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IN THE SYRIAN HAMSTER.

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UTERINE TRANSPLANTATION AND LUTEOLYSIS
IN THE SYRIAN HAMSTER

BY

BURTON VAUGHAN CALDWELL

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INTRODUCTION

The corpus luteum was first recognized as a regulator of sexual periodicity by Beard (1897). In attributing the functions of regulating the onset of labor and preventing the onset of the next ovulation to the corpus luteum, he reasoned that prevention of ovulation would avert the premature termination of pregnancy, and even in the non-pregnant animal, he suggested that the corpus luteum may act to regulate the onset of ovulation. In view of the paucity of data available to him, Beard, in the first attempt at assigning a function to this structure, was remarkably accurate. It remained for Loeb (1923) to provide an insight into the factors involved in regulating the corpus luteum. His now classic article, "The Mechanism of the Sexual Cycle," provided impetus for many investigations on the subject of ovarian periodicity, and pointed out for the first time the possible regulatory relationship between the uterus and corpus luteum. Working with the guinea pig, he found that total or subtotal excision of the uterus prolonged the functional life span of the corpus luteum from a normal interval of about fifteen to seventeen days after ovulation, to sixty or more days. He also noted the very important fact that hysterectomy in the absence of a preformed corpus luteum would not prevent the next ovulation, but was closely followed by a normal ovulation. The resulting corpus luteum

once formed, would then remain preserved for the unnaturally long period of time. He concluded that somehow activity of the uterus was at least partly responsible for the onset of regression in the corpus luteum, and that the relationship between the uterus and corpus luteum was reciprocal. The corpus luteum, he felt, "produces a substance which 'sensitizes' the uterus, and the uterus in return sends back an effect that causes the latter to regress." He continued his investigations by performing various subtotal hysterectomies and recorded results that suggested a direct relationship between the amount of uterine tissue removed and the prolonged survival of the corpus luteum.

Loeb's work on luteolysis has not been extended to any significant degree since publication of his original results. A number of factors to which he alluded have been clarified, e.g., isolation of hormones responsible for ovarian periodicity and accumulation of much additional morphological and physiological information, but today little more can be said about what factors are responsible for luteolysis.

The similarity between the effects of pregnancy and hysterectomy on the life of the corpus luteum suggested to Loeb the possibility that in pregnancy an inactivation of the uterus, interrupting its normal luteolytic effect, might be partially or wholly responsible for the prolongation of the life and function of the corpus luteum. Loeb also attempted the logical step of transplanting uterine tissue into hysterectomized animals hoping to re-establish a more

normal periodicity, but had no success in shortening the period of corpus luteum preservation by this technique. He tried to duplicate his results in laboratory rats, but was unable to demonstrate any relationship between hysterectomy and life span of corpora lutea.

Since Loeb's work with the guinea pig, thirteen additional mammalian species have been studied with respect to response of the ovary to hysterectomy, with reactions differing markedly from one species to another. A marked elongation of corpora lutea has been observed in cows and sheep (Witbank and Casida, 1956), and in the unmated pregnant pig (Du Mesnil du Buisson and Dauzier, 1959; Spies, Zimmerman, Self and Casida, 1960), where the luteal phase may be so prolonged as to exceed the gestation period of the species. In contrast, the operation has no effect on the ovaries of the ferret (Deanesly and Parks, 1933), the macaque (Burford and Diddle, 1936), or the opossum (Hartman, 1925). Changes in the ovarian cycle, similar in nature to those observed in the guinea pig but much less pronounced, have been described in the pseudopregnant rat (Bradbury, Brown and Gray, 1950) and rabbit (Asdel and Hammond, 1933; Loeb and Smith, 1936; Gillard, 1937; Mishell and Motyloff, 1941; Heckel, 1942; Chu, Lee and You, 1946). Hysterectomy during pregnancy in the rat (Bradbury, 1937; Bradbury et al., 1950), rabbit (Micale, 1940; Greep, 1941; Chu et al., 1946), and hamster (Klein, 1938), brings about a shortening

rather than prolongation of the life of the corpus luteum, especially when performed in the second half of gestation.

A study on some long-term effects has shown that hysterectomy leads eventually to ovarian degeneration involving increased follicular atresia in the rat (Spurny, 1959), mouse (Hall, 1934), rabbit (Sessums and Murphy, 1933; Mishell and Motyloff, 1941; Tenney, Parker and Robbins, 1958), dog (Cheval, 1934, 1935) and cat (Baidin, 1930).

