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Twenty-eight Columbia ewes were made available for study during their breeding and anestrous seasons. After the regularity of the estrous cycles had been established, eight of the ewes were sacrificed in pairs at four different stages of the estrous cycle. Eight additional ewes were sacrificed during anestrus. Pituitary glands, ovaries, uteri, thyroids and adrenals were grossly observed, removed and weighed. The anterior pituitaries were lyophilized and assayed on a unit weight basis for gonadotropic content by the use of hypophysectomized, immature, female rats. Mean gonadotropic activity of the anterior pituitaries, as indicated by the ovarian weight response in the rats, was higher during the anestrous season than during the breeding season. Mean ICSH activity, as indicated by stimulation of the ovarian interstitial tissue of the rats, was similar for both seasons, while mean FSH activity, as indicated by ovarian

follicle diameter in the assay rats, was significantly higher during anestrus than during the breeding season. Mean ovarian follicular activity in the ewes as indicated by measuring the diameter of the largest follicle appeared comparable between the two seasons. The pituitary gland was significantly heavier during anestrus, while the ovary and uterus were both significantly larger during the breeding season. Significant differences between seasons were not noted with respect to mean thyroid and adrenal weights of the ewes. The breeding season was found to be 235 days in length while anestrus was 130 days in length.

SOME HORMONAL FACTORS ASSOCIATED WITH THE REGULATION OF EWE REPRODUCTIVE PATTERNS

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TABLE OF CONTENTS

Table of Contents - continued

LIST OF TABLES

List of Tables - continued

SOME HORMONAL FACTORS ASSOCIATED WITH THE REGULATION OF EWE REPRODUCTIVE PATTERNS

I. INTRODUCTION

Recently, considerable interest has been directed toward increasing ewe prolificacy by producing two lamb crops per year. To accomplish this feat, the ewe must be capable of breeding and conceiving during the anestrous season[1/] as well as during the breeding season. Most domestic sheep of today exhibit an anestrous period during part of the year due to the influence of such factors as breed, nutrition, time of year, altitude and latitude. Many researchers in conducting studies on the anestrous season of the ewe have determined the basic environmental controlling factors, but the physiological changes which the ewe undergoes during this period are not clearly understood. The lack in knowledge concerning the physiological mechanisms involved may explain why a feasible method for inducing breeding in the ewe during the anestrous season has not yet been developed. By understanding the underlying mechanisms responsible for reproductive processes in the ewe, it will be easier to render new and better means of controlling reproductive activity.

It is generally agreed that ovarian hormones are responsible for estrous behavior and that ovarian activity is in turn controlled by hormones released into circulation by the anterior pituitary gland.

1/ The word season as used here refers to either the time of the year that ewes are exhibiting estrous cycles (breeding season) or the remaining portion of the year during which they do not show estrous cycles (anestrous season).

Studies that reflect or measure the functional activity of the pituitary during the estrous cycle as well as during anestrus are prerequisite to the understanding of physiological mechanisms responsible for anestrus. Information of this type may be gained by the measurement of individual hormonal activities in the pituitary and relating any variations to changes in ewe behavior and/or activity of reproductive structures.

The present report consists of a review of literature describing the breeding and nonbreeding seasons of the ewe, and also the results of a study which compares ewes during the two seasons with respect to differences in activities of reproductive and endocrine systems.

II. REVIEW OF LITERATURE

Sexual Season

It is generally accepted that the sexual season in sheep is inversely related to the length of daylight (McKenzie and Terrill, 1937; Hafez, 1951). Hammond (1944) and Yeates (1949) have both noted that the shortest day of the year is approximately the midpoint of the breeding season for the ewe. The domestic sheep is a seasonally polyestrous animal in which the number of annual consecutive estrous cycles ranges from seven in the Border Leichester (Hafez, 1950), to 20 in the Merino breed (Robinson, 1959).

In describing the estrous cycle, it is most useful to designate the various portions of the cycle according to ovarian changes. Estrus, the period of sexual receptivity, ends near the time of ovulation. The other parts of the cycle are usually discussed by regarding the first day of estrus as day 1 of the cycle. The post-estrous or metestrous portion of the cycle extends from day 1 to day 4 and includes the formation period for the corpus luteum (Warbritton, 1937). The luteal or diestrous phase during which time the corpus luteum is active extends from day 4 or end of post-estrus up to day 13 of the estrous cycle (Warbritton, 1937). The proestrous or early follicular phase has been defined as the portion of the cycle from diestrus or day 13 until the onset of estrus (Zarrow, Yochim and McCarthy, 1965).

Most researchers agree that the average length of the estrous cycle is approximately 17 days (McKenzie and Terrill, 1937; Underwood, Shier and Davenport, 1944; Barker and Wiggens, 1959; Santolucito, Clegg and Cole, 1960). During the first half of the sexual season the estrous cycle lengthens slowly and steadily (Hammond, 1944), and in the second half of the season the estrous cycle becomes more variable in length due to the increased silent estrous periods (Hafez, 1952). Age of ewe seems to have no effect on the length of estrous cycle (Briggs, et al., 1942; deBaca et al., 1954). However, the onset of the breeding season is earlier in yearling and adult ewes than in ewe lambs (Smith, 1966). It has been shown by Yeates (1949) that the length of the estrous cycle is not affected by reversal of sexual season by artificially changing the light to dark ratio.

McKenzie and Terrill (1937) reported that the duration of estrus in sheep ranged from 3 to 73 hours with a mean of 30 hours. This was in close agreement with the findings of Barker and Wiggins (1959) who reported the average duration of estrus to be 36 hours. McKenzie and Terrill (1937) also demonstrated that there were no differences in the number of ewes coming into estrus in the day or night. However, more heat periods started between midnight and noon than between noon and midnight.

Estrus in the ewe is less clearly defined by changes in physical behavior and in the external genitals than in the sow and cow.

In the cow estrus is marked in the absence of the bull by general restlessness and excitement, mounting or permitting mounting of other females (Hammond, 1927). Characteristic behavioral changes also occur in the sow at the time of estrus (McKenzie, 1926). Grant (1934) observed that the ewe coming into estrus ate less than usual, stood still with her head lowered for a time, and showed a tendency to separate from the flock. He also observed that some ewes coming into heat on hill farms left the flock and wandered for several miles apparently in search of a ram. All these changes in behavior are not consistant enough among ewes to be considered as any diagnostic value in determining the stage of the estrous cycle.

More definite changes are shown by the external genitalia with respect to the onset of estrus. Grant(1934) observed that during proestrus and early estrus the external labia of the vulva became swollen and congested. The congestion often spreads to the surrounding skin, and the entire tail region was sometimes involved. Even though external genital changes during the cycle are more definite than other physical responses, it is still difficult to use these changes as an indication of estrus. In rodents, the changes in the character of the vaginal smear are sufficiently diagnostic to allow determination of the stage of the estrous cycle (Zarrow, Yochim, and McCarthy, 1965). The vaginal smear method is not, however, a reliable indication of stage of cycle in the ewe (Grant, 1934). The

mutual behavior of the ewe and ram seems to constitute the best and most reliable indication of estrus. The ewe is expressing estrus when she will stand and permit mounting by the ram.

Cole and Miller (1935) and Santolucito, Clegg and Cole (1960) found that ovulation occurred in most ewes between 4 and 35 hours after the onset of estrus. The average time of ovulation was 30 hours after the onset of estrus, which is in close agreement with the end of estrus.

Factors Affecting Reproductive Activity

Light Influence

There can be no doubt that light is a controlling factor responsible for inducing the sexual season of sheep (Sykes and Cole, 1944; Godley, Wilson and Hurst, 1966; Robinson, 1951). All breeds of sheep exhibit to a greater or lesser extent a breeding season under the influence of light (Robinson, 1951). Yeates (1949) demonstrated that the onset of the sexual season in a group of Suffolk Border LeichesterxCheviot ewes was a response of the animal to decreasing daily amounts of daylight. Breeding activity occurred 13 to 16 weeks after the changes from increasing to decreasing length of day. The cessation of the breeding season was found to be a response to increasing daily amounts of light and occurred 14

to 19 weeks after the change from decreasing to increasing length of day.

Hart (1950) showed that a gradually decreasing plane of light and an increasing plane of darkness were not essential factors for stimulating the onset of estrus in sheep. A ratio of one part light and two parts dark given continuously for two weeks was sufficient to induce estrus in anestrous ewes. The estrous cycle induced by artificial light rhythms appeared to be regular in all respects, conforming to the average intervals between heat periods as observed during the breeding season and to be associated with the ovulation of typical ova. Hart (1950) also showed that sheep which had just entered the anestrous period could be brought back into the breeding period by change in the light ratio much faster than animals which were deep in anestrus.

Shelton, Maurice and Morrow (1965) studied ovulation rates and lambing performance of Rambouillet ewes in the Northern Hemisphere. The highest ovulation rate occurred in September, while the highest lambing rate occurred in response to December matings. It was suggested that the length of the photoperiod was the major factor affecting the occurrence of estrus and ovulation rate.

Yeates (1949) postulated that light waves are received by the eye of the ewe, from which impulses are passed to the hypothalamus along neutral pathways. Stimulation is then transmitted to the anterior pituitary by humoral means, via the hypophysial portal

vessles. The pituitary in turn releases gonadotropins which bring about changes in ovarian activity.

Genetic Influence

It has been generally concluded that the breeding season of sheep is controlled by inheritance as well as environmental factors. The mono-estrous behavior observed in wild sheep seems to have been modified by the improved shelter, selection, improved husbandry and better nutrition resulting from domestication. Hafez (1951) pointed out that extreme polyestrous behavior in ewes was found in the tropical and equatorial regions or after a high degree of domestication. However, some breeds of domestic sheep such as the Romanov of Russia and the Improved Whiteface mutton of Germany may breed essentially all year round even at high latitudes. In the United States, the Dorset Horn sheep have the most prolonged breeding season of any popular sheep breed; however, they do exhibit a short anestrous season (Hafez, 1950).

Schott, Phillips and Spencer (1939) found that Corriedale, Karakul and the crosses of these two breeds started their sexual season in late August or early September. Hampshires, Shropshires and Karakul x Black-Faced Highland crosses started their sexual season in mid-September and Southdowns entered their sexual season at the end of September.

Hafez (1950) reported that Blackface Mountain, Border Leichester and Welsh Mountain sheep had a restricted breeding season of 19 weeks average duration, and an average number of seven cycles per ewe. The Blackface Mountain, Border Leicester and Welsh Mountain breeds all have originated in the Scottish Highlands at about 2,000 feet above sea level. Romney Marsh and Suffolk ewes have a medium length breeding season of 26 weeks, and experience an average of ten estrous cycles. Both the Romney Marsh and Suffolk breeds were originated in the southern counties of England. Dorset Horns have a prolonged breeding season of 32 weeks with an average of 13 estrous cycles. The Dorset Horn breed probably originated partly from Merinos introduced from Spain (Hafez, 1950). It seems that there is a relationship between the duration of the breeding season and the latitude and altitude or origin of the breed.

Nutritional Influence

Darlow and Hawkins (1932) and McKenzie and Terrill (1937) found that severe undernourishment of ewes would retard the beginning of their sexual season. The duration of postparturient anestrus in Peppin Merino ewes in Western Queensland was shown to be influenced by inadequate nutrition (Smith, 1964). Sheep in Queensland are usually under a submaintenance plane of nutrition during part

of the year. This suboptimal diet increases the length of the anestrous season. However, work by Hafez (1951) showed that ewes placed on a submaintenance diet during late anestrus did not have a delayed breeding season.

Darlow and Hawkins (1932) demonstrated that on moderately good rations flushing did not appear to be important to the onset of estrus. Briggs et al. (1942) concluded that after several different flushing experiments no difference could be noticed between flushed and unflushed ewes as to the time of onset of the breeding season. It has been shown that underfed ewes have shorter estrous cycles (Hafez, 1952) and more 'silent heats' (McKenzie and Terrill, 1937; Briggs et al., 1942).

