ENDOCRINE AND REPRODUCTIVE CHANGES DURING THE ESTROUS CYCLE OF THE BOVINE

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ABSTRACT

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by Arthur James Hackett

Thirty-five Holstein heifers were slaughtered during the seventeenth month of age in groups of five at estrus (day 0), metestrus (days 2 and 4), diestrus (days 7, 11 and 18), and proestrus (day 20). Their body weight averaged 377 kg and previous estrous cycle length was 20.6 ± 0.2 days. The pituitary contents of prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were estimated by the pigeon crop assay, ovarian weight augmentation assay and the ovarian ascorbic acid depletion assay, respectively. And the hypothalamic luteinizing hormone releasing factor (LRF) content was also measured by the ovarian ascorbic acid depletion assay.

Pituitary contents of prolactin, FSH and LH were highest on days 0, 18, and 20, respectively. Pituitary prolactin decreased from 67 μg at day 0 to 19 μg at day 2 and then increased almost linearly to day 18, showing a slight decrease at mid-cycle. Between days 18 and 20, pituitary FSH content decreased 49 percent and further decreased 46 percent by day 0; i.e., 73 percent decrease

from day 18 to 0. Then FSH increased to day 2 and declined insignificantly at day 4, suggesting a biphasic pattern. Similarly, pituitary LH decreased 71 and 61 per cent between days 20 and 0 and between days 0 and 2, respectively; i.e., 89 per cent from day 20 to day 2. Again there was evidence of bimodality since, after an increase from day 2 to day 11, pituitary LH decreased between days 11 and 18.

Generally, changes in pituitary contents of prolactin, FSH and LH mimicked each other except that FSH release preceded prolactin release by 2 days and LH release preceded prolactin release by one day. Responses of all three showed evidence of bimodality during the estrous cycle.

The hypothalamic LRF content was highest on days 20, 0, 2, 4, and 7, a period which included the lowest pituitary LH. It was lowest on days 11 and 18, possibly because of high levels of plasma progesterone. In any event, high levels of hypothalamic LRF were associated with decreased levels (apparent release) of pituitary LH.

Size of the largest ovarian follicle was greatest at days 11 and 0. But the 11-day follicle was not as large as the ovulatory follicle, possibly because of insufficient FSH and LH. The ovulatory follicle probably ovulated because greater quantities of pituitary FSH and LH were released and plasma progesterone content was diminished at the time of estrus.

Progestin synthetic activity of the corpus luteum increased from day 2 to day 11, remained relatively constant to day 18 and then decreased dramatically as the next estrus approached. However, *in vitro* synthetic activity of corpora lutea homogenates remained high at day 20.

The length of the vagina, cervix, body and both horns of the uterus, and both oviducts were measured in addition to the epithelial cell heights of these organs. There were no differences in the lengths of these organs which could be attributed to physiological changes during the estrous cycle. However, heights of epithelial cells of the vagina, cervix, and uterus varied during the cycle. Whereas height of vaginal epithelial cell-layer was highest at estrus and lowest at mid-cycle, heights of cervical and uterine epithelial cells were biphasic--high at estrus and mid-cycle, suggesting stimulation by estrogens from the ovulatory follicle and from the mid-cycle follicle. Epithelial cell heights of oviducts followed a pattern similar to corpus luteum progesterone.

DNA, RNA, protein and lipid measurements were made for the vagina, cervix and uterus. Vaginal DNA, RNA and protein contents were high at day 11 and estrus, showing biphasic changes during the estrus cycle similar to those for the largest ovarian follicle. Cervical RNA also revealed a biphasic pattern, with peak values at estrus and day 11. In contrast to uterine epithelial cell height, which apparently was related to estrogen secretion, uterine RNA appeared more related to secretion of luteal progestogen.

In both the thyroid and thymus, there were no meaningful trends during the estrous cycle with respect to content of DNA, RNA, protein or lipid. Height of the thyroidal acinar cells did not change during the estrous cycle. Although these two glands are known to be associated with reproduction, there was no evidence of physiological changes in them during the estrous cycle in these heifers.

One must conclude that high content of hypothalamic LRF content is associated with release of pituitary LH at estrus, which in turn is responsible for ovulation and at least early growth (and function?) of the corpus luteum. FSH is necessary for maximal follicular growth--it is released during the 2 or 3 days before estrus. Prolactin may be part of a luteolytic complex in the bovine because pituitary content is greatest during luteal regression. That the mid-cycle follicle fails to ovulate may be due to an inhibitory amount of circulating progesterone which blocks release of FSH and LH. Several of the measured criteria suggested that the mid-cycle follicle may secrete estrogen, but in much smaller quantity than is secreted by the ovulatory follicle.

ENDOCRINE AND REPRODUCTIVE CHANGES DURING THE ESTROUS CYCLE OF THE BOVINE

By

Arthur James Hackett

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"Meminisse iuvabit"

Vergil's Aeneid

Dedicated to my parents

Walter and Elvira Hackett

BIOGRAPHICAL SKETCH

of

Arthur James Hackett

I was born in Guelph, Ontario, Canada on August 26, 1938 and received my public and high school education in that city. In September 1956, I entered the Ontario Veterinary College, University of Toronto and studied for my D.V.M., which I received in May 1961.

During my youth and collegiate days, I participated in most sports, notably track and field, cross country and hockey. I lettered in track and field and cross country for the five years I was an undergraduate. And while pursuing my Masters degree, I was again a member of the varsity cross country team. I was fortunate to receive the award for the outstanding member of the track and field team for two consecutive years.

Following graduation in May 1961, I worked for the Canadian Federal Government, testing cattle for tuberculosis. Beginning in August of that year, I worked for the Central Ontario Cattle Breeding Association at Maple, Ontario, Canada. While diagnosing and treating cases of bovine infertility I became more aware of the complexities of reproduction. So with the financial aid of the

Ontario Association of Animal Breeders Fellowship, I entered Graduate School at the University of Guelph. I received my M.Sc. from that institute in May 1965. My thesis was "Studies Related to the Freezability of Bovine Spermatozoa."

In September 1965, I received a graduate research assistantship in the Dairy Department, Michigan State University where I completed my Ph.D. in June, 1968.

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I should like to thank the Chairman and the staff of Dairy Department of Michigan State University for providing facilities for my training. But without the ever-guiding hand and encouragement of Dr. Harold Hafs, many of my personal endeavours would have been futile. I feel greatly indebted to him for his guidance and hope that in the future I shall be a source of some satisfaction to him.

I also owe sincere thanks to Dr. Allen Tucker who capably guided me through my first year at Michigan State University while Dr. Hafs was on sabatical at Harvard, and for his continued interest, advice and assistance throughout my sojourn in East Lansing. And I also am grateful for the cooperation given to me by members of my committee, Drs. E. P. Reineke, J. L. Gill, R. L. Anderson and S. D. Aust.

Because a large portion of the work required to obtain the data in this thesis was the result of team cooperation, I should also like to thank those involved. They include Mrs. Helga Hulkonen, Dr. L. J. Boyd, and my student colleagues, Claude Desjardins, Ken Kirton, Jock Macmillan, Max Paape, Yogi Sinha and Bill Thatcher.

The gifts of hormones from the Ayerst, Squibb and Upjohn Laboratories and the Endocrinology Study Section of the National Institutes of Health, as well as the financial support provided by the National Institutes of Health (grant number HD 01374) are recognized and appreciated.

I should like to thank my wife, Ellen, and children Lisa, Ruth, and A. J. for their tolerance and sacrifices while being without husband and father for much of the time I spent at the "lab."

For those who cannot read Latin, "Meminisse iuvabit" means "A pleasure to recall."

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INTRODUCTION

In 1967, infertility (85) ranked second as the Michigan dairyman's greatest financial loss. For maximum reproductive efficiency and economic production, a calving interval of one year to 13 months is required. Since the advent of artificial insemination techniques and calfhood vaccination against brucellosis, many transmissible and infectious diseases (109, 117) have been eliminated. In my opinion, and excluding poor management, hormonal imbalances are now the major causes of bovine infertility. Although we have been able to treat some cases successfully, we have been unsuccessful in determining the specific causes of these conditions.

To better understand endocrine imbalances, we must first quantify pituitary or gonadal and plasma levels of the pertinent hormones, luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen and progesterone during the normal estrous cycle. Most of our present day concepts of physiological control of reproduction and reproductive cycles are based on evidence (117) established 30 or 40 years ago. Within the last few years more specific and sensitive assays have allowed us to measure quantitatively both LH and FSH in an individual cow pituitary. For example, Rakha and Robertson (102) determined both the FSH and LH content in pooled pituitary glands of heifers at the 15th and 18th day of the estrus cycle, and during estrous and after ovulation. Prior to 1965, most investigators measured "total gonadotrophic" activity.

Although we base our working hypotheses of reporduction control on the pituitary-gonadal axis, we should also be cognizant of the regulation by the central nervous system and production and release of gonadotrophins. Using median-eminence extracts from rats, rabbits, and cattle, Nikitovitch-Winer (92) induced ovulation in "pentobarbital-blocked" proestrous rats and Campbell et al. (17) induced ovulation in female rabbits. At about the same time, McCann (79) induced release of LH in the rat by injection of median-eminence-stalk extract. Since that time, separate hypothalamic factors (45) have been demonstrated to control synthesis and release of each of the anterior pituitary hormones.

That FSH and LH act directly and indirectly on the reproductive tract is well established. FSH promotes follicular development and LH stimulates ovulation and formation of corpora lutea. Through the ovarian hormones (estrogen and progesterone), FSH and LH cause morphological changes in the reproductive tract. Now with the development of methods for measuring biochemical components such as deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein, we can quantify cell numbers and activity. These biochemical measurements can supplement studies of anatomical changes.

The chief objective of the present study was to measure the individual pituitary gonadotrophins and some meaningful biochemical components of the reproductive tract of dairy heifers during the estrous cycle. A second objective was to correlate these quantitative measurements of pituitary gonadotrophins with follicular and luteal growth. Finally, we wished to study the relationship of the luteinizing hormone releasing factor (LRF) with the synthesis and release of pituitary LH.

REVIEW OF LITERATURE

A. Nature of the Estrous Cycle

1. Length of the Estrous Cycle

Duration of the estrous cycle in cattle averages 21 days and somewhat less than 21 days in heifers (7, 11, 48, 73, 101, 117). Physiologically, infertile service, effects of feed level, season of year and age may alter estrual cycle length (32, 93, 109). Pathologically, diseases such as tuberculosis and Johne's disease may alter the estrous cycle and embryonic mortality may lengthen the estrous cycle (109). And experimentally, drugs also alter the length of the cycle. For example, oxytocin (7) administered during the first week of the estrous cycle shortens the length of the cycle. Daily injections of estradiol (200-300 μg) decrease the length of the cycle (100) and 5 to 10 mg of progesterone (54) shorten the length of estrus and reduce the time from the end of estrus to ovulation. Pregnancy (109) abruptly terminates cyclicity of the pituitary and reproductive tract.

2. Changes in the Bovine Reproductive Tract During the Estrous Cycle

The bovine estrous cycle is usually divided into proestrus, estrus, metestrus and diestrus (9, 30, 109, 117).

Under the influence of estrogen, produced by the growing Graafian follicle, proestrus (109) is a buildup phase for cells and cilia lining the oviduct as well as for vaginal vascularity and thickness of the vaginal mucosa. And the increasing amounts of estrogen cause an increase in uterine vascularity and stimulate muscular activity of the uterus causing it to be turgid.

The highlight of the cycle is estrus (117), a period lasting 12 to 18 hours and a time at which the female is receptive to the male. During metestrus, the cow ovulates, usually about 12 hours following "standing heat." Capillary haemorrhage, resulting from hyperemia over the caruncles, may occur during early metestrus, and the profuse secretion of cervical mucous ceases.

Throughout diestrus, progesterone exhibits its most marked effects. For example, hypertrophy of the endometrial glands and stricture of the cervix occur. Beginning at about day 18 the corpus luteum (31) regresses and progestin concentration decreases near the time of the next estrus.

3. Regulation of the Estrous Cycle

Throughout the estrous cycle, the anterior pituitary hormones (117), secreted into and carried by the blood to the ovaries, modify the action of these target organs. The follicle, growing rapidly during the 3 days prior to estrus (50, 117), is under the influence of FSH (102) which decreases by 27% in the pituitary about 18 hours before estrus. And the fluid of the follicle follicular phase (82) has ten times as much estrogenic activity than the luteal phase fluid. Although the levels of endogenous estrogen in the peripheral blood plasma (13) have not been unequivocably identified, the results indicate highest levels (82) during the follicular phase of the estrous cycle, which is in agreement with bioassayed levels in follicular fluid. In the rat (83), the increasing level of estrogen in the blood at the time of proestrus feeds back negatively on the pituitary, the hypothalamus, or both, blocking the release of FSH from the pituitary. At the same time, increasing amounts of circulating progesterone, produced by the maturing follicle, stimulate the release of LH, and this surge of LH induces ovulation. But in the cow there is no evidence for estrogenic feedback blocking the release of FSH; however, there is evidence of luteinization (27) of most follicles before estrus and that follicles obtained near ovulation in the cow contained 3 μg of progesterone (117) per ml of fluid. Small amounts of progesterone (54) administered at the beginning of estrus hasten ovulation. This suggests that a rise in progesterone levels prior to ovulation may contribute to the control of the estrous cycle. In the cow, pituitary LH decreases 61% just prior to ovulation

(102) and preliminary results indicate blood plasma levels (4, 61) of LH increase 6-7 hours before ovulation. So it appears likely that LH is the hormone responsible for ovulation in the cow. Moreover, LH stimulates growth of the corpus luteum.

B. Changes in the Hypothalamus and Pituitary Gland During the Estrous Cycle

1. Releasing Factors

That releasing factors (45, 120) exist in hypothalamic extracts of several (if not all) species has been firmly established by several groups of investigators. In fact, partial or complete purification (120) of bovine, ovine and porcine luteinizing hormone releasing factor (LRF) has been described. Column chromatography has been used to separate FSH-RF from LH-RF and to partially purify it (44, 62). Besides FSH-RF and LRF, other releasing factors (45) control synthesis and release of growth hormone, thyrotrophic hormone and ACTH. But prolactin systhesis and release (84) is chronically inhibited by prolactin inhibiting factor (PIF) in the hypothalamus.

Although releasing factors have been obtained from large animals (cow, sheep and pig), the LRF content (18) has been measured only in the rat during the estrous cycle. In this species, LRF content in the hypothalamus increased during late diestrus and decreased markedly during proestrus, synchronizing in an inverse relationship with the pattern of LH secretion during the estrous cycle.

2. FSH and LH

The total gonadotrophic content (39, 60, 113) of pituitaries decreased at the time of estrus in all species which have been studied. It was elevated during the remainder of the cycle, suggesting release of gonadotrophin from the pituitary at the time of estrus. Following development of more sensitive assay techniques to study pituitary FSH (131) and LH (96) contents separately, later studies (38, 59, 94, 97, 102, 110, 111, 112, 119, 122) disclosed that FSH was released shortly before LH at the time of estrus in the rat, pig, monkey, sheep, hamster and cow. And preliminary data revealed that blood plasma content of LH (4) increased rapidly about 12 hours prior to ovulation in the gilt, 6-17 hours in the cow, and 6-12 hours in the rat. Specifically, FSH and LH levels in the bovine pituitary decreased 27 and 61% respectively between the onset and end of estrus indicating that FSH as well as LH (102) may have a role in reproductive events near the time of ovulation in cattle.

3. Prolactin

Although an assay for prolactin (107) was developed in 1937, there are very few reports concerning levels of this hormone during estrous cycles. In the rat and guinea pig (106, 107) pituitary prolactin concentration is higher during estrus and proestrus than during metestrus and diestrus, and in the gilt (23), pituitary prolactin potency increases linearly with advancing stages of the estrous cycle. Prolactin (64) was measured by radioimmunoassay in blood plasma of the mouse and found to increase during proestrus and estrus. And prolactin reaches detectable levels in the blood of women (125) during the first five and last ten days of the menstrual cycle.

C. Ovarian Changes During the Estrous Cycle

1. Growth of the Follicle

Formation of oocytes (101) is completed at birth, and the nuclei of all oocytes have entered the pachytene stage of the first meiotic division. In sexually mature heifers, oocyte nuclei in the primordial follicles are also in the pachytene stage. Primordial follicles, consisting of an ovum and a single layer of epithelium, proliferate to form growing follicles. During this stage the theca forms from the multiplication of this single layer of cells surrounding the ovum and by a special development of the surrounding connective tissue cells (53). The theca develops into two layers, externa and interna and cells of the latter layer are believed to secrete the estrogenic hormones. Granulosa cells, derived from the proliferations of the single layer of cells surrounding the ovum, secrete the follicular fluid which results in the formation of an antrum in the follicle.

Large vesicular follicles are called Graafian follicles and the cells surrounding the ovum, known as the cumulus oophorus, eventually become separated from the granulosa cells lining the cavity of the follicle.

Preovulatory growth (50, 101) of the bovine Graafian follicle is most rapid during the 3 days prior to estrus. During this follicular phase (95) there is ten times as much estrogenic activity in the follicular fluid than in the luteal phase, and estrogens in the peripheral blood plasma (13) follow a similar pattern. But consistent results of estrogenic (82) activity in the urine of cycling cows have not been obtained. In the sow (66, 99) however, urinary estrogen excretion was greatest just before or at the onset of estrus. Apparently the large follicle at mid-cycle (101) in the bovine does not produce discernible changes in sexual behavior of the animal.

2. Ovulation and Corpus Luteum Formation

Although the exact mechanism is unknown, ovulation (50) involves the rupture of the largest follicle at the surface of the ovary and release of the ovum into the infundibulum of the oviduct. After ovulation there is some haemorrhage (9, 50) at the point of rupture, but any slight haemorrhage (9) in the follicle is confined between theca interna and the granulosa. Occasionally a clot (50) may form in the empty follicle. Also, following ovulation, the walls of the follicle (27) collapse and the granulosa and theca interna layers become deeply folded. Approximately two days after ovulation, the folds of the wall of the corpus luteum meet, and by the third day after ovulation, the cavity produced by these folds is obliterated. Cysts (11, 49, 101) of various size have been noted in bovine corpora lutea, but are usually part of normal development, or if they are of an abnormal condition, they are of little consequence. However, luteal cysts (80) over one cm in diameter may be an important cause of infertility.

Contrary to a previous report (49) suggesting corpora lutea are derived from granulosa cells only, more recent work (27, 36, 39, 81) supports the hypothesis that they are also derived from theca interna cells. Early luteinization of both layers occurs approximately 6 hours after the onset of estrus (and before ovulation), and mitotic activity is seen in both layers but more frequently in the granulosa layer. In addition to mitosis, the granulosa and theca interna cells also enlarge and have increasing amounts of lipid material.

The size of the corpus luteum increases between days 4 and 7 and reaches a maximum (72) by about day 11. At days 9 and 11, the only mitotic activity (36) appears in the connective tissue. Between days 11 and 17 (72) the weight of the corpus luteum is constant and it decreases (50) in size shortly before the cow returns to estrus.

3. Luteotrophic Process

The luteotrophic process (115) has been defined as the process "which promotes the growth of the corpus luteum and a rate of progesterone secretion at least sufficient to prevent ovulation and/or to permit implantation to occur." In the cow (51, 52, 127), LH promotes the growth of the corpus luteum and secretion of progesterone. When added to bovine corpus luteum slices incubated *in vitro* (78), LH enhanced synthesis of progesterone. In a similar experiment, Armstrong and Black (5) demonstrated this phenomenon occurred only in functional corpora lutea, i.e., corpora lutea capable of secreting progesterone *in vivo*. Bartosik *et al*. (14) demonstrated increased progesterone secretion from perfused luteal ovaries and contralateral "follicular" ovaries, following administration of LH *in vitro*. They also observed increased progesterone secretion from luteal ovaries, but not from contralateral "follicular" ovaries, following prolactin administration. These researchers suggested that prolactin as well as LH is involved in the luteotrophic process of the bovine. When bovine ovaries (43) were perfused *in vitro* with a combination of LH and prolactin, the corpus luteum synthesized greater amounts of progesterone than during treatment with either hormone alone. However, *in vivo* prolactin (7, 127) alone did not maintain the corpus luteum beyond its normal life span. Nor did daily injections of prolactin delay the onset of estrus. Some of these data are contradictory but it is at least conceivable that under certain circumstances, prolactin may indeed be luteotrophic or part of a luteotrophic complex in cows.

Prolactin (12, 29, 33, 34) has been conceded to be the luteotrophic hormone in rats and mice. But prolactin (77) administered to hypophysectomized rats, requires synergistic action of LH to maintain corpora lutea. Reminiscent of the effect of LH on progestin secretion in the rabbit ovary (57), intravenous injections (77) of LH increased secretion of progesterone 2.5 fold. And prolactin increased the secretion of 20α- hydroxy-pregn-4en-3-one, but to a lesser extent than the LH effect on progesterone secretion. This study also agrees with other investigators (8) who suggest LH aids in regulation of ovarian functions in the rat. Possible other synergisms exist in the luteotrophic process in rats. For example, progesterone or estrogen alone (116) maintain growth of rat corpora lutea for only 2 to 3 weeks, whereas the two hormones together maintain luteal growth and function for at least 4 weeks. Although corpora lutea respond to prolactin, even in the absence of other pituitary hormones, this does not mean that other pituitary hormones are not involved in the luteotrophic process in the rat. In the mouse (16), there is also evidence for LH-prolactin synergism.

In other species, the luteotrophic process varies. A complex (43) of prolactin, FSH, and LH is required for luteal maintenance in hamsters hypophysectomized during the first half of pregnancy. Exogenous prolactin alone does not maintain pregnancy but it does preserve the histologic integrity of the corpus luteum. But concurrent administration of FSH maintains functional corpora lutea which sustain pregnancy. If a small amount of LH is added to this minimal luteotrophic complex, the LH enhances corpora luteal function as shown by increased size and hyperemia of the corpora lutea.

Progesterone (2) injected into pigs from the time of ovulation until day 10 to 13 of pregnancy does not prevent formation of corpora lutea, whereas progesterone injections from days 12 to 16 of pregnancy result in regression of corpora lutea. Based on this evidence, Nalbandov (88) suggested that discharge of a pituitary luteotrophin over a relatively short time (2-3 days) maintains the life span of the corpora lutea during the estrous cycle. Furthermore, no additional release of luteotrophin occurs unless the female becomes pregnant, and, if such is the case, intrauterine events stimulate a secondary release of the pituitary luteotrophin which continues throughout gestation. Because exogenous prolactin (2) is not luteotrophic in pigs, perhaps LH is the luteotrophin. Neither FSH nor prolactin (19) increased progesterone synthesis in porcine corpora lutea in vitro, whereas porcine, ovine or bovine LH increased progesterone synthesis by 15%.

Except that the initial outpouring of luteotrophin (possibly LH) lasts longer (1), the guinea pig parallels the pig in formation and maintenance of corpora lutea. That prolactin (86, 132, 136) is luteotrophic in sheep has been questioned. Now most evidence indicates FSH or LH, or both, is luteotrophic (124).

4. Luteolytic Process

If one accepts the arguments (51) that LH is the major luteotrophic hormone, the question arises whether luteal regression is due to a decrease in plasma LH level. Accurate measurements of blood levels of LH during development and regression of the corpus luteum are required to determine whether lowered LH in the plasma is responsible for luteal regression. Regression of corpora lutea (129) and interruption of pregnancy occur in rabbits following treatment with rabbit anti-serum to ovine LH. These observations suggest anti-serum to ovine LH suppresses the secretion of endogenous estrogen, thereby prompting regression of corpora lutea, with a consequent shortage of progestin and interruption of pregnancy.

Bovine corpora lutea (5) lose their ability to produce increased amounts of progesterone in response to high levels of LH added _in vitro_ at the 18th day of the cycle. But homogenates (46) of corpora lutea obtained from cows at day 18 or 20 of a 21 day estrous cycle retain their ability to synthesize progesterone and the altered appearance of their blood vessels suggests that deficiencies of precursors account for decreased steroidogenesis _in vivo_.

