THE LIFE SPAN OF INITIALLY AND EXPERIMENTALLY INDUCED CORPORA LUTEA OF PSEUDOPREGNANT AND HYSTERECTOMIZED-PSEUDOPREGNANT RABBITS FOLLOWING HCG, LH AND ESTRONE TREATMENT

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INTRODUCTION

Artificial insemination, ova transfer and the need for greater labor efficiency reflect the importance of successfully controlling the estrous cycle. Successful estrous synchronization depends on an understanding of the mechanisms associated with corpora lutea maintenance and regression. Hypophysectomy, partial hysterectomy and induction of accessory corpora lutea have recently revealed many facts concerning corpora lutea life span and have opened new avenues for study. This experiment was designed to study factors influencing the life span of rabbit corpora lutea.

Corpora lutea were produced at various stages of pseudopregnancy and the life span of the different ages of corpora lutea was determined. Corpora lutea 5 days or older regressed immediately as a result of human chorionic gonadotropin (HCG) injection. FSH and LH, FSH alone or LH alone was given to determine the component of HCG which caused the original corpora lutea to regress. Miscellaneous groups involving atropine sulfate, copper acetate and low levels of LH were employed in a limited number of rabbits in an attempt to determine whether ovulation is necessary for HCG or LH to provoke luteal regression.

Estrogen was given surrounding HCG administration in an attempt to block regression caused by HCG or LH and the life span of both sets of corpora lutea was observed. Since estrogen was found to prevent regression of corpora lutea caused by HCG, an antiestrogenic compound was employed in an attempt to obtain additional evidence for an estrogen-LH interaction involved in the luteal regressive mechanism. Also, estrogen determinations by thin layer chromatography were attempted on ovarian tissue free of corpora lutea and on corpora lutea of does two days following HCG injection.

HCG was further tested by injecting it into pseudopregnant-hysterectomized does. Does hysterectomized 3 days following or 10 days prior to induction of pseudopregnancy served as controls. The two control groups also served to test the equality of life span of corpora lutea induced before and after hysterectomy.

REVIEW OF LITERATURE

Progesterone block of the hypophysis is established by 48 hours post-injection (Foote et al., 1958). This block established immediately following ovulation will not interfere with normal development and maintenance of gilt or guinea pig corpora lutea (Sammelwitz et al., 1961; Brinkley and Nalbandov, 1961; Brinkley et al., 1964a, b; Aldred et al., 1959, 1961). Hypophysectomy just prior to ovulation in the gilt (du Mesnil du Buisson and Leglise, 1963) and ewe (Denamur and Mauleon, 1963a, b), immediately following ovulation in the guinea pig (Dempsey, 1937; Perry and Rowlands, 1962) or one hour after mating in the rabbit (Fee and Parkes, 1929; Deanesly et al., 1930; Smith and White, 1931; Firor, 1933; White and Leonard, 1933) will allow normal corpora lutea development and maintenance for a time approximating a cycle length. This is assuming that normal life span of the corpora lutea in the rabbit, without naturally occurring pseudopregnancy, is about 4 days. A higher dosage of hormones is needed to produce ovulation during the first 4 days of pregnancy or pseudopregnancy (Snyder and Wislocki, 1931). This fact along with the failure of luteinizing hormone as discussed in the present paper and of hypophysectomy to produce corpora lutea regression until after day 4 indicates a possible 4 day luteal life span in the rabbit. This assumption suggests that the 4 day luteal life span would persist if naturally occurring pseudopregnancy could be prevented.

Progesterone blockage and hypophysectomy data indicate that if a hypophyseal luteotropic substance is necessary for maintaining corpora lutea of the cycle, it is released prior to or simultaneous with luteinizing hormone (Nalbandov, 1961; Brinkley et al., 1964a). Unless luteinizing hormone is luteotropin, it is difficult to visualize an initial shot of luteotropin being responsible for maintaining corpora lutea of the cycle since Kilpatrick et al. (1964) produced ovulation and corpora lutea formation in the rabbit by injecting luteinizing hormone two hours after hypophysectomy. Thus, in many species ovulation alone, or luteinizing hormone associated with ovulation, may be the only factor necessary for development and maintenance of the corpora lutea of the 4 day cycle. This suggests that there are two maintenance factors, the cyclic maintenance factor associated with ovulation and a second continuously released factor, luteotropin, which may be initially released near the end of the cycle if pregnancy or hysterectomy occurs.

Regression of corpora lutea at the end of the cycle may result from depletion of the cyclic maintenance factor since corpora lutea of the hypophysectomized ewe, sow and rabbit regress following a normal interestrual life span. Depletion of the cyclic maintenance factor in the gilt is supported by asynchronous regression of two sets of corpora lutea, the second set being introduced about 11 days following spontaneous ovulation (Neill and Day, 1964). However, if additional corpora lutea are induced on day 5, 9 or 13 post-ovulation in the ewe, synchronous regression with the naturally occurring corpora lutea results. The induced corpora lutea regress at the end of the normal estrous cycle even if the naturally occurring corpora lutea are excised two days following induction of the second set (Inskeep et al., 1963). One may postulate that there is either a luteolytic factor causing regression of

the second set of corpora lutea or a lack of sufficient maintenance stimulus to cause the induced corpora lutea to become fully functional.

Hypophysectomy on day 2 or 5 in the guinea pig will allow corpora lutea development and morphological maintenance well past normal estrual cycle length, but if hypophysectomy is performed on day 10 corpora lutea regress following their normal interestrual life span (Perry and Rowlands, 1962). Synchronous regression of two sets of corpora lutea in the ewe and the data on hypophysectomized guinea pigs may indicate a luteolytic mechanism, possibly mediated through the uterus. The mechanism, however, appears to be effective only after depletion of the maintenance factor for corpora lutea of the cycle.

Few means of regressing corpora lutea of the estrual cycle have been found. Regressive changes in bovine corpora lutea are brought about by injections of oxytocin on days 3 through 6 post-estrus (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Simmons and Hansel, 1962). Oxytocin injections are less effective in shortening estrous cycles if given concurrently with atropine (Armstrong and Hansel, 1959; Black et al., 1963) or reserpine (Armstrong and Hansel, 1959) and are completely ineffective in regressing the corpora lutea of hysterectomized cows (Armstrong and Hansel, 1959; Anderson and Bowerman, 1963). This indicates that oxytocin acts via the uterus, possibly by a neural or hormonal stimulus. Distending the uterus early in the estrual cycle shortens the interestrual period in the ewe (Moore and Nalbandov, 1953; Nalbandov et al., 1955; Inskeep et al., 1962), guinea pig (Donovan and Traczyk, 1960; Donovan, 1961; Moore, 1961) and cow (Hansel and Wagner, 1960), but not in the sow (Anderson and Melampy, 1962). A single injection (Wiltbank et al., 1961a) or continual injections (Greenstein et al., 1958; Loy et al., 1960; Rahlmann and Cupps, 1962) of estrogen initiated early in the cycle will shorten cycle length in the cow. Regressive changes in bovine corpora lutea

can be brought about by a single injection of progesterone early in the estrual cycle (Loy et al., 1960; Ray et al., 1961). Continual progesterone injections initiated during corpora lutea formation in the ewe will inhibit further growth but will not decrease weight or percent luteal cells below the values at the time injections were initiated (Zimbelman et al., 1959a). Corpora lutea develop in the ewe in the absence of the pituitary (Denamur and Mauleon, 1963a, b), but luteal development in the intact ewe is hampered by exogenous progesterone. This may mean that progesterone has a direct local effect in some species. The direct local effect of progesterone on corpora lutea is suggested in swine since addition of pregnenolone to an "in vitro" culture media increases progesterone production by luteal tissue slices (Duncan et al., 1960). However, one must not overlook the action progesterone may have on a luteotropin.

Sawyer et al. (1949) has shown that in the rat estrogen stimulates release of luteinizing hormone by its action on nerve centers. Ulberg and Lindley (1960) have suggested that estrogen facilitates release of luteinizing hormone to cause ovulation in the cow. It is also possible that the uterus alters estrogen and progesterone (de Jongh and Wolhuis, 1964) levels which may directly affect the corpora lutea or may indirectly act through a neural pathway to cause early regression.

From experiments on oxytocin and uterine distention one might postulate that the uterus is initiating or allowing release of the second maintenance factor, luteotropin. Once luteotropin is initiated, a continuous secretion is needed for corpora lutea maintenance. The corpora lutea may regress if at any time after its initiation luteotropin release or utilization is blocked. Thus, discontinuation of luteotropin release may explain regression following

neural or hormonal stimulus by oxytocin, and possibly estrogen and progesterone, in the cow and by uterine distention in various species.

The possibility exists that oxytocin, estrogen and uterine distention have their action by preventing corpora lutea formation. This is indicated since oxytocin injections and uterine distention must be applied early in the luteal phase of the estrual cycle to shorten the interestrual interval. Still, a cyclic maintenance factor appears to be associated with ovulation and to be capable of supporting the corpora lutea for a normal interestrual life span. If this is true, something must be acting on the corpora lutea to cause regression. A single injection of estradiol valerate as late as mid-cycle will cause early corpora lutea regression (Wiltbank et al., 1961). The action of estrogen at mid-cycle and the fact that 14 to 24 hours following oxytocin injection in the bovine progesterone concentration in the corpora lutea is higher than that in control corpora lutea (Mares and Casida; 1963) would favor a luteotropin initiation theory. Estrogen, progesterone and oxytocin injections may initiate release of luteotropin but fail to maintain luteotropin secretion.

Reports indicate that corpora lutea may be experimentally regressed once luteotropin has been released by pregnancy, pseudopregnancy or hysterectomy. Exogenous progesterone has no effect on the corpora lutea of the pregnant rat (Sammelwitz et al., 1956a, b, 1961), pregnant mouse (Selye, 1939), pseudopregnant rabbit (Ulberg, 1952) or cycling gilt (Sammelwitz et al., 1956a, b, 1961; Sammelwitz and Nalbandov, 1958). However, continuous exogenous progesterone causes regression of swine corpora lutea maintained beyond cycle length by pregnancy (Sammelwitz et al., 1956, 1961; Sammelwitz and Nalbandov, 1958; Spies et al., 1959; Dziuk and Baker, 1962) or by hysterectomy (Spies

et al., 1960). Bovine corpora lutea also regress following a single progesterone injection early in pregnancy (Zimbelman et al., 1961a).

Luteinizing hormone will destroy the nonfunctional persisting corpora lutea which follow hypophysectomy in the rat, while no effect is observed from follicle stimulating hormone, estrogen or progesterone (Bunde and Greep, 1936; Greep 1938a, b). Luteinizing hormone will also provoke regression of the corpora lutea of pseudopregnancy in rats bearing an accessory pituitary in the renal capsule (Rothchild, from de Jongh and Wolthuis, 1964).

Unfractionated pituitary extract causes a decrease in progesterone concentration of bovine corpora lutea in late pregnancy when injected intravenously (Zimbelman et al., 1961b). "In vitro" cultures of swine corpora lutea reflect a decrease in synthesis of progesterone when endometrial filtrates extracted from gilts on days 16-18 of the estrual cycle are added to the culture media. Addition of pituitary homogenates prepared from gilts in various stages of the estrual cycle to the "in vitro" cultures has no effect on progesterone synthesis (Duncan et al., 1961). Kiracofe et al. (1963) could detect no effect on corpora lutea of ewes receiving subcutaneous injections of uterine extracts prepared from ewes at various stages of the estrual cycle.

Release of luteotropin stimulus may have either neural or hormonal origin, both of which presumably may be working through the higher brain centers. Release of luteotropin is initiated by neural and hormonal changes in the pregnant uterus (Nalbandov, 1961) but may also be released by other factors in some species. Luteotropin release, causing a pseudopregnant condition, follows induced ovulation in the rabbit (Everett, 1961), cat (Foster and Hisaw, 1935) and ferret (Brambell, 1956) and spontaneous ovulation in the dog (Evans and Cole, 1931). Ovulation and resulting pseudopregnancy in the rabbit

appear to result from neural impulses to the hypothalamus since mating (Heape, 1905) and electrical stimulation (Zondek and Sklow, 1941) of cerebral or spinal nerves (Marshall and Verney, 1936) or of the hypothalamus (Markee et al., 1946; Hayward et al., 1964) will result in ovulation. Ovulation will not normally result from artificial insemination (Hammond, 1925) but will occur after mating even if the vagina and vulva are locally anesthetized (Fee and Parkes, 1930), sacral or abdominal sympathetic nerves removed, or genital region denervated (Brooks, 1937). Likewise, hysterectomy extended to include extirpation of the proximal one-half of the vagina will not prohibit ovulation in mated does (Brooks, 1937). One may assume that ovulation and resulting pseudopregnancy in the rabbit occur due to excitation of the higher centers but is neurally mediated since dibenamine, an adrenergic blocking agent, and atropine, a cholinergic blocking agent (Sawyer et al., 1949), as well as severance of the spinal cord (Marshall and Verney, 1936) will prevent ovulation. Nerves and the uterus are also implicated in the rat since mechanical stimulation of the cervix during estrus by sterile mating (Long and Evans, 1922) or a glass rod (Long and Evans, 1926) or stimulation of the nipples of a nonlactating rat by an active litter (Selye and McKeown, 1934) initiate pseudopregnancy. An electrical impulse on the cervix during estrus (Shelesnyak, 1931) or diestrus (Greep and Hisaw, 1938) or through the head (Harris, 1936) will also establish pseudopregnancy. If continual injections of estrogen are given (Wolfe, 1935; Selye et al., 1935a), the uterus is traumatized (Brshoff and Devel, 1943; Peckham and Greene, 1948) on day 4 or an accessory pituitary is grafted into the renal capsule (Mühlbock and Boot, 1959), pseudopregnancy results in the rat. Incidence of pseudopregnancy from cervical stimulation (Meyer et al., 1929) or from estrogen injection (Sawyer et al., 1949) can be decreased by anesthesia and by injection of dibenamine or atropine, respectively.

