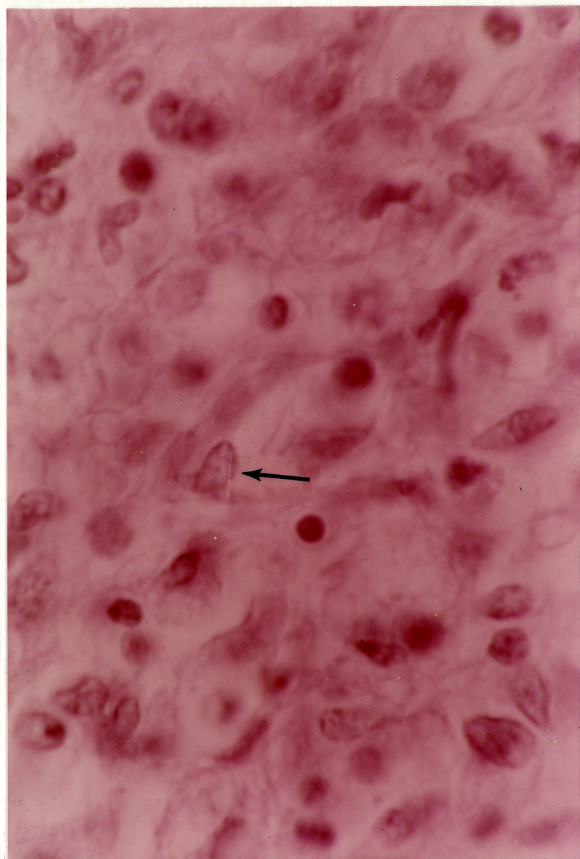


Fig. 1

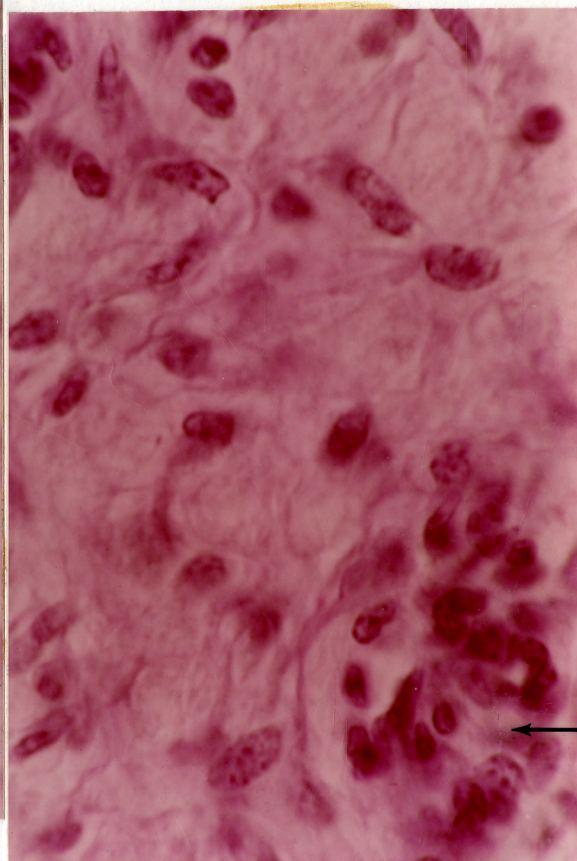


Fig. 2

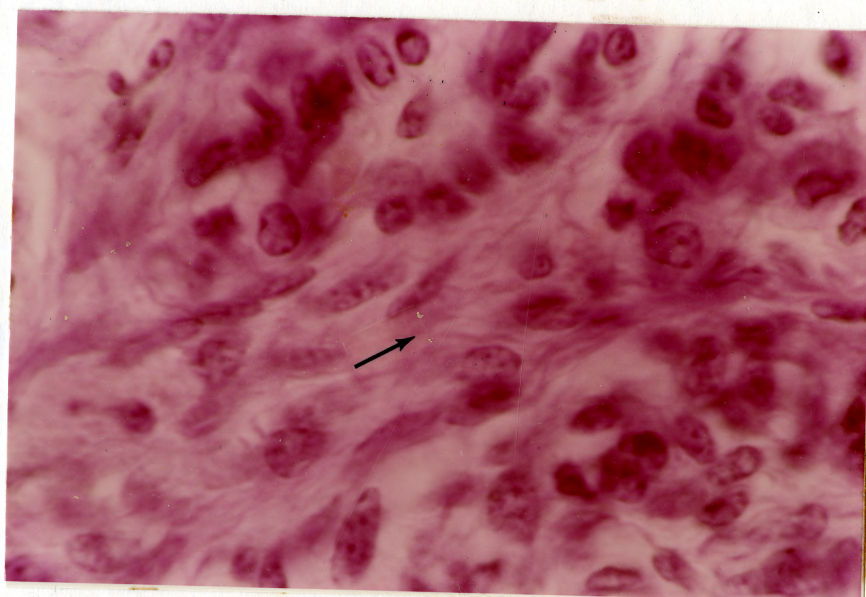


Fig. 3



#### EXPLANATION OF PLATE VII

Fig. 1. Photomicrograph of the cells of a regressing corpus luteum, 109 days post estrus. The figure is composed of blood vessels encompassed by cells of theca interna origin. (600X).

Fig. 2. Photomicrograph of the cells of a regressing corpus luteum, 127 days post estrus. Note the presence of an intensely eosinophilic substance between the blood vessels. (600X).

Fig. 3. Photomicrograph of the cells of a regressing corpus luteum, 141 days post estrus. The cells of the small blood vessels are disorganized as are the remaining cells of this corpus luteum in the final stage of regression. (600X).