In many species (3, 67, 71), including cattle, the uterus produces a luteolysin which appears to act locally on the corpus luteum. Corpora lutea formed prior to hysterectomy fail to regress following hysterectomy and contain as much progesterone 20 days after hysterectomy as those removed 9-11 days postestrus. In addition, they also contain significant levels of 20β-ol. However, hysterectomy does not affect the ability of the pituitary to secrete hormones required for follicular growth, ovulation, and corpus luteum formation. Estradiol (51) does not cause luteal regression in hysterectomized heifers to the same degree that it occurs in normally cycling animals. This evidence provides further support for the hypothesis that the uterus may elaborate a luteolytic factor at certain stages.

Recently, a luteolytic effect of the uterus has been demonstrated more directly. When acetone dried powder preparations of either late luteal or early estrual bovine uteri were injected intraperitoneally into pseudopregnant rabbits (134, 135), they induced luteal regression and development of follicles. During *in vitro* incubations of ovaries, these powders depressed incorporation of acetate into progesterone. For controls, the same dosage of a similarly prepared abdominal muscle powder was without effect. Although they hypothesized a uterine luteolytic hormone, affecting ovarian function without involving LH, these authors provide no evidence that their preparation was indeed a hormone. And their preparation was made up of an entire uterus which may contain other factors which could adversely affect the corpora lutea.

Because single-layer cultures of granulosa cells provide an ideal test system for luteolytic substances, Short (123) has tested various fractions of uterine washings. His preliminary data indicated uterine washings possessed no luteolytic substances. He attributed any death of the culture cells to toxic factors in the test substances.

D. Morphology of the Reproductive Tract

1. Vagina

As estrogen levels increase during the follicular phase of the estrous cycle, the superficial layer (9, 76) in the mucoid portion of the vagina becomes secretory. At estrus the cell membranes rupture, extruding the cell contents, leaving the underlying layer to continue secretion. During the luteal phase, the superficial layer of epithelium forms a layer of secretory low cuboidal cells. In the vestibular region, the number of leucocytes in the mucosa, increase and congestion and edema of the epithelial layers occur during proestrus. These changes subside by two days postestrus, but the cells of the middle layer increase markedly in size and number by day 8 to 11. At 10 days postestrus the superficial layers tend to become squamous, but true cornification does not occur (50).

2. Cervix

While the predominate cell type is the mucous-secreting cell (74), approximately 10% of the cells of the bovine cervical epithelium are ciliated. These mucous-secreting cells distend with mucous at estrus and, following its discharge into the folds of the cervix and into the cervical canal itself, become cuboidal cells. Thus these cells, unlike those in the vagina, are not lost. At the fifth day after estrus, the mucous cells become larger, and mucous accumulates in the cells. The peak of this stage occurs at the eighth day after estrus when the first wave of follicles developing in the ovaries is secreting estrogen. With secretions of this estrogen, the cells become smaller again and remain small until the 16th day of the cycle. The author suggests the discharge of mucin is controlled by estrogen and the accumulation of mucous is controlled by progesterone.

3. Uterus

Less than 1% of the uterine surface epithelium cells (75), obtained from heifers at estrus are ciliated and, of the remainder, cell height is lowest (15 μ) at estrus. By day 6 of the estrous cycle, the cell height increases to 30 μ. This high level remains until day 16, after which time it decreases. During proestrus and estrus the uterine glands are fairly quiescent and the lumina are straight. Two days postestrus, the glands are more coiled, and the lumina are filled with secretion. As the corpus luteum grows, the glands hypertrophy and reach maximum growth at about 12 days postestrus; then they regress after the fifteenth day.

4. Oviduct

Increased epithelial cell height in the oviduct accompanies an increase in the secretion from the fimbriated region during estrus (68, 108). Two days postestrus, more mucous and leucocytes are found than at any other time (9). By day 8, the epithelium becomes lower, and numerous globules of cytoplasm are found.

The cilia of the epithelial cells are more prominent during proestrus than at any other time.

E. Changes in Other Endocrine Glands

1. Thyroid

That the thyroid gland is necessary for reproduction has been established (21), because thyroidectomized animals show no behavioral signs of estrus. However, there are no reports (50) of changes of a cyclic nature in the thyroid associated with the estrous cycle in the cow. In sheep (69), diethylstilbestrol decreases total pituitary gonadotrophin content while increasing thyrotrophin content. This finding, coupled with the finding that increased thyrotrophin (6) content is correlated with increased thyroid activity, suggests changes may occur in the ruminant thyroid during estrus.

2. Thymus

Although the thymus (109) has been assumed to be an endocrine gland, its endocrine function is not well documented and little understood. In the heifer (25), thymus weight increases steadily from 1 to 12 months of age. Desjardins concluded that reproductive development in heifers was not related to changes in the thymus weight. These results are in contrast to those in female laboratory animals (24), in which thymus weight declines with advancing age. In the bull (70), the weight of the thymus gland increases dramatically from 1 to 4 months of age and then shows a decline to 7 months of age. Between 7 and 12 months there is a slight increase with age. Macmillan concluded that changes in the weight of the thymus gland in the Holstein bull do not appear to be associated with reproductive development. And these two studies show differences in the development of the thymus between the male and female.

F. DNA, RNA, Protein and Lipid Content of the Reproductive Tract, Thyroid and Thymus

To the best of my knowledge, no one has measured DNA, RNA, protein and lipid content of the bovine reproductive tract during the estrous cycle. However, the DNA, RNA, and lipid contents (126) have been measured in the rat uterus; DNA being maximal at proestrus or estrus, decreasing at metestrus and remaining at the same level during diestrus. The RNA content paralleled DNA. The ratio of RNA to DNA is often calculated to illustrate metabolic activity, and in the above study with rats, this ratio, in the uterus decreased from a maximum at estrus to a minimum at metestrus. Between metestrus and estrus, the ratio increased 27%. These results suggested increased metabolic activity at the time of estrus. Variations in lipid content also paralleled that of RNA:DNA ratio, suggesting that lipid synthesis was one of the earliest responses of the uterus to estrogen.

There have been no reports about DNA, RNA, protein and lipid content of the bovine thyroid and thymus during the estrus cycle. After 7 months of age (25), thyroid weight, total DNA and RNA paralleled each other. Between 6 and 12 months of age the thymus weight, total DNA and RNA increased five fold.

MATERIALS AND METHODS

A. Experimental Animals and Management

This experiment was designed to study endocrine and reproductive tract changes of dairy heifers during the estrous cycle and to ascertain whether or not cyclic changes occurred in the thyroid and thymus glands during the estrous cycle. Of a total of 35 Holstein heifers, five each were slaughtered on day 0 of the estrous cycle (estrus) days 2 and 4 (metestrus), days 7, 11, and 18 (diestrus) and day 20 (proestrus). These heifers were born from registered Holstein sires and production tested dams and came from Wisconsin or Michigan farms. To have as uniform a group as possible, we selected only animals born within the period between June 30 and July 19, 1964. They were transported, within one week of birth, to the Michigan State University Dairy Barn. Each animal received a physical examination, the results of which was recorded on its health record.

Initially they were housed in individual pens and fed an average of 8 lbs of milk, about 5 lbs of hay, and grain increasing to about 2 1/2 lbs per day. Gradually the milk was deleted and both hay and grain increased so that by four months of age they were fed

12 lbs of hay and 5 lbs of grain daily. From this age they were housed communally in box stalls. When they reached their sixth month of age, they occupied a loose housing arrangement and were fed corn silage and hay free choice. They remained there until slaughtered in their 16th or 17th month of age, the time at which most Holstein heifers are customarily mated for dairy purposes.

Beginning in August 1965, each heifer was observed for signs of estrus twice daily--between 7:30 a.m. and 8:30 a.m. and between 4:30 p.m. and 5:30 p.m. Dates of estrus were recorded for each animal. For determining estrus, the following criteria were used: (1) an animal standing when another animal mounted, (2) attempts to mount another animal, (3) swollen external genitalia, and (4) general uneasiness. Any post-estrus haemorrhage further substantiated the observed estrus.

B. Slaughtering and Retrieval of Samples

1. Method of Slaughter

On the day of slaughter, the heifers were transported to the University Meats Laboratory at approximately 5:30 a.m. and on arrival, each heifer was weighed. A captive-bolt pistol was used to stun the animal in the head, then the animal was exsanguinated. Usually, slaughtering began at about 7:00 a.m. and was completed by 11:00 a.m.

2. Hypothalamus and Pituitary

Within 5 minutes after stunning, the hypothalamus (including the surrounding median eminence and pituitary stalk) was removed, finely cubed, placed in a sample bottle, covered with a minimal amount of 0.1N hydrochloric acid (HCl) and rapidly frozen on Dry Ice. And at the same time a sample of cerebral cortex was similarly treated for a control. Immediately afterwards the pituitary gland was removed, trimmed, and weighed. Following the separation of the two lobes, the anterior lobe was weighed and placed in a polyethylene bag and frozen on Dry Ice.

3. Thyroid and Thymus

Concomitantly with the removal of the hypothalamus and pituitary, the thyroid gland was removed, trimmed free of fat and connective tissue and weighed. A small section (1-2 mm) was placed in Bouin's solution for subsequent histological examination and the remainder was covered in 0.25 M sucrose in a polyethylene bag and frozen on Dry Ice for subsequent analysis of DNA, RNA, protein and lipid content. Between 20 min and 45 min after stunning, the thymus gland was removed, trimmed and weighed and a portion was retained in 0.25 M sucrose for analysis of DNA, RNA, protein and lipid.

4. Reproductive Tract

Both ovaries* were removed within 10 min after stunning and placed in ice cold 0.9% NaCl. The corpus luteum was dissected from each ovary, weighed, a portion fixed in Bouin's solution for histological study and about 1 qm homogenized in about 4 ml Krebs-Ringer bicarbonate buffer in an all glass homogenizer. About 200 gm of this homogenate was frozen on Dry Ice and stored at -20°C for subsequent analysis of nucleic acids, sterols and steroids. Other portions of about 100 mg tissue (0.5 ml homogenate), after gassing with 95% O_2 and 5% CO_2, were incubated for 1 hr at 37°C in a final volume of 2.5 ml Krebs-Ringer bicarbonate buffer containing 1 mg/ml glucose and 5 μc cholesterol-7^{3}H with or without LH (4 μg/ml) and with NADPH (0.2 μmole/ml) or with NADPH generating system (NADP + glucose-6-phosphate, each 0.2 μmole/ml). When sterol, LH or cofactors were to be included, they were added to the incubation flask, lyophilized and stored desiccated at -79°C until buffer was added to the flask immediately before incubation was begun.

When present, the largest follicle on the ovary was isolated and its diameter measured. The follicle was then weighed with and without follicular fluid and by subtracting the weight of the follicular wall from

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*A study of the growth of the corpora lutea and progesterone synthesis has been reported by H. D. Hafs and D. T. Armstrong (46).

that of the total, the weight of the follicular fluid was obtained.

The vulva, vagina, cervix, uterus, and oviducts were removed intact approximately 30 min after stunning. After removing the connective tissue and fat, the vagina was opened on the dorsal surface from the dorsal commissure of the vulva to the dorsal fornix of the anterior vagina and the length of the vagina was measured from the ventral commissure of the vulva to the ventral fornix of the anterior vagina. After the vulva was removed from the vagina posteriorly and the cervix was detached anteriorly, the vagina was weighed, a small portion of tissue (1-2mm), taken just anterior to the urethral orifice, was fixed in Bouin's solution for histological examination, and the remainder was covered with 0.25 M sucrose in a polyethylene bag and frozen on Dry Ice for analysis of DNA, RNA, protein and lipid content. The cervical canal was exposed and its length measured. After severing the cervix from the uterus, the freed cervix was weighed, a small portion of tissue (1-2 mm) fixed in Bouin's solution for histological examination and the remainder frozen in 0.25 M sucrose for DNA, RNA, protein and lipid analysis. The body and both horns of the uterus were opened on the dorsal surface and, after taking their length, the entire uterus was weighed. A portion of tissue was taken from the middle of one uterine horn for a histological section, and the remainder was frozen in 0.25 M sucrose. After recording the length of each oviduct, a sample was fixed in Bouin's solution and the remainder discarded.

When slaughtering and dissecting had been completed, all samples frozen on Dry Ice were stored at -20°C until required for analysis. The histological specimens were processed immediately and kept imbedded in paraffin.

C. Bioassays of Pituitary Hormones and LRF

The anterior pituitaries were removed from storage (-20°C), thawed, weighed to the nearest 0.1 mg and homogenized in a Potter-Elvehjem homogenizer in small portions (about 100 mg) in pyrogen-free 0.85% saline. After adjusting the volume of homogenate to 50 mg pituitary equivalent per ml, the homogenate was centrifuged to remove cellular debris and the supernatant fluid was used in the assays for FSH and LH.

1. Follicle Stimulating Hormone

Because bovine pituitaries contain little FSH relative to LH or relative to pituitary FSH potencies in other species, doses of 50 and 100 mg equivalents of pituitary tissue were used for the FSH bioassay (25, 70, 73). In addition, each rat received 20 IU of human chorionic gonadotrophin (HCG-Upjohn Chorionic Gonadotrophin) as previously outlined in the ovarian weight augmentation bioassay (131). The unknowns were compared to 30 and 60 μg levels of NIH-FSH-S2 and to a dose of HCG alone. For each assay, five rats were used at each dose level of the unknowns and standard and the total dose was injected in 9 doses, given approximately 8 hrs apart over a three day period. Twenty-four hours after the last injection, the rats were killed, both ovaries removed, trimmed and weighed and the potencies were estimated by the slope ratio procedure (15).

2. Luteinizing Hormone

To measure the pituitary levels of LH, the ovarian ascorbic acid depletion method (OAAD) was used (96). At 25 days of age, the test rats (Sprague-Dawley strain from Spartan Research Animals, Haslett, Michigan) were injected with 50 IU of pregnant mare's serum (PMS-Ayerst Laboratories, "Equinex") and with 25 IU of HCG 60 hrs later. Six days after the HCG injection, each rat received 0.5 ml of either an unknown or standard preparation via the femoral vein. Two dose levels, 0.1 mg and 0.4 mg pituitary equivalent per rat, were used for each pituitary and five rats were used at each dose level. Four hours later, the left ovary was surgically removed, trimmed, weighed to the nearest 0.1 mg, homogenized in 2.5% meta phosphoric acid and brought to a concentration of 10 mg ovarian tissue/ml. This homogenate was filtered and the ascorbic acid concentration of the ovary was determined.

Each rat received approximately 30,000 IU of penicillin at the time of surgery and again 24 hrs later. Two days after surgery, the rats, which had been housed together in a large cage, were reallotted at random into groups of five and each rat again received 0.5 ml of either unknown or standard preparations via the femoral vein and the concentration of ascorbic acid was determined in the right ovary. The ovarian ascorbic acid depletion of these rats was compared with the ovarian ascorbic acid depletion of five rats at each level produced by 0.4 μg and 1.6 μg of NIH-LH-B2 per rat by the procedure for parallel-line assays (15).

3. <u>Luteinizing Hormone Releasing Factor</u>

Each hypothalamus was thawed and boiled in its own sample bottle to destroy any LH-like activity. After cooling, the samples were pooled according to day of cycle and each sample bottle was rinsed three times with 0.1 N HCI to remove any adhering material. All pools were brought up to 75 ml with 0.1 N HCl, placed in three dialysis bags (25 ml per bag), and dialyzed against 225 ml distilled water. The dialysate was neutralized with 2 N NaOH, frozen, lyophilized and stored at -20°C.

LH releasing activity of the hypothalamic extract was determined in rats prepared similarly to those for the LH bioassay. Each of the five rats in the low dose group received 0.2 equivalents of hypothalamus and those in the high dose group 0.8 equivalents; all in 0.5 ml solution. Four hours after injection of the test solutions, the ascorbic acid concentration response to the LRF was measured, and compared with the response obtained from rats injected with 0.5 ml 0.85% saline. Other controls were cerebral cortical extracts, treated similarly to those of the hypothalamus, and a dose level equivalent to 15 μg per rat of NIH-LH-S9 which had been boiled in 0.1 N HCl, dialyzed against distilled water, frozen and lyophilized. Since the lyophilized hypothalamic extract contained a large quantity of NaCl from the neutralization of HCl and NaOH, it was dissolved in distilled water and injected very slowly to prevent shock due to the hypertonicity of the test solution.

4. Biochemical Determinations

DNA, RNA, protein and lipid, were measured for the vagina, cervix, uterus, thyroid and thymus from homogenates containing 50 mg tissue per ml saline. For nucleic acid analyses, a modification (133) of the Schmidt and Thannhauser (121) method was employed. Lipid content was estimated from the weight of the residue after evaporation of the combined alcohol, methanol-chloroform and ether extractions obtained from the above nucleic acids analysis. And protein content was based on the Biuret reaction (41).

5. Histological Determinations

Sections from the vagina, cervix, uterus, oviducts, and thyroid were stained with Harris' hematoxylin and eosin and the heights of the luminal epithelial cells were measured with a calibrated occular micrometer.

RESULTS AND DISCUSSION

A. Statistical Analyses

The analysis of variance appropriate to most criteria of response in the heifers in this study included two sources of variation--stages of estrous cycle and heifers within stage of estrous cycle. To test for significant differences due to endocrine changes at various stages of the estrous cycle, I orthogonally partitioned the 6 degrees of freedom for stage of cycle as follows.

a. days 20 + 0 versus the rest--proestrus and estrus versus metestrus and diestrus

b. day 20 versus day 0--proestrus versus estrus

c. days 2 + 4 versus days 7, 11 and 18--metestrus versus diestrus

d. day 2 versus day 4--early metestrus versus late metestrus

e. day 7 versus day 11 and 18--early diestrus versus later diestrus

f. day 11 versus day 18--middle diestrus versus late diestrus.

Conclusions in the following sections are based largely upon these orthogonal contrasts.

B. Length of the Estrous Cycle

Since observation of the heifers for behavioral manifestations of estrus began when the heifers were about 13 months old, the exact age at which first estrus occurred is unknown. Desjardins (25) reported that first estrus occurred between 5 and 11 months, the mean being 7.4 ± 0.3 mouths. And the animals in this study were raised under the same management conditions, so we may assume first estrus occurred in the majority of these heifers between 7 and 8 months.

The mean length of the estrous cycle was 20.6 ± 0.2 days and this agrees very well with the values of 20.23 ± 0.05 and 20.5 ± 0.6 obtained by others (10).

C. Body Weights at Slaughter

The mean body weight at slaughter of 377 kg is comparable to that of 373 kg (87) for growing dairy heifers in their 16th month of age. Orthogonal contrasts revealed some significant body weight differences between days of the estrous cycle ($P < 0.01$), but as will be shown below, these differences were not associated with endocrine changes and therefore, appeared to be caused by other factors.

D. Hypothalamic and Pituitary Gland Changes During the Estrous Cycle

1. Luteinizing Hormone Releasing Factor--LRF

Recent work (18) has shown LRF content to be high in hypothalami of cycling rats in the evening of late diestrus. Early in proestrus there is a decline in the hypothalamic content of LRF and shortly thereafter there is a decline in the pituitary LH content, suggesting LRF stimulates release of LH. The data obtained in this study are the first known report of hypothalamic changes of LRF during the estrous cycle of the bovine.

Ovarian ascorbic acid depletion activity exhibited by the hypothalami was not depleted by injections of similarly treated cerebral cortical preparations. These results suggest that the responses produced by the hypothalami are not due to substances produced elsewhere in the brain.

Another group of rats were injected with NIH-LH-S9 which had been inactivated by boiling (105) for 10 min in 0.1 N hydrochloric acid and then removed by dialysis. If none of this LH had been inactivated and if all had been found in the dialysate, each rat would have received 15 μg LH. The rats receiving "inactivated LH" did not exhibit a depression in ovarian ascorbic acid concentration. In fact the average value of ascorbic acid was identical to that of the saline controls. This result suggests that inactivation of any LH which may have existed in the hypothalami was complete. Injections of 0.2 μg and 0.4 μg of NIH-LH-S9 depressed ascorbic acid concentration by 24 and 28 per cent respectively. Any LH contamination of the hypothalami before boiling and dialyzing would probably be minimal, so the possibility of LH contamination altering the data for the hypothalamic extracts is negligible (70).

The units of LRF activity were arbitrarily set as being the per cent depression of ovarian ascorbic acid by the hypothalamic extracts compared with ovarian ascorbic acid content of rats receiving saline injections.

Since injections were made at two dose levels (0.20 hypothalamus and 0.80 hypothalamus), we can study the effects of 2 estimates of LRF activity independently and then combine the two. LRF activity for the low dose (Table 1 and Fig. 1) was high from days 2 to 7 of the estrous cycle and decreased to days 11 and 18. Orthogonal contrasts revealed that the depression of hypothalamic LRF at days 11 and 18 was significant ($P < 0.01$).

The response of the high dose generally followed that of the low dose. Again LRF activity was high from day 20 to day 7 and low on days 11 and 18. Orthogonal contrasts revealed that LRF was greater during metestrus than during diestrus and declined significantly during diestrus (from day 7 to days 11 and 18, $P < 0.01$).

TABLE 1.--Hypothalamic LRF units[a] for the two dose levels.

Day of Estrous Cycle	Low Dose[b]	High Dose[b]	Average
0	6.2	18.5	12.4
2	13.1	16.5	14.8
4	10.8	20.5	15.7
7	15.0	18.4	16.7
11	8.3	8.6	8.4
18	6.4	6.8	6.6
20	10.1	16.9	13.5

[a]Per cent ovarian ascorbic acid depletion, relative to that caused by saline.

[b]Low and high doses were 0.2 and 0.8 hypothalamic equivalents per rat.

2. Anterior Pituitary

a. Weight.--Anterior pituitary weight did not vary greatly during the estrous cycle (Table 2). There was, however, a relatively small decline during mid-cycle and again at proestrus, but only the increase from day 11 to 18 was significant ($P < 0.05$). At all other stages of the cycle, average anterior pituitary weight ranged only from 1.500 to 1.558 gm. As will be shown below, pituitaries, prolactin, FSH and LH reach maximum levels late in the cycle but in view of the quantity of hormones found, it seems unlikely that these levels alone would be responsible

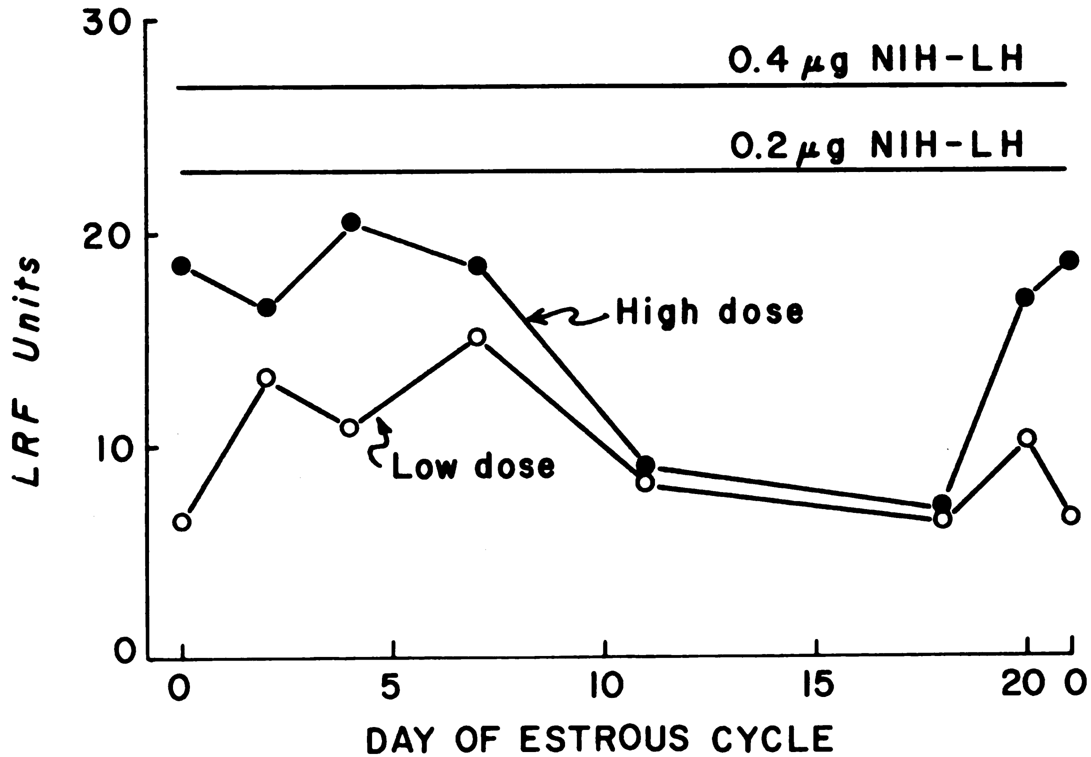

Figure 1.--Hypothalamic LRF during estrous cycle.