Latitude and Altitude Influence

Sheep show a graduation in length of sexual season according to the latitude of their place of origin (Yeates, 1949; Hafez, 1950). Sheep originating in countries with high latitudes have a shorter and more distinct sexual season than those originating nearer the equator. This variation has probably been developed through natural selection.

Sheep raised at high altitudes since the seventeenth century have a 100 percent fertility (Monge, 1943). In this work fertility is presumed to be percent lamb crop. When sheep were imported from sea level to high altitudes their average fertility dropped to

30 percent. The author indicated that the infertility was due mostly to the effect of high altitude on the ram and not on the ewe.

Temperature Influence

The breeding season varies in length during different years (Smith, 1966) due to different climatic conditions (Underwood, Shier and Davenport, 1944). Environmental temperature seems to have an influence on the number of estrous periods exhibited during a breeding season (Godley and Hurst, 1961; Godley, Wilson and Hurst, 1966). Dutt and Bush (1955) showed that ewes placed in air conditioned rooms came into estrus on the average of eight weeks earlier than those under the natural conditions.

It has been demonstrated by Dutt, Ellington and Carlton (1959) that fertilization rate was lower while percent of abnormal ova and embryo loss were significantly higher in ewes exposed to elevated air temperature ($90^{\circ}F$) before breeding. Work by Shelton, Maurice and Marrow (1965) showed that lambing results to June and September mating periods were substantially modified by high environmental temperatures.

Ram Influence

Observations have been made on the effect of the presence of a ram with ewes during the transition period from the anestrus to

the breeding season. Schinckel (1954) found that in the presence of a ram ovulation without estrus occurred earlier in a majority of the ewes which had not already started cyclic breeding activity. This effect started within six days after the rams were first introduced into the flock. Riches and Watson (1954) studied one group of ewes that had been running with vasectomized rams continuously and another group which was run with rams at regular intervals. They found that during the anestrus period the incidence of estrus did not decline as far in the changing group as in the constant group. The author concluded that the introduction of the ram with ewes had a stimulating influence causing the ewes to exhibit a more distinct estrus. Watson and Radford (1960) have experimentally demonstrated that smell and sound of a ram was sufficient stimulus to bring some ewes into cyclic activity. Ewes which had been held well separated from rams for four months were permitted at the end of the anestrous season varying degrees of association with rams for 11-12 hours daily for two weeks. Some of the ewes could smell, hear, see and had actual contact with the rams; others could smell and hear them only. These ewes, along with others which had remained well separated from rams during the same period, were then run together with vasectomized rams continuously. Almost all ewes experienced estrus during the following 26 days. However, most ewes which had previously had some association with rams experienced estrus

withing 17 days whereas most of those which had previously been kept separate from rams did so between 18 and 26 days.

From previous observations, it is difficult to limit the manner in which the presence of a ram acts as an exteroceptive factor. Schinckel (1954) believed that a stimulus was probably mediated through the central nervous system which was directed via the hypothalamus and hypophyseal portal vessels to the anterior pituitary, causing production of sufficient gonadotropins to bring about ovulation. It was postulated by Schinckel (1954) that many ewes ovulate within a few days after the introduction of the ram. But that the ewe failed to exhibit estrus because there was no waning corpus luteum from a previous ovulation and therefore no progesterone was produced, which is needed in conjunction with estrogen for expression of estrus.

Reproductive and Endocrine Structures During the Breeding Season and Anestrus

A study of the events in the ewe during the breeding and non-breeding seasons would not be complete without a consideration of the accompanying changes in the endocrine and reproductive organs. In describing the changes which occur in the endocrine and reproductive organs during the estrous cycle, it will be most helpful to keep in mind the various portions of the estrous cycle according to the ovarian changes as previously mentioned.

Vagina

Grant (1934) observed that during anestrus the vagina was in a state of quiescence in contrast to the vagina in ewes undergoing cyclic activity. It was also noted that the vagina epithelium during anestrus was much thinner than during the breeding season.

Histological changes in the vagina of the ewe during the estrous cycle have been studied quite extensively by McKenzie and Terrill (1937). They noted that the epithelium of the vagina reached its greatest height during estrus and post-estrus and decreased somewhat irregularly to the lowest height during late luteal and early proestrus stages. The average height of the epithelium during the luteal phase was less than one-half of that of the post-estrus phase. The height of the epithelium in early estrus was intermediate, indicating that growth was active during that time. Histological examination of the ewe vagina during the estrous cycle by Santolucito, Clegg and Cole (1960) indicated no changes during the different phases of the estrous cycle.

Cervix

In observing the cervix of the ewe, McKenzie and Terrill (1937) noted a build up of excretory secretions in the columnar epithelium during the luteal phase and the secretions were eliminated

during late luteal and proestrous stages. Secretions were stained with thionin in order to demonstrate mucus. The least amount of mucin material was seen in the epithelial cells during estrus and the greatest amount in the late luteal stages. The authors believed that the epithelial cells of the cervix undergo a definite secretory cycle during the course of the estrous cycle. The cells seemed to fill up during the luteal stages with secretory products which were secreted during late luteal and proestrus stages. Grant (1934) observed that during estrus there was a copious flow of clear, watery mucus from the cervix to the vagina. In contrast to the above work, Santolucito, Clegg and Cole (1960) observed no histological changes in the cervix during various stages of the estrous cycle.

Uterus

The uterus of the ewe during the anestrous season is quite different from that of the ewe during the breeding season. Cole and Miller (1935) observed that during anestrus portions of the uterine mucosa receded to a point not seen at any other stage of reproduction in the mature ewe. The number of uterine glands was also reduced and the diameter of the uterus was much smaller during the anestrous season.

McKenzie and Terill (1937) found no noticeable changes in the uterine mucosa that could be correlated with the estrous cycle.

However, they noted that edema of the stroma of both cotyledonary and intercotyledonary areas was greatest in estrus and post-estrous stages. Vascularity of stromal capillaries was also greatest in the estrus and post-estrous stages.

Casida and McKenzie (1932) and Cole and Miller (1935) in general agreed that there appeared to be an increase in growth and coiling of the uterine glands during metestrus. McKenzie and Terrill (1937) also noted that during metestrus there was an increase in the height of epithelium in the basal portions of the uterine glands. Santolucito, Clegg and Cole (1960) in contrast to the previous findings found no histological changes in the uterus during various stages of the estrous cycle.

Fallopian Tubes

Cyclic changes seem to be more definite in the fallopian tubes of ewes than in any other part of the reproductive system except for the ovary. McKenzie and Terrill (1937) observed that the height of the epithelium of the fallopian tube, though variable, was greatest during estrus and post-estrus . It was also noted that decrease in epithelial height during the luteal phase was at a low on day 14.

Ovary

Corpora lutea are absent from the ovaries of ewes during the anestrous season, indicating a lack of ovulation during this time (McKenzie and Terrill, 1937; Hutchinson and Robertson, 1960). Grant (1934) reported that the ovaries during anestrus were usually small and compact containing many follicles which were rarely more than 3 mm in diameter. During the breeding season the ovary had larger follicles in the range of 4 to 5 mm in diameter. Kammlade et al. (1952) and Hutchinson and Robertson (1960) found no difference in either the average diameter of all follicles or the diameter of the largest follicle between the anestrous season and the breeding season. Grant (1934) and Hutchinson and Robertson (1960) seemed to agree that there were a greater number of follicles in the ovary during anestrus than during the breeding season. However, Kammlade et al. (1952) observed a greater number of follicles in the ovary during the breeding season than during anestrus. There seemed to be no change in number or size of follicles 1 mm or greater than 1 mm in diameter during the anestrous season.

During the estrous cycle the ovary goes through a cyclic change, mainly one of growth and maturation of the Graafian follicles, the rupture of the follicles during estrus, the development of each of the ruptured follicles into a corpus luteum during post-estrus and subsequent atrophy of the corpus luteum (Grant, 1934). It was also

observed that follicle diameter at the time of rupture was about 9.5 mm and the mean number of follicles ovulated at this time was 1.87, with a decrease in the number of follicles ovulated late in the breeding season. No cyclic changes in the weight of the ovaries were noted.

Adrenal and Thyroid

McKenzie and Nahm (1933) observed changes in the fat and mitochondrial content of cortical and medulla cells of the adrenal during the estrous cycle. The changes in the fat content seemed to be continuous throughout the cycle, showing an increase during proestrus and early estrus and reaching the highest concentration during early metestrus. This was followed by a decrease in fat content through dioestrus. However, more recent histological examination of the adrenal by Santolucito, Clegg and Cole (1960) showed no changes associated with the various phases of the estrous cycle. They also found no difference in weight of the adrenal at various stages of the cycle.

Kammalade et al. (1952) found no significant difference between the weight of the thyroid glands in ewes during anestrus and the breeding season. There was no correlation between the thyrotropic potency of the pituitaries and the weights of the thyroids of the ewes.

Pituitary

A consideration of the pituitary gland is perhaps best begun by a brief description of its embryological origin. In all vertebrates the pituitary is derived from two sources. One of these is an ectodermal dorsal evagination of the buccal cavity, which extends as the pouch of Rathke toward the embryonic brain, giving rise to the glandular lobe or adenohypophysis (Hanstrome, 1966). The other source or origin is from a hollow ventral process of the floor of the diencephalon, the saccus infundibule, which gives rise to the neural lobe or neurohypophysis (Hanstrome, 1966).

The pituitary gland of the ewe lies in a deep depression in the sphenoid bone, the sella turcica, and is separated from the cranial cavity by a tough cartilaginous diaphragm through which passes the hypophyseal stalk (Hanstrome, 1966). Within the adenohypophysis are three subdivisions: the pars intermedia, pars tuberalis and pars distalis (anterior lobe) (Bloom and Fawcett, 1962; Hanstrome, 1966).

The blood supply of the hypophysis is intimately involved in the control of the secretory activity of the gland. Experiments indicate that neurohumoral substances released from the median eminence of the hypothalamus are carried in the blood via the hypophyseal-portal system to the anterior lobe of the pituitary where they

stimulate the pituitary cells into releasing specific hormones (Hanstrome, 1966).

Warbritton and McKenzie (1937) found that of three ewes slaughtered during the anestrous season, the pituitary of one ewe showed increased size and granulation of specific cells which were characteristic of the same type of pituitary cells from a ewe in the luteal phase of the breeding season. The other two ewes had pituitary cells not actually characteristic of any phase during the estrous cycle as the cells were much smaller in size.

Santolucito, Clegg and Cole (1960) observed no difference in weights of pituitaries during different stages of the estrous cycle. Warbritton and McKenzie (1937) arbitrarily classified the cells of the ewe pituitary into nine types. They found cyclic changes in numbers, size and granulation of cell types 2, 3 and 4. Cell types 2 and 3 were both acidophilic differing from each other mainly by the presence and absence of granules. The authors believed that the acidophilic cells were responsible for FSH secretion. Cell type 4, which was basophilic, was thought to be responsible for ICSH secretion. The cell types 2, 3 and 4 were observed having different patterns, due to size, number and granulation, characteristic of the four stages of the estrous cycle. The authors were of the opinion that an increase in number or size of specific pituitary cells was indicative of increased gonadotropic secretion. Robertson and

Hutchinson (1958) also made a histological study of the pituitary glands of ewes at various stages of the estrous cycle. An increase of peripheral basophilic cells, as indicated by periodic acid-Schiff (PAS) reaction was demonstrated during the early luteal phase of the cycle followed by a decrease until just before ovulation was observed.

Hormone Administration and Reproductive Activity Changes

Another method for study of the hormonal mechanisms involved in cyclic activity is by administration of exogenous hormones with subsequent observation on the changes that occur in the recipient animal. Most of the research in this area has been carried out with estrogens, progestogens and gonadotropins.