Length of the pseudopregnancy period resulting from uterine trauma is directly related to amount of tissue traumatized (Velardo et al., 1953; Melampy et al., 1964). Pseudopregnancy, as well as pregnancy, appears to result from release of luteotropin caused by neural and/or hormonal stimuli with both the uterus and higher centers being involved.

Hysterectomy may initiate luteotropin release prolonging corpora lutea life span in the guinea pig (Loeb, 1923, 1927; Rowlands, 1961), pseudopregnant (Bradbury, 1937; Melampy et al., 1964) or pregnant rat (Bradbury, 1937; Hechter et al., 1940), ewe (Wiltbank and Casida, 1956; Kiracofe and Spies, 1963), cow (Wiltbank and Casida, 1956; Anderson and Neal, 1961; Anderson et al., 1962), pig (Spies et al., 1958, 1960; du Mesnil du Buisson and Danzier, 1959; Anderson et al., 1961, 1963) and rabbit (Asdell and Hammond, 1933; Loeb and Smith, 1936; Gillard, 1937; Greep, 1941). Corpora lutea are maintained in the ewe even when hysterectomy is performed one day prior to expected heat (Kiracofe, unpublished). It is theorized that since this is the first time after ovulation that luteotropin release is initiated, the luteotropin can maintain the corpora lutea that were formerly under the influence of the cyclic maintenance factor.

It is also possible that by removing the uterus a regressive factor is removed, but this theory leaves one to answer the question of why the already regressing corpora lutea of the ewe 1 day prior to heat are maintained in near normal size following hysterectomy. Still, it is possible that the uterus produced an anti-luteotropin which is removed by hysterectomy thus allowing corpora lutea to persist.

Hysterectomy performed during the last half of pregnancy in the rabbit results in corpora lutea regression (Greep, 1941). Hysterectomy of immature (Shelesnyak and Schwarz, 1944; Ranney et al., 1947) or cycling rats (Durrant,

1926, 1927; Bradbury, 1937; Murphy, 1934; Perry and Rowlands, 1961) does not affect corpora lutea life span unless stimulated to pseudopregnancy during the process of hysterectomy (Perry and Rowlands, 1961). Hysterectomy has no effect on corpora lutea life span of the monkey (Burford and Diddle, 1936; van Wagenen and Catchpole, 1941), ferret (Deanesly and Parkes, 1933) and opossum (Hartman, 1925). Cycle length of the ferret and opossum already approximate the length of gestation.

In species where hysterectomy prolongs corpora lutea life span, the corpora lutea become larger and more functional (Rowlands and Short, 1959; Spies et al., 1960) as well as regress more slowly (Rowlands, 1961) following hysterectomy. Life span of corpora lutea in hysterectomized animals is more erratic than that of pseudopregnant and pregnant animals (Asdell and Hammond, 1933; Butcher et al., 1962b). This may indicate that the uterus causes luteal regression by metabolizing hormones present in the system, possibly estrogen or progesterone.

The effect of hysterectomy appears to be at least partially hormonal since attempts to denervate the uterus of the sow (Anderson and Melampy, 1962; du Mesnil du Buisson and Romants, 1963), ewe, rabbit and dog (Zhordania and Gotsiridze, 1964) have not altered corpora lutea life span. Autotransplants of uterine tissue in the rat (Hechter et al., 1940; Chu et al., 1946), rabbit (Chu et al., 1946; Zhordania and Gotsiridze, 1964), guinea pig (Butcher et al., 1962a), dog, ewe (Zhordania and Gotsiridze, 1964) and gilt (du Mesnil du Buisson and Romants, 1963; Anderson et al., 1963) result in re-established cycles. Corpora lutea life span following autotransplantation of uterine tissue is inversely related to amount of tissue transplanted (Butcher et al., 1962a; du Mesnil du Buisson and Romants, 1963). This inverse relationship is also noted when partial hysterectomy is performed in the guinea pig (Loeb, 1923;

Butcher et al., 1962b), rat (Melampy et al., 1964) and sow (Anderson et al., 1961). Presence of a sterile horn in the sow will cause luteal regression and termination of pregnancy, but if the sterile horn is removed corpora lutea and pregnancy persist (du Mesnil du Buisson, 1961b). Conservation of a piece of uterine horn 15 to 20 centimeters long near one ovary in the sow leads to regression of corpora lutea in that ovary while those in the other ovary persist (du Mesnil du Buisson, 1961a). Likewise, if all but a small portion of the nongravid uterine horn near the ovary of the unilaterally pregnant sow is excised, unilateral regression of corpora lutea will occur on the nonpregnant side while corpora lutea on the pregnancy side persist (du Mesnil du Buisson, 1961b). Thus, it appears that the uterus by its hormonal activity can exert a local effect directly on the ovary in some species as well as a systemic effect on the ovary, directly or via the hypothalamus, to provoke corpora lutea regression. Filtrates of uteri excised on days 16-18 have an inhibitory effect on progesterone production of "in vitro" cultures of swine corpora lutea (Duncan et al., 1961), again indicating a hormonal influence from the uterus on the corpora lutea. The uterus may be neurally or hormonally prohibited from exerting its effect. This inhibition may occur in the hysterectomized, pregnant or pseudopregnant animal.

Hypophysectomy during pregnancy causes regression of the corpora lutea in the ewe (Denamur and Mauleon, 1963a, b), sow (du Mesnil du Buisson and Leglise, 1963), mouse (Selye et al., 1933) and rabbit (White, 1932; Firor, 1933; Robson, 1937a) but corpora lutea of the cycling and pseudopregnant rat (Pencharz and Long, 1933; Astwood and Greep, 1938) and guinea pig (Pencharz and Lyons, 1934) persist in a nonfunctional state. Hypophysectomy causes regression of corpora lutea maintained by estrogen or hysterectomy in the ewe (Denamur and Mauleon, 1963b) and by hysterectomy in the sow (du Mesnil du Buisson

and Leglise, 1963). Hypophysectomy during early pregnancy causes termination of pregnancy in all species in which it has been tried (Allen, 1939). It appears that luteotropin release is continuously needed following ovulation since corpora lutea will regress or become nonfunctional following hypophysectomy at any stage of pregnancy, hysterectomy or pseudopregnancy.

Continual prolactin injections in intact rats will lengthen cycles to 13-16 days (Lahr and Riddle, 1936; Nathanson et al., 1937; Astwood, 1941), will favor production of uterine trauma (Astwood, 1941; Evans et al., 1941) and will maintain corpora lutea of hypophysectomized rats (Lyons et al., 1943) sufficiently to support pregnancy (Cutuly, 1941a, b). If prolactin injections are discontinued, the hypophysectomized rat will return to vaginal estrus (Nelson, 1946) although the corpora lutea persist in a nonfunctional state. Prolactin will also maintain the corpora lutea of rats given ergotoxine injections (Shelesnyak, 1958). Transplanting the anterior pituitary to the renal capsule or anterior chamber of the eye allows it to release prolactin continuously and maintain the corpora lutea for a greatly extended period of time (Everett, 1954, 1956). Retransplanting the anterior pituitary to the area of the median eminence will allow some rats to resume cycles (Nikitovitch-Winer and Everett, 1957, 1958a, b). Continual prolactin injections will also maintain the corpora lutea of mice (Dresel, 1935; Nathanson et al., 1937). It may be concluded that prolactin is luteotropin in the rat and possibly the mouse. However, prolactin is not luteotropin in the gilt (Sammelwitz and Nalbandov, 1958), heifer (Smith et al., 1957; Armstrong and Hansel, 1959; Simmons and Hansel, 1962), monkey (Hisaw, 1944), woman (Bradbury et al., 1949), guinea pig (Aldred et al., 1961; Rowlands, 1962) or rabbit (Hilliard et al., 1961) since it will not prolong corpora lutea life span. Early work by Moore and Nalbandov (1955) and Raeside (1958) showed prolactin to have a luteotropic

effect in a limited number of ewes, but later work has shown that prolactin will not maintain corpora lutea of cycling or hypophysectomized ewes (Denamur and Mauleon, 1963b).

Continual estrogen injections will maintain the corpora lutea of the pseudopregnant (Allen and Heckel, 1936) or pregnant (Heckel and Allen, 1939) rabbit, will maintain rat corpora lutea in an enlarged state typical of pregnancy (Wolfe, 1935; Selye et al., 1935) and will enlarge corpora lutea of immature (Wolfe, 1936) or hypophysectomized rats being maintained by unfractionated pituitary extracts (Selye and Collip, 1936). Estrogen will maintain the corpora lutea of the hypophysectomized rabbit (Allen, 1937; Robson, 1937b, 1939) sufficiently to maintain pregnancy (Robson, 1940), and if implanted into the corpus luteum, will cause local maintenance of even portions of the corpus luteum (Hammond and Robson, 1951). Contrary to Lyons et al. (1943), estrogen will not stimulate function of rat corpora lutea maintained in a nonfunctional state following hypophysectomy (Nelson, 1946). Estrogen is suggested to have its effect on the intact rat indirectly through release (Wolthuis, 1963; Wolthuis and de Jongh, 1963) or utilization (Kullander, 1961) of prolactin. A single injection of estrogen has been shown to lengthen or shorten the estrous cycle of the gilt depending on the time of administration (Kidder et al., 1955) but has no detectable effect on corpora lutea of the cycling hysterectomized (Spies et al., 1961) or hypophysectomized guinea pig (Rowlands, 1962). Continual estrogen injections started on day 11 were found to maintain corpora lutea until slaughter on day 34 in the gilt (Gardner et al., 1963) and to a maximum of 50 days in the ewe (Denamur and Mauleon, 1963b). It is interesting to note that this 50 days corresponds to the time the ewe will maintain pregnancy if ovariectomized. It appears that

estrogen indirectly affects the corpora lutea life span of many species, but is luteotropin only for the rabbit.

Human chorionic gonadotropin has been found to maintain the corpora lutea of the cow (Wiltbank et al., 1961b), monkey (Hisaw, 1944) and human (Bradbury et al., 1949). Human chorionic gonadotropin and pregnant mare serum both contain luteinizing hormone which has been shown to stimulate release of progesterone from ovaries of rabbits (Hilliard et al., 1961). Luteinizing hormone is capable of maintaining the corpora lutea of hypophysectomized rabbits (Foster et al., 1937; Kilpatrick et al., 1962, 1964). Kilpatrick et al. (1964) has suggested that luteinizing hormone causes estrogen release from interstitial cells and maintains the corpora lutea indirectly.

In addition to these hormone effects, Selye (1934) reported maintaining the corpus luteum of pregnancy in the rat by substituting paraffin for the products of conception, but this work could not be confirmed by Greene (1941) or Bradbury (1941). Davis et al. (1959) found a luteotropic substance in the subplacenta (between the foetal placenta and the basal decidua) of the guinea pig. "In vitro" cultures of swine corpora lutea show increased secretion of progesterone if pregnenolone, DPN (Duncan et al., 1960) or endometrial filtrates from gilts on day 12-13 of the estrual cycle are added to the media (Duncan et al., 1961).

Results of experiments on corpora lutea maintenance lead to the conclusion that luteotropin is not the same for all species, but whatever the luteotropin may be, it is needed in continuous release for the corpora lutea to be maintained.

Corpora lutea of hysterectomized, pseudopregnant or pregnant animals appear not to regress by age alone since rats with pituitaries transplanted to the renal capsule (Everett, 1954, 1956) and guinea pigs hysterectomized

early in the cycle (Rowlands, 1961) can retain functional corpora lutea for months. Thus, corpora lutea must either be destroyed in spite of available luteotropin or they must regress due to a lack of luteotropin. Corpora lutea regression in the rat bearing an accessory pituitary in the renal capsule (Mühlbock and Boot, 1959) and unilateral luteal regression in swine (du Mesnil du Buisson, 1961a, b) occur in spite of available luteotropin. This does not rule out regression due to an inability to utilize the available luteotropin which may occur due to hormonal influence at the level of the ovaries. Corpora lutea regression may also occur due to a lack of luteotropin release. This is known to be the case in animals hypophysectomized during hysterectomy or pregnancy.