## PLATE VII

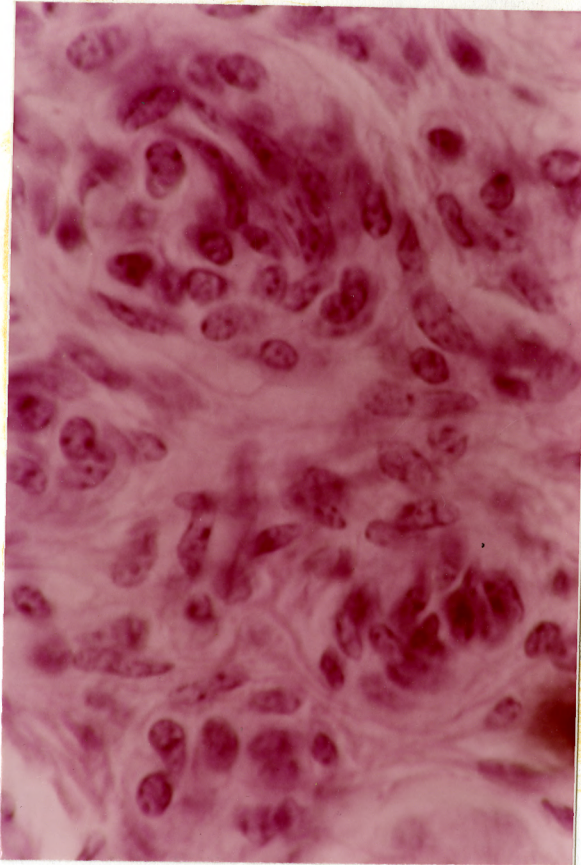


Fig. 1

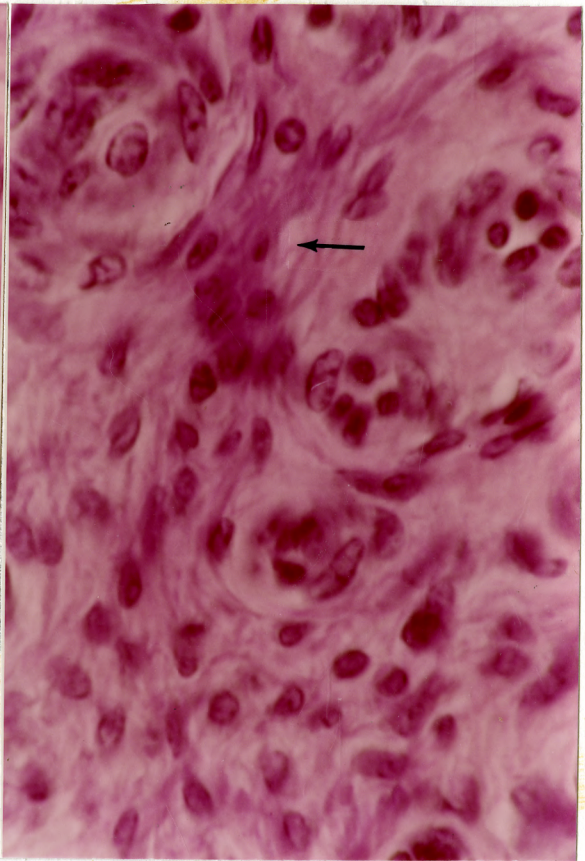


Fig. 2

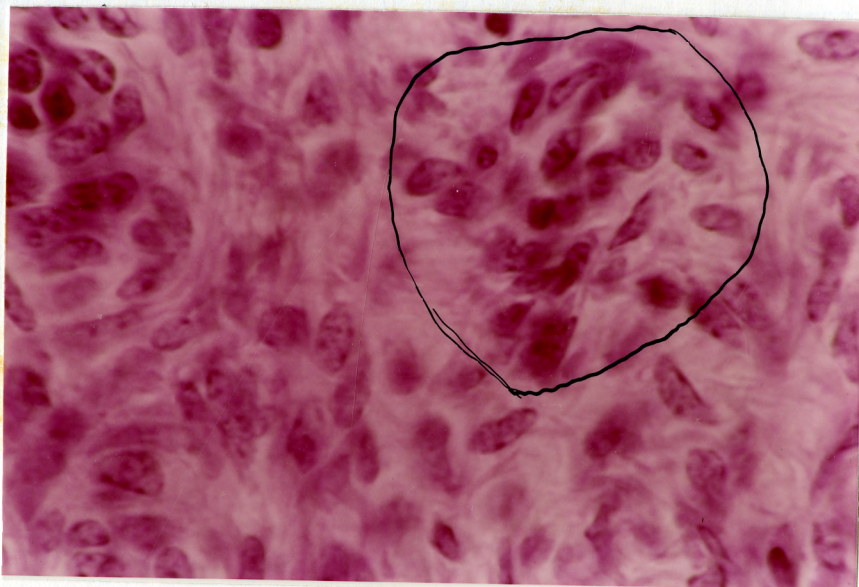


Fig. 3

#### EXPLANATION OF PLATE VIII

Fig. 1. Medial section of an ovary through a cystic corpus luteum. The cavity of the cyst is bordered by a well-formed connective tissue layer, isolating it from the luteal tissue. (2.5X).

Fig. 2. Photomicrograph of the cells of a regressing corpus luteum, 21 days post parturient. Cell types and arrangement are similar to the 33 day post estrus corpus luteum shown in Plate VI, figure one. (600X).

Fig. 3. Photomicrograph of the cells of a regressing corpus luteum, 433 days post estrus. This stage was included to show the retarding effects of pregnancy on the regression of the corpus luteum. This corpus luteum was formed during the estrous cycle immediately before conception. It had regressed slowly throughout pregnancy, and post parturition to a condition comparable to 127 days post estrus (Plate VII, Fig. 2). (600X).