The first proposed explanation for prolongation of luteal function due to hysterectomy hypothesizes the secretion of a specific substance by the uterus which abbreviates the life of the corpus luteum (Loeb, 1927; Bradbury, Brown and Gray, 1950; Mishell and Motyloff, 1941; Tenney, 1958; and Hechter et al., 1940). Hechter et al., (1940) found in rats that grafts of estrous uteri shortened the pseudopregnancies of hysterectomized animals to normal length. Implantation of similar tissue which had been killed by freezing had no such effect, nor did successful grafts of uteri from diestrous donors. Bradbury, Brown and Gray (1950) found that partially hysterectomized rats in which the remaining uterine tissue was continuous with the cervix, hence properly drained, experienced pseudopregnancies of normal length. However, when continuity was interrupted, uterine remnants becoming greatly distended and endometrium atrophied, the animals had prolonged pseudopregnancies. These investigators, having been successful in reversing

elongated pseudopregnant cycles by implanting estrous uterine tissue, view the effect as one induced by a direct corpora luteal antagonist, an anti-luteal factor, the removal of which allows persistence of the corpora lutea. Correspondingly, their data suggests that maintenance of the corpora lutea during pregnancy may depend upon removal or neutralization of the uterine antagonist. Hechter et al., have postulated that during pregnancy the placenta may be responsible for the ineffectiveness of the anti-luteal factor. They base their view on the fact that removal of both placenta and fetus causes corpora luteal regression, whereas if fetus alone is removed, no effect on corpora lutea is noted.

Velardo, Olsen, Hisaw and Dawson (1953) tried to duplicate the above experiments with no success, concluding that their rats did not show a significant prolongation of pseudopregnancy due to hysterectomy. However, their denial was based on operations performed later in the luteal phase than those of Bradbury et al., and of Hechter et al. Whereas the latter workers had operated in the range from the fourth to seventh days of pseudopregnancy, and many of Bradbury's animals lacked uteri when they entered pseudopregnancy, Velardo and associates excised the uteri on the ninth day. It seems very probable that this difference in time may be crucial, for by the ninth day of a twelve day pseudopregnancy the corpora lutea must be on the verge of regression, if that process has not already been initiated.

Under certain circumstances the endometrium of the rat

has been shown to be luteotrophic rather than luteolytic, for when deciduomas are induced by trauma, pseudopregnancies of otherwise normal animals are lengthened to twenty-two days or more (Ershoff and Duell, 1943; Velardo, Dawson, Olsen and Hisaw, 1953). This is not true in mice, however, and Kammel and Atkinson (1948) suggest that the reason may lie in the shorter life span of the decidual tissue in this species.

In most cases, nonspecific portions of the uterine horn, as long as the amounts exceed threshold, are adequate to produce luteal regression in the nonfertile cycle. The occurrence of estrous cycles in guinea pigs following uterine and endometrial autotransplants further suggests that the endometrium is the specific tissue involved in the cycle (Butcher et al., 1962). However, it has also been reported that uterine autotransplants have no effect on the occurrence of post-hysterectomy estrous in guinea pigs (Loeb, 1927), gilts (Spies, 1960) and rabbits (Inskeep, 1965). Evidence has been presented that the endometrium is directly involved in the regression of corpora lutea in the rat (Hill and Alpers, 1961). This endometrial activity is negated by transection of the sacral spinal cord, which effectively denervates motor secretory stimulation of the endometrial glandular epithelium.

Further evidence for endometrial involvement is seen in shortened estrous cycles in pseudopregnant hysterectomized rats injected with endometrial suspensions (Hansel, 1965), and shortening of the life span of the corpora lutea of rabbits to approximately that of pseudopregnancy by whole uterine

implants (Chu et al., 1946). Endometrial filtrates from days twelve and thirteen of the estrous cycle increased synthesis of progesterone by gilt luteal tissue in vitro, whereas filtrates from days sixteen and eighteen showed an inhibitory effect on this hormone production (Duncan et al., 1961). However, these factors influencing progesterone synthesis in vitro may not be involved with corpora lutea function in vivo. Bovine endometrial extract retarded ovarian atrophy following hysterectomy in the rabbit (Mishell and Motylloff, 1941) and similar effects were produced by administration of aqueous extracts of uterine tissue to the same species (Tenney et al., 1955).