It is known that corpora lutea life span is somewhat longer in hysterectomized than in pseudopregnant animals (rat: Bradbury, 1937) (rabbit: Loeb and Smith, 1936; Gillard, 1937) and may even exceed gestation (guinea pig: Loeb, 1923, 1927; Rowlands, 1961) (gilt: Spies et al., 1958; Anderson et al., 1961) (ewe: Kiracofe and Spies, 1963). Thus, the uterus and its contents may have some effect on the length of pseudopregnancy and pregnancy even if it is not necessary for regression to occur in most species.

The uterus metabolizes estrogen (Pincus, 1937) and has recently been shown to decrease circulating progesterone in the rat (de Jongh and Wolthuis, 1964), especially when traumatized. Estrogen is known to have a neural stimulus on the hypothalamus capable of increasing release of luteinizing hormone in the rat (Nelson, 1935; Hellbaum and Greep, 1946; Byrnes and Meyer, 1951; Sawyer et al., 1949; Hellbaum et al., 1961; Wolthuis, 1963; Wolthuis and de Jongh, 1963), rabbit (Smith and Smith, 1931) and cow (Ulberg and Lindley, 1960). The fetal membranes contained within the gravid uterus produce substances (Adler et al., 1934; Haterius, 1935; Astwood and Greep, 1938;

Deanesly and Newton, 1940) which are capable of supporting pregnancy following ovariectomy late in gestation in the cat (Courrier and Gros, 1935), guinea pig (Herrick, 1928), mare (Hart and Cole, 1934), monkey (Hartman, 1941), woman (Asdell, 1928), ewe (Casida and Warwick, 1945) and cow (McDonald, 1952, 1953). Some of the placental substances from the rat (Astwood and Greep, 1938), human and cow (Adler et al., 1934) appear to be progestational in their action. Thus, it appears that the uterus is having its main action on estrogen and progesterone, both of which can influence pituitary hormone secretions as stated earlier. Following hypophysectomy corpora lutea maintained by estrogen in the ewe (Denamur and Mauleon, 1963b) regress, suggesting that estrogen is not itself luteotropin in the ewe, but may work via higher centers and be mediated via the uterus. Estrogen stimulates growth of corpora lutea fragments cultured "in vitro" from rats (Kullander, 1961). It appears that estrogen and progesterone levels and ratios are important in corpora lutea regression by action directly on the corpora lutea or through the higher centers possibly by gonadotropin release.

MATERIALS AND METHODS

Experimental Procedure

One hundred twenty-six New Zealand White and Dutch Belted rabbits were purchased to study factors associated with corpora lutea maintenance and/or regression. Since an estrual condition was desired at treatment initiation, all does were individually caged for a minimum of 18 days pre-treatment. Individual caging was continued throughout the experiment. Artificial light, controlled by a time clock, was maintained from 6 a.m. to 8 p.m. Rabbits were fed a liberal amount of a complete ration pellet.

To initiate each treatment group corpora lutea were induced by injecting 100 IU of human chorionic gonadotropin (HCG) dissolved in 1 cubic centimeter (cc.) sterile saline into the marginal ear vein. Day of HCG injection was designated as day 0.

Pentobarbital sodium was used to anesthetize all animals prior to surgery. The anesthetic was diluted using two parts sterile saline to one part pentobarbital sodium and administered into the marginal ear vein. Artificial respiration or the respiratory stimulant, metrazol, was used when necessary.

The incision area was prepared by shaving the hair, moistening with 75% alcohol and wiping clean with sterile gauze. Incisions were sutured in two separate layers with 4-0 merselene using a modified continuous stitch. The inner layer included the peritoneum and three muscle layers when the paralumbar approach was used but only the peritoneum and linea alba when a mid-ventral incision was closed. The outer suture closed only the skin of all incisions.

Hysterectomy, when desired, was performed through a mid-ventral incision. A ligation using 4-0 merselene was placed at the anterior end of the vagina and all or most of both cervices were removed. Ferguson angiotribe forceps were used to crush the cut edges of tissue to prevent bleeding as the uterine horns and small portions of both oviducts were removed. Thus, hysterectomy included removal of the cervices, uterine horns and small portions of both oviducts.

The initially induced corpora lutea were marked with India ink through a paralumbar incision on day 2, 3 or 4 post-ovulation using a small syringe and a 26 or 27 gauge needle. Caution was taken to avoid disturbance of the ovarian blood supply which is known to cause premature corpus luteum regression (Loeb, 1923; Kiracofe et al., 1963). A needle was inserted into the

ovulation papilla of each young corpus luteum and a small amount of India ink released. Corpora lutea of control rabbits were marked to test the effect of India ink on luteal tissue life span. Number of marked corpora lutea and normal and blood filled follicles on each ovary were recorded for subsequent identification.

Treatments

Human chorionic gonadotropin (HCG), follicle stimulating hormone (NIH-FSH S-1-ovine standard),* luteinizing hormone (NIH-LH B-1-bovine standard),* estrone, estradiol benzoate, antiestrogen (U-11100A),** copper acetate and atropine were used in the various pseudopregnant and hysterectomized-pseudopregnant groups. HCG, FSH and LH were dissolved in sterile saline and kept refrigerated prior to injection.

A single intravenous injection of 100 IU of HCG dissolved in 1 cc. sterile saline was given to intact pseudopregnant does having marked corpora lutea. The HCG was administered to nine groups on day 3, 4, 5, 6, 7, 9, 11, 12 or 13 of pseudopregnancy (table 1). Two groups of pseudopregnant does with marked corpora lutea which either received an intravenous injection of 1 cc. sterile saline or remained uninjected served as controls for the HCG injected does and also for the FSH and LH treated groups in treatment 2.

FSH and LH were injected intravenously at varying combinations and levels on day 7 of pseudopregnancy (table 2). FSH was dissolved to a concentration

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*The FSH and LH were supplied through courtesy of the National Institute of Health, Bethesda, Maryland.

**The antiestrogenic compound, (1-{2-[P-(3,4 dihydro - 6 - methoxy - 2 - phenyl - 1 - naphthyl) phenoxy] ethyl}-pyrrolidine, hydrochloride), commonly called U-11100A was supplied by The Upjohn Company, Kalamazoo, Michigan.

of 1 milligram (mg.) per cc. LH was dissolved to concentrations of 1 and 0.05 mg. per cc. All LH injections involved between 0.7 and 2 cc. and all FSH injections 1 to 2 cc. of total solution. Combinations of FSH (1-2 mg.) and LH (0.2 - 1 mg.), LH alone (0.035 - 1 mg.) and FSH alone (1.5 mg) formed three groups used to determine the component of HCG causing luteal destruction. In the group receiving both FSH and LH two separate but simultaneous injections were given.

The drugs atropine and copper acetate were employed in an attempt to prove that ovulation is not necessary for exogenous HCG or LH to cause regression of corpora lutea. Four rabbits were primed with estrogen by giving 1 mg. of estrone subcutaneously on days 5 and 6. Two of the rabbits were given an intravenous injection of 1 cc. of 1% copper acetate while the other two, receiving no injections, served as controls. The 1% solution of copper acetate was prepared by making 2% solutions of copper sulfate and sodium acetate and mixing them in equal portions. The 2% solutions were prepared well before injection time but were mixed just prior to intravenous injection. Atropine sulfate pellets were dissolved in distilled water to a concentration of 20 mg. per cc. and given intravenously to two rabbits on day 7 of pseudopregnancy in doses of 20 and 30 mg. per kilogram of body weight 3 min. prior to an intravenous injection of 100 IU of HCG. A third rabbit died shortly after receiving atropine injected at 30 mg. per kilogram of body weight.

Estrogen was given surrounding HCG administration to block the observed regression caused by HCG and LH (table 3). Estrone and estradiol benzoate were dissolved in sesame oil at concentrations of 1 mg. per cc. and 32 micrograms (µg.) per cc., respectively, and injected subcutaneously. Both estrogenic compounds were warmed to get them into solution.

One mg. of estrone per day was given subcutaneously on days 5, 6, 7 and 8 surrounding an injection of 100 IU of HCG on day 7. Two control groups were given daily subcutaneous injections of 1 mg. of estrone for four consecutive days beginning on days 5 and 14. The day 14 estrone control group was added to determine the length of effect of four days of estrogen treatment on the life span of corpora lutea.

One-half mg. of cyclopentylpropionate (ECP) was injected subcutaneously on days 5, 6, 7 and 8 in two rabbits, one of which received an intravenous injection of 100 IU of HCG on day 7. An additional rabbit received four daily injections of 8 µg. of estradiol benzoate surrounding an intravenous injection of 100 IU of HCG on day 7.

An antiestrogenic compound (U-11100A) was given to three groups of rabbits in the third treatment (table 3). The antiestrogen was placed in distilled water and in sesame oil at concentrations of 10 mg. per cc. Neither solvent was successful in dissolving the antiestrogen, even when heated to 45°C., so the compound was injected in suspension. One of two rabbits in each group received the oil suspension and the other received the water suspension. All three groups received subcutaneous injections of the antiestrogen suspension twice on day 7 and once on days 8 and 9. Group 1 received only antiestrogen, group 2 received antiestrogen plus 1 mg. of estrone on days 6, 7, 8 and 9 and group 3 received antiestrogen plus estrone plus 100 IU of HCG on day 7.

Hysterectomized-pseudopregnant does having marked corpora lutea were given HCG in treatment 4 (table 4). Three treatment groups were injected intravenously with 100 IU of HCG on day 3, 7 or 13 following hysterectomy on day 2 or 3. Hysterectomy was performed and corpora lutea marked on day 2 or 3 post-ovulation in one control group, while in a second control group does

were hysterectomized 10 days prior to induction of pseudopregnancy. The two control groups also served to test the equality of life span of corpora lutea induced before and after hysterectomy.

Procedure of Analysis

Frequent unilateral or bilateral paralumbar laparotomies were performed as needed to follow changes in size and color of marked and later induced corpora lutea and follicular patterns in all groups. Control and treated rabbits were laparotomized on similar days to equalize any possible effect the operation may have had on corpora lutea.

Unilateral ovariectomy, which has no effect on corpora lutea life span (Butcher et al., 1962b; Brinkley et al., 1964c) was often employed to obtain ovarian tissue for histological or morphological analysis without destroying the rabbit for further study. Ovaries were removed through a paralumbar incision following a ligation of the ovarian blood supply.

Estrogen determinations by thin layer chromatography using the method of Veenhuizen et al. (1960) were attempted on ovarian tissue and corpora lutea from HCG injected and uninjected does. Corpora lutea of six rabbits, three of which received HCG injection on day 7, were carefully peeled from ovarian tissue excised on day 9 of pseudopregnancy. Estrogen determinations were run on grouped ovarian tissue and on grouped corpora lutea from the three HCG injected does. Like determinations were run on tissues from the three uninjected does.

Three methods of detecting corpora lutea regression were employed in this study. Method 1 used visual observation and "in vivo" ovarian photographs; method 2 used weight of individual corpora lutea; method 3 used histological preparations.

Visual regression of the corpora lutea was observed at laparotomy by noting their size and vascularity or color. Comparisons were often made between treatment and control corpora lutea of the same age. "In vivo" photographs of exposed ovaries were taken using a 35 millimeter Exacta camera with a bellows extension (plates I and II). Two 250 watt photoflood lamps were used to light the ovary while photographs were taken. Photographs were taken at 1/25 of a second and various focal lengths were used. Length of the bellows extension was variable, but normally ranged from 2.0 to 2.7 centimeters.

Weights of individual corpora lutea were taken as a proof of regression in some rabbits following ovariectomy or sacrifice. They were carefully peeled from surrounding ovarian tissue and weighed on a Mettler gram-atic balance to an accuracy of 0.1 mg. Comparisons were made between treatments and between days within treatments.

Histological preparations were made of ovarian tissue or, in a few cases, of individual corpora lutea which had previously been weighed. Ovaries were fixed in Bouin's fluid; washed with water; dehydrated with an alcohol series; cleared with xylol; embedded in paraffin; sectioned at eight microns; mounted on 2" x 3" slides using Mayer's albumen fixative; stained with PAS, hematoxylin plus acid fuchsin orange G mixture or Mallory's triple stain modified to include hematoxylin; and covered with number 2 coverslips using piccolyte. Slides were observed under a microscope with magnification ranging from 50 to 1000. Luteal cell counts per microscopic field under oil immersion at 1000 magnification were taken in each age group of corpora lutea present on the ovary. Marks in the initial set of corpora lutea were used to distinguish age differences (plate V, fig. 18). Only large fully distinct luteal cells were counted (plate III, fig. 13).

Luteal cells were counted from three individual fields of a single corpus luteum and the average number of luteal cells per field recorded for comparisons. Large luteinized interstitial cells were also counted in three fields of interstitial tissue and the average number recorded. Cell diameters were taken using an ocular micrometer on three nearly round luteal cells that possessed a distinct nucleus and on three similarly characterized interstitial cells. Cell diameters were averaged and the average diameter recorded.