## PLATE VIII

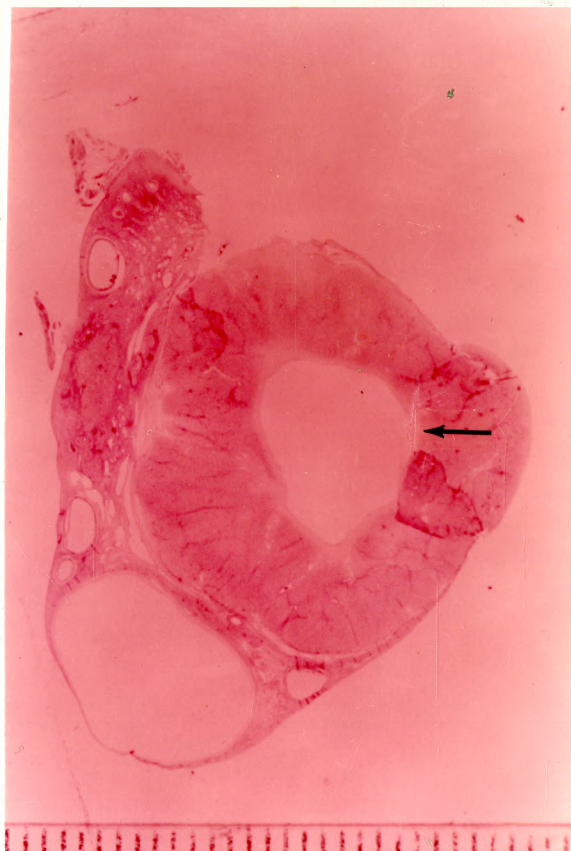


Fig. 1

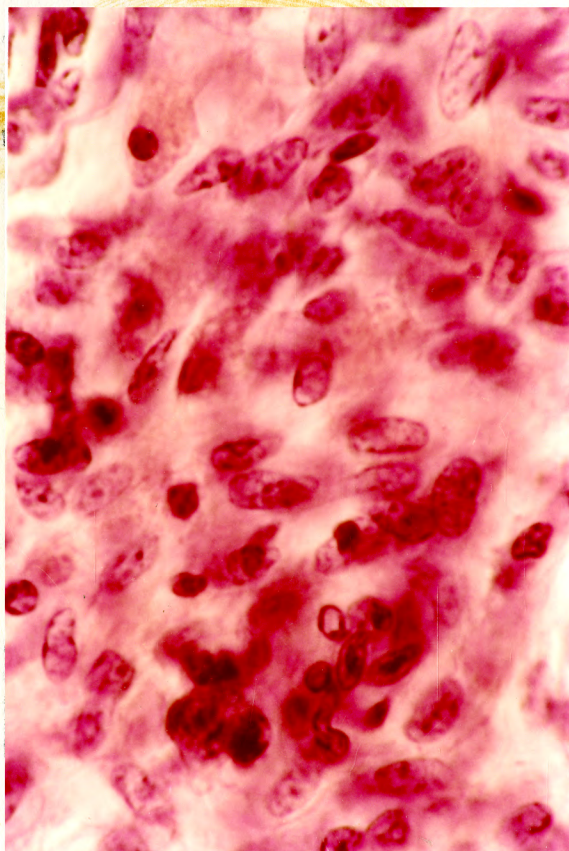


Fig. 2

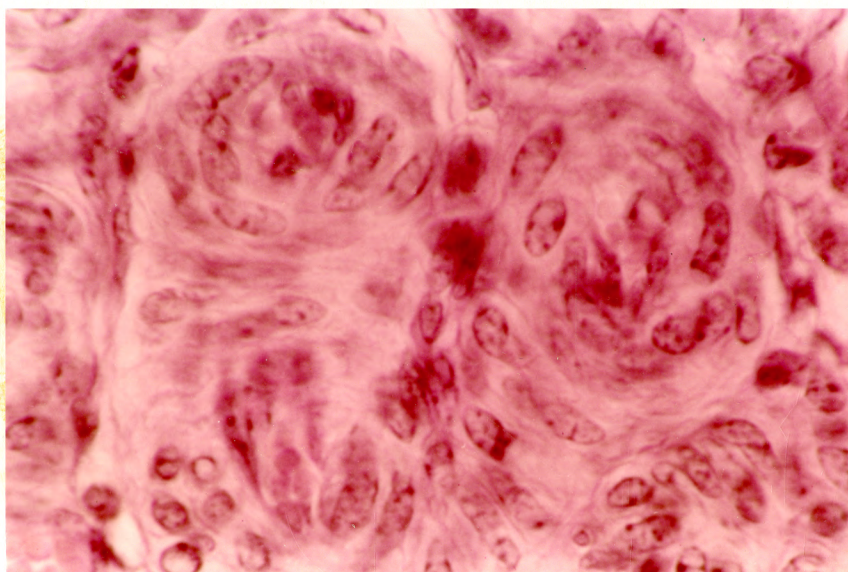


Fig. 3

#### EXPLANATION OF PLATE IX

Fig. 1. A 127 day post estrus regressing corpus luteum treated to show alkaline phosphatase. The most intense reaction occurred in the endothelial cells of the blood vessels with a slight reaction in the nuclei of the luteal cells. (600X).

Fig. 2. Low magnification photomicrograph of an ovary treated for the alkaline phosphatase reaction, showing an intense reaction in the theca interna of tertiary follicles. (5X).

Fig. 3. Alkaline phosphatase activity in the theca interna of a tertiary follicle. (600X).