In rats in which the pituitary gland was transplanted beneath the kidney capsule, the corpora lutea persisted for long periods of time (Everett, 1956). Following administration of estrogen, corpora lutea enlarged to about the size of those in the pregnant rat. Hansel (1965) further found that homotransplantation of hypothalamic tissue along with the pituitary resulted in luteal regression within eight days in a significant percentage of those animals tested. Brain cortex tissue transplanted as a control did not show the same results, indicating that the presence of the hypothalamus in close coordination with the pituitary may be an essential condition for luteolysis. Hansel's results suggest that it is the pituitary that produces the luteolytic substance and sheds little light on the presumed role of the uterus in the process.

Bradbury and associates (1950) concluded from their work and a review of the investigations reported at that time, that in some species there may be a luteolytic substance, especially located in the endometrium, which hastens the involution of the corpus luteum during nonfertile cycles. In the absence of the uterus, this luteolytic activity would not be present. Experimental results up to the present do not allow more specific statements concerning the role of endometrium in luteolysis.

In contrast to the view that the uterus may act as an endocrine organ with a specific hormone secretion effecting luteolysis, is the contention, supported by many competent investigators, that there is a direct neural control over the process of luteal regression. The ovaries and uterus can function in some species in a normal manner without a nerve supply; copulation, implantation, pregnancy and parturition proceeding normally in the absence of nervous connections. This fact, however, does not diminish the importance of nervous function in the normal reproductive process and results suggesting the involvement of neural influences modifying the estrous cycles of certain species should be considered as equally significant in evaluating all possible mechanisms (Nalbandov, 1963).

Uterine distention in the rat prolonged the period of gestation and corpora lutea function, when paraffin was substituted for the products of conception (Selye, 1934). This elongation by distention was thought to occur by the neural

stimulation of the anterior pituitary causing the persistence of corpora lutea perhaps by increasing or prolonging the release of LTH (Prolactin), the luteotrophic hormone in rats. A similar set of experiments with the same species, designed to duplicate these results, failed to show the above relationship between uterine distention and corpora lutea length, (Bradbury, 1941; Greene, 1941). The effect of plastic beads sutured in the uterus three days after the first signs of heat, and causing distention, showed a significant shortening of the estrous cycle. However, if the section of the uterus containing the beads is denervated, uterine distention induced by these objects is ineffectual in producing a significant shortening of the cycle. Similar denervation of a section of the uterus without inserting a bead had no effect on the estrous cycle, (Moore and Nalbandov, 1953). These authors reasoned that a neurogenic stimulus from the distended uterine horn probably acts in the regulation of the secretions of the anterior pituitary gland. They also found that the time of bead insertion was of importance, with placements later in the cycle not showing the same effect of shortening. This differential response of the cycle interval to the time of insertion may be attributed to humoral as well as neural factors, and may also help to explain the differing results obtained in investigations on the rat mentioned earlier. The mechanism by which this time-related effect may be inhibited could be through the change in circulating levels of estrogens or progesterone which vary at different

times of the estrous cycle. It is possible that the concentrations of these hormones may alter the capacity of the uterine nerves to respond to the stimulus of the bead, or perhaps change the ability of the hypothalamus or pituitary to respond to uterine stimuli.

Other investigators attempting to duplicate these findings in gilts were unsuccessful in demonstrating uterine denervation as a factor in regulating corpora lutea persistence (Anderson and Melampy, 1962). Earlier work (Huston and Nalbandov, 1953) and extensive work lately (Hawk, 1965) which indirectly may relate to this subject, indicates that the presence of a mechanical irritant in the oviduct, and various rings in the uterus, tends to block ovulation. The blockage in the fowl may be extended for as long as twenty days but ovulation may be brought about at any day by the injection of LH. Huston and Nalbandov feel that this phenomenon, like the plastic beads in the uterus of sheep, may involve a neural mechanism. However, definite work in this area is still lacking.

Experiments with rats and ewes have further shown that in both species there is a local action of the uterus relative to corpus luteum life span (Inskeep, 1965). The author concluded from these studies and those of Du Mesnil du Buisson, (1941), whose results he confirmed, that the ability of the uterus to limit corpus luteum life span may be dependent upon the proximity of the uterus to the ovary. The results, showing re-establishment of normal ovarian cycles with

autografts of uterine tissue in distant parts of the body seem to refute this possibility, at least in those species in which this has been accomplished (Hechter, 1940; Duby, 1965).

In the guinea pig, ewe, cow, and chicken, as mentioned above, there is evidence relating ovarian function and neural influences, possibly working through the control of secretions of the anterior pituitary. However, it has not been demonstrated to complete satisfaction of all that the regular occurrence of the estrous cycle is under direct neural control of stimuli originating in the uterus.