TABLE 2.--Pituitary weight and prolactin, FSH and LH.

Day of estrous cycle	Weight	Prolactin[a]		FSH[b]		LH[c]	
		Conc.	Content	Conc.	Content	Conc.	Content
	(mg)	(IU/mg)	(IU)	(μg/mg)	(μg)	(μg/mg)	(μg)
0	1,500	0.04	67.2	0.14	122	1.3	1,783
2	1,558	0.01	19.4	0.19	290	0.4[d]	689[d]
4	1,502	0.01	17.4	0.14[d]	184[d]	1.3[d]	1,693[d]
7	1,418	0.03	39.5	0.16	223	2.8[d]	3,995[d]
11	1,364	0.02	28.9	0.27	372	3.9	5,390
18	1,544	0.03	51.0	0.29	450	2.5	3,731
20	1,396	0.04	50.0	0.16	228	4.3	6,178

[a] IU NIH-P-S5 (Y.N. Sinha, 1967).

[b] μg-equivalents NIH-FSH-S9.

[c] μg-equivalents NIH-LH-B2.

[d] Four pituitaries assayed. All other values are means of five.

for a significant change in total pituitary weight during the cycle. In the rat (126), however, anterior pituitary glands gained weight during proestrus and estrus and lost weight during metestrus and diestrus.

b. Prolactin.--Pituitary prolactin content (126) paralleled prolactin concentrations (Table 2 and Fig. 2) throughout the estrous cycle. Because of large variations among animals, Sinha evaluated the data by regression analysis and obtained a significant slope ($P \cong 0.08$). Although the statistical validity is questionable, he used a t-test to compare the various stages of the estrous cycle such as day 2 vs day 20. Although the changes in prolactin concentration were of borderline statistical significance, he concluded that there were some valid trends. For example, from a minimum of 0.012 IU/mg at day 2 of the estrous cycle, prolactin concentration increased to a maximal value of 0.045 IU/mg on the day of estrus--an increase of 275 per cent. Day (24) also observed a significant linear increase in pituitary prolactin potency from day 2 to day 18-19 of the estrous cycle of sheep.

That prolactin is possibly luteotrophic in the bovine was discussed above in "Review of Literature." The additional evidence from the present heifers suggests that luteal function in the bovine may be related to prolactin as well as to LH in that changes in pituitary prolactin content follow by about 1 day changes in pituitary LH. Also, a recent

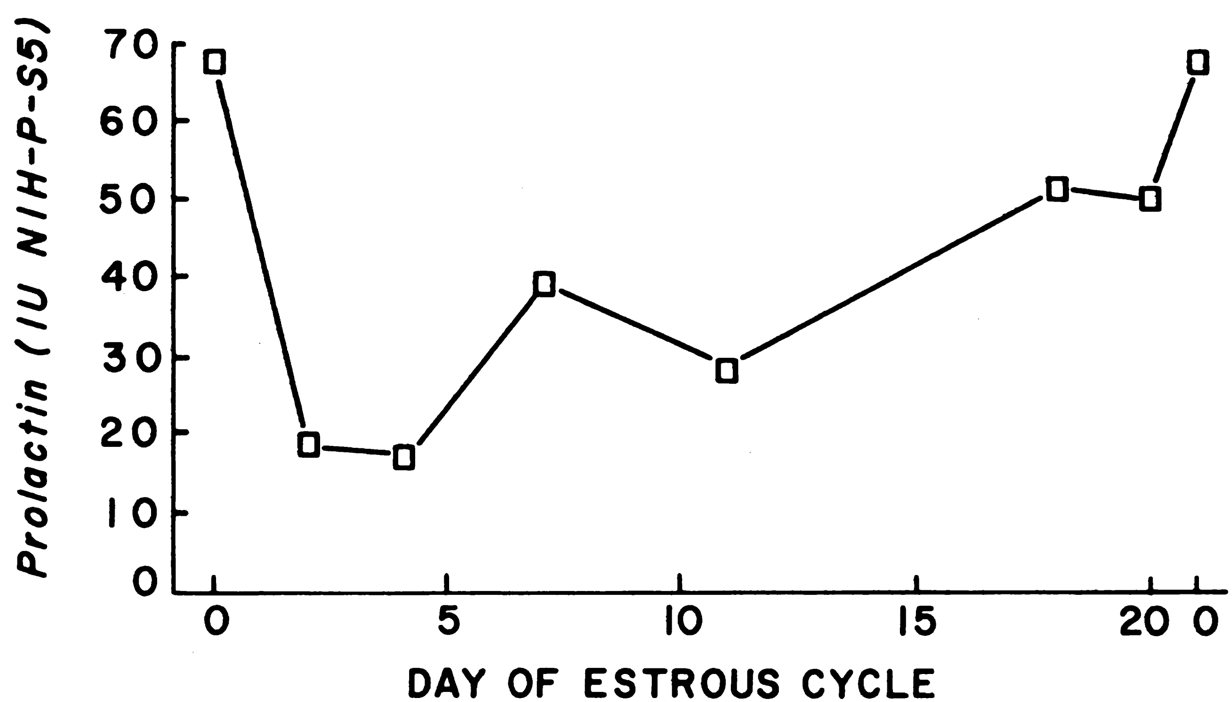

Figure 2.--Pituitary prolactin during estrous (Sinha, 1967).

report revealed that injections of antibovine LH serum (128) reduced corpus luteum weight and total progesterone but did not decrease progesterone content. Furthermore, in cows with severed pituitary stalks (56), progesterone secretion followed a curve identical to that for intact controls but at a reduced level. In pituitary stalk-sectioned sheep (132) progesterone secretion was comparable to intact animals during the estrous cycle. Thus, our knowledge is presently insufficient to determine the relationships of LH and prolactin to luteal function.

c. Follicle Stimulating Hormone--FSH.--Total content of pituitary FSH paralleled pituitary FSH concentration (Table 2 and Fig. 3). Pituitary FSH was highest on day 18 (late diestrus). Forty-nine per cent of pituitary FSH was lost from day 18 to day 20 and 46% of the FSH present on day 20 was lost by day 0. In other words, from day 18 to day 0 there was a decrease of 73 per cent in pituitary FSH. This stage of the cycle corresponds to the period of rapid growth of the follicle (9, 30, 54).

From day 4 to day 18 there was a linear increase in pituitary FSH and the maximum content was attained on day 18. There was little evidence of biphasic FSH release, although as Desjardins and Hafs (26) also reported, there was an insignificant depression in pituitary FSH on days 4 and 7.

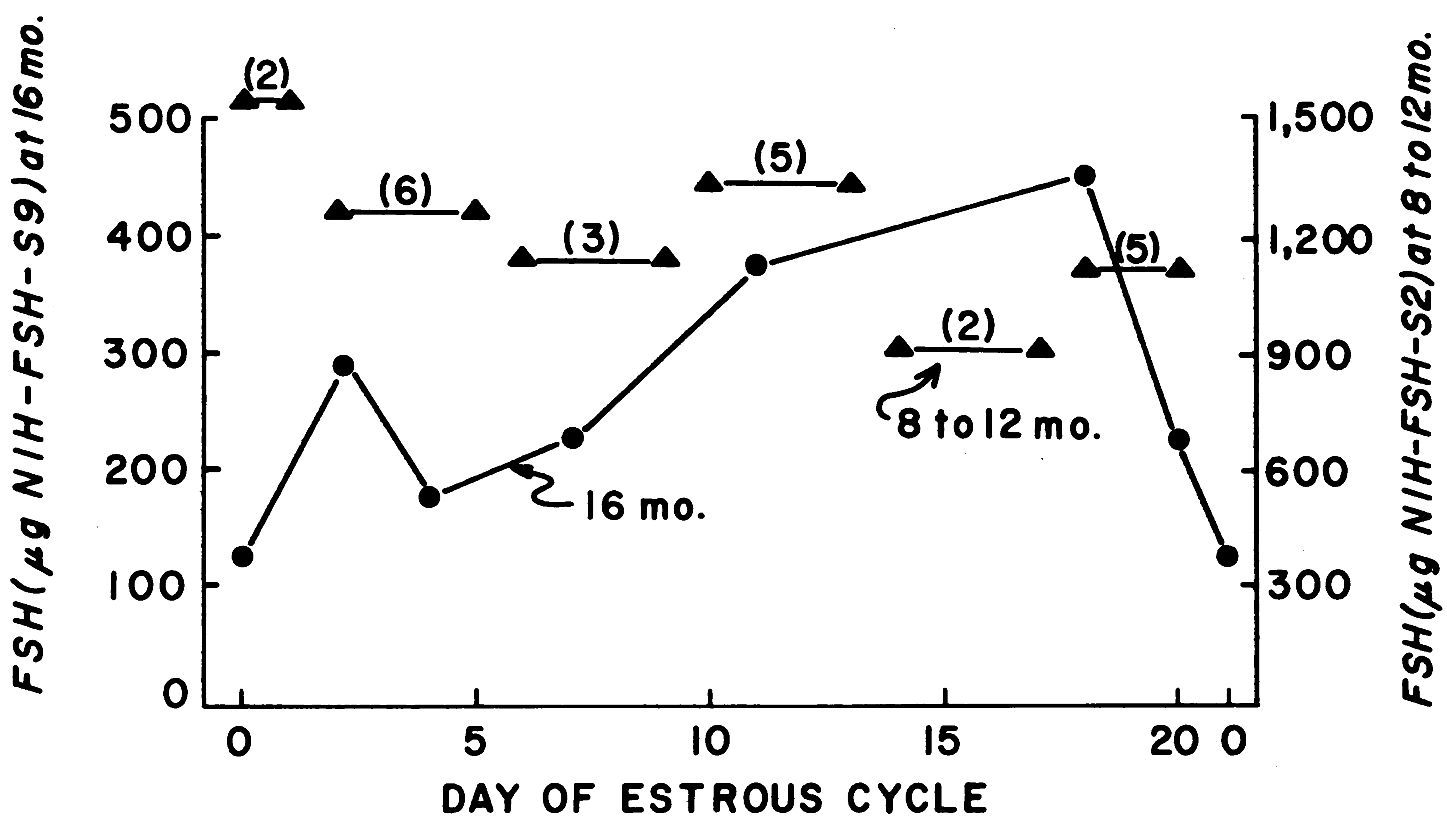

Figure 3.--Pituitary FSH during estrous cycle.

d. <u>Luteinizing Hormone--LH</u>.--As with prolactin and FSH, LH concentration and total LH content measured in individual pituitary glands paralleled each other (Table 2 and Fig. 4). Pituitary LH increased significantly from metestrus to diestrus ($P < 0.01$). It then decreased from day 11 to day 18 ($P < 0.05$) and increased to a maximum at day 20. But the most dramatic change in pituitary LH occurred from day 20 to day 0, when there was a significant decrease ($P < 0.01$) of 71 per cent in pituitary LH. It further declined 61 per cent from day 0 to day 2. Thus, pituitary LH decreased 89 per cent from day 20 to day 2.

Unfortunately at the time of this study, meaningful techniques for measuring LH in peripheral blood were unavailable. Therefore, no data on plasma LH levels were taken on these heifers. However, if LRF may be equated with LH release and pituitary LH reflects LH storage, then the ratio LRF/LH should be a rough measure of release/storage ratio. These ratios (x 1000) for days 0, 2, 4, 7, 11, 18 and 20 were 6.9, 21.5, 9.2, 4.2, 1.6, 1.8 and 2.1, respectively. Similar calculations could be done by equating LRF with LH synthesis, but the important conclusion is that the hypothalamic/pituitary relationship is relatively constant during the last half of the estrous cycle. It changes to a peak on day 2. Whether an increment in these ratios represents release or synthesis, it suggests more plasma LH between days 0 and 7. The low values at days 11, 18 and 20 may be due to high progesterone negative feedback on LRF.

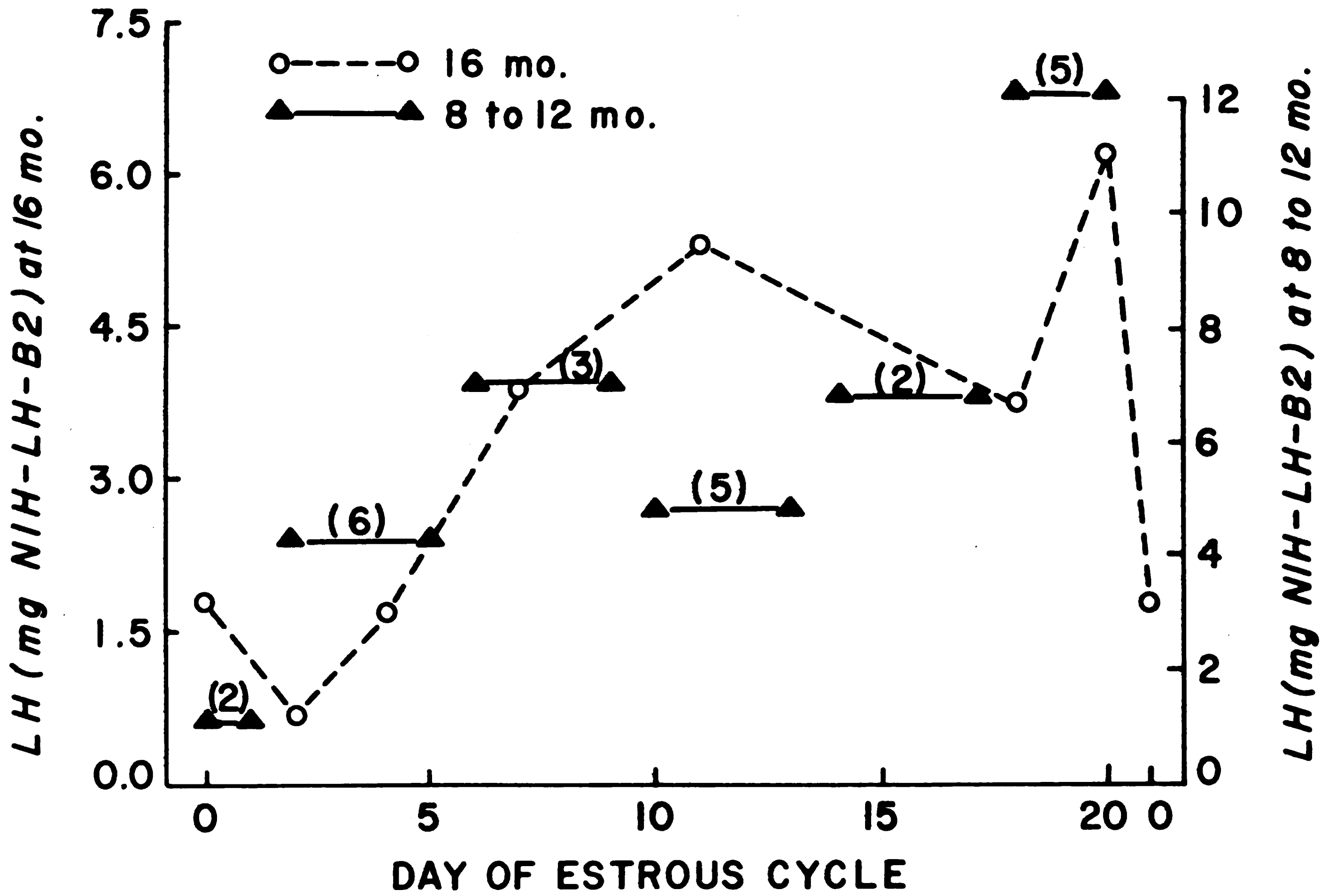

Figure 4.--Pituitary LH during estrous cycle.

E. Ovarian Changes During the Estrous Cycle

1. Follicle

Of the four criteria of the follicle measured (Table 3 and Fig. 5), the weight of the follicle wall probably gives the best estimate of follicular steroidogenic activity. The weight of the follicle wall increased from day 2 to day 7, levelled between day 7 and 11 and decreased significantly ($P < 0.05$) from 388 mg on day 11 to 252 mg on day 20. Follicles measured on day 20 may have been atrophying or they may have been the new preovulatory follicles. The most marked follicular growth occurred between day 20 and day 0. The periods of follicular wall growth (days 2 to 7 and 18 or 20 to 0, Table 3) coincide with loss of FSH from the pituitary (Fig. 3). When there was little follicular growth (days 0 to 2 and 7 to 18 or 20), FSH accumulated in pituitaries. Thus, follicular growth probably reflects stimulation by FSH which has been released from the pituitary gland between days 2 and 4 and 18 and 0.

Trends in follicular diameter, weight of follicle and weight of follicular fluid are somewhat similar to that of the weight of the follicle wall. Overall, the results suggest a buildup in the size of the follicle from day 2 to day 11 and degeneration to day 20. But the greatest change is the marked increase in size from day 20 to day 0.

TABLE 3.--Ovarian weight characteristics.

Day of Estrous Cycle	Ovarian Weight		Weight of Follicle	Weight of Follicle Wall	Weight of Follicular Fluid	Diameter of Follicle
	With CL and Follicle	Without CL and Follicle				
	(gm)	(gm)	(mg)	(mg)	(mg)	(mm)
0	7.22	3.21	3450	686	2764	19.4
2	8.18	4.71	214	73	163	6.6
4	7.32	3.91	598	175	425	10.4
7	8.44	4.13	1938	410	1528	16.0
11	9.39	3.78	1998	388	1590	16.4
18	12.05	5.91	1694	296	1369	16.2
20	9.25	4.85	1733	252	1453	15.2

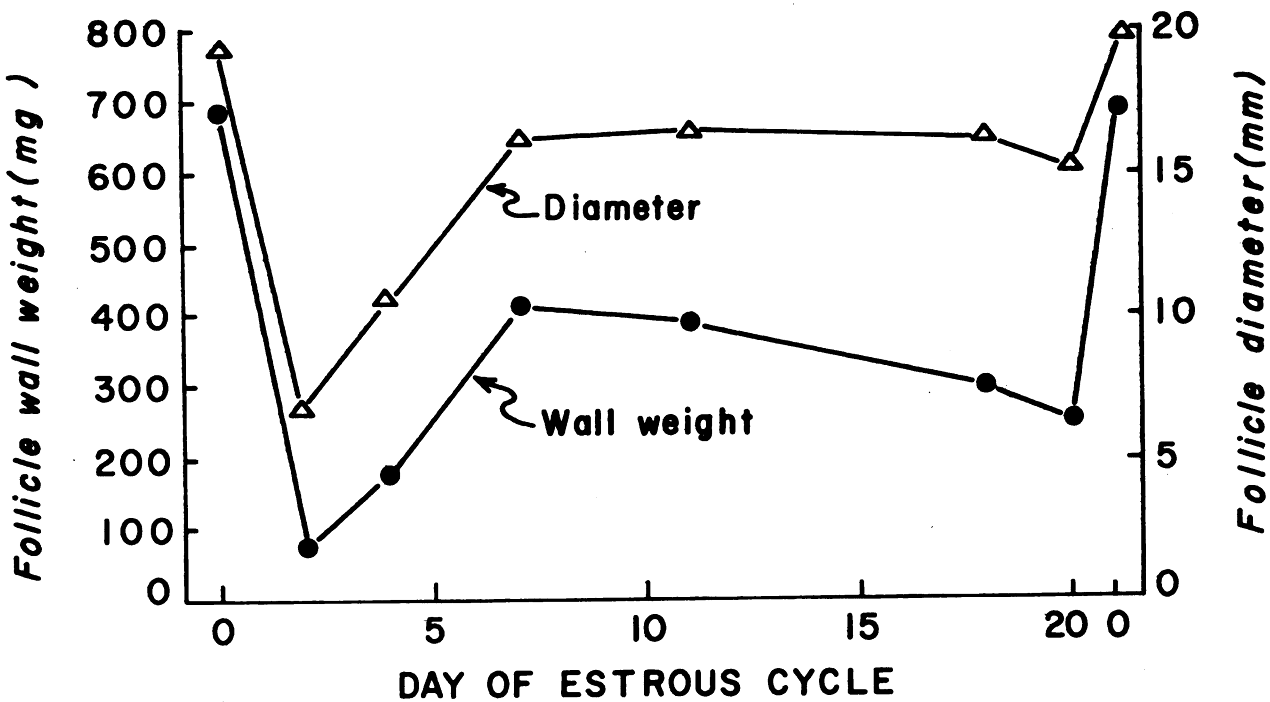

Figure 5.--Follicular size during estrous cycle.

2. Corpus Luteum

a. Growth.--A report (46) has been published on the corpora lutea obtained in this study and only highlights of those data will be given here. During the first half of the estrous cycle, weight changes followed a typical growth curve (Table 4) and estimated cell numbers (DNA) paralleled luteal weight during luteal growth. But the DNA concentration increased from 2.48 mg/gm at day 11 to 4.30 mg/gm at day 0. This suggests more cells in a smaller volume, presumably caused by aging of the corpora lutea. Ratios of RNA/DNA which estimate synthetic activity were greatest during the most rapid growth phase.

TABLE 4.--Corpus luteum weight and progesterone, 20 β-ol, progestin (progesterone plus 20 β-ol) content.

Day of Estrous Cycle	Weight of CL	Progesterone Content Per CL	20 β-ol Content Per CL	Progestin Content Per CL
	(mg)	—	(μg)	—
0	1,917	5.3	1.7	10.6
2	360	2.5	0.8	3.3
4	932	23.6	7.0	30.6
7	4,594	186.7	16.6	203.3
11	6,328	266.7	62.8	329.5
18	5,042	239.1	23.1	262.1
20	3,496	104.4	30.1	134.5

Histologically, large lutein cells which have been associated with progesterone synthesis (130) were sparse in corpora lutea on day 2 but increased through day 11 and remained as the most striking micromorphological feature through days 18 and 20. However, by day 18, these cells were rounded as compared with their previous irregular polyhedral shape and the small lutein cells had lost most of their identity by day 18. Arterioles (46) of corpora lutea on days 18 and 20 possessed thickened walls, and the endothelia appeared serrated.

b. Progesterone.--Progesterone concentration paralleled total progesterone (Table 4 and Fig. 6), and both of these parameters paralleled corpus luteum weight. These data generally agree with previous research (72) but reveal, for the first time, low progesterone concentration (7 μg/gm) in corpora lutea on day 2 and a significant increase from day 4 (25 μg/gm) to day 7 (41 μg/gm). At mid-cycle it is maximal, decreasing gradually toward the time of next ovulation.

However, net synthesis of corpus luteum progesterone (Table 5) increased progressively from 17 μg/gm at day 2 to 136 μg/gm at day 11. Synthesis *in vitro* was relatively constant from day 11 to day 20 but at day 0, it dropped to 46 μg/gm. There was greater synthesis of progesterone in incubations with NADPH ($P < 0.001$) than in incubations with the NADPH generating system.