Statistical comparisons were made on observations of luteal regression recorded at laparotomy. Corpora lutea were classed as functional (plate I, fig. 3) or regressing (plate II, fig. 9) and analyzed according to Fischer's exact probability test (Siegel, 1956). Corpora lutea weights and histological studies were used to confirm the visual observations.

RESULTS

Treatment 1

Results of human chorionic gonadotropin (HCG) administered on varying days of pseudopregnancy (table 1) are shown in tables 5 and 10 and in graphs 1 and 3. HCG administration on day 3 post-ovulation (group 1) resulted in new ovulation and formation of a second set of corpora lutea. Maintenance of both initial and day 3 induced corpora lutea was observed (plate I, fig. 1). Luteal cell counts taken from four ovaries removed on the 20th day of pseudopregnancy averaged 5.2 for initial and 5.2 for day 3 induced corpora lutea confirming the observations (table 10).

Regression of the initial (marked) and day 3 induced corpora lutea could not be morphologically or histologically differentiated (plate IV, fig. 16). Both sets of corpora lutea began regression about day 21 post initial

ovulation. Average luteal cell counts per microscopic field for six does, days 18-27, averaged 5.0 for marked and 5.2 for day 3 induced corpora lutea. These observations did not differ significantly.

Marked corpora lutea were regressing by day 19 in 6 of 6 control does (group 11, table 5), but in only 1 of 4 does with accessory corpora lutea induced on day 3 (group 1, plate IV, fig. 16 vs. fig. 17). This difference approached significance ($P \cong .06$). The observation was supported by luteal cell counts of marked corpora lutea averaging 5.2 for group 1 and 1.3 for group 11 (table 10). Thus, regression of initial and day 3 induced corpora lutea was sychronous and regression began with the mechanism established by the day 3 induced corpora lutea.

Corpora lutea of does receiving HCG injections on day 4 (group 2, table 5) never attained maximum size and appeared avascular in color (plate I, fig. 2). Histological studies revealed that partial regression had occurred by day 11 (table 10; plate V, fig. 18), this being the earliest day ovaries were taken for histological preparation. Marked corpora lutea, though small and avascular, remained until the later induced corpora lutea began their regression. Limited observations and histological material did not allow adequate comparison of initial and day 4 induced corpora lutea to draw definite conclusions as to synchronous or asynchronous regression.

HCG administration on day 5 (group 3, table 5) provoked rapid regression of marked corpora lutea in 4 of 6 does as revealed by laparotomy on day 9 or 10. Thus, regression of the marked corpora lutea was significantly (table 9, $P \leq .05$) faster than control does (group 11) in which none were observed regressing by day 10 (plate I, fig. 3). Regression in the two remaining does was observed in 7 of 11 and 8 of 11 marked corpora lutea (plate I, fig. 4; plate VI, fig. 20, 21), while the remainder of the corpora

lutea persisted in their normal state and were still present at day 23. Average luteal cell counts of 6.0 for marked and 4.7 for day 5 induced corpora lutea in an ovary taken on day 23 indicated that marked corpora lutea were regressing with the mechanism of the second set (plate VI, fig. 20). The fact that in two does some of the marked corpora lutea regressed while others persisted may indicate that corpus luteum maintenance can be locally influenced in this species.

HCG injected on or after day 6 (groups 4-9, table 5) provoked rapid regression of initial corpora lutea in all pseudopregnant does. Morphological (plate I, fig. 5) and histological (plate VII, fig. 22, 23) regression of marked corpora lutea were apparent by 3 days post-HCG injection (graph 1). Average lutea cell counts of marked corpora lutea were lowered from 7.1 to 4.1 and average corpora lutea weight dropped from 11.1 to 3.1 mg. by 3 days post injection of HCG (graphs 1, 3; table 10). Later induced corpora lutea, resulting from HCG treatment, groups 1-9, had a life span of approximately 17-18 days (graph 1; table 10; plate V, fig. 19). Interstitial cell counts ranged from 14-20 in ovaries containing functional corpora lutea, but dropped to 10-12 during regression in both induced and initially formed corpora lutea (table 10). Interstitial cell counts immediately following HCG administration were not taken.

Treatment 2

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) were given in treatment 2. Results of treatment 2 are displayed in tables 6 and 11. FSH and LH given at varying levels on day 7 post initial ovulation in groups 12 and 13 resulted in regression of marked corpora lutea in 7 of 7 does as revealed by laparotomy on day 9 or 10. Thus, time of regression of

initially formed corpora lutea in FSH and LH treated does was significantly different from control does (table 9, $P \leq .005$). These results were similar to results obtained by injecting HCG on day 7. Ovulation and the development of corpora lutea was noted in 6 of 7 FSH and LH treated does. The remaining doe, given 0.1 mg. of LH and 1.5 mg. of FSH, failed to ovulate, but marked corpora lutea regressed as a result of the treatment. Ovaries of this doe contained only marked well regressed corpora lutea and clear follicles at laparotomy on day 14. Results from this doe indicate that ovulation may not be necessary for HCG to cause luteal regression.

Group 14, given 1.5 mg. of FSH on day 7, possessed ovaries containing corpora lutea in varying stages of regression when viewed at laparotomy on day 9. No recent ovulations were observed, but histological study of one ovary removed on day 9 indicated that a new ovulation had occurred as a result of the treatment and that some marked corpora lutea were regressing while others were not (plate VIII, fig. 24). These limited observations showing asynchronous regression of corpora lutea of the same age following ovulation suggest that corpora lutea may in some cases persist in spite of ovulation.

Varying dosages of LH on day 7 resulted in regression of marked corpora lutea by day 9 or 10 in 10 of 11 LH-treated does (plate I, fig. 6; plate VIII, fig. 25). Corpora lutea were not regressing by day 10 in 6 of 6 controls (plate I, fig. 5). Thus, results of corpora lutea regression in LH-treated does (groups 15-18, table 6) differed significantly (table 9, $P \leq .01$) from results in untreated controls (group 11), but were similar to results obtained by injecting HCG on day 7 (plate I, fig. 6 vs. fig. 5). Average cell counts of marked corpora lutea on day 10 were approximately 7.0 for uninjected controls (group 11) and 4.3 for does receiving LH on day 7

(groups 15-18, table 11) confirming observations taken at laparotomy. Average weights of marked corpora lutea on day 10 of approximately 11.1 mg. for controls and 3.6 mg. for LH treated does also supported interpretations made at laparotomy. Ovulation had occurred and new corpora lutea were developing in 10 does observed to have regressing marked corpora lutea. One doe which did not ovulate possessed 3 marked corpora lutea which were morphologically normal and 2 which appeared avascular. The unovulated doe received 37.5 µg. of LH (group 18).

Realizing that corpora lutea of one doe in the FSH and LH treatment regressed without occurrence of ovulation and that some of the corpora lutea of 2 does receiving FSH treatment maintained in spite of ovulation preliminary studies were designed involving copper acetate and atropine. These studies were designed to test whether or not ovulation (presumably endogenous LH release) was directly involved in the luteal regression provoked by exogenous HCG or LH. Copper acetate was injected into two does on day 7 following estrone injections on days 6 and 7. No ovulations resulted from the copper acetate injection. Corpora lutea of one doe were small and nonvascular by day 13. Histological study indicated slight regressive changes had occurred (average luteal cell count of 5.7). However, marked corpora lutea in the second doe appeared normal on day 13 but were regressing on day 27. Luteal cell counts on day 27 averaged 4.3. Luteal life span was not altered in control does receiving only estrone on days 6 and 7. No definite conclusions were drawn but additional work is indicated.

As opposed to copper acetate, atropine was given in an attempt to block ovulation. Atropine was given to three estrual does 3 minutes prior to an HCG injection. Atropine failed to block ovulation in two does. The third doe died soon after the injections were given.

Treatment 3

Estrogens were given subcutaneously surrounding HCG administration in treatment 3 (tables 7 and 12). Group 19 received estrone on days 5, 6, 7 and 8 post-ovulation surrounding HCG injection on day 7. Laparotomy revealed that marked corpora lutea were not regressing by day 10 in any of 7 does in group 19 (plate II, fig. 7) although 5 of them had ovulated following HCG injection. Maintenance of marked corpora lutea in HCG injected does given estrone was significantly (table 9, $P \leq .025$) different from HCG injected non-estrone treated does, since all 6 does in the non-estrone treated group were regressing by day 10 (group 19 vs. 21). Histological counts of luteal cells from marked corpora lutea of ovaries removed on day 17 were 3.0 for HCG injected does given estrone surrounding the injection and zero for HCG injected does not receiving estrone. Marked corpora lutea on day 17 were too small to remove for weighing in the group receiving only HCG (group 21), but averaged 6.1 mg. when estrone was administered (table 12). Life span of marked corpora lutea in estrone plus HCG does was approximately 18-20 days (plate IX, fig. 26; plate II, fig. 8). This was similar to the life span of corpora lutea of estrone controls (18-20 days) receiving no HCG injection (table 7, group 20; plate IX, fig. 27; plate II, fig. 9). Corpora lutea formed by injection of HCG on day 7 persisted for a normal pseudopregnant life span (17-18 days). Thus, regression was asynchronous for the marked and later induced sets of corpora lutea (plate II, fig. 8) and (plate IX, fig. 26). It is possible that estrone maintained the marked corpora lutea, since estrone injected on days 14, 15, 16 and 17 (group 22) prolonged corpora lutea of control does to as long as day 25 (table 12). It was noted that during asynchronous regression in group 19 the marked corpora lutea were regressing

when interstitial cell counts remained above 14 while in controls (group 11) interstitial cell counts were approximately 10-12 when corpora lutea on the ovary were regressing (table 10). Use of an estrogenic compound with a shorter "in vivo" life is needed before drawing definite conclusions.

One doe received 1 mg. of ECP on days 5, 6, 7 and 8 surrounding an HCG injection on day 7. Repeated laparotomies revealed both the initial and day 7 induced corpora lutea were maintained until about day 40. Corpora lutea of a control ECP treated doe had a similar life span indicating that ECP maintained the corpora lutea far beyond injection. Interstitial cell counts of under 12 were noted while large vascular corpora lutea were present on the ovary. This may indicate direct maintenance of the corpora lutea from exogenous estrogen since low interstitial cell counts appear to be associated with corpora lutea regression.

Estradiol benzoate was given to one doe on days 6, 7, 8 and 9 surrounding HCG injection on day 7. HCG did not produce new ovulations or luteal regression. Marked corpora lutea were at about mid-regression when observed on day 20.

Knowing that estrone will maintain corpora lutea of HCG injected does, an antiestrogenic compound was injected simultaneously with estrone to determine the effect on corpora lutea maintenance. The antiestrogenic compound, U-11100A, was administered subcutaneously (table 3). Injection of the antiestrogenic compound suspended in sesame oil or distilled water twice on day 7 and once on days 8 and 9 in two pseudopregnant does (group 23) did not affect the marked corpora lutea. Corpora lutea life span remained unaffected in two does (group 24) when estrone was injected on days 6, 7, 8 and 9 along with the antiestrogen injections as described in group 23 (table 12). Estrone plus the antiestrogenic compound were again given in group 25 and an injection

of HCG was added on day 7 in two does. HCG resulted in ovulation and formation of a second set of corpora lutea. Marked corpora lutea were avascular in the doe receiving the antiestrogenic compound in distilled water suspension plus estrone (plate II, fig. 10), but did not affect marked corpora lutea in the doe receiving the antiestrogenic compound in oil suspension plus estrone. Further study using the antiestrogenic compound with lower dosages of estrone are needed before definite conclusions are drawn.

Treatment 4

Pseudopregnant does were hysterectomized in treatment 4 prior to administration of HCG (table 4). Results indicate that pseudopregnant-hysterectomized does respond similarly to uterine-intact, pseudopregnant does given HCG (tables 8, 13). HCG administered on day 3 (group 26) produced ovulation in all does and both marked and newly formed corpora lutea persisted in 5 of 6 does. The sixth doe had four corpora lutea which appeared regressing and four which did not at laparotomy on day 9 (plate II, fig. 11). The initially formed, and day 3 induced corpora lutea regressed synchronously. This observation was supported by luteal cell counts (plate X, fig. 28) from six ovaries taken in the regression range, day 22-26, averaging 4.8 for marked and 4.8 for the unmarked corpora lutea (table 13). Corpora lutea weights also confirmed this observation (marked corpora lutea averaged 6.8 mg. while unmarked averaged 14.4 mg.). Luteal regression was so variable in the hysterectomized rabbits that conclusions were not made as to which of the two sets of corpora lutea possessed a normal life span.