## PLATE IX

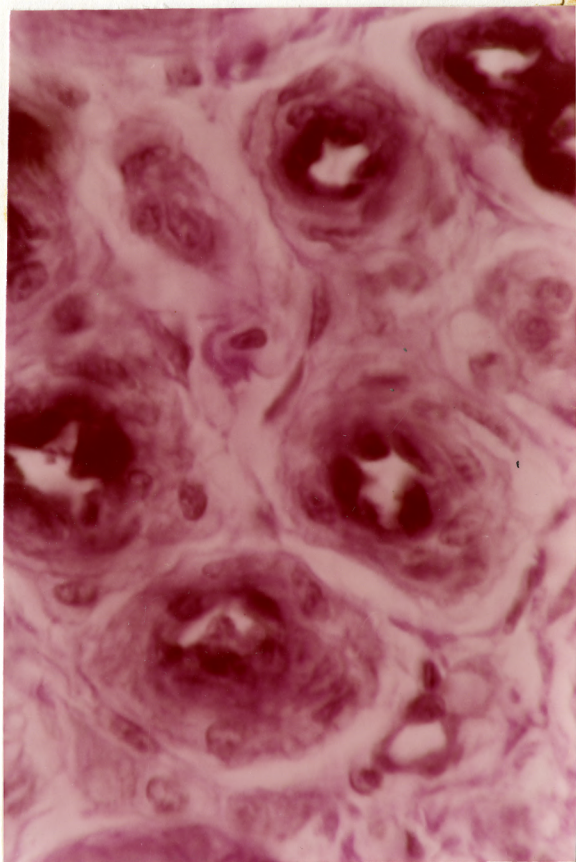


Fig. 1

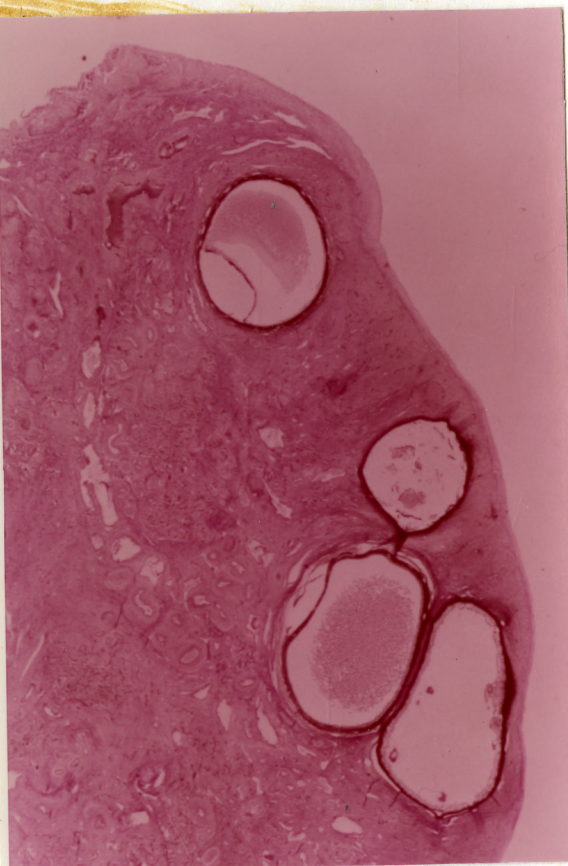


Fig. 2

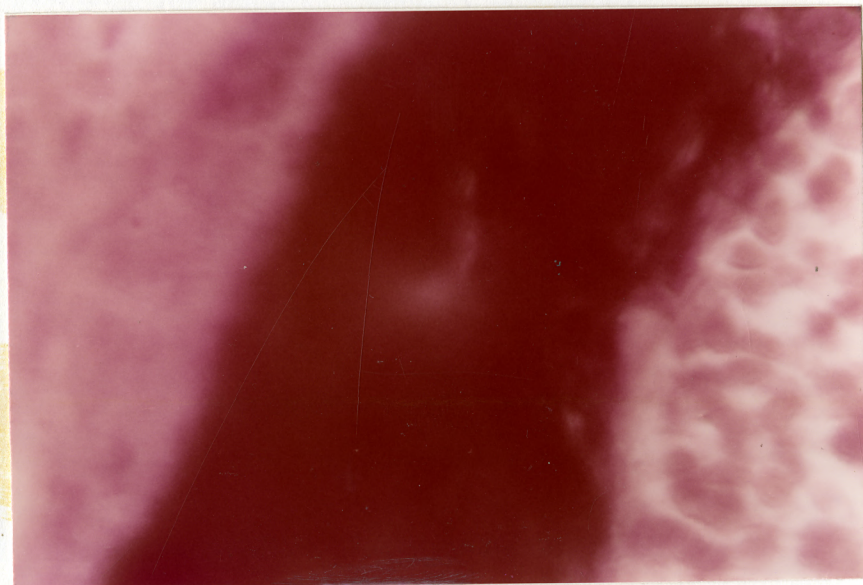


Fig. 3

#### EXPLANATION OF PLATE X

Fig. 1. Photomicrograph of a 7 day post estrus corpus luteum treated for alkaline phosphatase. The cells that are highly reactive appear black and are considered to be of theca interna origin. Those that are slightly reactive are probably of granulosa origin. (600X).

Fig. 2. Alkaline phosphatase marking the blood vessels in a functional corpus luteum. (5X).

Fig. 3. The theca interna of a cystic follicle showing the absence of alkaline phosphatase reactivity as opposed to that seen in a normal tertiary follicle.