Estrogens

It is generally accepted that estrogen does play a major role in controlling cyclic changes in the reproductive tract. Bell, Casida and Darlow (1941), by injecting estrogen into spayed and anestrous ewes followed by progesterone, induced the characteristic histological changes of the reproductive tract similar to the cyclic changes occurring during the luteal phase and at metestrus. The height of the cervical epithelium was increased by estrogen and maintained by progesterone. The increased height of the epithelium in the fallopian tube during estrus is believed to be brought about by

estrogen (McKenzie and Terrill, 1937).

Studies involving estrogen injected animals and effects on pituitary secretion seem to be complicated by dose levels and the period of time injected. Frank (1940), Greep (1961) and Everett (1961) generally agreed that short term, low dose estrogen administration would stimulate the release of gonadotropins while high dose, long term administration would inhibit gonadotropic secretion. This was in agreement with the report of Cole, Hart and Miller (1945) which showed that injections of estrogen in doses large enough to induce sexual receptivity in the ewe, given either alone or in conjunction with pregnant mare serum (PMS), inhibited ovarian activity. Cooper (1967) studied the effects of exogenous estrogen on the secretion of anterior pituitary hormones in gonadectomized male sheep. He found that estrogen treatment resulted in a significant decrease in plasma gonadotropic activity as determined by ovarian and uterine weight responses in assay rats. Ovarian follicular and interstitial tissue examination of the assay rats showed that the depression always involved follicle stimulating hormone, (FSH) and usually interstitial cell stimulating hormone (ICSH).

Progestogens

It is generally believed that ewes need a progestational influence prior to an estrogenic influence in order to express estrus.

Robinson (1951) and Yeates (1965) demonstrated that the first ovulation of the sexual season was without a physically apparent estrus. As described by the above authors, the evocation of heat with ovulation appears to be dependent on the presence of a waning corpus luteum. Yeates (1965) believed that a silent heat usually occurred at the first ovulation at puberty in ewes.

Everett (1948) demonstrated that progesterone could facilitate ovulation in the rat if administered in conjunction with estrogen. Dutt and Casida (1948) noted that estrus in the ewe was surpressed by treatment of 10 mg of crystaline progesterone. Hinds, Dziuk and Lewis (1964) found that estrus was suppressed by continued consumption of 20 mg or more of 6-methyl-17-acetoxyprogesterone (MAP) per day for 14 days by ewes previously experiencing normal estrous cycles. Research by Raeside and Lamond (1956) indicated that administration of progesterone to anestrous ewes had no effect on pituitary hormone content as indicated by the chick testicular weight response. Kawakami and Sawyer (1959) after employing characteristics of electroencephalogram (EEG) arousal and EEG after reaction to progesterone in rabbits, supported the view that progesterone exerts its effect on ovulation by way of the hypothalamic control of the anterior pituitary gonadotropins. Ellington, Contopoulos and Clegg (1964) studied the influence of exogenous progesterone in releasing pituitary gonadotropins in gonadectomized male sheep.

Their results revealed that a daily administration of 20 mg of progesterone increased the amount of gonadotropins released into circulation on the fifth and sixth day of treatment and again at approximately the same time interval following the withdrawal of treatment.

In considering the average progesterone level in ewe blood during the estrous cycle, Edgar (1953) obtained blood samples from the ovarian vein by laparotomy and assayed it for progesterone content. He found that during the first two days of the cycle, the amount of progesterone present was less than 0.1 μgm per ml of blood. Detectable amounts of progesterone were present from the third or fourth day and increased until the ninth day. The average quantity stayed relatively high until the sixteenth day at which time a sudden fall was observed.

Gonadotropins

The effects of gonadotropins on the cyclic activity of the female animal have been extensively studied during the past quarter of a century. It is generally accepted that FSH brings about growth and maturation of the ovarian follicle and LH causes ovulation and stimulates growth of interstitial tissue (Robertson and Hutchinson, 1962; Santolucito, Clegg and Cole, 1960).

Ovulation without heat can be induced regularly by the use of either PMS or anterior pituitary extract (Bell, 1941). In this work,

the number of ovulations per ewe was considered to be average in all ewes given PMSG, but was very low in ewes injected subcutaneously with anterior pituitary powder. Dutt (1953), Robinson (1954) and Raeside and Lamond (1956) found that injections of PMSG alone produced ovulation without estrus, but when PMSG was given following progesterone treatment, ovulation and estrus both occurred in 60% of the ewes. Robinson (1954) demonstrated that two PMSG injections 16 days apart brought about estrus in the treated ewes. The first injection caused ovulation with no estrus; the second injection brought about estrus in 61% of the ewes. It was also found that an injection of PMSG in addition to proportionate amounts of testosterone induced estrus in a low percentage of the ewes. Cole, Hart and Miller (1945) found that a single injection of PMSG would hasten the onset of the sexual season.

Posterior Pituitary Hormones

Recently there has been much interest in the possibility of associating posterior pituitary hormones with functional aspects of the anterior pituitary. Oxytocin has been suggested as a possible mediator for gonadotropic secretion (Shibusawa et al., 1955). Armstrong and Hansel (1958) injected 50-150 U.S.P. units of either a posterior pituitary extract or a more highly purified oxytocin preparation to cycling heifers once daily for seven days

beginning on the day of estrus. All heifers returned to estrus one to four days after injections were discontinued. When injections were administered on the fifteenth day post-estrus, all heifers came into estrus at the normal time. Again, all heifers were injected for seven days and they came back into estrus on the average of four days later. All animals ovulated following the induced estrus. These results were taken to indicate an involvement of oxytocin or a closely associated substance in the regulation of gonadotropic hormone secretion by the anterior pituitary.

Oxytocin treatment of the ewe during the breeding season by Milne (1963) and Braden and Moule (1964) failed to cause any shortening of the estrous cycle. Histological examination of the corpora lutea of the treated ewes (Milne, 1963) revealed a smaller number of active lutein cells, more connective tissue and a change from basophilic to eosinophilic staining reaction when compared to the corpora lutea of the untreated controls.

Guillemin, Jutisz and Sakiz (1963) partially purified in acetic acid an LH releasing factor (LRF) from the median eminence of ewes. A technique of molecular sieving involving the use of Sephadex G-25 was used for isolating the LRF. The zone where LRF activity was observed corresponded to the same region as α-melanophore stimulating hormone (α-MSH) and was well separated from arginine vasopressin or oxytocin. The material with LRF activity, as isolated from

median eminence tissue, was a small polypeptide which was heat stable and definitely not oxytocin or vasopressin.

ACTH and Adrenal Hormones

It has been observed by several researchers that under certain stress conditions a female animal can be induced to ovulate sooner than if kept under conditions of minimum stress. Edgar (1958), using rectal electrical stimulation caused ovulation and estrus in five of six ewes in early anestrus. The effect of this treatment, however, was progressively reduced in other ewes with the advancement of the anestrus season. Hafez and Sugie (1963) reported a frequent occurrence of ovulation unaccompanied by estrus in beef heifers transported long distances. Braden and Moule (1964) found similar results when anestrous ewes synchronously ovulated after being transported for 24 hours by rail. However, stresses during the breeding season seemed to lengthen the estrous cycle by a few days.

Braden and Moule (1964) found that injections of ACTH did not induce ovulation in anestrous ewes, but ACTH injections during the breeding season did seem to increase the length of the estrous cycle. They concluded that stress does not appear to cause ovulation through an effect of increase ACTH action on the ovary. Instead, it seems to cause increased gonadotropin release, as indicated by ovulation during anestrus, as well as ACTH release from the anterior

pituitary. Sawyer, Markee and Everett (1950) stimulated the release of ICSH from the rabbit hypophysis by intervenous injections of epinephrine. It is possible that the adrenergic activity associated with stress could be responsible for the ovulatory stimulating effect of certain stress-type stimuli.

Assay of Gonadotropic Activity

At the present time, it is the general consensus that FSH is concerned primarily with growth of the ovarian follicle beyond the stage of antrium formation. ICSH in addition to FSH is involved in full follicle maturation, while ICSH is considered to be the main hormone responsible for ovulation. Arising from this is the view that the differences in cyclic activity during various phases of reproduction may be related to differences in the rate of synthesis and/or release of the individual gonadotropins (Greep, 1961).

Pituitary Activity

Considerable work has been done on determining the variation in gonadotropin content of the anterior pituitary during anestrous and breeding seasons. This research has been carried out mainly as a means of investigating the underlying mechanisms of cyclic activity in the ewe. Cole and Miller (1935), using as a means of study the amount of pituitary tissue required to induce ovulation in mature

rabbits, found no difference in the total gonadotropic activity of pituitaries collected at anestrus or at the breeding season. Warwick (1946) assayed the gonadotropic activity of the ewe by using the ovarian weight response of 21 day-old female rats and the testicular weight response of day-old cockerels. They found that no difference existed in the total gonadotropic activity of the pituitary tissue between the breeding season and nonbreeding season. However, Warwick (1946) did note an increase in total gonadotropic activity in the pituitary after gonadectomy. Robinson (1950) reported that sheep pituitaries contain more gonadotropic hormone during the anestrous season than during the breeding season. Similar findings were reported by Kammlade et al. (1952), using the chick testicular weight response as the assay method. It seemed that the maximum potency during the estrous cycle approximated the anestrous average.

Using the same assay method used by Kammlade et al. (1952), Raeside and Lamond (1956) found a slight increase in gonadotropic activity during anestrus, just prior to the breeding season. They also found a slight increase in the number of ewe ovarian follicles (1-5 mm in diameter) over the same period of time. The authors interpreted these findings suggesting that during anestrus there might be increased gonadotropic potency due to increase FSH activity. Lamond, Radford and Wallace (1959), using the immature mouse uterine weight response, found no appreciable difference in

gonadotropic content of pituitary glands from ewes sacrificed during both seasons. Clegg and Ganong (1960) assayed specifically for ICSH and found that ewes which had entered their yearly anestrous season had a lower ICSH content than ewes during the breeding season. Hutchinson and Robertson (1960) observed that FSH and ICSH potencies, as indicated by the methods of Steelman and Pohley and Parlow, of the pituitary during the anestrous season were similar to those during the breeding season. The FSH potency during anestrus was as high as the maximal value found during the preovulatory phase of the estrous cycle, while the value for ICSH at anestrus was similar to the mean value for the cycle. Robertson and Hutchinson (1962) also found that the levels of pituitary FSH during anestrus were comparable to pituitary FSH during the breeding season. It was noted that relatively less ICSH than FSH was present in the pituitaries of anestrous ewes.

It is in general agreement that the ovaries of ewes during the anestrous season are fairly active in terms of follicular activity (Robinson, 1950; Kammlade et al., 1952; Hutchinson and Robertson, 1960). If the maintenance of the larger ovarian follicles present during anestrus is controlled by gonadotropins then release of gonadotropins from the pituitary must occur. There must also be production of FSH and ICSH to maintain gonadotropic levels found in the pituitary. Hutchinson and Robertson (1960) believe that the

absence of ovulation during the anestrous season may be due to the lack of sudden substantial release of ICSH and FSH. However, Robinson (1950) and Kammlade et al. (1952) suggest that ovulation does not occur during the anestrous season due to an imbalance between FSH and ICSH.

Kammlade et al. (1952), using chick testicular response, found that there was a linear increase in gonadotropic potency of the pituitaries in ewes from day 1 to day 17 of the estrous cycle. On day 1 and day 2 of the cycle, when the ewes were expressing estrus, the gonadotropic potency was the lowest. In swine, Robinson and Nalbandov (1951) found that the gonadotropic hormone content of both sow and gilt pituitaries was at a low during the two days of heat. This potency remained low until day 8 of the cycle when the gonadotropic activity increased suddenly and remained high until day 20. There was a complete correspondence between the rise or fall of gonadotropic potency in the pituitary and the rise or fall in the number but not the size of the ovarian follicles.