Hechter et al., (1940) pointed out that hysterectomy in the rat might lead to an accumulation of estrone which might be the cause of corpora lutea maintenance. It was reasoned that increased levels of estrone might accelerate and maintain the production of a pituitary luteotropin after the uterus is removed, and may be the mechanism for prolonging luteal function. This was the basis for the hypothesis advanced by Heckel (1942) who found in rabbits that the extent of luteal function by subtotal hysterectomy is roughly proportional to the amount of uterine tissue removed. He offered the suggestion that removal of the uterus had an "estrogen-sparing" effect. The anterior quarter of one horn in gilts and the body and cervix in heifers may approach the threshold quantity of uterine tissue necessary to metabolize the gonadal steroids and thereby reduce the circulating level as well as the physiological activity of the substances. This view is based on the assumption, then, that

the greater amount of estrogen available to the corpora lutea prolongs their life.

The same relationship has been demonstrated for a progesterone-sparing effect (Bradbury et al., 1950; Gomes and Erb, 1965), the rationale being that high titers of circulating progesterone may inhibit pituitary gonadotropins which are essential for initiation of follicular growth, maturation, and ovulation. The uterus may selectively metabolize this hormone at varying rates during specific points in the ovarian cycle which could be a mechanism for regulating luteal regression.

The experimental evidence supporting these hypotheses is not conclusive, but they have been presented in an attempt to explain luteolysis and should, therefore, be considered.

Gardner et al. (1963) demonstrated that estrogens, both synthetic and natural, can maintain functional corpora lutea in gilts. They noted that estrogen was able to increase progesterone concentration in, but not able to maintain the size of, the corpus luteum. This may indicate that there are two different mechanisms involved in maintenance of corpus luteum size and function. In rats there is evidence that estrogen can extend the functional life span of the corpus luteum by causing the increased production of prolactin (Selye et al., 1934; Lewis and Turner, 1941). In support of this contention, to these workers, are results obtained in rabbits and rats that corpora lutea can be maintained by injections of estrone (Robson, 1937; Chu et al. 1946;

Lyons et al., 1943). Pincus (1937) indicated that injections of estrone into the intact rabbit increased estriol in the urine, but injections in the hysterectomized animal did not produce this effect. Estriol is known to be a less active estrogen than is estrone, and this holds true for maintenance of the corpus luteum, (Gardner et al., 1963). Hechter et al. (1940) and Chu et al. (1946) observed regression of corpora lutea in rats and rabbits after transplants of uterine tissue into hysterectomized animals and suggested that estrogen may be converted to inactive forms in the presence of a functional uterus. Work with progesterone led them to believe that this hormone was able to speed the rate of estrogen conversion.

Sammelwitz et al. (1956) and Spies et al. (1957, 1959), reported that exogenous progesterone treatment resulted in regression of the corpora lutea in pregnant gilts. The latter investigators were able to show that progesterone was not exerting its luteolytic effect through the uterus by noting regression of corpora lutea in hysterectomized animals after its administration. Also, it probably was not acting directly since local injections into the ovary did not influence average corpus luteum weight. They assumed that the systemic administration of progesterone was blocking the release and/or production of a corpus luteum-maintaining substance from some source such as the pituitary gland. Evidence indicates that this substance is probably not prolactin, since 25 or 50 mg. daily failed to prevent the

regression of corpora lutea in the progesterone-treated pregnant gilt (Sammelwitz and Nalbandov, 1958). It is thought that progesterone may exert its effect by inhibiting the release of LH from the anterior pituitary. An excellent review article (Gomes and Erb, 1965), however, suggests that further work must be concluded before this relationship can be definitely accepted. If, however, it is assumed that this is indeed the mechanism for at least those species in which LH appears to be luteotrophic, (rabbit Kilpatrick et al., 1964 , cow Hansel et al., 1965), this would begin to explain a possible inter-regulation between pituitary and uterus, if the uterus were shown to be able to metabolize progesterone selectively at different times of sexual cycles. Preliminary investigation leading to a possible conclusion on this matter is being attempted, (Williams et al., 1965), but results are not yet definite.