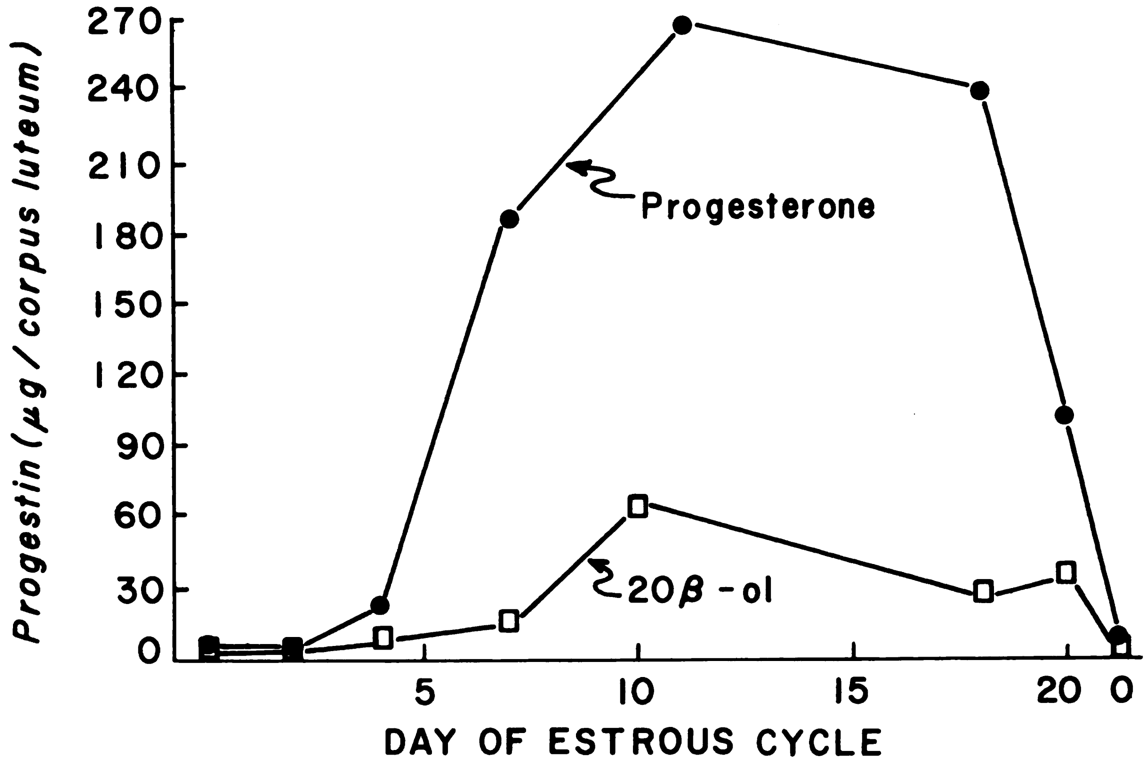

Figure 6.--Luteal progestin during estrous cycle.

TABLE 5.--Net synthesis of progesterone (prog) and 20 β-hydroxy-pregn-4-3n-3-one (20 β-ol) mass[a] during incubation[b] of corpus luteum homogenates.[c]

Day of Cycle	NADP + G-6-P						NADPH					
	No LH			LH			No LH			LH		
	Prog	20β-ol	Total	Prog	20β-ol	Total	Prog	20β-ol	Total	Prog	20β-ol	Total
2	-	-	-	-	-	-	18	3	21	16	3	19
4	55	2	57	54	2	56	57	12	69	54	4	58
7	91	14	105	85	14	99	102	10	112	99	11	110
11	115	40	155	136	40	176	144	14	156	150	14	164
18	128	26	154	126	26	152	161	4	165	162	4	166
20	139	34	173	136	35	171	158	10	168	167	8	175
0	36	0	36	39	0	39	54	1	55	56	4	60

[a]Each value for each steroid is the average (μg/gm corpus luteum) of 5 observations, except of 3 on day 0. Standard error for n=5 was 3 for days 2, 4, 7 and 0 for both steroids, 7 for days 11, 18 and 20 for progesterone, and 8 for days 11, 18 and 20 for 20 β-hydroxy-pregn-4-en-3-one.

[b]Incubation of about 100 mcg. corpus luteum for 1 hr. at 37° in 2.5 ml. Krebs-Ringer bicarbonate buffer with 1 mg/ml glucose and 5 μc cholesterol-$7^{3}H$.

[c]Hafs and Armstrong (49).

c. <u>20β-hydroxy-pregn-4-en-3-one (20β-ol)</u>.--Total 20β-ol content (Table 4 and Fig. 6) increased progressively from day 2 (0.80) μg/CL to day 11 (63 μg/CL). At day 18, 20β-ol content was 23 μg/CL, but unlike progesterone, the content increased to 33 μg/CL at day 20, declining to 5 μg at day 0. Hilliard <u>et al</u>. (58) suggested that 20α-ol, the α isomer of 20β-ol, acts as a positive feedback agent to prolong and heighten LH discharge in mated rabbits. Their results indicate that "coitus triggers a transient release of LH sufficient to activate the synthesis and release of 20α-ol and that this progestin acts as a positive feedback agent to prolong and heighten gonadotrophin discharge." In the rabbit, 20α-ol is a discreet hormonal entity derived from sterol precursors stored by the ovarian interstitial cells. However, in the present study 20β-ol was measured in the corpus luteum, and both the concentration and total content increased at day 20 when pituitary LH content was highest. Perhaps at this time sufficient LH was released to stimulate synthesis and release of further LH. 20β-ol measured at more frequent intervals and in ovarian venous effluent, rather than in the corpora lutea of the present heifers, would have been a better test of Hilliard's hypothesis.

From an average of 3 μg/gm at day 2, net synthesis of 20β-ol during incubation of corpus luteum homogenates increased to 26 μg/gm at day 11, remained relatively constant to day 20 (22 μg/gm), and then declined to 1 μg/gm (Table 5) at day 0. Average synthesis of 20β-ol on days 11, 18 and 20 was significantly ($P < 0.001$) greater in incubations with the NADPH generating system (19 μg/gm) than with NADPH (8 μg/gm). In other words, glucose-6-phosphate dehydrogenase was not rate limiting to synthesis of 20β-ol. But, this is in contrast to the results with progesterone synthesis. Total progestin (progesterone plus 20β-ol) synthesized with the NADPH generating system was quite similar to the amount synthesized with NADPH, and this was true at each stage of the estrous cycle.

3. Ovary Weight with CL and Follicle

The weight of the whole ovary which contained the corpus luteum and largest follicle (Table 3) increased progressively from day 2 (8.18 gm) to day 18 (12.05gm) and decreased to day 0 (7.22 gm). These changes are mainly due to the corpus luteum weight, the largest follicle weight and the weight of any other growing follicles. At day 18, both corpus luteum weight and the weight of the largest follicle are lower than at day 11, suggesting that the larger ovarian weight on day 18 may be due to increased interstitial tissue.

But this conclusion is untenable because one cannot be certain which "large follicle" was measured on day 18. And, besides the atretic mid-cycle follicle and new pre-ovulatory follicle on day 18, there are other follicles of varying size and weight which increase the overall ovarian weight. However, by day 20, the corpus luteum is rapidly declining in weight, and possibly other follicles are becoming atretic because the preovulatory follicle is growing at an extremely rapid rate. At day 0, there is a further decline by both the corpus luteum and smaller follicles which have given way completely to the ovulatory follicle.

4. Ovary Weight Without Corpus Luteum and Follicle

When weights of both the corpus luteum and largest follicle are removed from the weight of the ovary (Table 3), the remainder is largest on day 18, but as outlined above, this could be caused by structures other than interstitial tissue. The corpus luteum from the previous cycle further degenerates between day 2 and 4, reducing the weight of the ovary. The increase from day 4 to day 18 is probably due to the growth of smaller follicles which will eventually atrophy by day 20, possibly because of the increasing demands for FSH by the new follicle. And, as before, they will atrophy further by day 0.

From the preceding data, and the data on corpus luteum, progesterone and 20β-ol, it appears unlikely that changes in the ovarian interstitium cause significant changes on the estrous cycle; i.e., unlike the rabbit, the bovine ovarian interstitium probably does not produce any hormones. However, the progesterone and 20β-ol content should be measured in ovarian venous blood and interstitium to ascertain whether or not the interstitium of bovine ovaries does actively secrete gonadal hormones.

F. Vaginal Changes During the Estrous Cycle

1. Weight

The changes in weight of the vagina (Table 6) at various stages suggest a biphasic pattern. For example, the maximal weight of 207 gm occurred at day 2. Then it declined to 169 gm at day 7, but increased to 189 gm at day 11 in mid-cycle. There was a decline in weight by day 18 (178 gm) and then it increased to 201 gm at day 0 (estrus). Orthogonal contrasts revealed a significant ($P < 0.05$) decrease in vaginal weight from day 2 to 4 and from metestrus to diestrus (days 7, 11 and 18). The increase from days 7 to 11 approached significance ($P < 0.09$). Thus, the data suggest that weight increases occur at the time of estrus and around day 11, times when there are large follicles on the ovary.

2. DNA, RNA, Protein and Lipid

Vaginal DNA concentration was lowest on day 0 (1.55 mg/gm) and highest on day 11 (1.96 mg/gm) suggesting fewer number of cells per given volume during estrus, perhaps as a result of edema, possibly in the epithelial layer. But

TABLE 6.--Vaginal weight, DNA, RNA, protein and lipid content and epithelial cell-layer height.

Day of Estrous Cycle	Weight	DNA	RNA	Protein	Lipid	Epithelial Height
	(gm)	—	(mg)	—	(gm)	(μ)
0	201	310	622	31	58	57
2	207	287	655	33	57	39
4	182	326	675	30	49	31
7	169	282	551	23	41	22
11	189	371	692	34	45	21
18	178	331	598	31	37	27
20	184	296	588	33	40	29

total DNA (Table 6 and Fig. 7) was lowest on day 7 (282 gm) and highest on day 11 (371 gm). Thus, DNA flucturates during the estrous cycle. It is somewhat higher at estrus and mid-cycle. And this is further reflected by vaginal DNA/100 kg body weight which is highest at estrus and day 11 (92 and 94 mg/100 kg body weight respectively).

RNA concentration followed a pattern similar to DNA concentration but there were fewer significant changes between various stages of the estrous cycle. Changes in total RNA mimicked those for total DNA. Consequently, RNA also shows a biphasic pattern--high at estrus and around day 11 (Table 6 and Fig. 7).

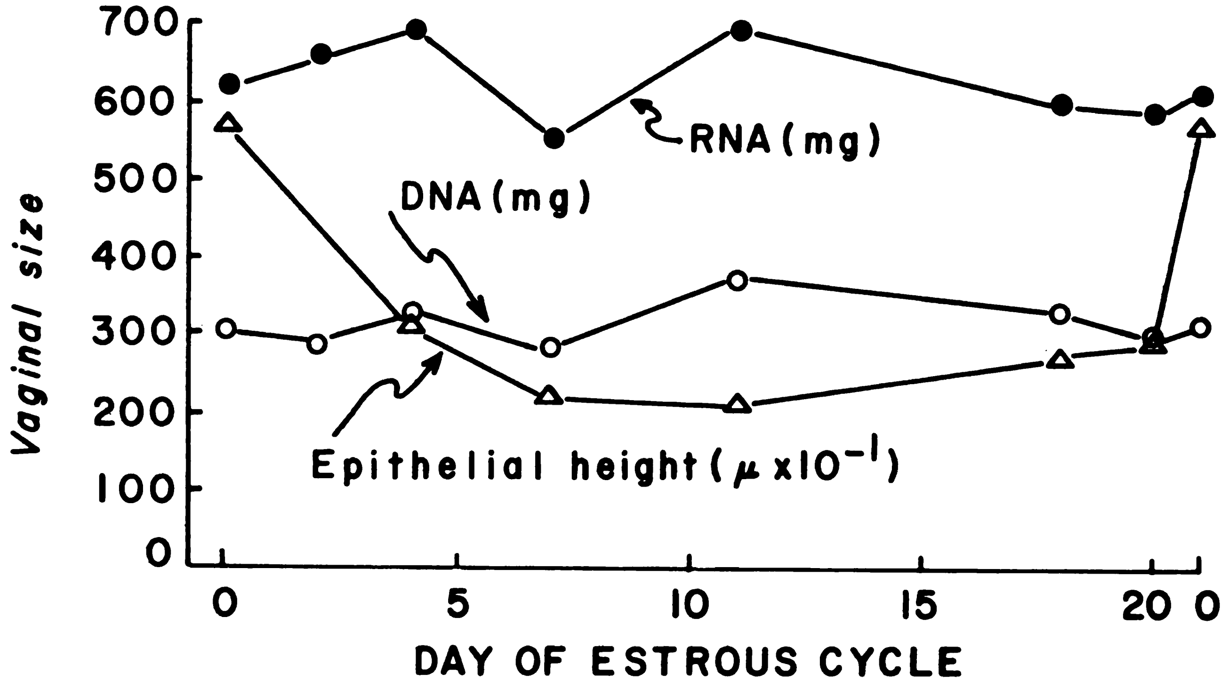

Figure 7.--Vaginal nucleic acids and epithelial cell-layer height.

As with DNA and RNA concentration, vaginal protein concentration does not follow the same trends as total proteins; being lowest on day 7 (0.139 mg/gm) and highest on day 19 (0.186 mg/gm). Total protein follows total DNA and RNA; being highest at day 11 (34 mg) and lowest on day 7 (23 mg).

Lipid concentration and total lipid content paralleled each other fairly well, being highest at estrus (283.2 mg/gm and 57.8 gm, respectively) and lowest at day 20 (222.4 mg/gm and 40.0 gm respectively). The total lipid content did not show the biphasic pattern as did DNA, RNA and protein. Rather, it was significantly greater ($P < 0.01$) from metestrus to diestrus.

3. Height of Epithelial Cell-Layer

At estrus, the height of the vaginal epithelial cell-layer reached a maximum of 57 μ (Table 6 and Fig. 7). Thereafter it decreased steadily to day 14 (21 μ), remained relatively constant to day 20, and doubled from day 20 to day 0. Thus, unlike some of the other vaginal criteria, there was no evidence from the height of the epithelial cell-layer to suggest appreciable estrogen secretion in mid-cycle. On the other hand, this response would be expected to require considerable time rather than being instantaneous. If this was the case in these heifers, the vaginal epithelium may have responded to mid-cycle estrogen secretion with increased thickness on days 12 to 15.

4. Length

The average length of the vagina was 29.4 cm (Table 7). Changes in length during the estrous cycle appeared to be random. At least no known physiological substance could be established for the minor changes in vaginal length apparent in Table 7.

TABLE 7.--Length of vagina, cervix, uterine body and horns and oviducts.

Day of Estrous Cycle	Vagina	Cervix	Uterus			Oviduct	
			Body	Right Horn	Left Horn	Right	Left
	(cm)						
0	28.6	5.6	2.0	28.8	31.6	21.2	19.8
2	29.8	5.4	2.6	29.6	29.2	21.0	21.2
4	28.8	5.2	2.4	31.0	31.6	20.4	19.8
7	28.6	5.0	2.6	33.8	34.8	22.4	21.4
11	33.0	6.0	2.6	32.4	32.0	21.4	22.2
18	29.8	6.0	2.6	35.0	33.6	24.0	21.6
20	27.2	5.2	2.8	30.0	30.4	21.4	23.2

5. Summary

Total DNA, RNA and protein content showed biphasic patterns; high at estrus and day 11. But the RNA/DNA ratio revealed no significant changes ($P > 0.05$), suggesting RNA increases paralleled those of DNA. Both the total lipid content and height of the epithelial layer were highest at estrus and lower for the remainder of the cycle.

G. Cervical Changes During the Estrous Cycle

1. Weight

Whether the changes in cervical weight reflected physiological changes or random variation were difficult to ascertain (Table 8). The highest value occurred during metestrus (42 gm) and the lowest on day 4 (30 gm). The only apparent physiological reason for this sudden increase was the growing corpus luteum with its increasing levels of progesterone. In fact, weight of the cervix follow a trend almost the inverse of progesterone content of the corpus luteum (Table 6).

2. DNA, RNA, Protein and Lipid

Total DNA content reached a maximal level at day 11 (Table 8) of 126 mg. It was lower at estrus (89 mg). At day 20, total DNA reached a second peak, suggesting a biphasic curve. The DNA/100 kg body weight did not follow this pattern; it was high at day 11 (31.7 mg/100 kg body weight) and decreased steadily to day 4 (20.1 mg/100 kg body weight). The significant decrease between day 2 and 4 (97 to 70 mg) for total DNA seemed to be due to the large difference in cervical weight between these two days ($P < 0.01$).

TABLE 8.--Cervical weight, DNA, RNA, protein and lipid content and epithelial cell height.

Day of Estrous Cycle	Weight	DNA	RNA	Protein	Lipid	Cell Height
	(gm)	——	(mg)	——	(gm)	(μ)
0	39	89	191	5.3	7.5	28.6
2	42	97	179	6.7	8.5	25.2
4	30	70	119	4.9	7.6	16.6
7	35	108	163	5.7	7.4	16.6
11	38	126	162	6.7	8.1	18.3
18	41	111	169	5.4	10.9	15.8
20	40	112	182	6.8	10.8	15.7

Basically, RNA concentration and total content of the cervix paralleled each other (Table 8 and Fig. 8). Total RNA was highest at estrus (191 gm) and declined to minimal levels at day 4 (119 gm). The significant decrease of the total RNA content reflected changes in the weight of the cervix ($P < 0.01$). The increase in RNA began at day 18 and continued through day 0. Although there was a significant decrease in RNA concentration throughout diestrus ($P < 0.01$), this was not reflected in the total RNA, as the weight of the organ icnreased steadily throughout this period. There was no evidence suggesting bimodality of total RNA during the estrous cycle.

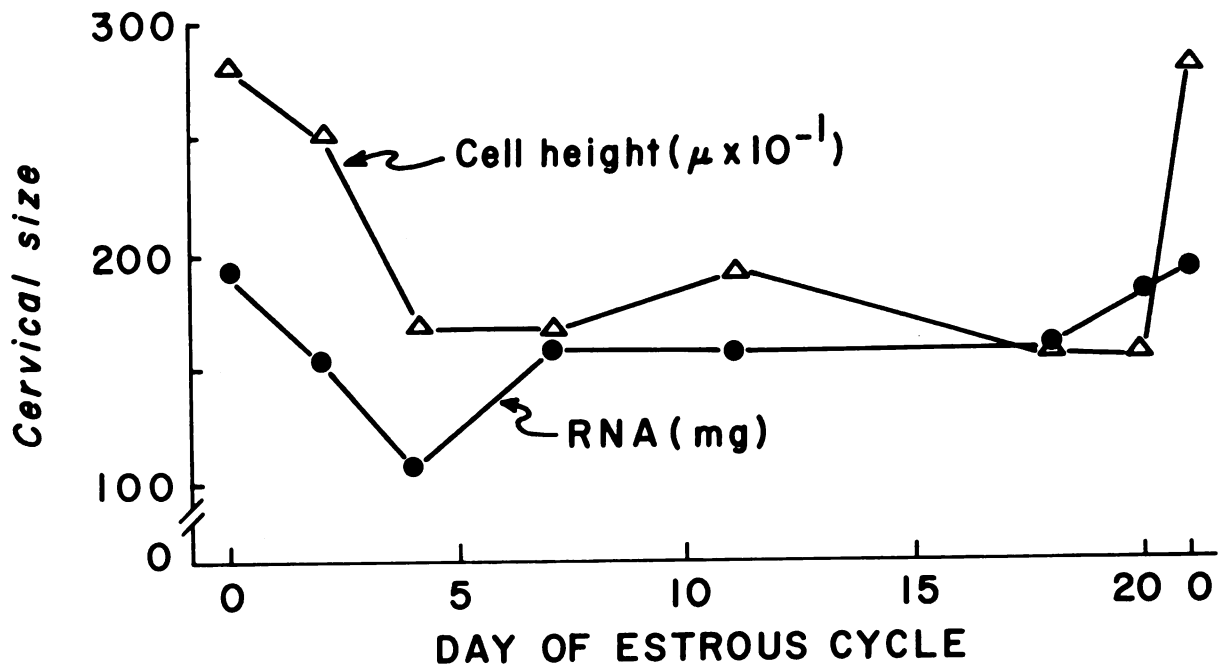

Figure 8.--Cervical RNA and epithelial cell height.

Except for the low value of total protein at day 4 (4.9 mg), protein concentration and total content paralleled each other. Total protein showed a triphasic pattern with high levels at days 2 (6.7 gm), 11 (6.7 gm) and 20 (6.8 gm). The lipid concentrations and total content also paralleled each other. The highest values occurred at day 18 and 20 (11.0 and 10.7 gm) followed by significant decreases at estrus ($P < 0.01$). During metestrus there was an increase, followed by a decrease to day 7 and then a steady increase through day 20.

3. Epithelial Cell Height

Between days 20 and 0, cervical epithelial cell heights almost doubled (15.7 μ to 28.6 μ respectively, Table 8 and Fig. 7). Following estrus there was a steady decrease to day 4 (16.6) and from day 4 there was a steady increase to day 11 when a second peak of 18.3 μ occurred; this was followed by a significant decrease to day 18 of 15.8 μ ($P < 0.05$). These data again showed a biphasic pattern.

4. Length

The average length of the cervix was 5.49 cm (Table 7). There do not seem to be any physiological differences in the length of the cervix throughout the estrous cycle.

5. Summary

Based upon epithelial cell height, greatest synthesis of mucus in the cervix took place at estrus. From estrus to mid-cycle (day 11), synthetic activity diminished and gradually increased from day 18 on. It followed the pattern of total RNA. Cervical epithelial cell heights and total DNA content revealed parallel biphasic curves, whereas protein showed a triphasic curve. Lipid content was maximal on day 20 and minimal at day 7.

H. Uterine Changes During the Estrous Cycle

1. Weight

The uterine weights suggested no meaningful cyclic pattern (Table 9).

2. DNA, RNA, Protein and Lipid

DNA concentration paralleled total DNA, reaching peak levels at day 2 and day 11 and minimal levels at days 7, 18 and 20. Here again there was a suggestion of a biphasic trend (Fig. 9), with peaks occurring around estrus and again at mid-cycle. There seemed to be a lag period from the time of expected maximal estrogen blood levels to the time of maximal DNA around estrus. Unfortunately, there were no other estimates between day 11 and 18 to see if such was the case at mid-cycle.

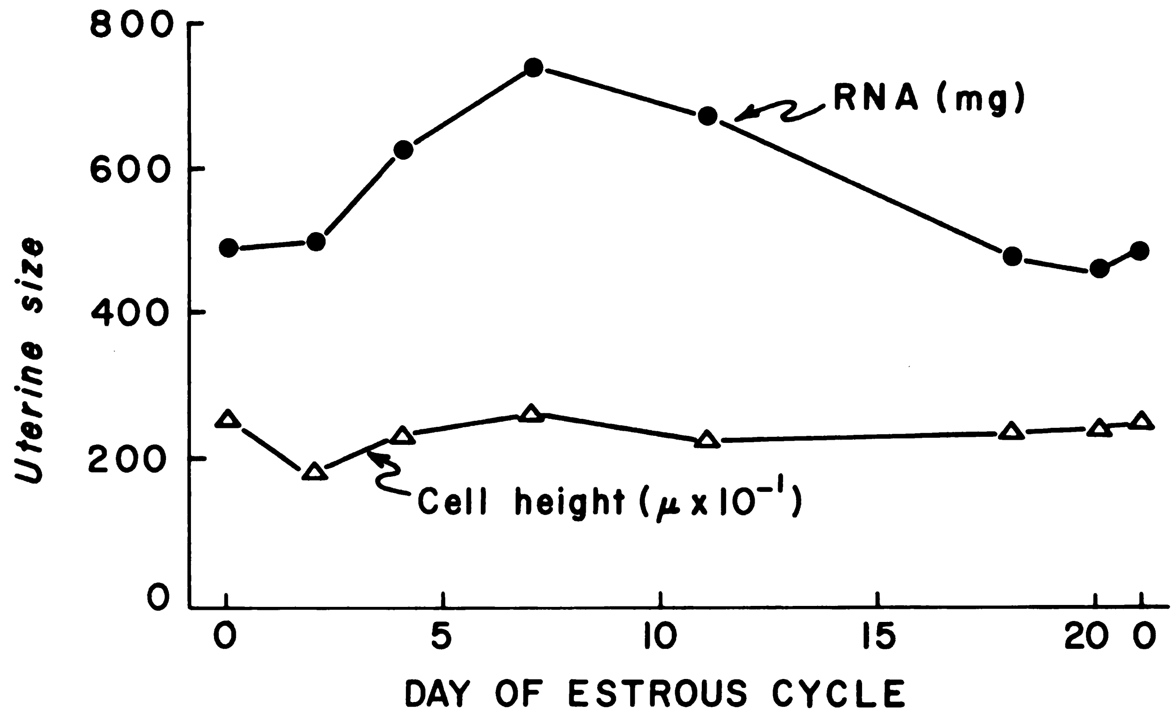

Figure 9.--Uterine RNA and epithelial cell height.

TABLE 9.--Uterine weight, DNA, RNA, protein and lipid content and epithelial cell height.