Bioassays of ewe pituitaries by Robertson and Hutchinson (1958) showed that there was a marked increase in FSH during the early luteal phase of the cycle and a steady fall from then until just prior to ovulation. In contrast to these findings, Santolucito, Clegg and Cole (1960) observed that FSH content was low after ovulation until the fifth day of the cycle at which time FSH content increased

rapidly and remained high until the next ovulation. Between the fourth and thirty-fifth hour after onset of estrus the FSH level remained low until the fifth day of the cycle.

Santolucito, Clegg and Cole (1960) found that the ICSH content of ewe pituitaries significantly declined between the fourth and thirty-fifty hour after estrus, followed by an increase in ICSH activity as the cycle advanced. The decline in ICSH between the fourth and thirtyfifth hour after estrus was believed to be associated with ICSH release. The work by Robertson and Hutchinson (1958) and Santolucito, Clegg and Cole (1960) indicated that the content of both FSH and ICSH drops in the pituitary during ovulation, indicating that both FSH and ICSH are needed for ovulation.

Using the pigeon crop assay, Santolucito, Clegg and Cole (1960) found the luteotropin (LTH) content of sheep pituitaries did not vary significantly among the various states of the estrous cycle.

Circulating and Urinary Gonadotropin Activity

An excellent method for studying gonadotropic regulation of reproductive events would be the determination of the levels of gonadotropic activity in the blood and/or urine. This would eliminate the necessity of sacrificing the donor animal and allow cyclic changes in gonadotropic activity to be determined in the same animal. Gonadotropic activity has been detected in the urine of castrated male and

ovariectomized female sheep (Robertson, MacGillivray and Hutchinson, 1963). Since no response was detected in intact sheep, seasonal or cyclic differences have not yet been noted by this procedure.

Blood collected in quantities as high as 500 ml from ewes at various times of the estrous cycle have failed to reveal any gonadotropic activity (Basset, Sewell and White, 1954). Ellington, Contopoulos and Clegg (1962), using the technique developed by McFarland, Clegg and Ganong (1960) for collecting blood from the cavernous sinus, found that in gonadectomized male sheep, FSH and growth hormone-like activities were higher in cavernous sinus plasma than in peripheral plasma.

Dierschke and Clegg (1965), using the previously mentioned cavernous sinus blood collecting technique, further investigated gonadotropin release. They found trends for increase levels of serum ICSH from 6 to 17 hours after the onset of estrus. They also found a significant depletion of pituitary content of both ICSH and FSH between 8 and 16 hours after the onset of estrus; however, no consistent correlation was found between ICSH concentration in plasma and ICSH activity in pituitary tissue collected from the same animal. These data seemed to indicate a preovulatory rise in blood ICSH with relatively small increases in the amount released over periods up to 12 hours. McDonald and Clegg (1966) again using the cavernous sinus technique of collecting blood and specific bioassays found that

in the ewe there was a trend toward increase ICSH activity in the serum during the anestrous season as compared with the breeding season. MacGillivray and Robertson (1963) found that in ovariectomized ewes the release of gonadotropins, as measured by mouse uterine weight, did not vary between seasons.

General Conclusions and Objectives of Present Study

From the foregoing survey of literature, it is concluded that there is general agreement among investigators that a ewe has an average estrous cycle length of 17 days and ovulates approximately 30 hours following the onset of estrus. The length of anestrus appears to be affected by genetic make-up of the ewe, light, temperature, altitude, latitude, nutrition and other external factors.

Some researchers find that the gonadotropic content of the pituitary is essentially the same during both seasons, while others report that gonadotropic activity is highest during the anestrous season. The discrepancy may in part be due to the different methods employed in assaying the pituitary. Further complications associated with the assay procedure involve the fact that many investigators measure total gonadotropic activity rather than the individual gonadotropic components. Probably the most difficult problem in determining hormone concentration of the anterior pituitary is interpreting the data in terms of secretory activity. The reason most

researchers have concentrated on the pituitary as a source of study for gonadotropins is due to the difficulty involved in detection of gonadotropic activity in other tissues and fluids of the intact ewe.

This study was designed to observe changes in endocrine and reproductive structures as associated with seasonal sexual activity. Total as well as individual gonadotropins in the anterior pituitary were measured. Determining individual gonadotropic activity facilitates a more precise interpretation of pituitary functional activity. From such data a more exact rendition of the secretory activity of the pituitary will be possible, especially when compared to variations in reproductive and other endocrine organs within the ewe. These interpretations may help to clarify some of the underlying mechanisms controlling the reproductive cycle of the ewe.

III. EXPERIMENTAL MATERIAL AND METHODS

Experimental Design

Twenty-eight, four-year old, grade Columbia ewes were made available from October 25, 1965, to January 1, 1967, for a study concerned with characterization of the breeding and anestrous seasons. All ewes had produced a lamb during the previous breeding season. The ewes were kept in an open barn with two vasectomized rams equipped with marking crayons and associated harnesses as described by Radford and Watson (1960). While on experiment, the ewes were provided with a daily ration of approximately three pounds of alfalfa hay and one-half pound of a grain mix. The concentrate contained 200 pounds whole oats, 80 pounds rolled barley, 50 pounds beet pulp and 12 pounds of linseed oil meal. None of the ewes in the experiment were exposed to an intact ram at any time during the study. The ewes were checked daily for occurrence of estrus which was noted by the marking of the crayon on the back or rump of the ewe.

The first day (day 1) of the estrous cycle was considered as the day the ewe was first receptive to the vasectomized ram. The length of the anestrous season for the individual ewes was figured from the date of last occurring estrus in the spring of 1966 to the first occurring estrus in the fall of 1966. After the regularity of the estrous cycles had been established for each ewe, eight of the

ewes were sacrificed in late December, 1965, in pairs at days 1, 3, 8 and 15 of the estrous cycle. By use of random sampling table, eight more ewes were sacrificed on June 15, 1966, when estrous activity had ceased in the remaining 20 ewes.

Slaughtering Procedures

The ewes were sacrificed by exsanguination. Immediately after death the head was severed from the body and opened to expose the brain. The brain was removed to expose the diaphragm covering the pituitary. By cutting around the lateral margins of the diaphragm and breaking the dorsum sellae, the pituitary was easily removed from the sella turcica. The entire gland was freed of surrounding tissue, weighed, and following removal of the posterior lobe, the anterior lobe was weighed. The anterior pituitaries were then frozen on dry ice for immediate preservation.

The thyroid glands, adrenal glands, ovaries and uteri were removed and their weights recorded. The largest possible diameter of the ovary as well as the largest follicle diameters and the presence of corpora lutea were also recorded. The adrenals were fixed in 10% formalin and the ovaries, uteri and thyroids were fixed in Bouin's solution so if further observations were necessary, the tissues would be available.

Assay Animals

The assay animals used were immature, Long-Evans, female rats which had been hypophysectomized at 26 to 28 days of age. The rats were allowed 14 days following hypophysectomy to adjust to their new environment and to allow pituitary endogenous hormone concentrations to become depleted. They were maintained on a diet of Purina rat pellets which was supplemented with sugar cubes during the actual injection period.

Preparation and Bioassay of the Pituitary

After the anterior pituitaries had been frozen by dry ice, they were lyophilized for 48 hours after which time the dry weights were taken. The pituitaries were then ground individually with a mortar and pestle. The resulting pituitary powder was stored in a desiccator at $0^{\circ}C$ until time of assay.

At the time of assay, the dry pituitary powder was suspended in physiological saline in such a way that the total dose for each bioassay rat would be in a 4 ml volume. All injections were made intraperitoneally once daily for four consecutive days with daily dosages for each rat consisting of a 1 ml volume. Between daily injections, the pituitary suspensions were stored under refrigeration at $9^{\circ}C$.

Each bicassay rat was ear marked by a convenient system

which yielded easy identification. Body weights were recorded immediately prior to the first injection and again at autopsy. The assay rats were autopsied under chloroform anesthesia 24 hours after the last injection. It was noted if the vagina were closed or open, and the tail length was measured from the anus to the tip of the tail. The oviducts plus the uterine horns, ovaries, adrenals, thymus and thyroids were then removed with the aid of a dissecting microscope. They were trimmed free of excess tissue and weighed. All structures were fixed in Bouin's solution except the adrenals which were fixed in 10% neutral formalin. After the structures of interest had been removed, measured, or macroscopically noted, the sella turcica of the rat was examined for any visible pituitary fragments. Data pertaining to incompletely hypophysectomized rats were discarded.

Determination of Hormonal Potency

The quanitative determination of gonadotropic potency in the pituitaries was based on increases in ovarian and uterine weights, and in addition, ovarian histological changes in the assay animals. Histological preparation of the ovaries was made by embedding one ovary of each rat into paraffin and then sectioning with a microtome at a thickness of 8 micra. Sections were mounted on slides and stained with hematoxylin and eosin. The FSH activity of the

pituitaries was determined by microscopic measurement of the diameter of non-atretic ovarian follicles of the rats with the aid of a calibrated ocular micrometer (Evans et al., 1939). The largest follicle diameter in 21 sections was taken as the reading for that assay animal. The ICSH activity of the pituitary was determined by microscopic examination of the ovarian interstitial tissue for evidence of stimulation (Simpson, Li and Evans, 1942). The same histological slides discussed for ovarian follicular measurements were used to evaluate the interstitial tissue. The ovaries of saline-injected hypophysectomized rats were considered as deficient or unstimulated controls. The degree of interstitial stimulation is defined as follows:

1. D (deficient)
   a. Nuclei small with chromatin in clumps
   b. Nuclei close together
   c. Cytoplasm not very evident

2. PR (partially repaired)
   a. Nuclei increased in size and slightly more rounded with the chromatin more evenly scattered
   b. Nuclei further apart and stained lighter with hematoxylin
   c. Cytoplasmic body increased in size and more eosinophilic

3. R (repair)
   a. Same characteristics as PR except more pronounced

4. H (hypertrophy)
   a. Nuclei far apart and lightly stained
   b. Nuclei large with even distribution of chromatin
   c. Cytoplasmic body increased in size and highly eosinophilic

The STH stimulating activity of pituitary tissue was based on the tibial epiphyseal plate response of the assay rat. In the method as developed by Greenspan et al., (1949), the tibia is stained by a silver nitrate staining procedure. Ten readings of each epiphyseal plate were recorded with the aid of a calibrated ocular micrometer at 100 X magnification. The average of these readings was converted to micra and taken as the reading for each assay rat. Concentrations of TSH and ACTH hormones in the anterior pituitary were determined by weight responses of the thyroids and adrenals in the assay animals.

Analysis of Data

The data concerned with measurements on the endocrine glands and reproductive organs of ewes were analyzed by the student t-test. The data concerning responses in the assay animals were analyzed by analysis of variance for detection of gross differences among treatment means.

IV. RESULTS

Length of Estrous Cycle

Data gained from checking for estrus served a threefold purpose: first, in determining length of successive estrous cycles; second, in determining length of anestrus; and third, in giving a reference date to follow in slaughtering ewes during respective stages of the estrous cycle.

Tables 1 and 2 show the lengths of the successive estrous cycles for individual ewes during the two breeding seasons studied. Extremely long estrous cycles, which are considered as cycles 24 days or longer in length, are underlined. In calculating the average estrous cycle length, all estrous cycles were used, but in computing the corrected average estrous cycle length the extremely long cycles were omitted.

The average length of estrous cycles observed between October 25, 1966, to January 1, 1967, was 20.6 days (Table 3). However, the average length of the estrous cycles after the long cycles had been omitted was 16.7 days. Several trends are noted in Table 1 concerning abnormally long estrous cycles. One is a tendency for more long estrous cycles during the last half of the breeding season than during the first half. It is also interesting to note that the extremely long estrous cycles seem to be repetative within certain ewes.