## PLATE X

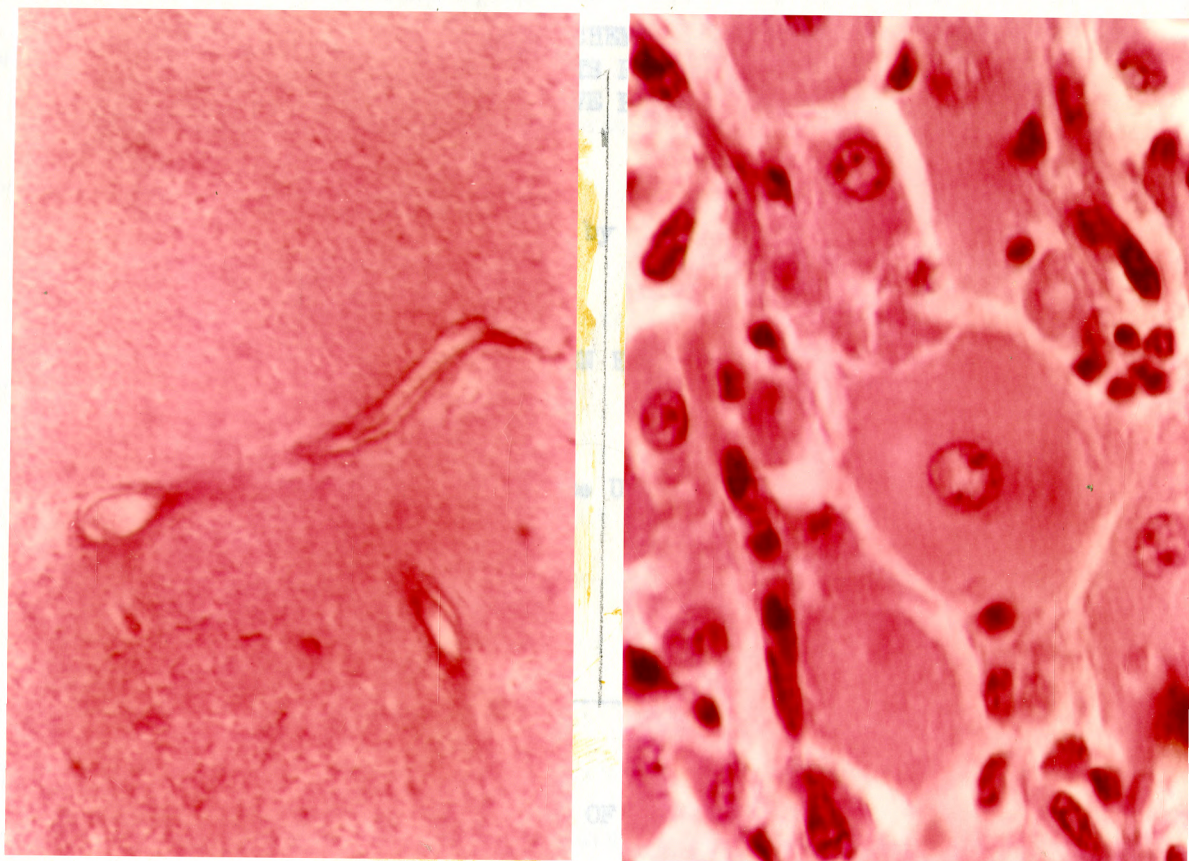


Fig. 1 omitted in partial fulfillment of the Fig. 2

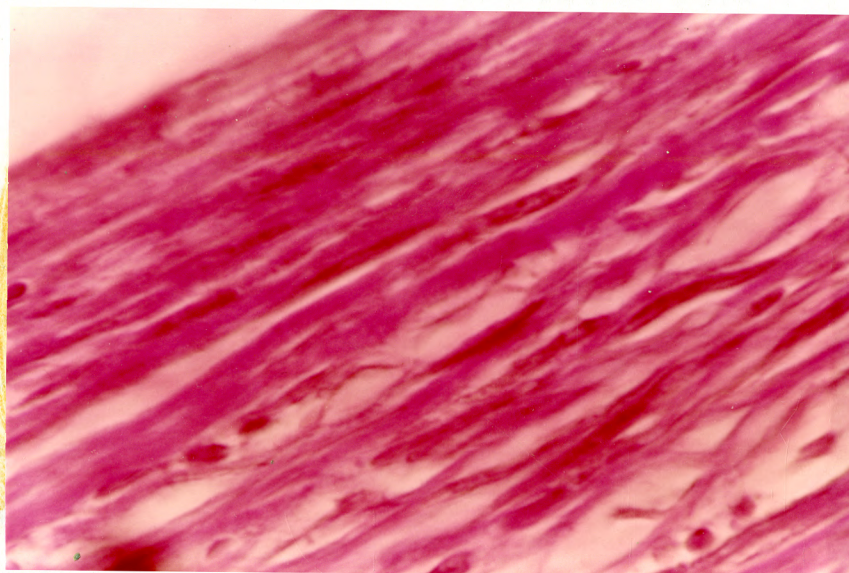


Fig. 3

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE BOVINE  
CORPUS LUTEUM AND ITS RELATIONSHIP TO PAST  
REPRODUCTIVE PERFORMANCE

by

BERT HAZEN ERICKSON

B.S., Utah State University, 1956

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE  
OF AGRICULTURE AND APPLIED SCIENCE

1957



The excised ovaries and reproductive histories of 72 cows culled from the Kansas State College herd were studied.

The tissues were processed by standard histological technics and alkaline phosphatase determinations were made by the technic of Gomori as published in 1939.

Descriptions of key stages of the development and regression of the bovine corpus luteum were presented in detail. The age of the regressing corpora lutea were determined by associating the number, size, and histological appearance of the corpora lutea present in the ovaries with recorded periods of estrus.

Following the study of the various stages of regression of the corpus luteum and the association of the number of regressing corpora lutea with recorded periods of estrus, it was found that the functional activity of the ovary and reproductive performance of the cow could readily be determined from the number and histological appearance of regressing corpora lutea.

The observations made of the developing corpus luteum indicate that it attains its maximum size in seven to nine days. The cellular arrangement and appearance of a 17 day post estrus corpus luteum was found to be highly similar to a corpus luteum at 240 days gestation.