Day of Estrous Cycle	Weight	DNA	RNA	Protein	Lipid	Cell Height
	(gm)	(mg)	(mg)	(mg)	(gm)	(μ)
0	168	905	486	18.8	36.0	25.1
2	173	1134	496	20.6	59.0	17.8
4	152	938	625	18.5	45.2	23.3
7	177	883	731	25.3	44.0	25.8
11	166	1106	672	19.9	42.2	22.1
18	190	870	484	22.8	46.0	24.4
20	169	872	460	23.4	43.4	24.0

Uterine RNA concentration and total were lower at proestrus and estrus than during the rest of the cycle and this was also true for DNA (Table 9 and Fig. 9). Highest RNA concentrations occurred at days 4, 7, and 11 (4.18, 4.01 and 4.06 mg/gm respectively), and this trend was also evident for total RNA. During early metestrus, RNA concentration was relatively low but increased markedly by day 4, and again this was evident for total RNA. From days 7 to 18, both RNA concentrations and total RNA decreased. By day 20, RNA concentrations began to increase rapidly to estrus (2.90 mg/gm). Partly due to the variation of the uterine weights, total RNA did not follow this trend, but it increased at estrus.

Protein concentrations and total protein generally paralleled each other, with peaks at days 7 and 20. Initially the curve followed that of RNA, but when RNA declined after day 11, total protein increased to day 20 (23.4 mg) and fell by the day of estrus.

Although there was some variation between lipid concentrations and total lipid content, the overall trends were similar. Lipid content was highest on day 2 (59.0 gm), declined to day 11 (42.2 gm), and remained relatively constant to day 20 (43.4 gm). Between days 20 and 0, there was a significant drop to 36.0 gm ($P < 0.05$), the lowest value during the estrous cycle.

3. Epithelial Cell Height

Uterine epithelial cell heights were maximum at estrus and day 7 (25.1 and 25.8 μ respectively). There was a significant increase in cell height during metestrus (from 17.8 μ on day 2 to 23.3 μ on day 4) which continued until day 7 ($P < 0.01$). This was followed by a drop at day 11 (22.1 μ) and a gradual increase to day 0 (25.1 μ). These data also showed some evidence for a biphasic change during the estrous cycle (Table 9 and Fig. 9).

4. Length

The average length for the uterine body was 2.51 cm (Table 7). The shortest length occurred at estrus, possibly because high estrogen titers increased the tone of the uterus. There was no important difference between lengths of right and left uterine horns; the right averaged 31.5 cm and the left 31.9 cm.

5. Summary

There seems to be no uniform pattern for all of the biochemical criteria measured in the uterus. This, in part, may be a result of the heterogeniety of this tissue which included endometrium, myometrium and connective tissue. But the data indicated there were cyclical changes in the individual parameters measured during the estrous cycle. With regards to the epithelial cell heights, the changes inversely followed the progesterone content of the corpus luteum. Actually, this criterion may have more accurately and directly reflected estrogen titers, but estrogens were not measured in this experiment. Uterine RNA and RNA/DNA, on the other hand, appeared directly related to progesterone content of the corpus luteum.

I. Changes in the Oviduct During the Estrous Cycle

1. Length

The average lengths of the right and left oviducts were 21.69 and 21.31 cm respectively; and the overall average was 21.50 cm (Table 7). There were no significant differences between lengths of left and right oviducts within any of the days of the estrous cycle ($P < 0.05$). And although there were significant differences in both groups when orthogonal comparisons were made for various stages of the estrous cycle, ($P < 0.05$) they have no known physiological or anatomical meaning.

2. Epithelial Cell Height

At proestrus, the epithelial cell heights (Table 10) were minimal (20.7 μ). From day 0 to 7, they increased to reach a maximum of 25.7 μ, and declined precipitously to 22.8 and 22.4 μ on day 11 and 18 respectively ($P < 0.01$). These results disagreed with another report (10) which cited that epithelial cells were highest (45.3 μ) at estrus and are minimal (26.6 μ) 6 days after estrus.

3. Summary

The lengths of the right and left oviducts were similar and varied from 19.8 to 24 μ. This agreed with the range of about 20 to 35 cm previously reported (10). There was disagreement between the present data and that of a previous report (10) concerning the epithelial cell heights and variations in heights during the estrous cycle. But the heights reported here agreed more closely to those of Desjardins (25), who measured these cell heights in heifers from birth to puberty. For example at 12 months, the epithelial cell heights averaged 28.4 ± 0.2 μ.

TABLE 10.--Oviducal epithelial cell height.

Day of Estrous Cycle	Cell Height
	(μ)
0	22.6
2	22.3
4	23.5
7	25.6
11	22.8
18	22.4
20	20.7

J. Changes in the Thyroid During the Estrous Cycle

1. Weight

Thyroid weight (Table 11) fluctuated throughout the estrous cycle, but in no orderly fashion, so it was concluded that there were no changes caused by the hormones of the estrous cycle.

2. DNA, RNA, Protein and Lipid

Unlike the reproductive organs, there were no patterns to indicate a cyclical pattern for the thyroid biochemical parameters. The overall tendency was for a slight increase in DNA, RNA, protein and lipid content around days 18 and 20, but this may have reflected the

TABLE 11.--Thyroid weight, DNA, RNA, protein and lipid content and acinar epithelial cell height.

Day of Estrous Cycle	Weight	DNA	RNA	Protein	Lipid	Cell Height
	(gm)	(mg)	(mg)	(mg)	(gm)	(μ)
0	16.3	70	103	3.2	3.3	11.6
2	17.8	68	112	3.7	3.7	10.7
4	13.6	61	99	2.7	2.1	11.3
7	16.3	76	90	3.6	2.8	10.9
11	14.6	65	93	3.0	3.0	12.4
18	18.1	85	124	3.7	3.8	11.6
20	17.4	80	111	3.3	4.0	10.9

slight increase in weight at this time which was significantly higher than at day 18 ($P < 0.01$). Otherwise there was no physiological pattern, nor for that matter any important physiological reason for increasing thyroid activity. But we must remember that a "normal" thyroid is required for reproduction (89).

3. Epithelial Cell Height

Essentially, thyroid acinar epithelial cell heights did not change during the estrous cycle.

4. Summary

Throughout the cycle, DNA, RNA, protein and lipid content practically paralleled each other and in no instance was there any reason to believe there was a cyclical thythm corresponding to follicular or luteal changes. The cell height data suggested there was no increased activity during the estrous cycle and the RNA/DNA ratio, although showing some differences, further indicated no increased activity.

K. Changes in the Thymus During the Estrous Cycle

As with the thyroid, the thymus did not seem to undergo any cyclic changes associated with the estrous cycle (Table 12). Although there was some variation in the weight of the thymus, this may have been due to difficulty in removing all of it. Because of anatomical variations, in some cases it was more difficult to remove it quantitatively than in others. And in some cases it was more involuted than in others. So it was concluded that, although gonadal hormones have an effect on thymus activity, the thymus is not involved to any great extent in the estrous cycle.

TABLE 12.--Thymus weight, DNA, RNA, protein and lipid content.

Day of Estrous Cycle	Weight	DNA	RNA	Protein	Lipid
	(gm)	(gm)	(gm)	(mg)	(gm)
0	474	24.5	3.7	82.7	94.2
2	464	22.3	3.2	78.6	77.4
4	580	27.1	4.2	104.0	102.4
7	436	22.9	3.2	82.4	72.8
11	553	26.9	4.0	94.8	94.2
18	516	27.9	4.0	88.0	83.8
20	484	22.0	3.3	93.1	90.8

GENERAL DISCUSSION

The relationship between the hypothalamus, pituitary and gonads is one of negative feedback of gonadal steroids on the pituitary, hypothalamus, or both (18) and possibly a positive feedback of 20α-ol (or in cattle 20β-ol) on pituitary LH release (58) through both the "long loop" and "short loop." By definition the long loop (35) is coupling of the target gland with that of the control unit, for example, gonadal steroids acting back on the hypothalamus, pituitary gland, or both. And the short loop refers the action of a pituitary gonadotrophin on its hypothalamic controller (20). Many of the details of these relationships have been described for rats, but not for other animals. For example, releasing factors (120) have been isolated from pig, sheep, and cattle hypothalami in relatively pure form but there are no known reported studies of changes involving hypothalamic levels during the estrous cycle in species other than rats. Hypothalamic LRF content (70) measured in young Holstein bulls from birth to puberty increased between 6 and 8 or 9 months of age. This increased hypothalamic LRF was associated with a marked increase in plasma LH, suggesting high levels of hypothalamic LRF were associated with LH release from the pituitary. In the present study, hypothalamic LRF content was high on days 20, 0, 2, 4, and 7 (proestrus, estrus and metestrus) and low on days 11 and 18 (diestrus). These data suggest that LH was released from the pituitary during proestrus, estrus and metestrus and that LH was responsible for ovulation and for formation and early growth (and function?) of the new corpus luteum.

Although the pituitary-ovarian relationship in rats is not necessarily similar to that in cows, results obtained from these animals may help to elucidate the possible controlling mechanism for the hypothalamo-pituitary-gonadal axis in other species. The peak LH-releasing activity (or LRF) of rat stalk median eminence occurred in the evening of late diestrus (18). By noon of the following day (proestrus) hypothalamic LRF had significantly ($P < 0.01$) declined. This lower level was maintained without any significant change throughout the remainder of the estrous cycle. Except for the duration of the high or low levels, these results are somewhat similar to those of the present study, in that high levels of hypothalamic LRF content are present just prior to estrus and ovulation. They differ, however, in the duration of the high levels; in the rat, high levels lasted for a very short time, whereas in the bovine they lasted somewhat longer. It is well known, in contrast to cows, that rats develop no functionsl corpora lutea during the estrous cycle, and consequently have a very short diestrous.

The acute decline of rat hypothalamic-LRF (18) was interpreted as being a release of stored releasing factor which may initiate an increase in LH release from the pituitary as indicated by plasma LH assays (103). And the authors suggested that a further decline in hypothalamic-LRF content on the afternoon of proestrus, when pituitary LH releasing is maximal, did not occur because synthesis was equivalent to release of LRF during this period. Since prolactin is luteotrophic in rats, is it possible that no further LH is required at this time so there is neither synthesis nor release of hypothalamic LRF? This may also explain why the level of hypothalamic LRF remained constant and low for the remainder of the rat estrous cycle.

The exact mechanism for control of hypothalamic LRF synthesis and release has not been completely mapped. Many researchers hypothesize that LH can act directly on the hypothalamus by negative feedback (18, 22, 103, 104), thereby blocking synthesis and release of LH. And estrogen from the blood plasma also can feedback negatively on both the hypothalamus and anterior pituitary. Furthermore, LRF activity (90) is detectable in plasma of long-term hypophysectomized rats but vanishes following destruction of the median eminence region--the site for storage of LRF. This result adds further support to the theory of negative feedback, since hypophysectomized rats also had atrophic uteri and ovaries incapable of secreting estrogen.

Hypothalamic LRF content, measured 21 days following castration in female rats (98), decreased to 30 percent of control values and daily injections of 0.8 μg of estradiol benzoate further depressed hypothalamic LRF content. Pituitary LH content increased about four-fold following castration and was significantly depressed by estrogen which was caused, the authors suggested, by depressed hypothalamic LRF content. And, since the reduction in hypothalamic LRF content after ovariectomy did not parallel the observed increase in pituitary LH content, they suggested that ovariectomy elicited a greater release of hypothalamic LRF than synthesis with consequent reduced levels of hypothalamic LRF. In similar experiments, Chowers and McCann (18) suggested that lowered steroid levels in blood plasma elevate hypothalamic LRF and high levels of LH in the blood counterbalance this effect, resulting in little if any change in hypothalamic LRF. Similarly, in steroid-treated rats, there would be an interaction between the two negative feedbacks; high steroid levels tending to decrease hypothalamic LRF, and low levels of LH in the blood tending to increase it. Overall, the net result of these two antagonistic forces would be little or no change in hypothalamic LRF.

Estrogen in very small doses (7, 37, 114) inhibits synthesis and release of folliculotrophin (FTH--a term used to specify LH and FSH but not prolactin), and a single injection of estrogen (37) may elicit LH release. This is known as the "Hohlweg effect." Furthermore, there is evidence suggesting the primary effect of progesterone is to suppress secretion of FTH, especially that of LH. And this inhibition may act at the basal tuberal region of the brain, at the anterior pituitary, or both. But, on the other hand, small doses of progesterone elicit or at least hasten ovulation in rats (91) and cows (40, 55). Maximal pituitary LH release occurs at proestrus, just prior to ovulation, and even though plasma LH is elevated, it can be further elevated by low levels of progesterone (91). Thus it appears, in the rat, that hypothalamic LRF may be regulated by circulating steroids (progestogens and estrogens), at least in certain ratios, and by LH by negative feedback.

When all nervous pathways to the hypophysiotrophic area (a term used to designate connection between the hypothalamus and anterior pituitary) of the median eminence in rats are severed (47), ovulation is inhibited, whereas testicular function is maintained. In reviewing this observation and those of his own research involving androgen sterilization in female rats, Gorski (42) suggested testosterone may eliminate the cyclic LH releasing properties of the preoptic area during the first postnatal week. Thus, he concluded that the ovarian hormones regulate the preoptic area as well as the ventromedial nucleus. And, in another review, Flerkó (35) concluded "that it is the preoptic-suprachiasmatic area in the normal cycling female which responds under proper environmental and hormonal circumstances by an activation of the more terminal hypophysiotrophic area to elicit an ovulatory discharge of FTH from the anterior lobe. In the absence of this mechanism, the hypophysiotrophic structures still function and FTH is secreted at a basal rate which evokes the constant vaginal estrus syndrome."

The exact influence which LRF exerts over the anterior pituitary gland is unknown, although an early hypothesis (118) suggested it involved both synthesis and release of pituitary LH. Recently, a conjecture has been made that "LRF somehow affects the enzymes involved in either the attachment or the synthesis of the carbohydrate moiety of the LH polypeptide chain and thereby controls the production of the glycoprotein, active LH." Using in vitro incubations of pituitaries from normal or castrated rats, recent work (118) has shown hypothalamic LRF failed to incorporate either ^{14}C-leucine or ^{14}C-glucosamine into LH. The authors concluded the main effect of the hypothalamic LRF was on LH release.

That follicle-stimulating hormone (FSH) releasing factor (63) is present in the hypothalami of various species has been reported by many investigators. *In vivo* results have shown FSH-RF acts directly on the anterior pituitary, causing a reduction of pituitary FSH and an increase in serum FSH. And release of FSH from rat pituitaries cultured *in vitro* followed addition of an extract of rat hypothalami to the incubation media. In ovariectomized rats injected with estrogen, extracts of beef stalk-median eminence depleted the pituitary of FSH by 26 percent. Measurements of serum FSH indicated that it increased as pituitary FSH fell. These results indicated that FSH-RF obtained from cattle stimulated release of FSH from rat pituitaries. There is some evidence for a negative feedback between estrogen in the blood and FSH secretion from the pituitary, and this is possible mediated through hypothalamic FSH-RF. Although these results suggest a role for FSH-RF in controlling FSH release from the pituitary gland, similar to LRF in controlling LH secretion, the effects of FSH-RF, extracted from the hypothalami of domestic animals on reproductive function remain to be investigated. There are no reports of changes in hypothalamic FSH-RF content during the estrous cycle in any species.

In general, the experiments described above have only outlined some of the cyclic changes occurring in the hypothalamus and pituitary gland of rats during the estrous cycle. And they only suggest a possible approach to elucidate the changes occurring in the bovine hypothalamus and pituitary during the estrous cycle. For example, hypothalamic LRF content remained high for a longer period at the time of estrus in the bovine than for the corresponding period of estrus in the rat. If we assume from a previous experiment (118) that LRF mainly causes release of LH, and that LH is luteotrophic or at least necessary for the initial growth of the corpus luteum in the bovine, this may be one function related to the persistently high levels of hypothalamic LRF after estrus. On the other hand, in rats, after the surge of LH which is required for ovulation, no further LH may be required, and therefore, there may be no further need for a high level of hypothalamic LRF. This hypothesis assumes that high levels of hypothalamic LRF indicate synthesis of LRF is at least equivalent to release of LRF. This reasoning differs from that of Chowers and McCann (18) who suggested the reason for no further decline in hypothalamic LRF on the afternoon of proestrus was the result of synthesis of LRF keeping up with the release of LRF and both were proceeding at a rapid pace although the hypothalamic level was low.

In the present heifers, beginning at proestrus, pituitary LH fell about 89 percent from day 20 to day 2, and at day 20 the hypothalamic LRF content was high, having increased from day 18 (Fig. 11). The suggestion that high levels of hypothalamic LRF are conducive to pituitary LH release in the bovine seems to be supported by the fact that from day 20 to day 2 most of the pituitary LH was released. Now it has also been shown that antibovine luteinizing hormone (128) administered daily on day 2 through day 6 following estrus significantly ($P < 0.01$) reduced corpus luteum weight and progesterone ($P < 0.01$) in corpora lutea removed on day 11 of the cycle. So a continuing release of LH must be required to stimulate the growth of the new corpus luteum, which increases rapidly both in weight and in progesterone concentration and content between days 4 and 7. However, as the corpus luteum is growing, the synthesis of LH exceeds release from day 2 to day 11, and pituitary LH content increases. The data in this thesis suggest that, during metestrus and early diestrus, circulating LH and progesterone may each feedback negatively on the hypothalamus reducing release of hypothalamic LRF and thereby reducing release of pituitary LH. The hypothalamic LRF, measured at this time, may very well be that which is synthesized in the hypophysiotrophic center responsible for tonic release of LRF, if indeed in the bovine a site exists such as has been advanced in rats (42).

Progesterone (100 mg) administered daily (65) for 35 days starting at day 7 of the estrus cycle (estrus being day 1) to dairy heifers curtailed release of both FSH and LH. These results substantiate the view that LH release is inhibited by large amounts of progesterone, but there is no indication in that report whether the action occurs at the hypothalamic or pituitary level, or both. And although these doses of progesterone may not be physiological, the results reveal that progesterone can feedback negatively on the pituitary and block synthesis release, or both, of LH and FSH.

By day 11 of the cycle of the present heifers, the hypothalamic LRF content was low, pituitary LH and progesterone concentration and content of the corpus luteum were high, and there was a relatively large mid-cycle follicle. The important question is, why does the follicle not undergo final maturation and ovulate? There may be several valid reasons. But first let us review the effects of various hormonal treatments on the estrus cycle. Firstly, a single injection of progesterone (10 mg), administered during estrus in the cow (40, 43, 55, 58), reduced the time to next ovulation by about 10 hours, probably acting through the hypothalamo-pituitary axis. Secondly, daily injections (40) of progesterone (50 mg) inhibited ovulation. Thirdly, as mentioned in the preceding paragraph, high levels of progesterone (100 mg) blocked synthesis, release, or both of FSH and LH. These results suggest that minimal amounts of progesterone are required to enhance release of gonadotrophin, but larger amounts are inhibitory. In fact, ratios of progesterone to estradiol of 12.5:1 to 75:1 appeared to be optimum for the induction of estrus in ovariectomized cows (40). Fourthly, oxytocin (7), given early in the estrous cycle, inhibited formation and function of the corpus luteum resulting in precocious estrus and ovulation at about day 12. This result suggested that reduction of progesterone altered the ratio of progesterone to estradiol in such a manner as to promote ovulation. Finally, antibovine luteinizing hormone (128) reduced corpus luteum weight and progesterone content. These treatments indicate to some degree what possible relationships may exist during the estrous cycle under normal physiological circumstances.

The mid-cycle follicle does not normally attain size as large as that of the ovulatory follicle. In part, this may be the result of lower plasma FSH levels in the early stages of the cycle. Pituitary FSH content decreased insignificantly early in the cycle (Fig. 3) and a similar trend was evident in a previous study (25), but by mid-cycle pituitary FSH was relatively high and the increasing levels of circulating progesterone at this time may block further synthesis, release, or both of FSH, expecially release. Consequently, the mid-cycle follicle does not grow as rapidly as the pre-ovulatory follicle which more than doubles in size between day 20 and estrus under the influence of greater quantities of plasma FSH (as evidenced by the precipitous decrease in pituitary FSH between day 18 and 0). And the failure of final follicular growth at day 11 may limit the amount of available estrogen which is needed to form the correct ratio of progesterone to estradiol, thereby failing to trigger pituitary LH release. In addition, the high levels of circulating plasma progesterone possibly block release of pituitary LH at the hypothalamic or pituitary level, or both. The synthesis and secretion of estrogen from the mid-cycle follicle will be discussed later.

There was a significant decrease in pituitary LH between days 11 and 18 and an increase from day 18 to day 20 ($P < 0.01$), but we do not know from the present data how great the depression in pituitary LH was, nor exactly when the depression occurred because of the long period between days 11 and 18 when no heifers were studied. A similar depression around mid-cycle (Fig. 4) has been reported previously. Although not designed to measure cyclic changes in pituitary FSH and LH, the study by Desjardins (25) revealed decreased pituitary LH levels around the middle of the estrous cycle. On a limited number of observations (61), blood levels of LH increased around day 8, suggesting that increased amounts of LH may indeed be released during the mid-cycle; but in most cases, blood levels of LH were too low to measure precisely.

The controlling mechanism of pituitary LH release at day 11 is complicated. Many factors may be involved. From the previous discussion, the release of pituitary LH in mid-cycle does not appear to be associated with any dramatic change in hypothalamic LRF, which is low at this time. But our assay for LRF is insensitive relative to the biological potency of LRF. In the rat (18), estradiol benzoate (2-50 μg/day) did not alter hypothalamic LRF, but decreased pituitary LH content. So perhaps small amounts of estrogen may act directly on the pituitary causing release of pituitary LH. And as the mid-cycle follicle atrophies, the depressing effect of estrogen is removed, and LH synthesis becomes greater than release, resulting in further increase in pituitary LH between days 18 and 20.

Recently, a functional role (58) for 20α-hydroxy-pregn-4-en-3-one in the rabbit has been proposed. The results suggest "that this progestin acts as a positive feedback agent to prolong and heighten LH discharge in the mated rabbit." Unfortunately, 20β-ol was not measured at various intervals between day 20 and day 0--the period comparable to that after mating in rabbits, so there was no indication as to whether 20β-ol may be related to LH release in cattle. Perhaps 20β-ol has a function in the dairy heifer similar to that proposed for 20α-ol in rabbits and, if so, perhaps the lower levels of 20β-ol observed at day 11 (compared to day 20) may be another factor responsible for failure of sufficient pituitary LH release for ovulation.

Dramatic endocrine changes occur after day 18. Pituitary FSH and corpus luteum progesterone content decrease precipitously between days 18 and 0. Following presumed increased plasma FSH, the follicle more than doubles in size and activity between days 20 and 0. Between days 18 and 0, both hypothalamic LRF and pituitary LH increase, but pituitary LH decreases by 89 percent during the next 3 days, presumably due to stimulation by LRF. Heifers exhibit psychic manifestations of estrus on day 0. About 18 hours later, under the influence of the "LH surge," she ovulates and the whole chain of events recycles.

As an alternate method to illuminate the relationship between pituitary LH and corpus luteum activity, correlation coefficients were calculated between pituitary LH and total progestogen (progesterone and 20β-ol) content of the corpus luteum within each of the 7 selected days of the estrous cycle (Table 13). On day 2 there was a significant correlation of -0.90 ($P < 0.10$). At this time pituitary LH was low, having been released from day 20 through day 2, while the corpus luteum was increasing in size and synthetic activity presumably under the influence of higher levels of blood plasma LH. Although the correlation coefficient of 0.83 at day 4 was not significantly different from 0 ($P > 0.10$), it revealed a relationship between pituitary LH and corpus luteum progestogen exactly opposite that which existed on day 2. Comparison of pituitary LH levels revealed net release of LH between days 20 and 2, but net storage of LH between days 2 and 11. And indeed, it appeared that pituitary LH synthesis exceeded LH release around day 2 as evidenced by an increase in pituitary LH after this time. Because corpus luteum progestogen content is an indicator of plasma progestogens, it seems quite likely that the increasing level of plasma progestogens at day 4 negatively affects the hypothalamus, the pituitary, or both, and thereby blocks or diminishes release of LH from the pituitary, thereby causing an increase in (or storage of) pituitary LH. However, it is likely that some LH is still released from the pituitary, as

TABLE 13--Correlations between gonadotrophins and luteal progestins or follicle size.

Day of estrous cycle	LH vs progestin	FSH vs follicle wall weight
0[b]	0.14(3)[a]	-0.90(3)
2	-0.90(4)	0.80(4)
4	0.83(4)	-0.13(5)
7	-0.40(4)	-0.04(5)
11	0.14(5)	-0.83(5)
18	0.19(5)	-0.62(5)
20	-0.89(5)	-0.13(5)

[a]Numbers in parentheses indicate number of samples.