HCG, day 7 (group 27) or day 13 (group 28), resulted in regression of marked corpora lutea by 4 days post-injection in 7 of 7 and 8 of 8 does, respectively. Corpora lutea were not regressing in any of the hysterectomized

controls by day 11. Thus, regression of marked corpora lutea by day 11 was significantly (table 9, $P \leq .005$) faster for groups 27 and 28 than for hysterectomized controls (group 29). HCG injection in hysterectomized does produced new ovulations and new corpora lutea developed. Life span of the set of corpora lutea initiated by HCG, day 7 or 13, was 22-29 days (plate II, fig. 12). This was similar to corpora lutea life span in hysterectomized controls (plate X, fig. 29). Control does hysterectomized 10 days prior to induction of pseudopregnancy (group 30) also exhibited a 23-29 day life span indicating no difference from corpora lutea life span of control does hysterectomized 2 or 3 days following induction of pseudopregnancy (table 8, group 29).

DISCUSSION

Ovulations have previously been induced in the pseudopregnant (Jares, 1932) and pregnant rabbit (Hill and Parkes, 1931; Wislocki and Snyder, 1931; Wolfe, 1931; Snyder and Wislocki, 1931; Jares, 1932; Hill and Parkes, 1932; Snyder, 1934), but little has been mentioned concerning the effect on corpora lutea life span. Snyder and Wislocki (1931) induced five sets of corpora lutea in one doe within a 27 day period but made no mention of effects on existing corpora lutea.

Data from the present experiment indicate that induction of a second set of corpora lutea by an injection of HCG on day 3 of pseudopregnancy in the rabbit resulted in ovulation followed by formation and persistence of both sets of corpora lutea. The two sets of corpora lutea regressed synchronously with the later induced set of corpora lutea having a normal life span and the life span of initial corpora lutea being prolonged about 3 days.

It seems appropriate to compare the corpora lutea of the day 3 induced doe with the corpora lutea of the cycling ewe and sow since data would indicate that rabbit corpora lutea may have approximately a 4 day life span if naturally occurring pseudopregnancy could be prevented. Corpora lutea develop normally for about 4 days in the hypophysectomized rabbit before they begin to diminish in size (Smith and White, 1931; White and Leonard, 1933). Corpora lutea also last for normal cycle length following hypophysectomy in the ewe (Denamur and Mauleon, 1963a, b) and sow (du Mesnil du Buisson and Leglise, 1963). One might postulate a 4 day luteotropin free maintenance period associated with ovulation in the rabbit comparable to the 17 and 21 day period of the ewe and sow. Corpora lutea of the doe may persist until luteotropin is released at about day 4 initiating actual pseudopregnancy. Growth of corpora lutea in the rabbit is fairly slow before day 4 after which growth is rapid (Greep, 1941) suggesting day 4 may be the approximate time of luteotropin release.

The fact that in the rabbit a second set of corpora lutea induced by HCG injection on day 3 were maintained along with the initial set is in agreement with results of inducing a second set of corpora lutea during the luteal phase of the estrous cycle in the ewe (Inskeep et al., 1963) and sow (Neill and Day, 1964). Like the doe, the two sets of corpora lutea regress synchronously in the ewe. However, in the ewe it is the original set of corpora lutea which has the normal life span while the later induced corpora lutea have a shortened life span. The sow differs from both the doe and ewe since both sets of corpora lutea have a normal life span. Thus, regression of the two sets of corpora lutea in the sow is asynchronous.

Evidence indicates that once luteotropin release occurs the corpus luteum may become dependent upon its continuous secretion. If at any time after its initial secretion luteotropin is not supplied, the corpora lutea may regress.

HCG injection on day 3 in the doe may not cause existing corpora lutea to regress because they are not yet luteotropin dependent. Initial release of luteotropin may have been delayed about 4 days in the intact doe by HCG injection on day 3. Initial corpora lutea had an extended life span and regressed with the day 3 induced corpora lutea. When initial release of luteotropin occurred, marked corpora lutea, although 7 days old, might still have been capable of responding to luteotropin. That the life span of corpora lutea near the end of cycle maintenance can be prolonged is indicated in the ewe where hysterectomy one day prior to expected heat results in maintenance of corpora lutea (Kiracofe, unpublished). Luteotropin may never be released in the cycling sow or ewe or may be an entirely different substance than luteotropin in the rabbit. One or both of these factors may account for the difference in regression patterns of the two sets of corpora lutea among the three species.

Estrogen (Allen, 1937; Robson, 1937b, 1939) and LH (Foster et al., 1937; Kilpatrick et al., 1962, 1964) are both capable of maintaining corpora lutea in the hypophysectomized rabbit. Estrogen will support a corpus luteum, or even a portion of one, if implanted in or adjacent to the corpus luteum (Hammond and Robson, 1951). Estrogen will maintain rabbit corpora lutea in the absence of interstitial tissue (Westman, 1934) while LH may be working on interstitial cells to cause estrogen release which in turn results in corpora lutea maintenance (Everett, 1961). Thus, data indicate that estrogen is

probably luteotropin in the rabbit and probably originates from interstitial tissue.

The present experiment revealed that when the second set of corpora lutea were induced by injecting HCG on day 4 the initial set of corpora lutea lost color and never attained maximum size but remained in a reduced form until later induced corpora lutea regressed. HCG or LH given on or after day 5 resulted in rapid regression of initial corpora lutea following the injection. These results would agree with the observation of Hill and Parkes (1932) who noted that the presence of a second set of corpora lutea accelerated the shrinkage and decay of corpora lutea present at the time of injection. They also noted that the induced corpora lutea passed through the normal histological changes more rapidly than corpora lutea of pregnancy and never reached the maximum size attained by corpora lutea of pregnancy. Induced corpora lutea which developed as a result of the ovulating injection in the present experiment had a normal pseudopregnant life span. Snyder (1934) found that by injecting antuitin S on day 25 he could prolong parturition until about day 40 presumably due to a 15 day life span of the induced corpora lutea.

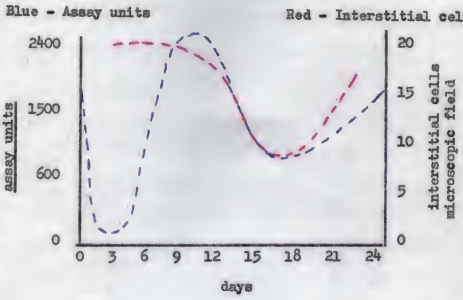
HCG or LH interrupts luteotropin stimulus initiated by day 5 causing luteal regression in most does. Greep (1941) reported that luteotropin will not stop regressive changes in rabbit corpora lutea, although changes occur more slowly upon luteotropin stimulus. However, estrone given surrounding HCG administration on day 7 prevented luteal regression which occurred in HCG injected does receiving no estrone. Thus, HCG presumably provoked luteal regression by interfering with endogenous estrogen production. Maintenance of initial corpora lutea with exogenous estrogen in does injected with HCG

resulted in both sets of corpora lutea having approximately normal life spans and regressing asynchronously.

The fact that LH will cause regression of corpora lutea in the intact doe but will maintain corpora lutea in the hypophysectomized doe would indicate that LH may have its action through the pituitary. However, Kilpatrick et al. (1964) provoked ovulation and noted old appearing and younger corpora lutea following an LH injection 2 hours after hypophysectomy. New ovulations did not occur and initial corpora lutea were maintained rather than decaying if LH injections were delayed until 12 hours after hypophysectomy. Therefore, LH may also have its antiluteotropic effect in the hypophysectomized doe by acting directly on the interstitial tissue of the ovaries. The reason LH is later luteotropic in the hypophysectomized doe may be a function of dosage level. It should be noted that LH in the present experiment was injected intravenously and would reach the corpora lutea in sudden quantity while in the work of Kilpatrick et al. (1964) maintenance injections of LH were given subcutaneously twice daily. High levels of LH may destroy interstitial tissue secretion or prompt an abundant luteotropin secretion which cannot be maintained later. Following hypophysectomy endogenous LH would gradually be depleted and the only LH reaching the ovary would have to be exogenously supplied. This could explain the differences observed due to time of injection following hypophysectomy. Of course, other hormones are endogenously depleted following hypophysectomy which could influence the effect of LH on the ovary.

Suspecting that LH can be luteotropic at low levels and then antiluteotropic at higher levels by its action on interstitial cells, it is interesting to review a graph on gonadotropic content of the rabbit pituitary during the pseudopregnancy cycle (Hill, 1934). Interstitial cell counts per

microscopic field taken from this paper along with the assay for ovulation-producing ability in estrual does run on the rabbit pituitaries by Hill are compared in the graph.



This data suggest there is a relationship between LH and interstitial cells. Kilpatrick et al. (1964) noted that LH injections affect size of interstitial cells in hypophysectomized does. It would be possible that LH by its action on interstitial cells may be responsible for both maintenance and regression of rabbit corpora lutea.

Corpora lutea of hysterectomized and intact does responded similarly to exogenous HCG. Induction of corpora lutea on day 3 in hysterectomized does was followed by persistence of both sets of corpora lutea, but induction of corpora lutea at day 7 or 13 resulted in rapid regression of the initial corpora lutea. The two sets of corpora lutea in the day 3 group regress synchronously after a 22 to 33 day life span. The day 7 or 13 induced luteal tissue also persisted about 23-29 days. Thus, life span of corpora lutea

in hysterectomized does was variable but longer than that of corpora lutea in the intact pseudopregnant doe.

Interstitial cell counts remained high in hysterectomized does longer than was observed in normal pseudopregnant does and may account for the extended corpora lutea life span resulting from hysterectomy. Whether the uterus is acting directly on the ovarian interstitial tissue or through a hypothalamic-pituitary pathway is not known.

SUMMARY

Initial corpora lutea were induced (day 0) in 126 estrual rabbits by intravenous injection of 100 IU of human chorionic gonadotropin (HCG). Corpora lutea were marked on day 2, 3 or 4 with India ink for subsequent identification and hysterectomy, when desired, was performed on day 2 or 3. A second set of corpora lutea was induced in 38 intact pseudopregnant does with HCG (100 IU, days 3-13, excluding days 8 and 10), in 7 does with combinations of follicle stimulating hormone (FSH, 1 - 2 mg.) and luteinizing hormone (LH, 0.2 - 1.0 mg.) on day 7 and in 11 does with LH alone (0.035 - 1.0 mg.) on day 7. FSH (1.5 mg.) was administered intravenously on day 7 in 2 intact pseudopregnant does. Saline injected and uninjected rabbits having marked corpora lutea served as controls. A second set of corpora lutea was induced in 25 hysterectomized-pseudopregnant does with HCG (100 IU) on days 3, 7 and 13. Ten does hysterectomized 3 days after and 10 days prior to induction of pseudopregnancy served as controls. Repeated laparotomies and unilateral ovariectomies were performed as needed to follow corpora lutea activity.

Marked corpora lutea of the pseudopregnant controls had a life span of about 17-18 days. Injections of HCG in normal pseudopregnant and

hysterectomized does, FSH and LH combinations, or LH alone in pseudopregnant does caused rapid regression of the initial set of corpora lutea when injected on or after day 5. The second set of corpora lutea produced by HCG injections had a normal 17-18 day life span in intact does and a 23-29 day life span in hysterectomized does. Injection of HCG on day 3 into intact and hysterectomized does resulted in two sets of corpora lutea in which the time of morphological and histological regression could not be distinguished in 10 of 11 does. Average luteal cell counts on day 23 were 5.0 for the initial and 4.7 for the induced corpora lutea in the intact pseudopregnant does. The initial set of corpora lutea of intact does, therefore, appeared to have a prolonged life span. Initial corpora lutea of two does reovulated on day 4 were slightly smaller and showed histological signs of regression by day 11 as indicated by luteal cell counts of 3.0 for marked vs. 9.0 for newer corpora lutea. Initial corpora lutea of rabbits reovulated on day 5 rapidly regressed in five of six does.

Initial corpora lutea of two does given an intravenous injection of FSH on day 7 (1.5 mg.) were in varying stages of regression by day 9. Some marked corpora lutea were histologically regressing while the remainder were not. Average luteal cell counts were 4.6 and 8.7 for the two types of marked corpora lutea, respectively. Histological evaluation also revealed that new ovulations were invoked by FSH injection.

One mg. estrone in 1 cc. of sesame oil was subcutaneously injected on days 5, 6, 7 and 8 surrounding an HCG injection (100 IU) on day 7. Does receiving estrone injections on either days 5, 6, 7 and 8 or on days 14, 15, 16 and 17 served as controls. Estrone prevented immediate regression and allowed the initial corpora lutea to have a normal life span. The second set

of HCG induced corpora lutea also had a normal life span making regression of the two sets of corpora lutea asynchronous.

HCG or LH injections on or after day 5 appeared to cause corpora lutea regression by interfering with endogenous estrogen, possibly via ovarian interstitial tissue.

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APPENDIX

Table 1. HCG* injection into pseudopregnant does

Treatment 1

Group	No. of rabbits	Day of HCG injection	Dosage
1	5	3	100 IU in 1 cc. of sterile saline
2	2	4	" " " " " " "
3	6	5	" " " " " " "
4	2	6	" " " " " " "
5	6	7	" " " " " " "
6	2	9	" " " " " " "
7	3	11	" " " " " " "
8	2	12	" " " " " " "
9	7	13	" " " " " " "
10	2	None (control)	1 cc. of sterile saline
11	9	None (control)	none

* All HCG injections were administered into the marginal ear vein. Initial corpora lutea of all does were marked with India ink on day 2, 3, or 4 post initial ovulation.