Definite evidence of regression in the corpus luteum of the cycle was first noted at 18 days and a corpus luteum at 21 days post parturition was noted to have undergone regression comparable to a 33 day corpus luteum of the cycle.

Seven cases of cystic corpora lutea were found and the reproductive histories of the affected cows following the parturition before slaughter were listed. The affected cows had cycled regularly prior to slaughter and each had been inseminated at least three times before slaughter. All cows were slaughtered within 35 days post insemination and found not pregnant. In two cases



remnants of fetal membranes were recovered.

From the evidence presented it was postulated that a cystic corpus luteum will cause early embryonic death. This was thought to be due to progesterone insufficiency.

Two cases of multiple cystic follicles were found. The affected cows in each case had undergone long periods of anestrus prior to slaughter. The follicles were judged cystic by the absence of granulosa cells from the follicular wall.

The study of the reproductive records and ovaries of the 72 cows revealed no instance in which anestrus had been caused by a retained corpus luteum.

Alkaline phosphatase was found in the cytoplasm of the granulosa cells of primary and secondary follicles, but was found only in the cytoplasm of the theca interna cells in tertiary follicles.

In the early corpus luteum, the deposition of alkaline phosphatase clearly differentiated the cells of granulosa and theca interna origin. This observation was used as a basis for concluding that the bovine corpus luteum is composed of freely intermingling cells of theca interna and granulosa origin.