Table 1. Estrous cycles in Columbia ewes from October 25, 1965, until the end of breeding season or termination of ewe.

Ewe no.	Length of successive[a] estrous cycles, days	Av. estrous cycle length, days	Corrected av.[b] estrous cycle length, days	Date slaughtered
304	19;15;18;17;18;16;16;12	16.4	16.4	
306	18;16;16;17;19;16;18;11	16.4	16.4	
308	15;18;14;17;32;16;16	18.3	16.0	
310	16;17;17;17;18;13	16.3	16.3	
312	19;19;16;14;22;17;11	16.9	16.9	
314	16;17;15;18;16;42;17;30	21.4	16.5	
316	17;17	17.0	17.0	12-24-65
318	19;16	17.5	17.5	12-20-65
320	17;37;17;19;18;18;12;27;15	20.0	16.6	
322	31;14;23;17;19;13;17;43	22.2	17.2	
326	19;17;20;15;22;21	19.0	19.0	
328	14	14.0	14.0	12-20-65
330	18;17;18;17;17;17;16;11;12;22	16.5	16.5	
332	16;18;19;17;28;39;17;44	24.8	17.4	
334	16;17	16.5	16.5	12-21-65
336	35	35.0	--	12-20-65
338	49;19;16;15;34;53	31.0	16.7	
340	14;20	17.0	17.0	12-20-65
342	16;19;17;32;22;51;27	26.0	18.5	
344	30;17;17;19;82	33.0	17.7	
346	18;15	16.5	16.5	12-20-65
348	18;16;35;17;16;18;16;32;14	20.0	16.4	
350	19;37;14;18;19;13;21;14;22	19.6	17.5	
352	15;19;14;17;32;18;42	22.4	16.6	
354	10;20;16;20;14;14;17	15.9	15.9	
356	17;10	13.5	13.5	12-20-65
358	35;16;17;17;18;68;12	26.1	16.0	
360	16;18;17;17;20	17.6	17.6	

[a] An underlined number infers an abnormally long cycle, probably consisting of two or more cycles.

[b] Cycles over 24 days in length were excluded.

Table 2. Length of estrous cycles in Columbia ewes from beginning of breeding season 1966, to January 1, 1967.

Ewe no.	Length of successive[a] estrous cycles, days	Av. estrous cycle length, days	Corrected av.[b] estrous cycle length, days	Comments
306	11;22;21;18;17;21	18.0	18.0	
312	19;20;18;20;34	22.2	19.3	
320	15;18;19;19;16;20;34	20.1	17.8	
322	38;8	23.0	8.0	Removed from study[c]
330	15;18;17;17	16.7	16.7	
338	15;21;16;16	17.0	17.0	
342	16;18;17	17.0	17.0	
344	33	33.0	--	Removed from study[d]
348	16;17;34	22.3	16.5	
350	18;18;18;17	17.8	17.8	
354	18;18	18.0	18.0	

[a, b] See footnotes a, b to Table 1.

[c] Removed from study due to poor health.

[d] Removed from study for preliminary assay trial of pituitary.

Table 3. Summary on length of estrous cycles.

No. of ewes	No. of estrous cycles	Average length of estrous cycle, days	No. of corrected estrous cycles	Average length[a] of corrected estrous cycle, days
28	206	20.6	165	16.8

[a] Cycles over 24 days in length were excluded.

Length of Anestrus

The dates of the last estrous periods for the 1965-66 breeding season and first estrous periods for the 1966-67 breeding season are given in Table 4. A wide variation in the dates of last estrus of the first breeding season is clearly apparent. The date of the last estrus ranges over a 4 month period, from January 18, 1966, to May 19, 1966. The average date for the end of the breeding season and beginning of anestrus, which for convenience is considered as the date of last estrus, was April 9, 1966, for the 20 Columbia ewes.

Since nine ewes were slaughtered during mid-anestrus, only 11 ewes were available for determining the end of the anestrous season. As can be seen in Table 4, the end of the anestrous season had a more definitely defined boundary than the beginning of anestrus. Of the 11 ewes observed, the average date for the end of anestrus was September 3, 1966, with a range of 25 days. The average length of anestrus was 130 days. From the length of anestrus the length of the breeding season was calculated to be approximately 235 days.

Endocrine and Reproductive Structures

Data concerning the ewe reproductive and endocrine structures collected at the time of autopsy are summarized in Table 5, 6 and 7. It can be seen in Table 5 that there is no striking differences in ovarian weight during various stages of the estrous cycle. However,

Table 4. Dates on beginning and end of the anestrous season for individual ewes.

Ewe No.	Date of last estrus, 1966	Date of first estrus, 1966	Length of anestrous season, days
304[a]	3-11-66	------	---
306	4-14-66	9-13-66	142
308[a]	3-19-66	------	---
310[a]	2-17-66	------	---
312	3- 3-66	8-22-66	173
314[a]	4-14-66	-------	---
320	4-29-66	8-22-66	115
322	4- 6-66	8-22-66	138
326	4-14-66	9- 8-66	147
330[a]	4-11-66	------	---
332[a]	5-19-66	-------	---
338	5-19-66	9-10-66	113
342	5- 9-66	8-27-66	109
344	5-10-66	9-17-66	129
348	4-26-66	9-10-66	127
350	4-30-66	9- 4-66	127
352[a]	4-11-66	-------	---
354	3-20-66	9- 7-66	161
358[a]	5- 9-66	-------	---
360[a]	1-18-66	-------	---
Mean:	4- 9-66	9- 3-66	130

[a]Slaughtered during the anestrous season

from Table 6 it can be noted that the mean ovarian weight during the breeding season is higher ($P<.01$) than it is during the nonbreeding season. The ovarian diameter shows no apparent differences within the estrous cycle (Table 5). However, the ovarian diameter is greater ($P<.05$) during the breeding season than during anestrus (Table 6). The diameter measurement seems to be correlated with ovarian weight in that as ovarian weight increases so does the ovarian diameter.

From Table 5 it can be seen that there is a tendency for the largest ovarian follicle diameter to occur on day 1 of the estrous cycle, with a drop in diameter occurring between day 1 and 3. After day 3 there seems to be an increase in diameter up to day 8, at which time follicle diameter plateaus off until day 15. It should be noted that these means have not been statistically analyzed with respect to the stage of estrous cycle due to the lack in numbers of observations at each of the four times. Even though the ovarian follicle diameter appears to be the largest during the breeding season, statistical analysis revealed that it did not differ significantly from that of the anestrous season (Table 6). However, the day 1 ovarian follicle diameter was considerably larger than any follicle diameters during anestrus (Table 5 and 6). Corpora lutea were found to be present only during the breeding season. The average number of visible corpora lutea per ewe was 1.8.

The mean uterine weight during the estrous cycle (Table 5)

indicates that the uterus is enlarged on day 1 of the estrous cycle. The remaining stages are poorly defined by their mean weights, but could be observed as showing a fall in weight after day 1 and leveling off at this level until the next successive estrous cycle. There is definitely a decrease in weight of the uterus during the anestrous season. As is noted in Table 7, the uterine weight is higher ($P<.01$) during the breeding season than during the nonbreeding season.

The mean thyroid weight differences (Table 5) during the estrous cycle are difficult to interpret. However, it does appear that the thyroid weight increases as the estrous cycle progresses. No statistical difference was noted between the thyroid weights of the breeding and nonbreeding season and the adrenal weights show no apparent statistical difference between the breeding season and anestrus (Table 7).

Pituitary weights of the donor ewes during different stages of cyclic activity are summarized in Tables 8 and 9. The whole pituitary wet weight, anterior pituitary wet weight and the anterior pituitary dry weight do not show striking differences among the various stages of the estrous cycle (Table 8). The wet weight of the entire pituitary (Table 9) was greater ($P<.05$) during the anestrous season than during the breeding season. The wet weight of the anterior pituitary was also higher ($P<.05$) during anestrus than during the breeding season. The same trend was observed in the dry weight of the anterior pituitary but there was no statistical

Table 5. Uterine weight, ovarian weight, ovarian diameter, ovarian follicle diameter and thyroid weight observations on donor ewes during different stages of the estrous cycle.

Day of estrous cycle[a]	Uterine[b] wt., gm	Ovarian wt., gm	Ovarian[c] diameter, mm	Largest ovarian follicle diameter, mm	Thyroid wt., gm
1[d]	76.5 ± 1.5[e]	3.22 ± 0.45	19.1 ± 0.5	7.5 ± 0.5	6.0 ± 4.2
3	66.4 ± 5.6	4.46 ± 1.41	18.0 ± 0.9	4.0 ± 0.0	6.4 ± 0.9
8	66.9 ± 0.2	3.67 ± 0.32	21.0 ± 1.8	6.5 ± 0.5	8.1 ± 0.7
15	60.4 ± 5.1	3.78 ± 0.00	20.0 ± 2.0	6.5 ± 0.5	9.4 ± 3.3

[a] Two ewes observed at each of the days noted in table.

[b] Uterine weight includes uterine horns and uterine body (excluding cervix)

[c] Diameter taken from widest part of ovary

[d] Day one of the estrous cycle is considered as the first day estrus is expressed.

[e] Mean ± S. E. of sample mean

Table 6. Ovarian observations on ewes during the breeding and anestrous seasons.

Observation[a]	Season	Mean + S. E.[b]	t value
Total ovarian weight, gm	Breeding	3.8 ± 0.3	3.06**
	Anestrus	2.8 ± 0.2	
Ovarian diameter[c], mm	Breeding	19.5 ± 0.6	2.71*
	Anestrus	17.3 ± 0.5	
Largest ovarian follicle diameter, mm	Breeding	6.1 ± 0.5	1.10
	Anestrus	5.4 ± 0.3	
Number of corpora lutea	Breeding	1.8	
	Anestrus	0.0	

[a]Each observation consists of 8 ewes for each season concerned.

[b, c]See footnote e, b to table 5.

**$P < .01$

*$P \leq .05$

Table 7. Uterine, adrenal and thyroid weights of donor ewes during breeding and anestrous seasons.

Observation[a]	Season	Mean + S. E.[b]	t value
Uterine weight[c], gm	Breeding	68.5 ± 2.6	9.5**
	Anestrus	32.4 ± 2.6	
Adrenal weight, gm	Breeding	4.9 ± 0.3	0.6
	Anestrus	5.2 ± 0.4	
Thyroid weight, gm	Breeding	7.7 ± 0.9	1.8
	Anestrus	9.7 ± 0.6	

[a]See footnote a to table 6.

[b, c]See footnote e, b to table 5.

** $P < .01$

Table 8. Mean pituitary weights of donor ewes during different stages of the estrous cycle.

Day of Estrous Cycle[a]	Whole pituitary wet weight, gm	Anterior pituitary wet weight, gm	Anterior pituitary dry weight, gm
1[b]	1.50[c]	1.37	0.300
3	1.78	1.68	0.416
8	1.52	1.42	0.422
15	1.58	1.46	0.354

[a] See footnote a to table 5.

[b] Day 1 of the estrous cycle is considered as the first day estrus is expressed.

[c] See footnote e to table 5.

Table 9. Pituitary weights of donor ewes during the breeding and anestrous season.

Observation [a]	Season	Mean + S. E. [b]	t value
Whole pituitary wet weight, gm	Breeding	1.59 ± 0.34	2.68*
	Anestrus	2.03 ± 0.12	
Anterior pituitary wet weight, gm	Breeding	1.48 ± 0.11	2.67*
	Anestrus	1.91 ± 0.12	
Anterior pituitary dry weight, gm	Breeding	0.37 ± 0.09	1.67
	Anestrus	0.46 ± 0.03	

[a, b] See footnotes a, b to table 6.

*P < .05

difference between the two seasonal weights.