[b]The corpus leutem was 21 days old.

evidenced by the increasing size of the corpus luteum. The correlation coefficients were not significantly different from 0 for days 7, 11, and 18 ($P > 0.10$). This lack of statistical significance may be due to the small number of heifers involved in each correlation (3 to 5) or it may reflect LRF/LH ratios which are relatively low at this time.

At day 20, for the negative correlation of -0.89, there may be two different explanations. Firstly, by this time some animals may have been close to estrus and the corpus luteum was degenerating and in view of its degenerate vascular system, probably incapable of responding to LH, at a time when pituitary LH content reached a maximum. Secondly, in synergism with FSH, the LH probably stimulates the preovulatory follicle causing it to produce increasing levels of estrogen which in turn probably hasten degeneration of the old corpus luteum.

Actually, it is surprising that such large correlation coefficients were obtained because there is undoubtedly a lag between pituitary LH release and formation and steroidogenic activity of the corpus luteum. And when a correlation coefficient was calculated for all the data, i.e., across all days and heifers, it was nonsignificant, further supporting the hypothesized lag from LH release to progesterone synthesis.

Luteal regression began about day 18. It was precipitous after day 20. This period coincided with the period of greatest pituitary prolactin content. If one assumes that high pituitary prolactin reflects high blood levels, as has been suggested (64) in rodents (and exactly the reverse of the relationships between pituitary and blood levels of LH or FSH), then prolactin may be associated with leuteolysis in heifers. Whether such an effect of prolactin may be directly on the aging corpus luteum or mediated through estrogen from the growing preovulatory follicle is entirely speculatory. It could even be mediated through a possible effect of estrogen upon the uterine luteolytic factor (134, 135).

Although the large follicle present at mid-cycle (101) does not produce discernible changes in sexual behavior, there are several signs that several parts of the sexual system display mid-cycle changes typically caused by estrogen. For example, there was some evidence for a biphasic increase in the weight of the vagina--at estrus and at mid-cycle. Also, total vaginal RNA/DNA and protein showed similar biphasic patterns. However, the epithelial layer while thickened at estrus was not affected at mid-cycle, probably because the lower estrogen levels at day 11 than at day 0 did not stimulate the entire cell layer to any great extent. In contrast, the single epithelial cell layer in the cervix was significantly stimulated at estrus and at day 11, revealing a biphasic pattern in the cervix which also typified cervical DNA and paralleled follicular size.

In the uterus and oviduct, neither the biochemical parameters nor epithelial cell heights reflected patterns indicative of bimodality corresponding to estrogen secretion by the mid-cycle follicle. This does not come as any great surprise, since these two organs seem to be more under the control of the luteal secretions while the cervix and vagina seem more under the control of follicular secretions.

Correlation coefficients between pituitary FSH and follicle wall weight within each day of the cycle (Table 13) were computed and none was significant ($P > 0.05$), probably because of the limited number of heifers. However, 6 out of 7 were negative and when the correlation was computed over the entire group, the correlation of -0.40 was significant ($P < 0.01$). These data suggested low pituitary FSH resulted in high follicle wall weight. In other words, release of pituitary FSH stimulated follicle wall growth.

A significant correlation of 0.42 between follicle wall weight and vaginal epithelial cell-layer suggested stimulation of the epithelial layer by estrogen ($P < 0.01$) although the vaginal epithelial cell-layer did not increase between day 2 and day 18 (Fig. 7). However, this may have been due to the inability of small amounts of estrogens to significantly stimulate the entire layer.

Correlations between total pituitary gonadotrophins and total gonadal hormones and the various biochemical parameters and cell heights measured in the reproductive tract were computed within the days of the estrous cycle. Most of these were not significant ($P > 0.05$), and those that were significant could not be interpreted from a sound physiological point of view mainly because of two factors: 1) most of the parameters measured are the result of stimulation and interaction of two or more hormones and 2) a variable lag period between endocrine stimulation of an organ and response is normally expected. So it seems most likely that any relationship between the pituitary gonadotrophins and gonadal hormones and the biochemical parameters and cell heights can most readily be interpreted by studying the various graphs and taking into account both the interactions and lag periods involved (Fig. 10, 11, 12, 13).

For the first time, prolactin, FSH, and LH were assayed in individual bovine anterior pituitary glands (Fig. 10) and LRF was assayed in pooled hypothalami

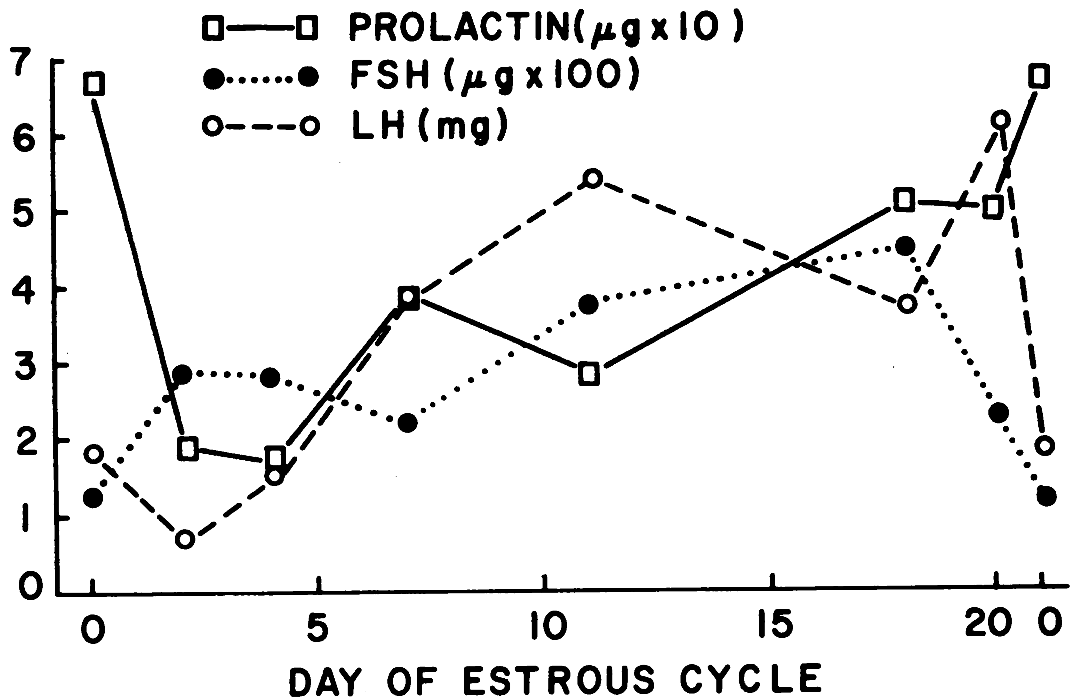

Figure 10.--Pituitary LH, FSH, and prolactin.

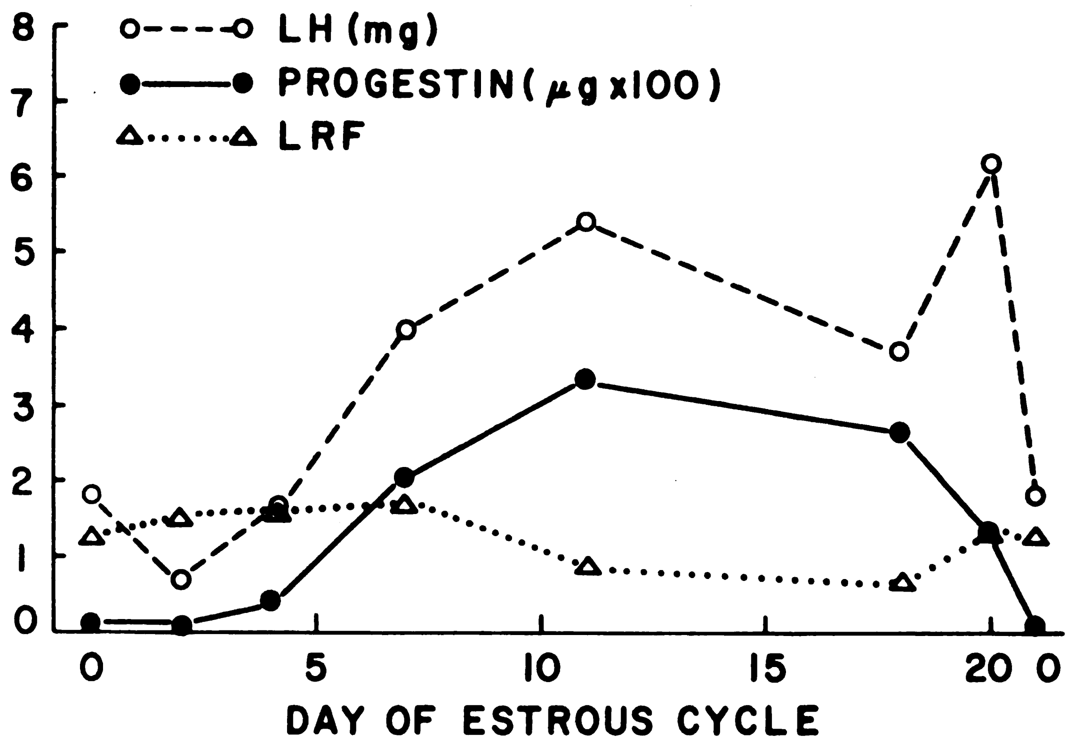

Figure 11.--Hypothalamo-pituitary-luteal relationships.

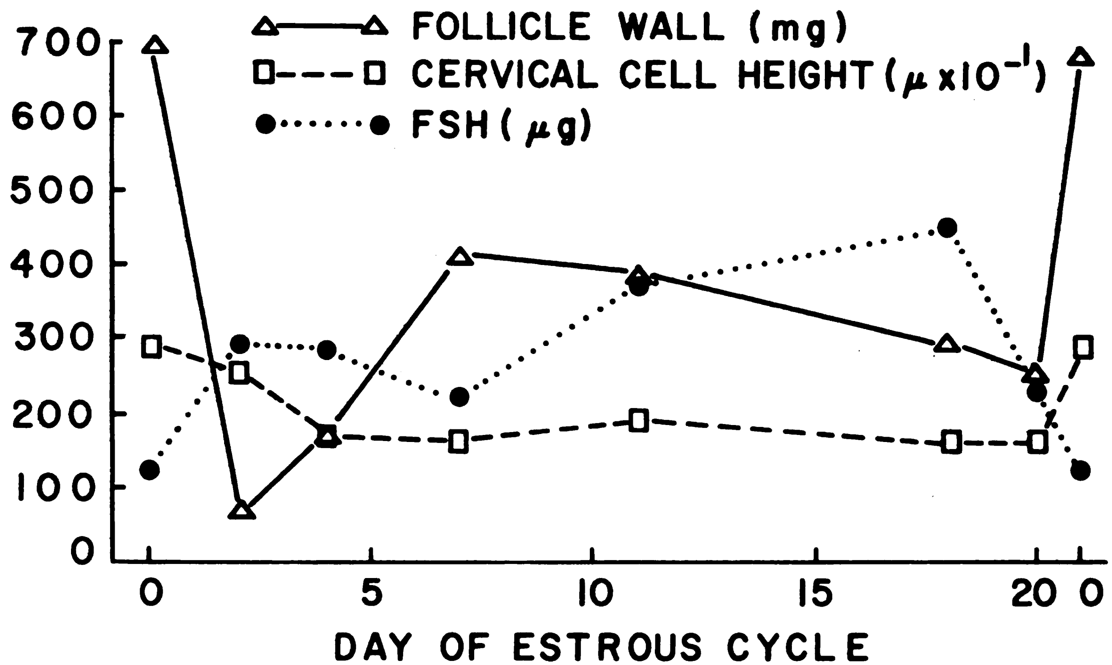

Figure 12.--Pituitary-follicular-cervical relationships.

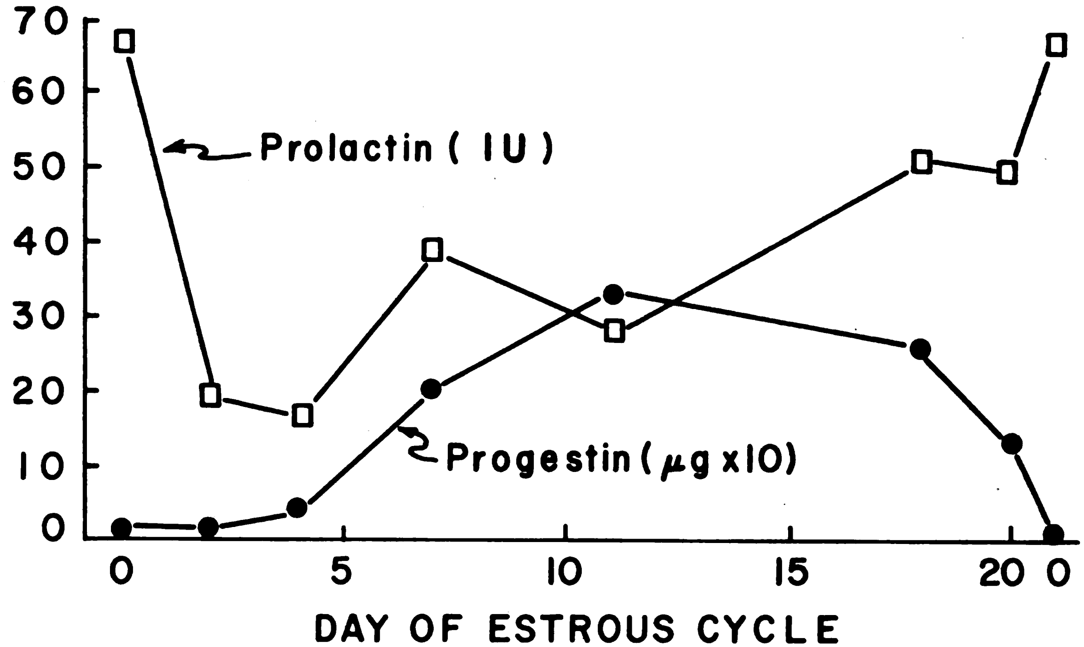

Figure 13.--Pituitary prolactin and luteal progestin.

during the estrous cycle. And at the same time, ovarian follicular and luteal activity was measured. From these results a mechanism for the regulation of the bovine estrous cycle is proposed.

Sometime after day 18, blood plasma levels of progesterone decrease thus freeing the hypothalamus, pituitary, or both, from the depressing action of progesterone. FSH-RF stimulates release of pituitary FSH which in turn synergizes with low plasma levels of LH to stimulate growth of the preovulatory follicle. This follicle more than doubles in size between day 20 and estrus and secretes ever increasing amounts of estradiol and smaller amounts of progesterone. The increasing level of estradiol blocks further pituitary FSH release. As follicular progesterone secretion increases, the ratio of progesterone to estradiol is such that around day 20 it stimulates increased hypothalamic LRF, which stimulates release of pituitary LH. Also around day 20, 20β-ol content is high and perhaps also stimulates hypothalamic LRF production. This surge of LH causes ovulation, and stimulates mitosis of the granulosa and theca interna cells for 2 to 4 days to form the corpus luteum. As the newly formed corpus luteum continues to grow, it secretes ever increasing amounts of progesterone, possibly under influence of prolactin (as well as LH) which is also released from the pituitary after day 0. These increasing levels of progesterone negatively feedback on the hypothalamus, pituitary, or both, decreasing LH release to a constant level, which is controlled by the tonic releasing center of the hypothalamus. Between days 7 and 18, the high level of progesterone inhibits release of gonadotrophins thereby causing the quiescent stage known as diestrus. Of course, after day 18, the events recycle.

Suggestions for Future Research

Unfortunately there were no sensitive assay procedures available at the time this study was performed to measure blood levels of the gonadotrophins. So it is impossible to relate the exact blood levels of these hormones to those in the pituitary throughout the estrous cycle and blood levels are more important than pituitary levels for controlling the estrous cycle. But, by observing the effects on target organs, especially the ovary, the pituitary hormone levels reflected fairly accurately the proposed role of these hormones in regulating the estrous cycle. In future experiments, measuring blood levels of the pituitary gonadotrophins by radio-immunoassay will further elucidate pituitary-gonadal relationships and provide valuable clinical methods upon which means of treatment of some endocrine disorders can be based.

When more sensitive assays for the blood levels of pituitary gonadotrophins become available, it will be possible to obtain more precise measurements of the releasing factors. For example, one possible method of measuring hypothalamic LRF would be to use the "Parlow rat" and measure blood levels of the rat's circulating pituitary hormone before and after injection of the test substance. Both the rat's pituitary LH and ovarian ascorbic acid content could also be measured to add precision to the assay. And possibly when better extraction and purification procedures become available, releasing factors for individual hypothalami can be measured. These improvements in techniques would add considerable impetus to endocrine research.

Further possible additions to the present study would be measurements of estrogen, progesterone and 20β-ol from ovarian venous blood, or possibly peripheral blood, especially around day 11 and around day 20. These measurements would, among other things, enable researchers to study the failure of the mid-cycle follicle to ovulate. Also by labeling gonadal steroids and gonadotrophins, the site of action of these hormones at the ovarian, pituitary and hypothalamic levels could be elucidated.

SUMMARY AND CONCLUSIONS

Thirty-five Holstein heifers, 16 months of age, were killed in groups of 5 at days 2, 4, 7, 11, 18, and 20 of the estrous cycle and at estrus (day 0).

The mean length of the estrous cycles (avg. 20.6 days) body weight at slaughter (avg. 377 kg) were similar to those previously reported for dairy heifers. Therefore these animals were representative of "normal" dairy heifers at the age most are first mated commercially.

Hypothalamic LRF was high during proestrus (day 20), estrus (day 0) and metestrus (days 2 and 4) and low during diestrus days 11 and 18. Although there was no significant correlation between hypothalamic LRF and pituitary LH ($P > 0.01$), the high level of hypothalamic LRF was associated with pituitary LH release as evidenced by declining levels of pituitary LH from day 20 to day 2 and formation and growth of the corpus luteum from day 2 through day 11. Thus it was concluded that high levels of hypothalamic LRF were associated with pituitary LH release.

Pituitary prolactin content was highest on day 0 (67 IU per mg fresh pituitary) and lowest on day 4 (18 IU per mg fresh pituitary). These results suggest prolactin may be part of a luteotrophic complex.

Changes in pituitary FSH during the estrous cycle appeared to be biphasic with peaks on days 18 and 2 and depressions on days 0 and 4. Pituitary FSH was significantly correlated with follicle wall weight, when taken over all days and hiefers ($r = -0.40$, $P < 0.01$). These results suggested that release of pituitary FSH on days 18 and 4 stimulated growth of the follicles, but the follicle at mid-cycle failed to ovulate. Thus it appeared that there was insufficient release of FSH around day 11 to maximally stimulate the mid-cycle follicle to maturity and in turn this follicle failed to secrete sufficient estrogen to cause the dramatic changes associated with estrus.

Pituitary LH declined 89% from 6,178 μg at day 20 to 689 μg at day 2, increased to 5,390 g at day 11 and decreased to 3,731 μg at day 18. Thus, like FSH, changes during the estrous cycle in pituitary LH appeared to be biphasic and pituitary LH was released during proestrus, estrus, and metestrus and possibly to a lesser extent in mid-cycle. The pituitary gonadotrophin data indicated that FSH release precedes LH release by about 2 days and LH release precedes prolactin release by about 1 day (Fig. 10). Other than these delays, changes in pituitary levels of these three gonadotrophins during the cycle were quite similar.

Corpus luteum total progestogen content increased from 3.3 μg at day 2 to 329.5 μg at day 11. But it declined from 268.1 μg at day 18 to 10.6 μg at day 0. Luteal progestogen was negatively correlated with pituitary LH on day 2 ($r = -0.90$) and positively correlated on day 4 ($r = 0.83$). The secretory pattern of 20β-ol differed from that for progesterone in that it was increased on day 20 as well as on day 11.

Follicle wall weight more than doubled between proestrus and estrus, during which period LH and FSH disappeared from the pituitary. It also increased to a lesser extent between days 4 and 7, just after the second depression in pituitary FSH. Weight of the wall of the largest follicle declined between days 7 and 18, suggesting that the large mid-cycle follicle degenerated and another, which was destined to ovulate, replaced it as the largest after day 18.

Vaginal DNA, RNA and protein content showed biphasic changes during the estrous cycle; high at estrus and day 11. These results suggested that increases in vaginal DNA, RNA and protein reflected estrogen secreted by the mid-cycle follicle as well as by the ovulatory follicle. Both cervical RNA and cervical epithelial cell heights revealed a similar biphasic pattern; with peak values at estrus and day 11.

Uterine epithelial cell heights were maximal on days 0 and 7, when follicle wall weights were greatest. In contrast, uterine RNA appeared more related to luteal progestogen secretion than to follicular estrogen secretion. Thus, the activity of the uterus appeared related to both follicular and luteal influences.

Oviducal epithelial cell heights followed a pattern during the cycle similar to corpus luteum progesterone content. No changes in thyroid or thymus were indicative of follicular or luteal changes during the estrous cycle.

The data on vagina, cervix and uterus all suggest that the largest mid-cycle follicle secretes some estrogen, but not at large levels typical of the ovulatory follicle because heifers do not normally show signs of estrus at mid-cycle. Release of pituitary FSH on day 4 may cause growth of the mid-cycle follicle. Although pituitary LH declined slightly after day 11, failure of the mid-cycle follicle to mature is undoubtedly associated with high levels of progesterone at mid-cycle. In contrast, final growth of the ovulatory follicle is associated with reduced levels of progesterone elevated hypothalamic LRF, and marked loss of LH and FSH from the pituitary.

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APPENDICES

APPENDIX TABLE 1.--Age, weight and estrous cycles.

Day of Estrous Cycle	Animal No.	Age	Weight	Estrous Cycles	
				Length	Avg.
		(mo)	(kg)	(days)	(days)
0	16	16.3	367.3	17, 19, 19	18.33
	44	15.8	348.2	21, 20, 19	20.00
	77	16.1	347.6	22, 20	20.33
	88	16.0	307.3	20, 19	19.50
	90	16.5	320.5	- - -	-
	Mean ± SE		337.4 ± 10.6		
2	91	16.7	351.8	20 - -	20.00
	61	16.2	396.4	22, 22, 20	21.33
	87	17.4	468.2	21, 22	21.50
	4	16.6	410.0	19, 21, 16, 19	18.75
	11	16.2	416.4	19, 23, 19	20.33
	Mean ± SE		408.6 ± 18.7		
4	83	15.9	360.0	22, 21	21.50
	71	16.2	352.7	21, 20, 19	20.00
	743	-	270.9	- -	-
	10	16.0	375.5	22, 24	23.00
	72	16.4	358.2	25, 19, 22	22.00
	Mean ± SE		351.5 ± 21.6		
7	58	15.8	342.7	26, 18, 19	21.00
	74	16.4	390.9	39, 23	31.00
	78	16.4	383.6	20, 20	20.00
	60	15.9	393.6	21, 19, 20	20.00
	81	16.3	322.7	20, 18, 22	20.00
	Mean ± SE		366.7 ± 14.3		
11	27	15.9	386.4	22, 22	22.00
	86	16.4	378.2	13, 20	16.50
	12	15.9	409.1	19, 18, 21	19.33
	69	16.0	400.0	36, 24	30.00
	76	16.4	411.8	18, 21, 20	19.66
	Mean ± SE		397.1 ± 6.5		
18	28	16.3	416.4	5, 19	12.00
	85	16.5	399.1	22, 23	22.50
	80	16.2	360.0	22, 23	22.50
	84	16.5	415.5	21, 21	21.00
	41	15.9	363.6	19, 20, 21	20.00
	Mean ± SE		390.9 ± 12.3		
20	73	16.6	352.7	22, 21	21.50
	75	16.5	424.5	19	19.00
	29	16.3	390.1	21, 19	20.00
	5	16.0	374.5	22, 21	21.50
	79	16.4	349.1	34, 23	28.50
	Mean ± SE		386.2 ± 12.3		

APPENDIX TABLE 2.--Pituitary weight and prolactin, FSH and LH concentration.