Table 2. FSH and/or LH injection into pseudopregnant does

Treatment 2 (FSH and LH)*

Group	No. of rabbits	Day of FSH & LH injection	Dosage
12	3	7	1-2 mg. LH and 1-2 mg. FSH
13	4	7	0.1 mg. LH and 1.5 mg. FSH
		FSH alone	
14	2	7	1.5 mg. FSH
		LH alone	
15	2	7	1 mg. LH
16	2	7	.075 mg. LH
17	2	7	.050 - .056 mg. LH
18	5	7	.035 - .045 mg. LH

* FSH and LH were mixed in sterile saline and injected into the marginal ear vein. Does receiving both FSH and LH were given separate but simultaneous injections.

Table 3. Estrone and antiestrogen treatment of HCG injected pseudopregnant does

Treatment 3 (Estrone* and Antiestrogen**)

Group	No. of rabbits	Day of HCG injection	Days of estrone injection	Dosage
19	7	7	5, 6, 7 & 8	100 IU of HCG in 1 cc. of sterile saline plus 1 mg. of estrone in 1 cc. of sesame oil
20	5	none	5, 6, 7 & 8	1 mg. of estrone in 1 cc. of sesame oil
21	6	7	none	100 IU of HCG in 1 cc. of sterile saline
22	4	none	14, 15, 16 & 17	1 mg. of estrone in 1 cc. of sesame oil
23	2	none	none	20 mg. antiestrogen twice on day 7 and once on days 8 and 9
24	2	none	6, 7, 8 & 9	20 mg. antiestrogen twice on day 7 and once on days 8 and 9 plus 1 mg. estrone
25	2	7	6, 7, 8 & 9	20 mg. antiestrogen twice on day 7 and once on days 8 and 9 plus 1 mg. estrone plus 100 IU HCG

* All estrone injections were subcutaneous and all HCG injections were intravenous.

** One in each antiestrogen treated group was given the anti-estrogenic compound, U-11100A, in an oil suspension subcutaneously while the other rabbit received it in a sterile water suspension subcutaneously.

Table 4. HCG injections into hysterectomized pseudopregnant does

Treatment 4 (HCG in hysterectomized does*)

Group	No. of rabbits	Day of HCG injection	Dosage
26	6	3	100 IU of HCG in 1 cc. sterile saline
27	10	7	100 IU of HCG in 1 cc. sterile saline
28	9	13	100 IU of HCG in 1 cc. sterile saline
29	8	none (control)*	none
30	2	none (control)**	none

* Does were hysterectomized days 2, 3 or 4 post initial ovulation.

** Does were hysterectomized 10 days prior to induction of the initial corpora lutea.

Table 5. Observations on the corpora lutea made at repeated laparotomies

Treatment 1 (HCG)

Group	No. of rabbits	Day of HCG injection	Results
1	5	3	Ovulated, both sets of C.L. maintained to about day 21
2	2	4	Marked C.L. lost color & size, but remained until newer C.L. regressed about day 21
3	5	5	Marked C.L. regressed in 4 of 6 and part of marked were regressed in the other 2, newer C.L. had normal life span, marked C.L. remaining regressed with the newer set
4	2	6	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
5	6	7	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
6	2	9	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
7	3	11	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
8	2	12	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
9	7	13	Marked C.L. regressed and newer C.L. had a normal 17-18 day life span
10	2	none (control)	Had a normal 17-18 day life span
11	9	none (control)	Had a normal 17-18 day life span

Table 6. Observations on the corpora lutea made at repeated laparotomies

Treatment 2 (FSH & LH)

Group	No. of rabbits	Day of FSH & LH injection	Results
12	3	7	Ovulated, marked C.L. regressed, 2nd set of C.L. had normal 17-18 day life span
13	4	7	Ovulated, marked C.L. regressed, 2nd set of C.L. had normal 17-18 day life span
		FSH alone	
14	2	7	Ovaries had C.L. in many stages of regression at day 9 and new ovulations had occurred (fig. 24)
		LH alone	
15	2	7	Ovulated, marked C.L. regressed, 2nd set of C.L. had normal 17-18 day life span
16	2	7	Ovulated, marked C.L. regressed, 2nd set of C.L. had normal 17-18 day life span
17	2	7	Ovulated, marked C.L. regressed, 2nd set of C.L. had normal 17-18 day life span
18	5	7	Ovulated and marked C.L. regressed in 4 does, the other did not ovulate and maintenance was questionable. Second set of C.L. had a normal 17-18 day life span.

Table 7. Observations on the corpora lutea made at repeated laparotomies

Treatment 3 (Estrogen and Antiestrogen)

Group	No. of rabbits	Day of hormone injections	Results
19	7	HCG day 7, estrone days 5, 6, 7 & 8	Ovulated, marked C.L. had an 18-20 day life span, newer C.L. had a normal life span
20	5	Estrone days 5, 6, 7 & 8	Had a nearly normal 18-20 day life span
21	6	HCG day 7	Ovulated, marked C.L. regressed, newer C.L. had a normal life span of about 17-18 days
22	4	Estrone days 14, 15, 16 & 17	Marked C.L. had an extended life span of 22-25 days
			(Antiestrogen)
23	2	U11100A days 7, 8 & 9	Had a normal life span (17-19 days)
24	2	Estrone days 6, 7, 8 & 9, U11100A days 7, 8 & 9	Had a normal life span (17-18 days)
25	2	HCG day 7, estrone days 6, 7, 8 & 9, U11100A days 7, 8 & 9	Marked C.L. regressed in the doe receiving the water suspension, but maintained in the doe getting the oil suspension. Newer C.L. had a normal 17-18 day life span.

Table 8. Observations on the corpora lutea made at repeated laparotomies

Treatment 4 (HCG in hysterectomized does)

Group	No. of rabbits	Day of HCG injection	Results
26	6	3	Ovulated, the two set of C.L. regressed synchronously with a life span of 22-29 days
27	10	7	Ovulated, marked C.L. regressed, newer C.L. had normal life span of 22-29 days
28	9	13	Ovulated, marked C.L. regressed, newer C.L. had normal life span of 22-29 days
29	8	none (control)	Had normal 23-29 day life span
30	2	none (control)	Had normal 23-29 day life span

Table 9. Statistical significance of experimental groups

Group	Marked corpora lutea were regressing in No. of No. does by day			Significantly different than group at P level	
		Treatment 1 (HCG)			
11 control	0	7	10		
1	0	5	10	11	
3	4	6	10	11	$P \leq .05$
5	6	6	10	11	$P \leq .005$
11 control	6	6	19		
1	1	4	19	11	$P \cong .06$
		Treatment 2 (FSH and LH)			
11 control	0	7	10		
12 & 13	7	7	10	11	$P \leq .005$
15 - 18	10	11	10	11	$P \leq .01$
		Treatment 3 (Estrogen and Antiestrogen)			
20 control	0	5	10		
21 control	6	6	10	20	$P \leq .025$
19	0	5	10	21	$P \leq .025$
19	0	5	10	20	
		Treatment 4 (HCG into hysterectomized does)			
29 & 30 control	0	8	10		
26	1	6	10	29 & 30	
27	7	7	10	29 & 30	$P \leq .005$
29 & 30 control	0	8	17		
28	8	8	17	29 & 30	$P \leq .005$

Table 10. Average luteal and interstitial cell counts and diameters and average corpora lutea weights of HCG treated pseudopregnant does

Treatment 1 (HCG)

		Histology of the Corpora Lutea					Histology of Interstitial Tissue			Corpora Lutea Weights		
			Av. Luteal Cells					Av. Interstitial Cells			mg.	
			counts/field		diameter(u)			counts/				
Group	Day data were taken	No. of rabbits	Marked	Newer	Marked	Newer	No. of rabbits	field	diameter (u)	No. of rabbits	Marked	Newer
1	18	(1)	6.7	8.3	27.3	25.9	(1)	10	22.6			
	20	(4)	5.2	5.2	24.4	22.5	(4)	13.2	22.7			
	27	(1)	2.7	2.0	16.1	16.1	(1)	17.0	21.5			
2		(1)	3.0	9.0	19.6	31.0	(1)	12.3	23.3			
	23	(1)	1.0	1.3	21.7	22.4				(2)	4.1	5.8
3	8	(1)	1.7		25.6	11.6						
	16									(1)	1.0	9.7
	22	(1)	0	5.0	0	23.3	(1)	9.0	16.5	(1)	1.0	1.7
	23	(1)	6.0	4.7	31.5	25.4	(1)	16.3	16.3			
	24									(1)	0	3.6
	25	(2)	.5	1.3	14.0	16.9	(2)	13.3	21.7	(1)	0	4.0
4	21	(1)	3.0	5.0								
5	10	(1)	3.0	9.0	18.0	15.6	(1)	20.8	17.5			
	14									(1)	0	11.2
6	21	(1)	1.5	7.5	16.8	31.5	(1)	14.3	19.4			
7	20	(1)	1.7	7.0	15.5	30.1	(1)	20.7	17.0			
	30	(1)	0	1.8	0	19.8	(1)	19.0	17.3			
8	27	(1)	.5	7.5	18.6	32.0	(1)	10	21.5			
	28									(1)	0	11.2
9												

Table 10. (Continued)

		Histology of the Corpora Lutea					Histology of Interstitial Tissue			Corpora Lutea Weights		
			Av. Luteal Cells					Av. Interstitial Cells			mg.	
			counts/field		diameter(u)			counts/				
Group	Day data were taken	No. of rabbits	Marked	Newer	Marked	Newer	No. of rabbits	field	diameter (u)	No. of rabbits	Marked	Newer
10	3	(1)	11.3		14.7		(1)	20	14.4			
	19	(1)	1.3		18.2		(1)	10.0	17.5	(1)	7.0	
	21	(1)	.3		17.5		(1)	17.0	17.7	(1)	3.7	
11	8	(1)	7.3		25.7					(1)	12.0	
	13	(1)	6.8		26.4					(1)	11.1	
	17	(1)	2.7		22.2		(1)	9.7	23.6	(1)	4.6	
	19									(1)	3.9	

Table 11. Average luteal and interstitial cell counts and diameters and average corpora lutea weights in FSH and LH treated pseudopregnant does

Treatment 2 (FSH and LH)

		Histology of the Corpora Lutea					Histology of Interstitial Tissue			Corpora Lutea Weights		
			Av. Luteal Cells					Av. Interstitial Cells			mg.	
Group	Day data were taken	No. of rabbits	counts/field Marked	counts/field Newer	diameter(u) Marked	diameter(u) Newer	No. of rabbits	counts/ field	diameter (u)	No. of rabbits	Marked	Newer
12												
13												
14	9	(1)	8.7 4.6	17.3	22.4	13.8 26.1	(1)	22.0	15.6			
	15	(1)	2.3	12.0	19.1	29.6	(1)	18.7	16.8			
15												
16										(2)	1.0	4.5
17	10									(1)	3.3	
	27	(1)	0	2.3	0	21.7	(1)	14.3	20.1			
	36	(1)	0	0	0	0	(1)	7.3	21.7			
18	10	(4)	4.3	14.0	24.1	14.7	(4)	15.4	19.6	(3)	3.1	2.0
	20	(1)	1.7	7.0	26.3	28.2	(1)	15.3	17.5			
	27									(1)	0	3.9

Table 12. Average luteal and interstitial cell counts and diameters and average corpora lutea weights of estrogen and antiestrogen treated does

Treatment 3 (Estrogen and Antiestrogen)

		Histology of the Corpora Lutea					Histology of Interstitial Tissue			Corpora Lutea Weights		
			Av. Luteal Cells					Av. Interstitial Cells			mg.	
			counts/field		diameter(u)			counts/				
Group	Day data were taken	No. of rabbits	Marked	Newer	Marked	Newer	No. of rabbits	field	diameter (u)	No. of rabbits	Marked	Newer
19	17	(3)	3.0	7.0	23.2	23.2	(2)	15.7	20.6	(2)	6.1	10.4
	20	(1)	2.3	6.7	21.0	22.6	(1)	18.0	14.4			
	21	(1)	5.7	7.0	28.0	23.3	(1)	17.7	16.5	(1)	8.9	11.4
	25	(1)	2.0	7.7	21.0	26.6						
	28	(1)	0	7.3	0	24.0		14.0	21.2	(1)	0	7.5
20	17	(3)	5.1		26.5		(1)	21.0	15.1	(2)	6.6	
	21	(1)	1.7		25.2		(1)	10.3	23.1			
	25	(1)	0		0		(1)	13.6	18.9			
	28	(1)	0		0		(1)	15.7	17.5			
			6.7		23.0							
21	10	(1)	3.0	9.0	18.0	15.6	(1)	20.8	17.5			
	14									(1)	0	11.2
22	20	(2)	4.5		25.1		(1)	16.3	18.6	(1)	9.9	
	21	(1)	6.7		26.1		(1)	14.0	18.6	(1)	9.5	
	24	(2)	3.7		20.1		(1)	20.0	18.6	(2)	7.0	
	25	(3)	3.0		23.9		(3)	14.1	19.5	(2)	7.6	
23	10	(2)	7.3		24.2					(1)	13.0	
	14	(1)	7.3		27.0							
	17									(1)	5.0	
24	11	(2)	6.8		29.2		(2)	13.5	20.2			
	14	(1)	6.7		26.8		(1)	14.3	17.9			
	17	(1)	3.0		28.0					(1)	11.6	
25	11	(1)	7.3	12.7	25.9	16.8	(1)	13.7	17.0			
	14	(1)		7.0		24.2	(1)	12.3	20.5			
	17	(1)	4.7	8.3	26.1	24.0				(1)	9.2	