Pituitary Hormonal Concentration During the Estrous Cycle

Gonadotropic responses in immature, hypophysectomized, female rats to 6 and 12 mg total dosages of pituitary homogenates given over a four day period are summarized in Table 10. The data in Table 10 are averages of the mean responses at the 6 and 12 mg levels. The recipient rats exhibit a peak mean ovarian weight in response to pituitaries from ewes slaughtered on the first day of the estrous cycle. Ewe number 346, which was in a preovulatory state of estrus, exhibited the highest pituitary activity according to recipient rat ovarian weight response of any ewe pituitary assayed. However, ewe number 318, which was in the same day of the estrous cycle but in a postovulatory state, exhibited pituitary activity much lower than ewe 346. The pituitary activity responsible for ovarian weight increase of the recipient rats is at a peak on day 1 of the estrous cycle with activity decreasing on day 3. A trend toward increasing activity continues up until estrus occurs again (Table 10). There appears to be no difference in total gonadotropic pituitary activity at different stages of the estrous cycle as indicated by the oviduct and uterine weight response of the assay rats (Table 10).

The ovarian follicle diameter response of the recipient rats which is indicative of FSH activity of the pituitary is shown in

Table 10. Gonadotropic responses in immature, hypophysectomized, female rats to 6 and 12 mg total dosages of pituitary homogenates from ewes at various stages of the estrous cycle.

Day of estrous cycle	Ewe no.	Number of rats	Ovarian wt., mg	Oviduct and uterine wt., mg	Largest ovarian follicle diameter, μ	Ovarian interstitial tissue[e]
1[a]	346[b]	6 + 6	15.0[d]	30.7	338	5.4
	318[c]	5 + 6	11.5	25.9	376	5.4
average			13.3	28.3	357	5.4
3	316	6 + 6	11.2	28.8	356	4.2
	336	6 + 6	9.9	27.3	352	4.3
average			10.5	28.0	354	4.3
8	334	6 + 6	11.8	28.8	340	5.6
	340	6 + 6	9.6	26.5	321	4.9
average			10.7	27.7	331	5.3
15	328	6 + 6	11.2	32.4	358	5.0
	356	6 + 6	10.8	26.8	348	5.1
average			11.0	29.6	352	5.1

[a] See footnote d to Table 5.

[b] Ewe was in preovulatory state.

[c] Ewe was in postovulatory state.

[d] Mean based on $\frac{\text{mean}_{6\text{ mg}} + \text{mean}_{12\text{ mg}}}{2}$.

[e] Based on numerical scale from 1 to 10 correlated with increasing degrees of interstitial cell stimulation.

Table 10 to be at a peak on day 1 of the estrous cycle. The other three periods studied during the estrous cycle all seem to be slightly lower in pituitary FSH activity than day 1 of the estrous cycle. ICSH activity of the pituitary, as deduced by ovarian interstitial tissue stimulation appears to be at a low on day 3 with the other three stages being higher and comparable to each other (Table 10).

Seasonal Variation in Pituitary Hormonal Concentrations

Gonadotropic responses in immature, hypophysectomized, female rats to 6 and 12 mg doses of pituitary homogenates collected during the breeding and anestrous seasons are summarized in Table 11. The ovarian weight response in the recipient rats, which is an indication of total gonadotropic activity, appears slightly higher during anestrus than during the breeding season (Table 11). However, statistical analysis revealed that ovarian weight responses did not differ significantly between seasons. The oviduct and uterine weight, which is another indication of total gonadotropic activity, also exhibits no statistical difference between seasons.

The ovarian follicle diameter of the recipient rats is an indication of FSH activity in the pituitary of the donor ewe. From Table 11 it can be seen that the FSH activitity in the pituitary is higher ($P<.05$) during anestrus than during the breeding season. The interstitial tissue responses are identical between the breeding season and

Table 11. Gonadotropic responses in immature, hypophysectomized, female recipient rats to pituitary homogenates from ewes during the breeding season and anestrus.

Observation[a]	Season	Mean[b]	F value
Ovarian weight, mg	Breeding	11.3	2.11
	Anestrus	12.1	
Oviduct and uterine weight, mg	Breeding	28.4	0.34
	Anestrus	28.9	
Follicle diameter, μ	Breeding	348.0	4.21*
	Anestrus	365.0	
Interstitial[c] tissue repair	Breeding	5.0	0.00
	Anestrus	5.0	

[a] Each observation contains data from 8 ewe pituitaries, dosages were at 6 mg and 12 mg levels, the data from both levels were pooled $\left(\frac{\text{mean}_{6\text{ mg}} + \text{mean}_{12\text{ mg}}}{2}\right)$

[b] See footnote d to table 10

[c] See footnote e to table 10

* $P < .05$

Table 12. Nongonadal responses in immature, hypophysectomized, female recipient rats to pituitary homogenates from ewes during the breeding season and anestrus.

Observation[a]	Season	Mean Response[b]	F value
Body weight gain[c] gm	Breeding	8.4	.64
	Anestrus	7.5	
Tibial epiphyseal width, μ	Breeding	262.0	1.64
	Anestrus	252.0	
Adrenal weight, mg	Breeding	10.2	.30
	Anestrus	10.0	
Thyroid weight, mg	Breeding	7.5	.20
	Anestrus	7.7	

[a] See footnote a to table 11.

[b] See footnote d to table 10.

[c] Gain made between the time of the first injection of test material and autopsy.

anestrus (Table 11).

Nongonadotropic activities of the pituitaries are shown in Table 12. Body weight and epiphyseal width responses show no statistical difference between seasons. Pituitary activity associated with adrenal weight and thyroid weight responses of the assay rats were not significantly different between seasons.

Gonadotropic Activity of Pooled Pituitaries

This next phase of the experiment (Table 13) was designed to determine gonadotropic activity of pooled pituitaries collected from ewes during the breeding season and anestrus at much higher dosages (100 and 200 mg total doses over 4 days). Because of the lack of pituitary tissue, all tissue remaining after the previous assay was pooled into two respective groups according to season. Amounts of pituitary tissue which were pooled from each ewe varied from 15 to 180 mg.

The ovarian weight response is higher during the anestrus season at both the 100 and 200 mg doses than during the breeding season. However, the oviduct and uterine weight responses in the assay rats were the same for anestrus and the breeding season at both 100 and 200 mg doses.

Histological examination of the rat ovaries demonstrated a higher FSH activity in the pooled pituitaries during the anestrous season. The mean follicle diameter is higher at both dosages during

anestrus than during the breeding season. Interstitial tissue response is essentially the same during the breeding season and anestrus.

Table 13. Gonadotropic responses in female hypophysectomized rats to pooled pituitary homogenates collected during the breeding and nonbreeding seasons.

Season	Total[a] Dosage, mg	Ovarian wt, mg	Oviduct and uterine wt, mg	Follicle diameter, μ	Interstitial tissue
Breeding	100	13.4[b]	38.7	381	7.0[c]
	200	15.5	44.6	447	7.3
Anestrus	100	14.4	38.4	406	7.0
	200	20.8	45.7	523	7.7

[a] Three recipient rats were injected at each dose level.

[b] Mean of sample.

[c] See footnote e to table 10.

V. DISCUSSION

Length of Estrous Cycles, Breeding Season and Anestrus

The average length of the estrous cycle in the 28 Columbia ewes studied in this experiment was 20.6 days; however, when extremely long estrous cycles (over 24 days) were omitted, the average cycle length was 16.8 days. Estrous cycles that were approximate multiples of one estrous cycle were a result of either a failure of the ewe to express estrus or of the method for estrus detection. Williams et al. (1956), using vasectomized rams as a means of detecting estrus in ewes, noted that approximately 16% of all estrous cycles were longer than 19 days in length. McKenzie and Terrill (1937), Underwood, Shier and Davenport (1944), Barker and Wiggens (1959) and Santolucito, Clegg and Cole (1960) found that the average length of estrous cycles with the extremely long cycles excluded was 17 days. In the present study estrous cycles greater than 24 days in length occurred more often toward the end of the breeding season than at the beginning. It was also noted that long estrous cycles occur in certain ewes at a higher frequency than in others. In accordance with above data, Hafez (1952) had also noted more "silent heats" in the second half of the breeding season than during the first half.

The initiation of the breeding season in all Columbia ewes

studied was within a range of 25 days. The average date for the beginning of the breeding season was found to be September 3. This is approximately the same time in which Corridale, Karakual and crosses of these two breeds start their sexual season (Schott, Phillips and Spencer, 1939). Most mutton breeds start their breeding season from mid-September to the beginning of October (Schott, Phillips and Spencer, 1939).

Since the present study was initiated during the breeding season in 1965, and was continued until approximately the same stage of the breeding season in 1966, it was possible to calculate the length of the breeding season by subtracting the length of anestrus from 365. The average length of the breeding season was calculated to be 235 days or 14 estrous cycles long. According to Hafez (1950), this is a prolonged breeding season quite similar to that observed in the Dorset Horn sheep. The prolonged breeding season observed in the Columbia and Dorset Horn sheep probably indicates that both breeds originated partly from breeds introduced from Spain. It is known that the Columbia breed originated from a Lincoln x Rambouillet cross. The Rambouillet, a descendent of the Merino breed, partially explains the prolonged breeding season expressed in the Columbia breed. Another factor contributing to the long breeding season in this specific case could also be due to the continual presence of the vasectomized rams. It has been shown by various workers that the

presence of a ram will initiate sooner and extend longer the breeding season of the ewe (Shinchel, 1954; Riches and Watson, 1954).

The present study indicates that the beginning of anestrus is not as well defined as the beginning of the breeding season. The dates of last observed estrus in the Columbia ewes ranged over a four month period with the average date for the beginning of anestrus being April 9. Thus, the average length of anestrus was 130 days. This variation in onset of anestrus could again be partially due to the presence of the vasectomized rams acting as an external stimulus causing ewes which might have otherwise stopped cyclic activity, to return to heat. This in turn might be a partial explanation for the increase in extended estrous cycles occurring during the latter half of the breeding season. McKenzie and Terrill (1937) have also noted in ewes a large variation in the last occurring estrus of the breeding season.

Variation in Reproductive and Endocrine Structures of the Ewe

The wet weight of the whole pituitary and the wet weight of the anterior pituitary were found heavier during the anestrous season than during the breeding season. Although the same trend was indicated with respect to anterior pituitary dry weight, it was not statistically different. One factor which may have an influence on the weight of the pituitary is differences in circulating levels

of progesterone activity in the system associated with the different seasons. It has been demonstrated by Van Rees (1959) that the weight of the anterior pituitary decreases under the influence of large dosages of progesterone. During the breeding season, the progestogen activity would be expected to be higher due to the presence of corpora lutea, while during anestrus, corpora lutea are not formed, and thus, the progestogen level in circulation should be lower.

There was no striking variation in mean ovarian weight or ovarian diameter observed within the estrous cycle. This is in agreement with findings of McKenzie and Terrill (1937) who demonstrated that no significant trends were found in the changes of size of the ovaries during the breeding season. Mean ovarian weights and ovarian diameters were shown to be significantly higher during the breeding season than during anestrus. This tendency could be due to a lack of sufficient amounts of gonadotropins being released from the pituitary during the anestrus season. To further clarify this point, other ovarian changes seem helpful. The follicle diameter of the ewe ovaries during the two seasons was not significantly different. However, there was a tendency for larger follicles to occur during the breeding season. The FSH in circulation of the ewes as indicated by their ovarian follicular activity was noted to be at a high on day 1 of the estrous cycle. A decline in FSH activity was noted on day 3 which increased again on day 8 and leveled off

until the beginning of the next estrous cycle.

The present study shows that the uterus of the anestrous ewe is distinctly in a regressive state as compared to the uterus during the breeding season. Kammlade et al. (1952) have described the uterine epithelium during anestrus to be of the castrate type. It is interesting to note that the uterine weight of the ewes regresses linearly starting at day 1 of the cycle until a low is reached on the fifteenth day. This decrease in uterine weight is no doubt associated with declining estrogenic activity as the estrous cycle progresses. The estrogen activity may be traced back to the release of FSH and ICSH in the proper quantity (Martini et al., 1959). There were no significant differences noted in adrenal or thyroid weights with respect to season. No changes were observed in thyroid weights within the estrous cycle.