Day of Estrous Cycle	Animal No.	Weight		Prolactin[a]			FSH			LH		
		Total	Ant pit	Potency	SE	Lambda	Potency	SE	Index of Precision	Potency	SE	Lambda
		(gm)	(gm)	(µg/mg)			(µg/gm)			(µg/gm)		
0	16	1.56	1.25	0.36	0.21	0.54	-	-	-	0.94	0.23	0.18
	44	1.87	1.42	0.70	0.37	0.51	0.11	0.14	5.3	4.61	0.62	0.12
	77	2.00	1.55	6.12	5.13	0.63	0.13	0.10	3.2	0.30	0.20	0.32
	88	1.94	1.50	1.01	1.67	0.63	-	-	-	0.41	0.31	0.43
	90	1.69	1.31	0.81	0.50	0.59	0.18	0.09	2.2	0.36	0.28	0.43
2	91	1.56	1.25	0.17	0.13	0.59	0.04	0.10	10.7	-	-	-
	61	1.61	1.19	0.31	0.18	0.54	0.32	0.09	1.2	0.47	0.34	0.43
	87	2.06	1.65	1.21	0.81	0.63	0.11	0.10	3.8	0.74	0.50	0.43
	4	2.71	2.18	0.32	0.23	0.63	0.05	0.14	12.9	0.19	0.33	0.58
	11	2.17	1.65	0.38	0.21	0.51	0.44	0.15	1.4	0.33	0.15	0.22
4	83	1.89	1.38	0.30	0.20	0.59	0.15	0.09	2.7	-	-	-
	71	1.78	1.34	0.94	0.40	0.51	0.32	0.09	1.3	2.70	0.37	0.12
	743	0.63	1.19	0.59	0.37	0.59	-	-	-	0.73	0.38	0.32
	10	1.95	1.40	0.28	0.21	0.63	-	-	-	0.85	0.29	0.22
	72	1.76	1.32	0.58	0.32	0.54	0.08	0.14	7.4	0.82	0.88	0.58
7	58	1.47	1.09	0.73	0.45	0.59	-	-	-	-	-	-
	74	1.80	1.36	0.28	0.19	0.59	0.23	0.09	1.8	2.92	0.70	0.20
	78	1.95	1.33	4.09	3.13	0.63	0.06	0.10	7.1	2.50	0.65	0.22
	60	1.32	1.47	0.31	0.18	0.54	0.13	0.10	3.4	1.93	0.81	0.32
	81	1.89	1.49	0.60	0.32	0.51	0.36	0.14	1.7	3.93	1.00	0.22
11	27	1.83	1.36	0.26	0.16	0.54	0.30	0.09	1.3	6.73	1.76	0.22
	86	1.86	1.24	0.47	0.25	0.51	0.45	0.11	1.0	4.44	0.60	0.12
	12	1.99	1.64	0.31	0.18	0.54	0.26	0.14	2.3	3.35	0.81	0.32
	69	1.78	1.20	0.31	0.21	0.59	0.19	0.10	2.1	0.39	0.30	0.43
	76	1.90	1.38	2.91	2.11	0.63	0.17	0.09	2.3	4.59	2.58	0.43
18	28	2.06	1.55	0.48	0.30	0.59	0.15	0.09	2.6	3.11	0.74	0.20
	85	2.08	1.52	0.16	0.11	0.54	0.32	0.09	1.2	2.73	0.52	0.18
	80	2.09	1.65	2.11	1.47	0.63	0.04	0.10	9.6	1.48	0.41	0.22
	84	1.92	1.32	0.84	0.44	0.51	0.41	0.15	1.5	4.49	1.79	0.32
	41	2.34	1.79	2.63	1.53	0.54	0.51	0.16	1.2	0.74	0.81	0.58
20	73	1.49	1.20	0.40	0.26	0.59	0.14	0.09	2.8	2.34	0.57	0.20
	75	2.10	1.55	1.77	0.99	0.54	0.08	0.14	8.0	4.21	0.79	0.18
	29	1.84	1.37	1.77	0.94	0.51	-	-	-	5.91	1.53	0.22
	5	1.88	1.45	1.21	0.63	0.51	0.31	0.14	1.9	7.72	2.06	0.22
	79	1.85	1.49	1.80	1.24	0.63	0.27	0.10	1.6	1.52	0.91	0.43

[a]Data from Sinha (126).

APPENDIX TABLE 3.--Ovarian weight characteristics, corpus luteum weight and progesterone, 20 β-ol and progestin (progesterone plus 20 β-ol) content.

Day of Estrous Cycle	Animal	Ovarian Weight: With CL and Follicle	Ovarian Weight: Without CL and Follicle	Weight of Follicle	Weight of Follicle Wall
		(gm)	(gm)	(mg)	(mg)
0	16	6.24	3.10*	2,510	422
	44	6.92	3.02*	3,650	980
	77	8.95	4.25*	3,280	764
	89	8.39	1.51*	5,920	878
	40	5.58	4.16	1,890	388
	Mean ± SE	7.22 ± 0.90	3.21 ± 0.44	3,450 ± 689	686 ± 120
2	91	-	-	-	-
	61	10.01	3.68	261	56
	87	-	7.40*	-	-
	4	lost	5.22*	-	118
	11	6.36	2.52*	166	45
	Mean ± SE	8.18 ± 1.82	4.70 ± 1.06	214 ± 15	73 ± 23
4	83	6.92	3.74*	650	146
	71	9.38	5.05*	1,120	331
	743	4.94	2.87*	370	175
	10	8.27	3.90*	371	107
	72	7.37	3.97*	480	114
	Mean ± SE	7.38 ± 0.71	3.91 ± 0.23	589.2 ± 137	175 ± 41
7	58	6.92	3.17*	1,880	336
	74	7.76	3.39	1,780	315
	78	9.26	3.35*	2,880	496
	60	6.12	5.17	2,030	620
	81	12.36	5.59	1,120	283
	Mean ± SE	8.48 ± 1.10	4.13 ± 0.51	1,938 ± 282	410 ± 64
11	27	7.74	2.87	2,240	496
	86	9.73	4.45*	1,610	290
	12	9.81	4.13*	2,490	448
	69	10.52	4.30*	1,520	355
	76	9.16	3.15*	2,130	353
	Mean ± SE	9.37 ± 0.46	3.78 ± 0.32	1,998 ± 187	388 ± 37
18	28	lost	-	-	280
	85	10.70	4.75*	1,110	300
	80	10.92	5.08	3,140	540
	84	8.60	4.70	1,540	193
	41	17.97	9.10	985	166
	Mean ± SE	12.05 ± 2.01	5.91 ± 0.95	1,694 ± 272	296 ± 66
20	73	9.69	5.13	1,390	193
	75	8.27	4.22	2,680	407
	29	11.91	5.41	1,900	282
	5	lost	5.42*	-	140
	79	7.13	4.06	960	236
	Mean ± SE	9.25 ± 1.03	4.85 ± 0.29	1,733 ± 286	252 ± 45

*Ovary had no corpus luteum or follicle. All others had corpus luteum, follicle, or both, but weights of these structures were subtracted from total ovarian weight to give the weights listed in this column.

Weight of Follicular Fluid	Diameter of Follicle	Weight of CL	Progesterone Content per CL	Content per CL	Progestin Content per CL
(mg)	(mm)	(mg)	(μg)	(μg)	(μg)
2,088	18	1,500	18.0	0	18.0
2,670	19	2,610	5.2	5.2	10.4
2,516	19	1,640	3.3	0	3.3
5,042	24	cystic	-	-	-
1,502	17	1,410	-	-	-
2,764 ± 604	19.4 ± 1.2	1,917 ± 349	5.3 ± 3.8	1.7 ± 1.7	6.0 ± 3.1
-	10	35	0.7	0	0.7
205	8	310	3.7	1.6	5.3
-	3	330	2.0	0	2.0
lost	8	400	2.8	2.0	4.8
121	4	410	3.3	0.4	3.7
163 ± 42	6.6 ± 1.3	360 ± 19	2.5 ± 0.5	0.8 ± 0.4	3.3 ± 0.9
504	10	590	12.4	3.5	15.9
789	14	1,590	35.0	22.3	57.2
195	9	710	14.2	5.7	19.9
270	9	1,150	39.1	2.3	41.4
366	10	670	17.4	1.2	18.6
425 ± 105	10.4 ± 0.9	932 ± 193	23.6 ± 5.6	7.0 ± 3.9	30.6 ± 8.0
1,544	17	4,170	216.8	12.5	229.4
1,465	15	6,720	228.5	13.4	241.5
2,384	19	3,930	133.6	7.9	141.5
1,410	18	4,100	184.5	41.0	225.5
837	11	4,050	170.1	8.1	178.2
1,528 ± 247	16.0 ± 1.4	4,594 ± 533	186.7 ± 16.9	16.6 ± 6.2	203.3 ± 18.8
1,744	18	6,700	301.5	120.6	422.1
1,320	15	10,080	201.6	80.6	282.2
1,942	18	4,700	267,9	42.3	310.2
1,165	14	5,300	333.9	31.8	365.7
1,777	17	4,860	228.4	38.9	276.3
1,590 ± 147	16.4 ± 0.8	6,328 ± 1,002	266.7 ± 23.9	62.8 ± 16.7	329.5 ± 28.5
lost	17	4,980	44.8	29.9	74.7
810	16	4,860	306.2	4.9	311.0
2,600	19	1,700	20.4	0	20.4
1,347	16	6,450	477.3	51.6	528.9
719	13	7,220	346.6	28.9	375.4
1,369 ± 438	16.2 ±1.0	5,042 ± 947	239.1 ± 88.8	23.1 ± 9.4	262.1 ± 94.7
1,197	14	5,400	194.4	75.6	270.0
2,273	18	3,720	37.2	37.2	74.4
1,618	16	1,610	0	0	0
lost	13	2,960	32.6	14.8	47.4
724	15	3,790	257.7	22.7	280.5
1,453 ± 308	15.2 ± 0.9	3,496 ± 617	104.4 ± 51.0	30.1 ± 12.9	134.5 ± 58.6

APPENDIX TABLE 4.--Net synthesis of progesterone (prog) and 20 β-hydroxy-pregn-4 en-3-one (20 β-ol) mass during incubation of corpus luteum homogenates.[b]

Day of Estrous Cycle	Animal No.	NADP + G - 6 - P					
		No LH			LH		
		Prog	20 β-ol	Total	Prog	20 β-ol	Total
0	16	20	0	20	22	0	22
	44	78	0	78	80	0	80
	77	10	0	10	14	0	14
	88	-	-	-	-	-	-
	90	-	-	-	-	-	-
	Mean ± SE	36 ± 21	0 ± 0	36 ± 21	39 ± 21	0 ± 0	39 ± 21
2	91	-	-	-	-	-	-
	61	-	-	-	-	-	-
	87	-	-	-	-	-	-
	4	-	-	-	-	-	-
	11	-	-	-	-	-	-
	Mean ± SE						
4	83	35	1	36	39	5	44
	71	52	3	55	47	0	47
	743	39	6	45	43	4	47
	10	73	0	73	75	3	78
	72	76	0	76	64	0	64
	Mean ± SE	55 ± 27	2 ± 1	57 ± 8	54 ± 7	2 ± 1	56 ± 7
7	58	149	5	154	128	6	134
	74	76	4	80	84	6	89
	78	74	9	83	78	7	85
	60	86	43	129	71	28	99
	81	72	10	82	62	23	85
	Mean ± SE	91 ± 15	14 ± 7	105 ± 15	85 ± 11	14 ± 5	99 ± 9
11	27	92	10	102	160	11	151
	86	49	17	66	49	34	83
	12	145	123	268	156	94	250
	69	156	16	172	174	19	193
	76	132	32	164	142	39	181
	Mean ± SE	115 ± 19	40 ± 21	155 ± 35	136 ± 22	40 ± 15	176 ± 27
18	28	180	18	198	170	19	189
	85	106	8	114	101	8	109
	80	62	0	62	64	0	64
	84	158	27	185	158	28	186
	41	134	79	213	138	74	212
	Mean ± SE	128 ± 21	26 ± 14	154 ± 29	126 ± 20	26 ± 13	152 ± 28
20	73	180	71	251	176	74	250
	75	132	18	150	156	10	166
	29	0	0	0	0	0	0
	5	223	11	234	214	11	225
	79	158	69	227	134	81	215
	Mean ± SE	139 ± 38	34 ± 15	173 ± 31	136 ± 36	35 ± 17	171 ± 45

[a]Incubation of about 100 mg corpus luteum for 1 hr at 37° in 2.5 ml Krebs-Ringer bicarbonate buffer with 1 mg/ml glucose and 5 μc cholesterol - 7^3H.

[b]Data from Hafs and Armstrong (46).

NADPH					
No LH			LH		
Prog	20 β-ol	Total	Prog	20 β-ol	Total
38	0	38	42	0	42
106	4	110	106	12	118
18	0	18	20	0	20
-	-	-	-	-	-
-	-	-	-	-	-
54 ± 28	1 ± 1	55 ± 28	56 ± 26	4 ± 4	60 ± 30
12	0	12	12	0	12
4	13	17	6	11	17
28	0	28	22	0	22
28	3	31	23	4	27
20	0	20	18	1	19
18 ± 5	3 ± 3	21 ± 4	16 ± 3	3 ± 2	19 ± 3
43	2	45	45	4	49
44	41	85	49	7	56
49	11	60	40	3	43
91	3	94	75	3	78
60	2	62	60	3	63
57 ± 9	12 ± 8	69 ± 9	54 ± 6	4 ± 1	58 ± 6
151	3	154	135	4	139
100	1	101	104	6	110
88	4	92	88	6	94
84	35	119	80	29	109
88	6	94	90	9	99
102 ± 12	10 ± 6	112 ± 12	99 ± 10	11 ± 5	110 ± 8
140	5	145	135	10	145
58	-	58	59	10	69
169	32	201	181	27	208
209	6	215	216	7	223
146	14	160	159	14	173
144 ± 25	14 ± 6	156 ± 28	150 ± 25	14 ± 4	164 ± 27
178	2	180	184	2	186
150	2	152	156	3	159
98	0	98	76	0	76
187	7	194	178	4	182
192	11	203	218	10	228
161 ± 17	4 ± 2	165 ± 19	162 ± 24	4 ± 1	166 ± 25
230	10	240	250	10	260
144	7	151	152	7	159
0	0	0	0	0	0
237	14	251	255	5	260
178	20	198	178	16	194
158 ± 43	10 ± 3	168 ± 46	167 ± 46	8 ± 3	175 ± 48

APPENDIX TABLE 5.--Vaginal characteristics.

Day of Estrous Cycle	Heifer No.	Weight	DNA			RNA	
			Conc	Content	per 100 kg	Conc	Content
		(gm)	(mg/gm)	(mg)	(mg/100 kg)	(mg/gm)	(mg)
0	16	210	1.6	327.0	[illegible]	2.9	613.4
	44	205	1.5	303.3	[illegible]	3.2	666.6
	77	184	2.0	361.5	109.2	3.3	[illegible]
	88	152	1.3	189.8	61.6	[illegible]	538.5
	90	250	1.5	365.7	114.1	[illegible]	691.5
	Mean ± SE	200.5±16.2	1.5±0.1	310.4±32.0	[illegible]	3.1±0.1	[illegible]
2	91	193	1.4	[illegible]	[illegible]	[illegible]	[illegible]
	61	196	1.6	[illegible]	[illegible]	3.7	[illegible]
	87	248	1.5	371.7	[illegible]	[illegible]	557.7
	4	[illegible]	[illegible]	211.0	[illegible]	2.4	[illegible]
	11	218	1.5	[illegible]	[illegible]	[illegible]	743.4
	Mean ± SE	207±11.6	1.4±0.1	[illegible]	[illegible]	[illegible]	[illegible]
4	83	147	2.0	[illegible]	[illegible]	[illegible]	614.4
	71	164	1.7	[illegible]	[illegible]	[illegible]	[illegible]
	743	163	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	10	177	1.6	[illegible]	[illegible]	[illegible]	[illegible]
	72	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	182.3±20.2	1.8±0.2	[illegible]	[illegible]	[illegible]	[illegible]
7	58	128	1.8	[illegible]	[illegible]	[illegible]	[illegible]
	74	154	[illegible]	[illegible]	[illegible]	4.0	[illegible]
	78	230	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	60	174	1.6	[illegible]	[illegible]	[illegible]	[illegible]
	81	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	169.5±16.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
11	27	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	86	172	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	12	188	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	69	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	76	175	1.6	273.1	[illegible]	[illegible]	[illegible]
	Mean ± SE	[illegible]	2.0±0.1	[illegible]	[illegible]	[illegible]	[illegible]
18	28	[illegible]	1.6	[illegible]	[illegible]	[illegible]	[illegible]
	85	149	2.0	[illegible]	[illegible]	[illegible]	[illegible]
	80	[illegible]	1.6	[illegible]	[illegible]	[illegible]	[illegible]
	84	159	1.5	[illegible]	[illegible]	[illegible]	406.3
	41	172	2.6	[illegible]	[illegible]	3.1	[illegible]
	Mean ± SE	178.3±13.0	1.9±0.2	[illegible]	[illegible]	[illegible]	[illegible]
20	73	179	1.6	[illegible]	74.6	[illegible]	[illegible]
	75	190	1.8	[illegible]	77.6	[illegible]	[illegible]
	29	221	1.7	[illegible]	[illegible]	3.7	[illegible]
	5	195	1.3	[illegible]	[illegible]	2.5	[illegible]
	79	136	1.6	211.8	60.7	[illegible]	[illegible]
	Mean ± SE	183.9±13.9	1.6±0.1	295.6±30.0	75.1±6.4	[illegible]	[illegible]

RNA/DNA	Protein		Lipid		Epithelial Cell-layer Height	Length
	Conc	Content	Conc	Content		
	(mg/gm)	(mg)	(mg/gm)	(gm)	(μ)	(cm)
1.9	0.16	[illegible]	[illegible]	[illegible]	42.4	30
2.2	0.14	[illegible]	[illegible]	76	39.8	30
1.7	0.17	31.7	[illegible]	53	[illegible]	19
2.8	[illegible]	19.1	[illegible]	[illegible]	[illegible]	[illegible]
1.9	[illegible]	[illegible]	[illegible]	76	[illegible]	34
2.1+0.2	0.15+0.01	30.6+3.4	[illegible]	[illegible]	57.0+8.0	[illegible]
1.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.4	0.14	27.4	[illegible]	[illegible]	[illegible]	[illegible]
2.3	[illegible]	[illegible]	[illegible]	51	[illegible]	[illegible]
2.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.3+0.1	0.16+0.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.1	0.17	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	33
2.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.3	0.17	[illegible]	[illegible]	[illegible]	47.3	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.1	0.13	17.0	[illegible]	[illegible]	[illegible]	[illegible]
1.8	0.13	19.9	[illegible]	[illegible]	17.5	27
2.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.8	0.17	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.0+0.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.7	0.17	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.9+0.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.5	0.19	32.4	[illegible]	[illegible]	47.5	33
1.7	0.21	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.3+0.2	0.19+0.004	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.8	0.19	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.2	0.17	[illegible]	[illegible]	[illegible]	33.8	[illegible]
2.1	0.19	41.4	150	[illegible]	[illegible]	[illegible]
1.9	0.17	[illegible]	235	[illegible]	24.4	[illegible]
1.8	0.19	[illegible]	[illegible]	39	27.7	[illegible]
2.0+0.1	0.18+0.05	33.3+2.5	222.4+19.4	40.0+2.1	[illegible]	[illegible]

APPENDIX TABLE 6.--Cervical characteristics.

Day of Estrous Cycle	Heifer No.	Weight	DNA Conc	DNA Content	DNA per 100 kg	RNA Conc	RNA Content
		(gm)	(mg/gm)	(gm)	(mg/100 kg)	(mg/gm)	(mg)
0	16	31.0	3.2	100.2	27.3	5.1	157.1
	44	48.5	3.0	146.8	[illegible]	5.2	252.9
	77	53.0	1.5	79.6	23.2	4.3	262.9
	88	31.5	2.2	67.1	22.2	[illegible]	119.6
	90	31.0	1.6	49.6	[illegible]	5.4	166.3
	Mean ± SE	39.0±4.9	2.3±0.4	88.9±16.6	[illegible]	[illegible]	191.4±27.9
2	91	34.0	2.1	71.4	[illegible]	[illegible]	132.1
	61	35.9	2.9	78.2	[illegible]	4.4	171.0
	87	55.0	2.1	116.5	[illegible]	3.3	184.2
	4	38.9	3.0	116.9	29.5	5.4	205.4
	11	44.0	2.3	100.9	[illegible]	5.7	237.3
	Mean ± SE	42.0±3.6	2.3±0.2	97.1±9.3	[illegible]	[illegible]	179.8±14.1
4	83	43.5	2.2	107.0	[illegible]	4.9	[illegible]
	71	28.5	2.4	68.5	19.4	5.9	111.7
	743	18.5	2.6	48.8	[illegible]	[illegible]	72.4
	10	33.0	2.3	75.0	[illegible]	4.1	135.6
	72	21.0	2.5	51.5	[illegible]	4.5	60.4
	Mean ± SE	29.9±5.3	2.4±0.1	70.4±[illegible]	[illegible]	[illegible]	118.6±20.4
7	58	32.5	3.3	105.5	[illegible]	[illegible]	171.9
	74	28.0	3.9	108.5	[illegible]	[illegible]	153.8
	78	38.5	3.4	130.5	[illegible]	[illegible]	[illegible]
	60	35.5	1.9	73.4	[illegible]	[illegible]	[illegible]
	81	36.3	3.3	119.7	36.9	[illegible]	[illegible]
	Mean ± SE	34.8±2.0	3.1±0.3	107.5±9.6	[illegible]	[illegible]	[illegible]
11	27	37.5	3.2	119.2	30.9	[illegible]	[illegible]
	86	42.5	3.1	129.7	[illegible]	[illegible]	[illegible]
	12	38.0	2.0	75.6	19.5	[illegible]	136.2
	69	38.7	3.8	146.0	36.5	[illegible]	230.4
	76	35.0	4.5	158.4	[illegible]	[illegible]	[illegible]
	Mean ± SE	38.3±1.2	3.3±0.4	125.6±14.2	41.7±5.5	[illegible]	161.4±14.8
18	28	49.0	2.1	104.9	[illegible]	4.0	193.6
	85	38.0	3.2	120.8	30.4	3.7	141.9
	80	43.1	3.0	130.4	30.2	[illegible]	244.6
	64	40.0	2.0	79.6	19.2	3.1	122.6
	41	33.5	3.6	118.9	34.7	4.6	154.3
	Mean ± SE	40.7±2.6	2.8±0.3	110.9±8.8	28.7±3.0	4.2±0.4	169.4±20.0
20	73	36.5	2.4	87.8	22.4	4.1	148.8
	75	52.5	2.6	137.2	32.3	5.5	259.8
	29	38.8	2.5	96.0	24.6	4.3	167.0
	5	42.4	2.2	94.5	25.2	3.1	131.2
	79	32.1	4.5	143.2	41.0	5.4	172.3
	Mean ± SE	40.5±3.4	2.8±0.4	111.8±11.8	29.1±3.4	4.5±0.8	[illegible]

RNA/DNA	Protein Conc	Protein Content	Lipid Conc	Lipid Content	Epithelial Cell-layer Height	Length
	(mg/gm)	(mg)	(mg/gm)	(gm)	(μ)	(cm)
1.6	0.12	3.5	155	4.6	22.4	6
1.7	0.14	6.9	241	11.7	24.0	6
3.3	0.10	5.3	135	7.2	33.4	6
1.8	0.19	5.3	173	5.8	31.2	6
3.4	0.15	4.6	262	8.1	31.4	5
2.34±0.4	0.14±0.01	5.3±0.5	195.2±24.5	7.5±1.2	28.6±2.1	5.8±0.07
1.8	0.15	[illegible]	249	3.5	[illegible]	5
2.2	0.15	5.4	165	6.4	[illegible]	5
1.6	0.15	5.2	[illegible]	[illegible]	[illegible]	6
1.8	0.17	6.8	[illegible]	[illegible]	[illegible]	[illegible]
2.1	0.17	7.5	157	5.5	[illegible]	6
1.9±0.1	0.16±0.005	[illegible]	[illegible]±38.0	[illegible]	[illegible]	[illegible]
1.7	0.16	7.8	[illegible]	14.1	19.7	6
1.6	0.17	4.2	241	6.6	17.9	4
1.6	0.17	3.1	247	4.4	16.7	6
1.8	0.13	5.9	277	6.8	[illegible]	5
1.6	0.14	2.9	257	6.0	15.5	5
1.7±0.1	0.16±0.007	4.9±0.9	250.8±16.4	[illegible]±1.7	[illegible]	[illegible]
1.6	0.15	5.5	[illegible]	9.5	[illegible]	[illegible]
1.7	0.14	[illegible]	151	8.1	[illegible]	4
1.2	0.15	5.7	134	7.1	14.3	4
2.0	0.19	7.3	[illegible]	[illegible]	[illegible]	[illegible]
1.4	0.17	6.0	147	5.3	16.1	6
1.6±0.1	0.16±0.01	5.7±0.5	[illegible]	[illegible]	[illegible]	[illegible]
1.0	0.21	7.9	311	11.7	[illegible]	[illegible]
1.3	0.20	3.5	166	[illegible]	[illegible]	[illegible]
1.8	0.15	5.6	244	9.5	[illegible]	6
1.4	0.16	6.2	162	6.3	[illegible]	6
1.2	0.15	5.1	174	[illegible]	[illegible]	7
1.3±0.1	0.17±0.01	6.7±0.7	[illegible]±29.3	[illegible]±1.1	[illegible]	[illegible]
1.8	0.11	5.6	315	15.4	17.9	6
1.2	0.12	4.4	296	11.7	14.3	6
1.8	0.12	5.2	234	10.1	20.8	6
1.5	0.13	5.4	268	10.7	12.9	5
1.3	0.18	6.2	221	7.4	14.8	6
1.5±0.1	0.13±0.01	5.4±0.3	266.8±17.8	11.0±1.3	15.8±1.4	[illegible]
1.7	0.16	5.9	298	10.9	18.1	[illegible]
2.1	0.17	5.9	269	14.1	[illegible]	[illegible]
1.7	0.15	5.7	329	17.8	[illegible]	[illegible]
1.4	0.20	8.5	230	9.8	[illegible]	[illegible]
1.2	0.17	5.2	197	5.3	[illegible]	6
1.6±0.2	0.17±0.01	6.8±0.8	262.6±22.5	10.7±2.1	15.7±0.3	5.7±0.4

APPENDIX TABLE 7.--Uterine characteristics.