Table 13. Average luteal and interstitial cell counts and diameters and average corpora lutea weights of HCG injected hysterectomized pseudopregnant does

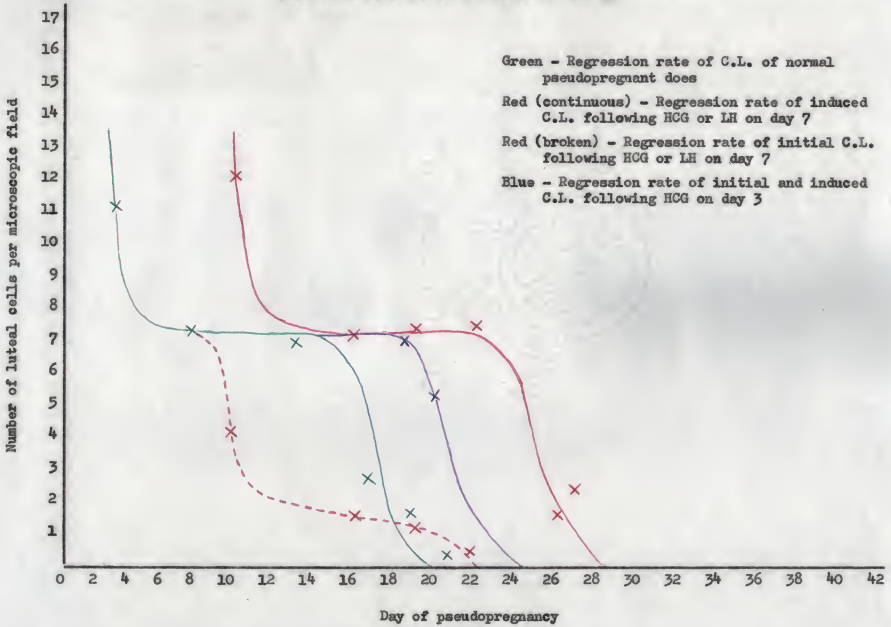
Treatment 4 (Hysterectomy and HCG)

		Histology of the Corpora Lutea					Histology of Interstitial Tissue			Corpora Lutea Weights		
			Av. Luteal Cells					Av. Interstitial Cells			mg.	
			counts/field		diameter(u)			counts/				
Group	Day data were taken	No. of rabbits	Marked	Newer	Marked	Newer	No. of rabbits	field	diameter (u)	No. of rabbits	Marked	Newer
26	22	(1)	6.7	7.3	28.5	22.2				(1)	10.6	7.5
	23	(3)	5.0	4.7	26.6	26.0	(2)	11.2	24.4	(1)	5.6	8.0
	26	(2)	3.7	3.8	19.8	23.7	(2)	23.0	18.4	(2)	8.9	5.9
	30									(4)	5.7	2.7
	37									(1)	5.7	3.4
27	11	(1)	1.0	8.0	18.0	16.3	(1)	15.0	14.7			
	21	(1)	0	7.0	0	25.6	(1)	9.0	27.5			
	26	(1)	.3	4.7	9.1	23.8	(1)	15.3	21.6			
	27	(1)	1.0	3.7	12.8	28.0	(1)	14.3	17.0	(1)	0	9.5
	29									(1)	0	7.5
	34	(1)	0	6.0	0	21.7				(1)	0	3.8
	36	(2)	0	3.3	0	21.5	(2)	13.1	20.4			
	41									(1)	0	4.4
28	15	(1)	3.7	17.0	24.0	11.6	(1)	15.3	19.4			
	17	(1)	2.3	8.3	25.2	16.6	(1)	22.5	17.5			
	32	(1)	0	7.3	0	27.3	(1)	12.0	18.7			
	35	(1)	0	4.0	0	24.5				(1)	0	5.0
	36	(1)	0	3.3	0	28.5				(1)	0	11.7
	37	(1)	0	7.3	0	26.4	(1)	12.3	21.2	(1)	0	3.1
	38									(1)	0	4.5
	39	(1)	0	2.7	0		(1)	13.0	19.8	(1)	0	4.4
	41	(1)	1.7	7.7	16.8	21.7	(1)	14.3	17.0	(1)	0	7.6

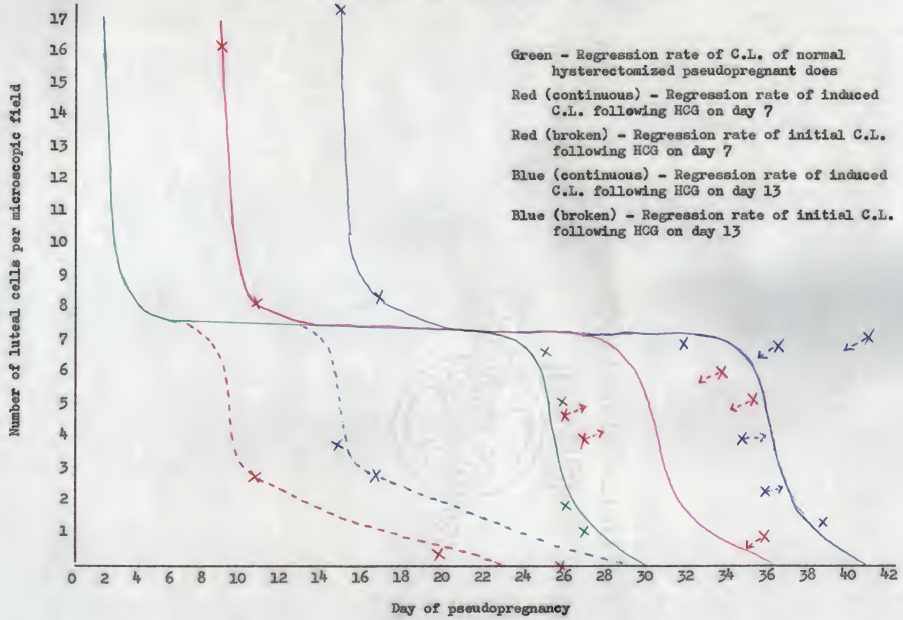
Table 13. (Continued)

Group	Day data were taken	Histology of the Corpora Lutea: No. of rabbits	Av. Luteal Cells counts/field: Marked	Newer	Av. Luteal Cells diameter(u): Marked	Newer	Histology of Interstitial Tissue: No. of rabbits	Av. Interstitial Cells counts/field	diameter (u)	Corpora Lutea Weights mg.: No. of rabbits	Marked	Newer
29	22									(1)	9.1	
	23									(1)	7.0	
	25	(1)	6.7		25.4		(1)	14.3	21.9	(1)	8.2	
	26	(2)	2.8		22.3		(2)	10.3	19.9	(2)	8.2	
	27	(1)	1.0		14.0		(1)	14.3	19.1			
	30									(1)	3.1	
	31	(1)	0	0	0	0	(1)	12.0	20.3			
30	22	(2)	4.7		23.2							
	23									(1)	7.0	
	28	(1)	2.3		14.7					(1)	3.4	
	30	(1)	1.7		23.3					(3)	3.3	

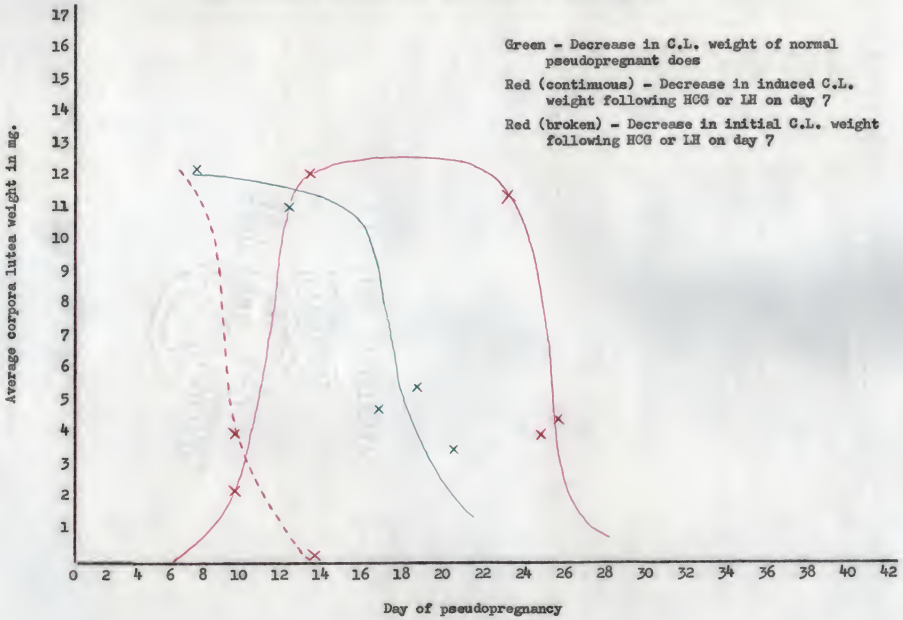
Graph I. Rate of decrease of luteal cells for pooled HCG and LH treated pseudopregnant does drawn from data listed in tables 10 and 11



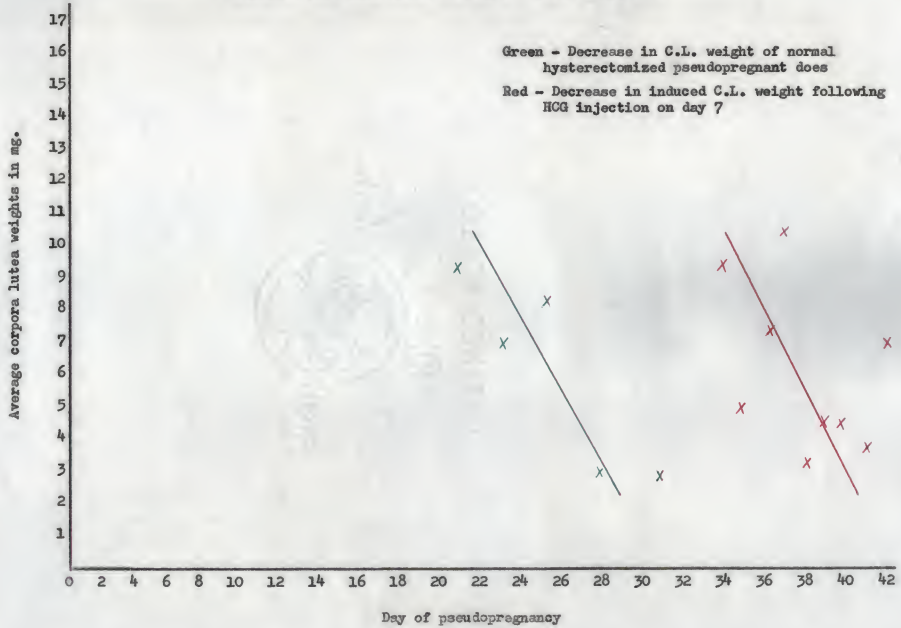
Graph II. Rate of decrease of luteal cells for HCG treated hysterectomized pseudopregnant does drawn from data listed in table 13



Graph III. Rate of decrease in corpora lutea weight for pooled HCG and LH treated pseudopregnant does drawn from data listed in tables 10 and 11



Graph IV. Rate of decrease in corpora lutea weight of HCG and LH treated pseudopregnant does drawn from data listed in table 13



EXPLANATION OF PLATE I

Fig. 1. HCG administered on day 3 (Group 1). Photograph shows that original (marked) corpora lutea as well as HCG induced (unmarked) corpora lutea are normal at day 12.

Fig. 2. HCG administered on day 4 (Group 2). Photograph indicates that original (marked) corpora lutea are small and nonvascular while HCG induced (unmarked) corpora lutea appear normal at day 11.

Fig. 3. Uninjected control. Note that corpora lutea are marked and appear normal on day 10.

Fig. 4. HCG administered on day 5 (Group 3). This photograph taken on day 9 shows the presence of different stages of regression in the original (marked) corpora lutea noted in 2 does of this group following HCG injection and new ovulation.

Fig. 5. HCG administered on day 7 (Group 5). Note that original (marked) corpora lutea are regressing by 3 days post-HCG and that new ovulations have occurred.

Fig. 6. LH administered on day 7 (Group 15-18). Note that original (marked) corpora lutea are regressing by 3 days post-HCG and that new ovulations have occurred. Also note that effects are very similar to those of HCG treatment (Fig. 5).