Pituitary Activity During the Breeding Season and Anestrus

In determining gonadotropic hormone levels of the anterior pituitary, many researchers have used techniques which allow only total gonadotropic activity to be detected. In this study, by utilizing hypophysectomized, immature, female rats as assay animals, pituitary levels of total gonadotropic activity were quanitated by the ovarian and uterine weight responses. Histological study of the ovaries of the assay animals allowed for quantitative determination

of FSH and ICSH (Evans et al., 1939; Simpson, Li and Evans, 1942).

The total gonadotropic potency of the anterior pituitary as indicated in this study was at a high during day 1 of the estrous cycle with a decrease in activity taking place between day 1 and day 3 or after ovulation. Between day 3 and day 8, activity again increased and leveled off until the next estrous cycle. These findings are somewhat contrary to the findings of Kammlade et al. (1952); Santolucito, Clegg and Cole (1960) who all state that gonadotropic activity of the pituitary drops during day 1 of the estrous cycle or during ovulation. Ewe 346 was in a preovulatory state when slaughtered on day 1, which partially explains the high gonadotropic activity demonstrated on day 1 of the estrous cycle. Ewe 318 was in a postovulatory state and showed a low pituitary gonadotropic activity on day 1. This conflict in data with previous authors may actually not be in disagreement but a difference due to the fact that in this study one ewe on day 1 was in a preovulatory state, thus, showing pituitary gonadotropic activity at its highest peak prior to ovulation. There is a substantial drop in pituitary total gonadotropic activity after ovulation as noted by day 3 rat ovarian weight responses. This indicates the pituitary might be releasing gonadotropins soon after or during the time of estrus. Pituitary FSH activity as indicated by follicle diameter of the assay animals seems to be at the same concentration throughout the estrous cycle. Interstitial tissue stimulation, which

is the basis of an assay for ICSH, shows a drop between day 1 and day 3. Santolucito, Clegg and Cole (1960) found that the ICSH content of ewe pituitaries declines significantly between the fourth and thirty-fifthhour after estrus, followed by an increase in ICSH activity as the cycle advances. They interpreted the drop at this time to be associated with pituitary release of ICSH.

Seasonal differences in gonadotropic potency of the pituitary were quite evident from data collected in this study. The ovarian weight differences of the recipient animals at all dosages (6, 12, 100 and 200 mg) indicated more gonadotropic activity in the pituitary during anestrus than during the breeding season. The ovarian weights of the assay rats also indicated less variation in gonadotropic activity during anestrus than during the breeding season. These findings are in agreement with data obtained by Kammlade et al. (1952); Raeside and Lamond (1956). This indicates that there is probably not a cyclic change, at least not to the extent observed in an estrous cycle, in hormonal release from the pituitary of the anestrous ewe as postulated by some workers.

In all studies performed, pituitary FSH activity was higher during anestrus than during the breeding season, as noted by ovarian follicle diameter of the assay rats. The interstitial cell stimulation and oviduct and uterine weight responses in the recipient animals were found to be identical during the two seasons, thus indicating

ICSH content of the pituitary remains constant between seasons. The pituitary assays were based on absolute weight and not percent weight of the pituitary. Since the pituitaries were significantly larger during the anestrous season, the total gonadotropic concentration of the pituitary during this period could possibly be higher than indicated by the results of this study.

The present paper has presented results obtained from a series of assays concerned primarily with determining FSH and ICSH levels in the pituitary of the ewe at precise stages of the estrus cycle and anestrus. An attempt will now be made to correlate these findings with conditions of the reproductive structures.

Assuming that the pituitary content of any hormone is the algebraic sum of synthesis, storage and liberation, it is reasonable to believe that pituitary content would not necessarily have to reflect secretory activity under all conditions (Ellington, 1963). An indication as to which of the three above processes the pituitary is exhibiting is possible by comparing a rise or fall in gonadotropic activity of the pituitary to changes in physiological functions which are under gonadotropic control.

After ovulation, the ICSH content of the pituitary is low, and this is believed associated with secretion (Santolucito, Clegg and Cole, 1960). The average ICSH content during the breeding season and anestrus is the same, while several changes occur in the

reproductive organs of the ewe between the breeding season and anestrus. In anestrus, ovulation does not occur, the uterus regresses, and the ovary decreases in size. These findings, in conjunction with ICSH activity of the pituitary indicate that amounts of ICSH released into circulation during anestrus are not sufficient to carry out the physiological occurrences of the breeding season. This information could be interpreted as implying the pituitary is maintaining a relatively constant storage of ICSH and probably releasing little if any into circulation.

Data in this report confirm the findings of Robertson and Hutchinson (1958) and Santolucito, Clegg and Cole (1960) that not only do ICSH levels but also FSH levels of the pituitary decline over the period of estrus. The previous authors interpret these findings as indicating that both FSH and ICSH are important in ovulation.

The FSH content of the pituitary during the anestrous season is higher than during the breeding season while the ewe ovarian follicle size prior to ovulation is larger during the breeding season than at any time during anestrus. This may indicate an increase synthesis of FSH during anestrus while release of FSH remains constant during both seasons. Increased synthesis and storage of hormones in the pituitary during anestrus may also account for the increased size of the pituitary during this period.

It might be questioned why blood collecting techniques such as those developed by McFarland, Clegg and Ganong (1960) and Folz,

Johnson and Nelson (1966) were not employed in this study to determine gonadotropic activity in circulation. By using one of the above mentioned blood collecting techniques, it has been shown possible to determine FSH and ICSH activity in gonadectomized animals. Granted data concerning gonadotropic activity in circulation would clearly answer many questions. However, one link is missing yet in using these procedures in combination with intact ewes. Preliminary studies with plasma indicated that a major problem was being able to concentrate the plasma sufficiently to allow detection of the low level of gonadotropic activity without having so much protein as to be toxic to the assay animals. There has been some work done with fractionated plasma and Sephadex gel filtration on intact ewe plasma, but a good method of concentrating plasma from intact ewes in high enough quantities to detect gonadotropic activity is lacking. It is evident that until a satisfactory technique is developed to determine gonadotropic activity in the plasma of intact ewes, researchers must continue to study the pituitary activity in conjunction with other physical features in order to further understand mechanism controlling reproduction.

VI. SUMMARY

Twenty-eight Columbia ewes were made available for a study concerned with the characterization of their breeding and anestrous seasons. Results were obtained from a series of assays concerned primarily with gonadotropic activity of the pituitary of 16 of the ewes during various phases of cyclic activity. The gonadotropic activity of the pituitary was discussed in relation to conditions of the reproductive and other endocrine structures. Dates and length of cyclic activity were also noted.

Pituitaries were assayed in hypophysectomized, immature female rats. It was seen that ICSH activity was lowest in the pituitary after ovulation, whereas changes in FSH activity during the estrus were not immediately apparent. However, it was shown that ovarian follicle diameter in the ewe is at a high during estrus and drops immediately after estrus. In contrast to gonadal responses of the assay animals, no marked trends were established with respect to tail length, epiphyseal width, adrenal weight, thymus weight and thyroid weight responses.

During the anestrous season, the pituitaries were higher in gonadotropic activity on the average than during the breeding season. The ICSH activity of the pituitary was shown to remain the same regardless of season. However, the FSH activity was higher during anestrus than during the breeding season. The ovarian follicle

diameter of the ewes during anestrus was comparable in diameter to those during the breeding season, indicating that FSH was being secreted during anestrus. However, preovulatory follicle diameter during the breeding season seemed to be larger than at any time during anestrus. The lack in size of the ovary and regression of the uterus indicate that ICSH was lacking in circulation. One might explain the cause of anestrus from these findings as a lack of substantial ICSH being secreted into circulation to induce ovulation and estrogen production.

In contrast to the gonadal and pituitary weight differences between the ewes of the two seasons, no marked differences were noted in the adrenal weights or thyroid weights. There were no differences with respect to tail length, epiphyseal width, adrenal weight, thymus weight and thyroid weight reponses of the assay animals injected with ewe pituitary tissue obtained during anestrus and the breeding season.

The length of the corrected estrous cycles was found to average 16.8 days. The length of the breeding season for this group of Columbia ewes was found to be approximately 33 weeks, which is considered an extremely long breeding season for most sheep breeds in the United States.

BIBLIOGRAPHY

Armstrong, D. T. and W. Hansel. 1959. Alteration of the bovine estrous cycle with oxytocin. Journal of Dairy Science 42:533-542.

Barker, Hal B. and Earl L. Wiggins. 1959. Estrual activity in open Rambouillet ewes. (Abstract) Journal of Animal Science 18:1547-1548.

Bassett, E. G., O. K. Sewell and E. P. White. 1954. Sex hormone studies on sheep. New Zealand Journal of Science and Technology 36:437-449.

Bell, Donald T. 1941. Production of heat and ovulation in the anestrous ewe. Journal of Agricultural Research 62:619-625.

Bell, O. T., L. E. Casida and A. E. Darlow. 1941. Effects of estrogen and progesterone upon the genital tract of the ewe. Endocrinology 28:441-449.

Bloom, William and Don W. Fawcett. 1962. A textbook of histology. Philadelphia, W. B. Saunders Company. 720p.

Braden, A. W. H. and G. R. Moule. 1964. Effects of stress on ovarian morphology and oestrous. Australian Journal of Agricultural Research 15:937-949.

Briggs, H. M. et al. 1942. The influence of nutrition on the reproduction of ewes. Stillwater. 30p. (Oklahoma. Agricultural Experiment Station. bulletin no. B-225)

Casida, L. E. and Fred F. McKenzie. 1932. The oestrus cycle of the ewe; Histology of the genital tract. Columbia. 28p. (Missouri. University. Agricultural Experiment Station. Research bulletin no. 170)

Clegg, M. T. and W. F. Ganong. 1960. The effect of hypothalamic lesions on ovarian function in the ewe. Endocrinology 67: 179-180.

Cole, H. H., G. H. Hart and R. F. Miller. 1945. Studies on the hormonal control of estrous phenomena in the anestrous ewe. Endocrinology 36:370-380.

Cole, H. H. and R. F. Miller. 1935. Changes in the reproductive organs of the ewe with some data bearing on their control. American Journal of Anatomy 57:39-97.

Cooper, Gordon Jay. 1967. Estrogen regulation of pituitary function in the gonadectomized sheep. Master's thesis. Corvallis, Oregon State University. 90 numb. leaves.

Darlow, A. E. and L. E. Hawkins. 1932. The influence of nutrition on the oestrous cycle in the ewe. In: Proceedings of the American Society of Animal Production, 25th annual meeting, p. 173-176.

de Baca, R. C. et al. 1954. Factors associated with the onset of estrus in ewes. Corvallis. 29p. (Oregon. Agricultural Experiment Station. Technical bulletin 29)

Dierschke, Donald F. and M. T. Clegg. 1965. Gonadotropins in anterior pituitary and cavernous sinus serum of cycling ewes. (Abstract) Federation Proceedings 24:321.

Dutt, R. H. 1953. Induction of estrus and ovulation in anestrual ewes by use of progesterone and pregnant mare serum. Journal of Animal Science 12:515-523.

Dutt, R. H. and Leon F. Bush. 1955. The effect of low environmental temperature on initiation of the breeding season and fertility in sheep. Journal of Animal Science 14:885-896.

Dutt, R. H. and L. E. Casida. 1948. Alteration of the estrual cycle in sheep by use of progesterone and its effect upon subsequential ovulation and fertility. Endocrinology 43: 208-217.

Dutt, R. H., Earl F. Ellington and W. Carlton. 1959. Fertilization rate and early embryo survival in sheared and unsheared ewes following exposure to elevated air temperature. Journal of Animal Science 18:1308-1318.