Day of Estrous Cycle	Heifer No.	Weight	DNA			RNA		RNA/DNA
			Conc	Content	per 100 kg	Conc	Content	
		(gm)	(mg/gm)	(mg)	(mg/100 kg)	(mg/gm)	(mg)	
0	16	195.0	4.4	864.0	235.2	3.6	710.5	0.82
	44	156.0	5.1	795.1	228.3	3.6	568.4	0.72
	77	194.0	5.2	1011.5	294.4	2.2	432.8	0.43
	88	118.0	6.3	748.3	243.5	2.7	313.9	0.42
	90	176.0	6.3	1105.3	344.9	2.3	403.8	0.37
	Mean±SE	167.8±14.4	5.5±0.4	904.8±67.0	269.3±22.2	2.9±0.3	485.9±69.4	0.55±0.10
2	91	184.0	5.0	924.9	262.9	2.6	479.8	0.52
	61	166.0	9.3	1538.0	358.0	2.7	450.1	0.29
	87	214.0	6.9	1482.8	316.7	4.1	871.9	0.59
	4	150.0	5.7	853.1	208.1	1.9	286.6	0.34
	11	153.0	5.7	870.7	209.1	2.6	391.3	0.45
	Mean±SE	173.4±11.8	6.5±0.8	1133.9±154.4	277.0±34.3	[illegible]±0.4	495.9±99.6	0.44±0.05
4	83	178.0	5.2	931.0	258.6	[illegible]	[illegible]	0.47
	71	128.0	6.7	851.3	241.4	4.6	[illegible]	0.69
	743	169.0	6.3	107.7	395.6	4.7	790.3	0.74
	10	135.0	6.4	867.8	231.1	4.9	[illegible]	0.76
	72	153.0	6.3	967.0	242.8	[illegible]	[illegible]	0.68
	Mean±SE	152.4±9.5	6.2±0.3	937.7±39.5	273.9±30.7	4.2±0.4	[illegible]	0.67±0.05
7	58	199.0	6.3	1253.3	[illegible]	5.5	[illegible]	0.87
	74	179.0	6.3	1125.0	[illegible]	5.4	[illegible]	0.56
	78	188.0	4.6	881.4	[illegible]	[illegible]	[illegible]	1.02
	60	168.0	0.6	483.5	[illegible]	[illegible]	[illegible]	0.63
	81	153.0	4.3	655.3	207.1	2.5	393.1	0.57
	Mean±SE	177.4±8.0	4.9±0.6	882.9±141.9	[illegible]±40.7	[illegible]	[illegible]±201.1	[illegible]
11	27	168.0	6.2	1034.3	[illegible]	[illegible]	[illegible]	0.65
	86	202.0	5.8	1169.7	776.1	[illegible]	756.1	[illegible]
	12	175.0	7.7	1354.5	[illegible]	4.7	[illegible]	0.61
	69	155.0	7.0	1078.6	515.0	3.4	[illegible]	0.48
	76	129.0	6.9	893.8	549.7	4.5	[illegible]	0.62
	Mean±SE	165.5±11.9	6.7±0.3	1106.2±76.4	672.4±63.0	4.1±[illegible]	[illegible]	0.61±0.03
18	28	235.0	3.4	796.3	191.2	2.4	[illegible]	0.70
	85	176.0	7.0	1227.9	537.7	1.9	[illegible]	[illegible]
	30	240.0	4.8	1143.0	317.5	5.6	[illegible]	[illegible]
	84	162.0	3.5	566.7	136.4	1.9	313.0	0.55
	41	138.0	4.5	613.6	165.7	2.5	337.2	0.55
	Mean±SE	190.2±20.4	4.6±0.7	869.5±135.2	224.3±37.1	2.5±[illegible]	[illegible]	0.57±[illegible]
20	73	154.0	5.0	768.8	195.6	2.4	362.0	0.47
	75	206.0	3.0	623.5	146.9	1.7	[illegible]	0.57
	29	196.0	5.2	1027.9	263.5	4.3	[illegible]	[illegible]
	5	159.0	6.4	1022.0	272.9	2.0	311.0	[illegible]
	79	132.0	6.9	916.0	262.4	3.3	431.0	0.47
	Mean±SE	169.5±13.8	5.3±0.7	871.6±77.8	228.3±24.6	2.7±0.5	459.6±96.6	[illegible]

Protein		Lipid		Epithelial Cell Height	Body	Length Horn	
Conc	Content	Conc	Content			Right	Left
(mg/gm)	(mg)	(mg/gm)	(gm)	(μ)	(cm)	(cm)	(cm)
0.12	22.6	201	[illegible]	21.0	2	32	37
0.11	17.8	151	28	25.6	2	[illegible]	34
0.11	20.4	[illegible]	50	-	2	30	33
0.11	13.4	[illegible]	27	28.3	2	25	25
0.11	19.9	[illegible]	[illegible]	-	2	28	[illegible]
0.11±0.002	18.8±1.6	[illegible]	36±[illegible]	25.1±2.3	2±0	[illegible]±1.2	31.6±2.1
0.12	22.6	[illegible]	[illegible]	-	[illegible]	27	[illegible]
0.11	17.8	[illegible]	[illegible]	17.2	[illegible]	27	[illegible]
0.11	20.4	[illegible]	[illegible]	[illegible]	[illegible]	31	[illegible]
0.11	13.4	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]
0.11	19.9	[illegible]	[illegible]	13.2	[illegible]	[illegible]	30
0.11±0.002	18.8±1.6	[illegible]±40.[illegible]	[illegible]	17.[illegible]	2.6±0.4	[illegible]±1.1	[illegible]±1.[illegible]
0.12	21.[illegible]	[illegible]	[illegible]	30.6	[illegible]	[illegible]	24
0.12	15.6	246	[illegible]	23.[illegible]	[illegible]	[illegible]	[illegible]
0.11	18.2	[illegible]	[illegible]	-	[illegible]	[illegible]	[illegible]
0.15	19.6	[illegible]	[illegible]	[illegible]	2	[illegible]	[illegible]
0.11	17.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	30
0.12±0.006	18.5±0.9	[illegible]±31.[illegible]	[illegible]	[illegible]	[illegible]±0.1	31.[illegible]	31.6±[illegible]
0.15	29.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.13	24.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.18	34.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.12	20.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.12	18.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.14±0.011	25.3±[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±1.7
0.12	20.3	[illegible]	[illegible]	[illegible]	[illegible]	30	[illegible]
0.11	22.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.12	21.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.12	18.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.13	16.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	31
0.12±0.002	19.9±1.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.12	27.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.13	22.5	247	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.11	26.8	205	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.13	21.5	205	33	[illegible]	3	[illegible]	[illegible]
0.11	15.7	345	47	[illegible]	2	40	[illegible]
0.12±0.004	22.8±2.1	249.4±25.5	46.0±4.1	24.4±2.5	2.5±0.1	[illegible]	33.6±[illegible].7
0.16	23.8	199	[illegible]	19.2	2	[illegible]	[illegible]
0.18	37.2	265	55	21.7	3	41	[illegible]
0.10	20.3	337	66	[illegible]	2	[illegible]	[illegible]
0.11	18.2	175	[illegible]	[illegible]	3	[illegible]	[illegible]
0.13	17.3	[illegible]	39	25.7	4	[illegible]	[illegible]
0.14±0.014	23.4±3.6	251.6±30.7	43.4±7.5	24.0±1.9	2.8±0.4	30.0±3.0	[illegible]

APPENDIX TABLE 8.--Oviducal lengths and epithelial cell height.

Day of Estrous Cycle	Heifer No.	Length		Epithelial Cell Height
		Right	Left	
		(cm)	(cm)	(µ)
0	16	22	16	33.9
	44	19	19	15.7
	77	21	21	21.3
	88	21	21	23.5
	90	23	22	18.2
	Mean ± SE	21.2 ± 0.7	19.8 ± 1.1	22.6 ± 3.[illegible]
2	91	21	23	20.6
	61	21	21	23.2
	87	18	18	31.4
	4	24	24	18.2
	11	21	20	22.7
	Mean ± SE	21.0 ± 1.0	21.2 ± 0.8	23.3 ± [illegible]
4	83	24	23	24.3
	71	15	15	24.2
	743	18	15	20.0
	10	20	21	25.9
	72	25	25	22.8
	Mean ± SE	20.4 ± 1.9	19.8 ± 2.1	23.5 ± 1.0
7	58	23	22	23.8
	74	23	22	21.8
	78	19	19	27.2
	60	21	21	29.5
	81	26	23	25.4
	Mean ± SE	22.4 ± 1.2	21.4 ± 0.7	25.6 ± 1.3
11	27	21	23	26.4
	86	19	22	25.6
	12	21	22	24.9
	69	25	23	16.8
	76	21	21	27.0
	Mean ± SE	21.4 ± 1.0	22.2 ± 0.4	22.8 ± 1.[illegible]
18	28	28	21	23.0
	85	21	20	23.8
	80	26	25	25.9
	84	20	20	18.5
	41	25	22	22.4
	Mean ± SE	24.0 ± 1.5	21.6 ± 0.9	22.4 ± 1.[illegible]
20	73	21	23	21.5
	75	17	17	15.9
	29	22	27	27.0
	5	20	22	21.6
	79	27	27	17.5
	Mean ± SE	21.4 ± 1.6	23.2 ± 1.9	20.7 ± 1.9

APPENDIX TABLE 9.--Thyroid characteristics.

Day of Estrous Cycle	Heifer No.	Weight	DNA Conc	DNA Content	DNA per 100 kg	RNA Conc	RNA Content
		(gm)	(mg/gm)	(mg)	(mg/100 kg)	(mg/gm)	(mg)
0	16	13.1	4.7	61.9	16.9	[illegible]	[illegible]
	44	17.7	3.3	59.1	17.6	[illegible]	[illegible]
	77	19.5	3.8	74.7	[illegible]	[illegible]	[illegible]
	88	16.4	4.9	78.5	[illegible]	5.6	92.4
	90	14.9	5.0	74.9	[illegible]	7.6	[illegible]
	Mean±SE	16.3±1.1	4.3±0.3	69.3±3.9	[illegible]	[illegible]	[illegible]
2	91	[illegible]	4.1	[illegible]	[illegible]	[illegible]	[illegible]
	51	[illegible]	3.3	[illegible]	[illegible]	[illegible]	[illegible]
	47	[illegible]	3.8	[illegible]	[illegible]	[illegible]	[illegible]
	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	11	16.4	3.3	[illegible]	[illegible]	[illegible]	[illegible]
	Mean±SE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	83	10.4	3.7	[illegible]	[illegible]	[illegible]	[illegible]
	71	10.4	4.3	[illegible]	[illegible]	[illegible]	[illegible]
	743	10.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	10	17.6	4.6	[illegible]	[illegible]	[illegible]	[illegible]
	72	19.2	4.5	[illegible]	[illegible]	[illegible]	[illegible]
	Mean±SE	13.6±2.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7	58	13.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	74	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	78	[illegible]	[illegible]	74.0	[illegible]	[illegible]	[illegible]
	60	14.7	4.4	[illegible]	[illegible]	[illegible]	[illegible]
	81	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean±SE	16.3±1.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
11	27	14.6	5.0	[illegible]	[illegible]	[illegible]	[illegible]
	66	14.6	5.3	[illegible]	[illegible]	[illegible]	[illegible]
	12	11.0	3.7	41.3	[illegible]	[illegible]	[illegible]
	69	15.8	3.5	55.7	[illegible]	[illegible]	[illegible]
	76	17.1	4.6	79.0	[illegible]	[illegible]	[illegible]
	Mean±SE	14.6±1.0	4.4±0.4	[illegible]	[illegible]	[illegible]	[illegible]
18	28	17.7	4.6	[illegible]	[illegible]	[illegible]	[illegible]
	85	16.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	80	19.2	4.1	[illegible]	[illegible]	[illegible]	[illegible]
	84	19.7	4.7	[illegible]	[illegible]	[illegible]	[illegible]
	41	17.9	4.4	[illegible]	[illegible]	7.4	[illegible]
	Mean±SE	18.1±0.6	4.7±0.2	[illegible]	[illegible]	[illegible]	[illegible]
20	75	14.0	[illegible]	71.7	[illegible]	[illegible]	[illegible]
	29	16.1	4.4	[illegible]	[illegible]	[illegible]	[illegible]
	5	16.3	3.9	63.2	16.9	[illegible]	[illegible]
	79	21.2	5.4	114.1	[illegible]	7.3	[illegible]
	73	19.6	4.0	77.8	[illegible]	[illegible]	[illegible]
	Mean±SE	17.4±1.3	4.6±0.3	79.7±5.9	[illegible]	[illegible]	[illegible]

RNA/DNA	Protein		Lipid		Acinar Epithelial Cell Height
	Conc	Content	Conc	Content	
	(mg/gm)	(mg)	(mg/gm)	(gm)	(u)
1.8	0.17	2.2	198	2.6	14.5
1.5	0.20	3.6	-	-	11.3
1.5	0.20	3.8	-	-	12.3
1.2	0.24	4.0	243	4.0	10.9
1.5	0.17	2.6	-	-	9.1
1.5 ± 0.1	0.20 ± 0.01	3.2 ± 0.4	220.5 ± 22.4	3.3 ± 0.7	11.6 ± 0.8
1.4	0.15	2.4	-	-	10.1
1.6	0.20	3.6	206	3.7	13.6
1.4	0.22	3.4	237	3.7	10.1
1.9	0.22	4.9	-	-	8.7
1.9	0.24	4.0	-	-	10.9
1.6 ± 0.1	0.21 ± 0.02	3.7 ± 0.4	221.5 ± 15.5	3.7 ± 0.0	10.7 ± 0.7
1.5	0.19	2.0	216	2.2	11.3
1.2	0.20	2.1	197	2.0	10.0
1.2	0.20	2.1	201	2.1	13.9
2.0	0.25	4.3	-	-	11.3
1.8	0.15	3.0	-	-	7.7
1.6 ± 0.2	0.20 ± 0.01	2.7 ± 0.4	204.7 ± 5.8	2.1 ± 0.0	11.3 ± 1.1
1.4	0.20	2.9	191	2.7	9.4
1.0	0.21	4.0	-	-	[illegible]
1.4	0.23	4.5	-	-	[illegible]
1.2	0.20	2.9	191	[illegible]	[illegible]
1.0	0.26	3.7	-	-	[illegible]
1.2 ± 0.1	0.22 ± 0.01	3.6 ± 0.3	194.0 ± 3.0	[illegible]	[illegible]
1.4	0.21	3.0	[illegible]	[illegible]	[illegible]
1.4	0.22	3.2	203	[illegible]	7.9
1.5	0.20	2.2	[illegible]	2.2	[illegible]
1.6	0.21	3.3	231	3.6	[illegible]
1.4	0.20	3.4	[illegible]	[illegible]	13.0
14. ± 0.1	0.21 ± 0.00	3.0 ± 0.2	204.2 ± 9.8	[illegible]	[illegible] ± 1.4
1.4	0.21	3.7	205	3.6	[illegible]
1.1	0.18	3.0	214	3.4	[illegible]
1.4	0.22	4.2	-	-	13.4
1.7	0.21	4.1	207	4.1	[illegible]
1.7	0.20	3.6	216	3.9	[illegible]
1.5 ± 0.1	0.20 ± 0.01	3.7 ± 0.2	210.5 ± 2.7	2.75 ± 0.01	11.6 ± 1.1
1.3	0.19	3.8	243	3.4	[illegible]
1.5	0.19	2.7	-	-	[illegible]
1.7	0.17	2.8	189	3.1	[illegible]
1.4	0.20	3.2	-	-	[illegible]
1.2	0.21	4.3	278	5.4	[illegible]
1.4 ± 0.1	0.19 ± 0.01	3.3 ± 0.3	270.0 ± 44.6	3.97 ± 0.2	10.9 ± 0.8

APPENDIX TABLE 10.--Thymus characteristics.

Day of Estrous Cycle	Heifer No.	Weight	DNA			RNA	
			Conc	Content	per 100 kg	Conc	Content
		(gm)	(mg/gm)	(gm)	(gm/100 kg)	(mg/gm)	(gm)
0	16	407.3	49.6	20.2	[illegible]	7.3	3.0
	44	504.0	53.5	27.0	7.7	7.9	4.0
	77	359.5	44.3	[illegible]	4.6	[illegible]	2.6
	88	616.2	53.8	33.2	10.8	[illegible]	5.3
	90	480.5	54.6	26.2	[illegible]	7.4	3.6
	Mean ± SE	473.5±44.0	51.2±1.9	24.5±3.0	7.4±[illegible]	[illegible]	3.7±0.5
2	91	538.0	49.9	26.9	[illegible]	7.6	4.1
	61	175.5	43.1	7.6	1.9	[illegible]	1.6
	67	565.0	45.9	25.9	[illegible]	7.0	[illegible]
	4	570.3	48.2	[illegible]	[illegible]	[illegible]	[illegible]
	11	473.5	49.9	23.6	[illegible]	[illegible]	[illegible]
	Mean ± SE	464.5±74.2	47.4±1.3	22.3±3.7	[illegible]	[illegible]	3.2±0.5
4	83	715.0	48.9	35.0	9.7	[illegible]	[illegible]
	71	448.6	42.4	19.0	[illegible]	7.3	[illegible]
	743	750.0	40.5	[illegible]	11.5	[illegible]	[illegible]
	10	595.6	50.3	[illegible]	[illegible]	[illegible]	[illegible]
	72	392.5	53.5	21.0	[illegible]	[illegible]	[illegible]
	Mean ± SE	580.3±70.6	47.1±2.4	[illegible]	[illegible]	[illegible]	[illegible]
7	58	359.8	50.3	[illegible]	[illegible]	[illegible]	[illegible]
	74	478.2	48.9	[illegible]	[illegible]	[illegible]	[illegible]
	78	439.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	60	576.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	81	324.0	54.6	17.7	[illegible]	[illegible]	[illegible]
	Mean ± SE	436.0±44.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
11	27	557.7	46.6	[illegible]	[illegible]	[illegible]	[illegible]
	66	468.1	53.8	[illegible]	[illegible]	[illegible]	[illegible]
	12	369.7	[illegible]	[illegible]	[illegible]	[illegible]	2.9
	69	857.9	45.6	[illegible]	[illegible]	[illegible]	[illegible]
	76	572.0	45.7	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	[illegible]	47.9±1.6	26.1±3.5	[illegible]	[illegible]	[illegible]
18	28	744.1	53.1	[illegible]	[illegible]	[illegible]	[illegible]
	85	483.4	[illegible]	25.3	[illegible]	[illegible]	[illegible]
	80	484.0	57.7	27.9	[illegible]	[illegible]	[illegible]
	84	353.2	52.4	[illegible]	[illegible]	[illegible]	[illegible]
	41	514.3	54.6	[illegible]	7.7	[illegible]	[illegible]
	Mean ± SE	515.8±63.5	54.0±1.0	[illegible]	[illegible]	[illegible]	[illegible]
20	73	363.6	55.3	[illegible]	[illegible]	[illegible]	[illegible]
	75	541.3	46.2	25.0	[illegible]	7.3	[illegible]
	29	612.7	46.9	28.7	7.4	7.0	4.3
	5	364.7	34.2	12.5	[illegible]	[illegible]	[illegible]
	79	540.1	44.0	23.5	[illegible]	[illegible]	[illegible]
	Mean ± SE	484.5±50.9	45.3±3.4	22.0±2.8	5.7±0.7	[illegible]	[illegible]

RNA/DNA	Protein Conc	Protein Content	Lipid Conc	Lipid Content
	(mg/gm)	(mg)	(mg/gm)	(gm)
0.15	0.15	[illegible]	[illegible]	81
0.15	0.18	[illegible]	[illegible]	[illegible]
0.17	[illegible]	71.6	[illegible]	111
0.16	[illegible]	116.0	[illegible]	[illegible]
[illegible]	[illegible]	74.2	[illegible]	75
0.15 ± 0.005	0.17 ± 0.010	[illegible]	205.8 ± 27.2	94.2 ± [illegible]
0.15	0.15	50.1	132	71
0.14	[illegible]	34.2	[illegible]	34
0.15	0.20	113.2	[illegible]	118
0.14	[illegible]	63.0	[illegible]	[illegible]
0.12	0.17	[illegible]	[illegible]	65
0.14 ± 0.006	0.17 ± 0.011	78.6 ± 12.7	[illegible]	77.4 ± 14.5
0.17	0.19	[illegible]	[illegible]	[illegible]
0.17	0.16	[illegible]	[illegible]	[illegible]
0.15	0.19	[illegible]	[illegible]	[illegible]
0.16	[illegible]	106.9	[illegible]	[illegible]
0.13	0.16	[illegible]	[illegible]	[illegible]
0.16 ± 0.007	0.18 ± 0.007	[illegible]	[illegible]	[illegible]
0.14	0.13	[illegible]	[illegible]	[illegible]
0.15	[illegible]	[illegible]	[illegible]	[illegible]
0.15	[illegible]	[illegible]	[illegible]	[illegible]
0.13	[illegible]	[illegible]	[illegible]	[illegible]
0.12	[illegible]	[illegible]	[illegible]	[illegible]
0.14 ± 0.005	[illegible]	[illegible]	[illegible]	[illegible]
0.15	[illegible]	114.9	[illegible]	[illegible]
0.13	0.19	[illegible]	[illegible]	[illegible]
0.16	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.15	[illegible]	[illegible]	[illegible]	[illegible]
0.15 ± 0.006	[illegible]	[illegible]	[illegible]	[illegible]
0.17	0.17	[illegible]	[illegible]	[illegible]
0.13	[illegible]	[illegible]	[illegible]	[illegible]
0.16	0.19	[illegible]	[illegible]	[illegible]
0.14	0.17	[illegible]	[illegible]	[illegible]
0.12	0.17	[illegible]	[illegible]	[illegible]
0.14 ± 0.007	[illegible]	[illegible]	[illegible]	[illegible]
0.12	0.15	[illegible]	[illegible]	[illegible]
0.16	0.18	97.1	[illegible]	[illegible]
0.15	0.21	129.7	[illegible]	[illegible]
0.13	0.13	67.3	[illegible]	[illegible]
0.18	[illegible]	117.5	174	[illegible]
0.15 ± 0.009	0.19 ± 0.012	93.1 ± 14.4	[illegible]	[illegible]