PLATE I

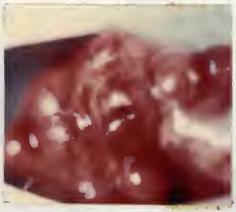


Fig. 1

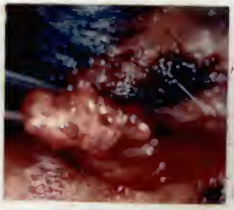


Fig. 2

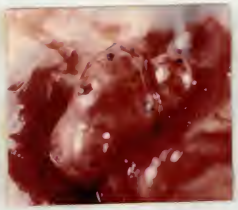


Fig. 3

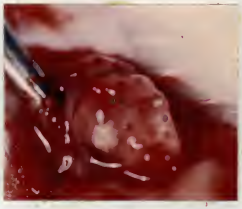


Fig. 4

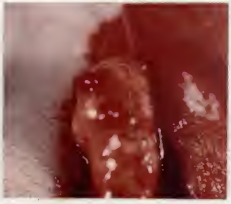


Fig. 5

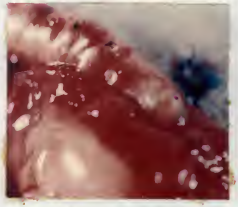


Fig. 6

EXPLANATION OF PLATE II

Fig. 7. Estrone injected surrounding HCG administration on day 7 (Group 19). At laparotomy on day 15 both initial (marked) and HCG induced (unmarked) corpora lutea appear normal.

Fig. 8. Estrone injected surrounding HCG administration on day 7 (Group 19). Photograph taken on day 20 indicates that initial (marked) corpora lutea are regressing prior to regression of HCG induced corpora lutea (unmarked).

Fig. 9. Estrone control (Group 20). Photograph shows marked corpora lutea of estrone control doe in late regression on day 20.

Fig. 10. Estrone and antiestrogen in sterile water suspension given surrounding HCG administration on day 7 (Group 25). Photograph taken on day 14 shows that initial (marked) corpora lutea are regressing and HCG induced corpora lutea (unmarked) are normal.

Fig. 11. HCG administered into a hysterectomized doe on day 3 (Group 26). Photograph shows that part of the initial (marked) corpora lutea are normal while others are regressing. This was the only doe which had regressing marked corpora lutea following HCG injection on day 3.

Fig. 12. HCG administered into a hysterectomized doe on day 13 (Group 28). Photograph taken on day 31 indicates that initial (marked) corpora lutea are completely regressed and that HCG induced corpora lutea are not regressing.

PLATE II

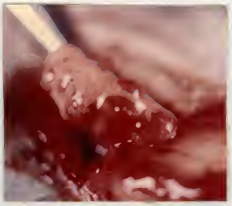


Fig. 7

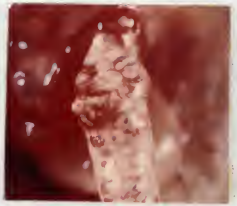


Fig. 8

Fig. 9

Fig. 10

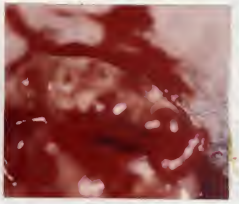


Fig. 11

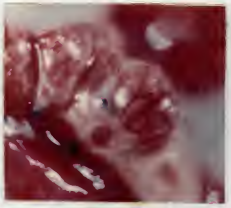


Fig. 12

EXPLANATION OF PLATE III

Fig. 13. Photograph shows a regressing corpus luteum. Only one luteal cell would be counted in this field. (970 x magnification)

Fig. 14. Photograph shows a functional corpus luteum. Ten luteal cells would be counted in this field. (970 x magnification)

Fig. 15. Photograph shows a forming corpus luteum. Seventeen luteal cells would be counted in this field. (970 x magnification)

PLATE III

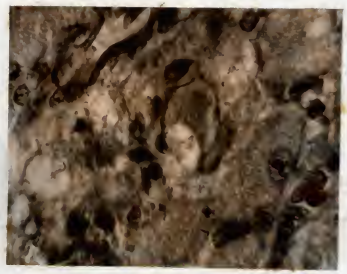


Fig. 13

Fig. 14

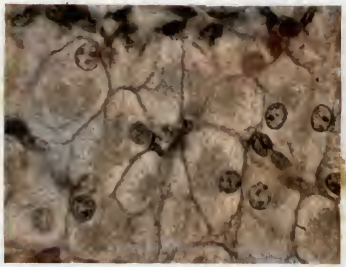


Fig. 15

EXPLANATION OF PLATE IV

Fig. 16. HCG administered on day 3 (Group 1). Photograph shows that both initial (marked) and 3-day HCG induced corpora lutea are regressing on day 20. (100 x magnification)

Fig. 17. Control (Group 11). Photograph shows that initial (marked) corpora lutea of control does are regressing on day 18. (100 x magnification)

PLATE IV

Fig. 16

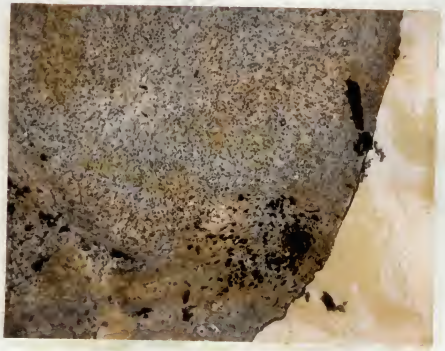


Fig. 17

EXPLANATION OF PLATE V

Fig. 18. HCG administered on day 4 (Group 2). Photograph taken of an ovary removed on day 11 shows that initial (marked) corpora lutea are regressing while day 4 HCG induced corpora lutea appear functional. (100 x magnification)

Fig. 19. HCG administered on day 7 (Group 5). Note that initial (marked) corpora lutea are fully regressed on day 21, but day 7 induced corpora lutea are still functional in appearance.

PLATE V

Fig. 18

Fig. 19

EXPLANATION OF PLATE VI

Fig. 20. HCG administered on day 5 (Group 3). Photograph shows condition of luteal cells in initial (marked) corpora lutea of one of the two does which maintained part of the initial (marked) set of corpora lutea following the HCG injection. Ovary was removed for histological preparation on day 23. (430 x magnification)

Fig. 21. HCG administered on day 5 (Group 3). Photograph shows that not all marked corpora lutea on the ovary possessing the corpus luteum shown in plate VI, fig. 20 were maintained. The initial marked corpora lutea shown here is fully regressed while the day 5 HCG induced corpus luteum appears functional.

PLATE VI

Fig. 20

Fig. 21

EXPLANATION OF PLATE VII

Fig. 22. HCG administered on day 7 (Group 5). Photograph from an ovary removed on day 10 shows initial (marked) corpora lutea are regressing and that 7 day HCG induced corpora lutea are forming. (100 x magnification)

Fig. 23. HCG administered on day 7 (Group 5). Photograph is taken from the regressing initial (marked) corpora lutea shown in fig. 22 (this plate). (970 x magnification)

PLATE VII

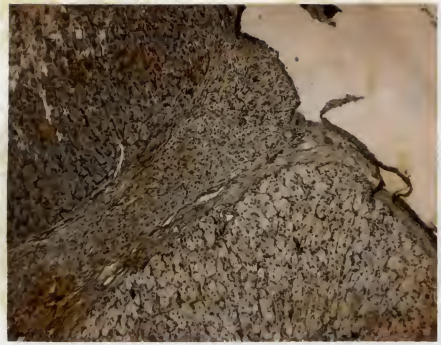


Fig. 22

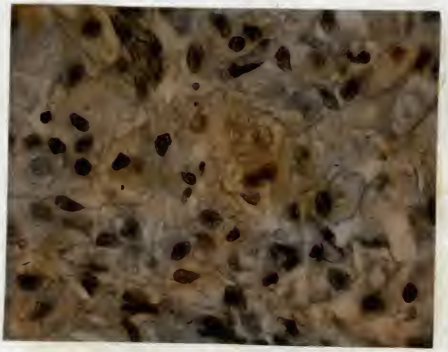


Fig. 23

EXPLANATION OF PLATE VIII

Fig. 24. FSH administered on day 7 (Group 14). Photograph taken from an ovary removed on day 9 shows the difference in stage of regression of two initial (marked) corpora lutea and reveals that a new ovulation has occurred. (100 x magnification)

Fig. 25. LH administered on day 7 (Group 15-18). Photograph shows that by 3 days post-LH injection initial (marked) corpora lutea are regressing and LH induced corpora lutea are forming. (100 x magnification)

PLATE VIII

Fig. 24

Fig. 25

EXPLANATION OF PLATE IX

Fig. 26. Estrone injected surrounding HCG administration on day 7 (Group 19). Photograph taken from an ovary removed on day 20 shows that initial (marked) corpora lutea are now beginning to regress while corpora lutea resulting from the day 7 HCG injection are functional in appearance.

Fig. 27. Estrone control (Group 20). Photograph shows that corpora lutea of an estrone control are regressing on day 21.

PLATE IX

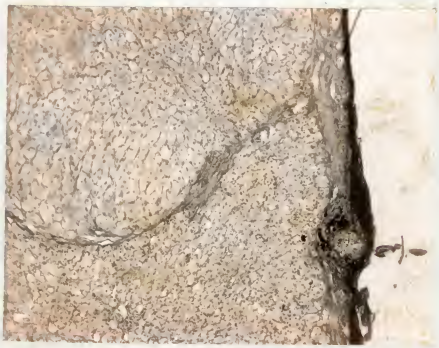


Fig. 26

Fig. 27

EXPLANATION OF PLATE X

Fig. 28. HCG administered into a hysterectomized doe on day 3 (Group 26). Photograph shows that initial (marked) and day 3 HCG induced corpora lutea of this doe are regressing synchronously on day 26.

Fig. 29. Hysterectomized control (Group 29). Photograph shows that marked corpora lutea of this hysterectomized control are regressing on day 26.

PLATE X

Fig. 28

Fig. 29

THE LIFE SPAN OF INITIALLY AND EXPERIMENTALLY INDUCED CORPORA LUTEA OF PSEUDOPREGNANT AND HYSTERECTOMIZED-PSEUDOPREGNANT RABBITS FOLLOWING HCG, LH AND ESTRONE TREATMENT

by

LARRY LEE COON

B. S., Kansas State University, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1964

Initial corpora lutea were induced (day 0) in 126 estrual rabbits by intravenous injection of 100 IU of human chorionic gonadotropin (HCG). Corpora lutea were marked on day 2, 3 or 4 with India ink for subsequent identification and hysterectomy, when desired, was performed on day 2 or 3. A second set of corpora lutea was induced in 38 intact pseudopregnant does with HCG (100 IU, days 3-13, excluding days 8 and 10), in 7 does with combinations of follicle stimulating hormone (FSH, 1 - 2 mg.) and luteinizing hormone (LH, 0.2 - 1.0 mg.) on day 7 and in 11 does with LH alone (0.035 - 1.0 mg.) on day 7. Saline injected and uninjected rabbits having marked corpora lutea served as controls. A second set of corpora lutea was induced in hysterectomized-pseudopregnant does with HCG (100 IU) on days 3, 7 and 13. Does hysterectomized 3 days after and 10 days prior to induction of pseudopregnancy served as controls. Repeated laparotomies and unilateral ovariectomies were performed as needed to follow corpora lutea activity.

Marked corpora lutea of the pseudopregnant controls had a life span of about 17-18 days. Injections of HCG in normal pseudopregnant and hysterectomized does, FSH and LH combinations or LH alone in pseudopregnant does caused rapid regression of the initial set of corpora lutea when injected on or after day 5. The second set of corpora lutea produced by HCG injections had a normal 17-18 day life span in intact does and a 23-29 day life span in hysterectomized does. Injection of HCG on day 3 into intact and hysterectomized does resulted in two sets of corpora lutea in which the time of morphological and histological regression could not be distinguished in 10 of 11 does. Average luteal cell counts on day 23 were 5.0 for the initial and 4.7 for the induced corpora lutea. The initial set of corpora lutea of intact does appeared to have a prolonged life span. Initial corpora lutea of two does reovulated on day 4 were slightly smaller and showed histological

signs of regression by day 11 as indicated by luteal cell counts of 3.0 for marked vs. 9.0 for newer corpora lutea. Initial corpora lutea of rabbits reovulated on day 5 rapidly regressed in five of six does.

One mg. estrone in 1 cc. of sesame oil was subcutaneously injected on days 5, 6, 7 and 8 surrounding an HCG injection (100 IU) on day 7. Does receiving estrone injections on either days 5, 6, 7 and 8 or on days 14, 15, 16 and 17 served as controls. The late estrone control group was used to determine the length of corpora lutea life following four days of estrone administration. Estrone prevented immediate regression and allowed the initial corpora lutea to have a normal life span. The second set of HCG induced corpora lutea also had a normal life span making regression of the two sets of corpora lutea asynchronous.

HCG or LH injections on or after day 5 appeared to cause corpora lutea regression by interfering with endogenous estrogen production.