Edgar, D. G. 1953. Progesterone secretion in ewes. (Abstract) Nature 173:639-640.

Edgar, D. G. 1958. The induction of ovulation and oestrus in ewes during anoestrus. Proceedings of the New Zealand Society of Animal Production 18:97-98.

Ellington, E. F. 1963. Discussion on levels of gonadotropins. In: The sixth animal reporductive symposium on gonadotropins, their chemical and biological properties and secretory control, Oregon State University, Corvallis, 13 August, 1963, ed. by H. H. Cole. San Francisco, S. H. Freeman. p. 108-112.

Ellington, E. F., A. N. Contopoulos and M. T. Clegg. 1962. Pituitary gonadotropic and growth hormone levels in sheep plasma. Proceedings of the Society for Experimental Biology and Medicine 110:704-707.

Ellington, E. F., A. N. Contopoulos and M. T. Clegg. 1964. Progesterone regulation of the production and release of pituitary gonadotrophins in gonadectomized sheep. Endocrinology 75:401-410.

Evans, H. N. et al. 1939. Biological studies of the gonadotropic principles in sheep pituitary substance. Endocrinology 25: 529-546.

Everett, J. W. 1948. Progesterone and estrogen in the experimental control of ovulation time and other features of the estrous cycle in the rat. Endocrinology 43:389-405.

Everett, J. W. 1961. The mammalian female reproduction cycle and its controlling mechanisms. In: Sex and internal secretions, ed. by W. C. Young. 3d ed. Vol. 1. Baltimore, Williams and Wilkins. p. 497-555.

Foltz, F. M., D. C. Johnson and D. M. Nelson. 1966. Methods for obtaining the venous outflow from the hypothalamus and hypophysis. Proceedings of the Society for Experimental Biology and Medicine 112:223-227.

Frank, R. T. 1940. The sex hormones: their physiological significance and use in practice. Journal of the American Medical Association 114:1504-1512.

Godley, W. C., R. L. Wilson and Victor Hurst. 1961. Effect of light, temperature, and hormones on the reproductive performance of ewes. Journal of Animal Science 20: 693-698.

Godley, W. C., R. L. Wilson and Victor Hurst. 1966. Effect of controlled environment on the reproductive performance of ewes. Journal of Animal Science 25:212-216.

Grant, R. 1934. Studies on the physiology of reproduction in the ewe. Transactions of the Royal Society of Edinburgh 58:1-47.

Greenspan, F. S. et al. 1949. Bioassay of hypophyseal growth hormone: the tibia test. Endocrinology 45:455-463.

Greep, R. O. 1961. Physiology of the anterior hypophysis in relation to reproduction. In: Sex and internal secretions ed. by W. C. Young. 3d ed. Vol. 1. Baltimore, Williams and Wilkins. p. 240-301.

Guillemin, R., M. Jutisz and E. Sakiz. 1963. Purification partielle d'un facteur hypothalamque (LRF) stimtant la secretion de l'hormone hypophysaire de luteinisation (LH). Comptes Rendus de l'Academi des Sciences, Paris 256:504-507.

Hafez, E. S. E. 1950. Sexual season of the ewe and daylight environment. Nature 166:822-823.

Hafez, E. S. E. 1951. The influence of environment and heredity on the breeding season of the ewe. Experientia 79:353-354.

Hafez, E. S. E. 1952. Studies on the breeding season and reproduction of the ewe. Journal of Agricultural Science 46:13-265.

Hafez, E. S. E. and T. Sugie. 1963. Behavioural oestrus and ovulatory cycle in beef cattle with a note on the clay model techniques. Acta Zoologica 44:57-71.

Hammond, J. 1927. The physiology of reproduction in the cow. Cambridge, University Press. 226p.

Hammond, J., Jr. 1944. On the breeding season in the sheep. Journal of Agricultural Science 34:97-105.

Hanstome, Bertil. 1966. Gross anatomy of the hypophysis in mammals. In: The pituitary gland, ed. by G. W. Harris and B. T. Donovan. Vol. 1. Berkeley, University of California, p. 2-51.

Hart, D. S. 1950. Photoperiodicity in Suffolk sheep. Journal of Agricultural Science 40:143-149.

Hinds, F. C., P. J. Dziuk and O. M. Lewis. 1964. Control of estrus and lambing performance in cycling ewes fed 6-methyl-17-acetoxyprogesterone. Journal of Animal Science 23: 782-786.

Hutchinson, J. S. M. and Hamish Robertson. 1960. Effect of season on the follicle stimulating hormone and luteinizing hormone potency of sheep anterior pituitary glands. Nature 188: 585-586.

Kammlade, W. G. et al. 1952. Pituitary activity of sheep in relation to the breeding season. Journal of Animal Science 11:646-655.

Kawakami, M. and C. H. Sawyer. 1959. Neuroendocrine correlates of changes in brain activity thresholds by sex steroids and pituitary hormones. Endocrinology 65:652-668.

Lamond, D. R., H. M. Radford and A. L. Wallace. 1959. Bioassay of sheep anterior pituitary glands. Nature 183:1597-1598.

MacGillivray, A. J. and H. A. Robertson. 1963. Total gonadotropic activity in the urine of the ovariectomized, normal and pregnant ewe and in that of the ram. Journal of Endocrinology 26:125-132.

Martini, L. et al. 1959. Neurohypophysial hormones and release of gonadotrophins. Journal of Endocrinology 18:245-250.

McDonald, P. G. and M. T. Clegg. 1966. Some factors affecting gonadotropin levels in sheep. Proceedings of the Society for Experimental Biology and Medicine 121:482-485.

McFarland, L. Z., M. T. Clegg and W. F. Ganong. 1960. Concentration of ACTH in cavernous sinus and peripheral blood collected from unanesthetized sheep. Proceedings of the Society for Experimental Biology and Medicine 103:538-539.

McKenzie, Fred F. 1926. The normal estrous cycle in the sow. Columbia. 41p. (Missouri. Agriculture Experimental Research. Bulletin no. 86)

McKenzie, Fred F. and Clair E. Terrill. 1937. Estrus, ovulation and related phenomena in the ewe. Columbia 88p. (Missouri. Agricultural Experiment Station. Research bulletin no. 264)

Milne, J. A. 1963. Effects of oxytocin on the oestrous cycle of the ewe. Australian Veterinary Journal 39:51-52.

Monge, Carlos. 1943. Chronic mountain sickness. Physiological Reviews 23:166-184.

Radford, H. M., R. H. Watson and G. F. Wood. 1960. A crayon and associated harness for the detection of mating under field conditions. Australian Veterinary Journal 36:57-66.

Raeside, J. T. and D. R. Lamond. 1956. Effects of progesterone and pregnant mare serum (PMS) administration in the anestrus ewe. Australian Journal of Agricultural Research 7:591-600.

Riches, J. H. and R. H. Watson. 1954. The influence of the introduction of rams on the incidence of oestrus in Merino ewes. Australian Journal of Agricultural Research 5:141-147.

Robertson, Hamish and J. S. M. Hutchinson. 1958. Gonadotropic hormone levels of the pituitary gland of the ewe during the reproductive cycle. Acta Endocrinologica Supplementum 38:55.

Robertson, H. A. and J. S. M. Hutchinson. 1963. The levels of FSH and LH in the pituitary of the ewe in relation to follicular growth and ovulation. Endocrinology 24:143-151.

Robertson, H. A., A. J. MacGillivray and J. S. M. Hutchinson. 1963. Gonadotropins of pituitary origin in the urine of sheep after castration and ovariectomy. Acta Endocrinology 42:147-152.

Robinson, G. E., Jr. and A. V. Nalbandov. 1951. Changes in hormone content of swine pituitaries during the estrual cycle. Journal of Animal Science 10:469-478.

Robinson, T. J. 1950. The control of fertility in sheep. Part I. Hormonal therapy in the induction of pregnancy in the anestrous ewe. Journal of Agricultural Research 5:730-736.

Robinson, T. J. 1951. Reproduction in the ewe. Cambridge Philosophical Society, Biological Reviews 26:121-157.

Robinson, T. J. 1954. Fertility of anoestrous ewes following injection of progesterone and pregnant mare serum (PMS). Australian Journal of Agricultural Research 5:730-736.

Robinson, T. J. 1959. The estrous cycle of the ewe. In: Reproduction in domestic animals, ed by H. H. Cole and P. T. Cupps. Vol. 1. New York, Academic Press. p. 291-333.

Santolucito, J. A., M. T. Clegg and H. H. Cole. 1960. Pituitary gonadotropins in the ewe at different stages of the estrous cycle. Endocrinology 66:273-279.

Sawyer, Charles H., J. E. Markee and John W. Everett. 1950. Activation of the adenohypophysis by intravenous injections of epinephrine in the atropinized rabbit. Endocrinology 46:536-543.

Schinchel, P. G. 1954. The effect of the presence of the ram on the ovarian activity of the ewe. Australian Journal of Agricultural Research 5:465-469.

Schott, R. G., R. W. Phillips and D. A. Spencer. 1939. The occurrence of estrus in sheep and its relation to extra-seasonal production of lambs. In: Proceedings of the American Society of Animal Production, 32nd annual meeting, p. 347-353.

Shelton, Maurice and John T. Morrow. 1965. Effect of season on Rambouillet ewes. Journal of Animal Science 24:795-799.

Shibusawa, K. 1955. Neurosecretion of oxytocin stimulates the release of the pituitary gonadotropin. Endocrinology (Japan) 2:183-188.

Simpson, M. E., C. H. Li and H. M. Evans. 1942. Comparison of methods for standardization of pituitary interstitial-cell-stimulating hormone (ICSH). Endocrinology 30:977-984.

Smith, I. D. 1964. Postparturient anoestrus in the Peppin Merino in Western Queensland. Australian Veterinary Journal 40: 199-201.

Smith, I. D. 1966. The onset of the breeding season in Southdown ewes in subtropical Australia. Journal of Agricultural Science 66:295-296.

Sykes, J. F. and C. L. Cole. 1944. Modification of mating season in sheep by light treatment. Quarterly Bulletin of the Michigan Experiment Station 26:250-252.

Underwood, E. J., F. L. Shier and N. Davenport. 1944. The breeding season of Merino, crossbred and British breed ewes in the agricultural districts. Journal for the Department of Agriculture of West Australia 21:135-143.

Van Rees, G. P. 1959. The effect of progesterone on the ICSH and FSH content of anterior pituitary and blood serum. Acta Physiologica et Pharmacologi Neerlandica 8:180-210.

Warbritton, Virginia and Fred F. McKenzie. 1937. The pituitary glands of ewes in various phases of reproduction. Columbia. 59p. (Missouri. University. College of Agriculture. Research bulletin no. 257)

Warwick, E. J. 1946. Gonadotropic potency of ewe pituitary glands as affected by spaying, season and breed. Proceedings of the Society for Experimental Biology and Medicine 63:530-533.

Watson, R. H. and H. M. Radford. 1960. The influence of rams on onset of oestrus in Merino ewes in spring. Australian Journal of Agricultural Research 11:65-67.

Williams, S. M. et al. 1956. Variations in the length of estrous cycles and the breeding season in ewes. Journal of Animal Science 15:984-989.

Wilson, R. L., W. C. Godley and Victor Hurst. 1961. Effect of light, temperature and hormones on the reproductive performance of ewes. Journal of Animal Science 20:693-697.

Yeates, N. T. M. 1949. The breeding season of the sheep with particular reference to its modification by artificial means using light. Journal of Agricultural Science 39:1-43.

Yeates, N. T. M. 1965. Modern aspects of animal production. Washington, D. C. Butterworths. 371p.

Zarrow, M. X., J. M. Yochim and J. L. McCarthy. 1965. Experimental endocrinology, a sourcebook of basic techniques. New York, Academic Press. 519p.