It has been found recently that estrogen is inactivated by uterine preparations in vitro (Klebanoff, 1965). The uterus of the rat contains an enzyme which in the presence of H_2O_2 can catalyze the inactivation of estradiol as well as certain other estrogens. This enzyme (called uterine peroxidase) is present only under certain experimental conditions. It is absent from ovariectomized animals but appears following estrogen administration. Progesterone decreases accumulation of the enzyme in the uterus, and its presence in the uterus in response to ovarian hormone concentrations is most noteworthy. Eosinophils have been shown to be the carriers of

this enzyme and their appearance during only specific times in the estrous cycle, correlating with high estrogen concentrations, compels consideration of their role in discussion of possible mechanisms operating in vivo. It has been demonstrated by the same author that human endometrial scrapings also contain peroxidase activity at various times.

It is evident that the uterus may be important in regulating levels of circulating gonadal steroids and may act indirectly, through the anterior pituitary, to influence the rate of secretion and/or production of gonadotropins from that gland. Varying gonadotropin levels in turn affect the life span of various ovarian structures, including the corpus luteum.

From this review of existing evidence and suggestions for elucidating the factor, or factors, responsible for regulation of corpora lutea during various ovarian cycles, it is clear that crucial work in this area is still lacking. The present work is designed to test the relationship of the endometrium to the ovarian cycle in the Syrian hamster (Mesocricetus auratus), and purports to increase the body of evidence supporting the existence of a luteolytic factor originating in the proliferative endometrium.

The evolution of a new technique has been realized through the kind cooperation of the investigators at the University of Massachusetts (Duby, 1965) and our methods are essentially modifications of theirs, suited to our specific requirements. The cheek pouch as a site for tissue trans-

plantation was first used by Patt, Handler, and Lutz (1951) and since then has been found applicable to many endocrine studies. The cheek pouch as a site for demonstrating ovarian transplantation and its relationship to the uterine-pituitary axis, if one does exist, is unique with this laboratory.

MATERIALS AND METHODS

A. General:

The Syrian hamster (Mesocricetus auratus) was selected as the experimental subject primarily because of the unique immunological relationship of its cheek pouch. The unusual nature of the pouch is due in part to the fact that it is alymphatic and composed chiefly of intermembranous loose connective tissue which may retard inert particles from diffusing out of the area (Shepro et al., 1953). Shepro and associates postulated that the retardation of transplantation antigens may be the cause of its excellence as a transplant site for both homologous and heterologous tissues. It is an easily accessible area, resistant to infection and inflammation, and relatively rich in blood supply. The latter attribute allows for rapid vascularization of implants, facilitates a high percent of transplant success, and augurs well for acceptance of relatively large amounts of tissue. Black (1965) had unusual success in transplanting oviduct and pituitary, making it entirely possible that ovarian and uterine tissue might be transplanted successfully also.

All animals were housed singly in 12½" by 15½" opaque, plastic cages covered with 1" wire screening. An ample supply of shavings was provided and aided in curtailing infection. Food and water were always available. Purina laboratory chow (suitable for hamsters) was supplemented

weekly with "Hamster Food" (Geisler) and forage.

The temperature of the animal room was maintained between 65^oF. and 75^oF., an attempt being made to regulate it at 72^oF., the optimum (Short, 1963). Another important factor in the general health of the colony is the relative humidity. Values below 45/mm/Hg and above 55/mm/Hg have been shown to affect the mating habits of the animals, necessitating a close surveillance of this parameter. Attempts to regulate the humidity at 50/mm/Hg, the optimum, were often thwarted due to lack of control over the moisture content of the entering air. A reasonable success was achieved in this venture by varying the moisture content of the room and using an exhaust fan to regulate the air circulation.

One of the major advantages in using opaque cages for this investigation is that it restricts the opportunity for inter-cage observation of the characteristic mating behavior. Although it has not been demonstrated that visual excitation does occur, neighbor interest in the proceedings when all wire cages were used was noted in previous work. A great amount of sexual stimulation is olfactory, and a degree of isolation in this respect is also achieved by using a housing system of this type.

In the natural habitat the hamster comes into estrus about five hours after sunset, and under laboratory conditions, about five hours after the lights have gone out (Orsini, 1961). For convenience, the light-dark schedule was regulated as suggested by Duby (1965), allowing observation

of estrus at a reasonable hour. Each morning at 3:30 the lights were turned off automatically, and on at 3:30 each afternoon. The animals were examined for estrus between the hours of 8:30 and 10:30 A.M. corresponding to approximately five to seven hours after sunset under normal habitat conditions. All operations were done between 9:00 A.M. and 1:00 P.M., laboratory time, corresponding to evening hours for the hamster. The animals adjusted completely to the re-arranged schedule within one week, as evidenced by the regularity of estrous behavior and the normal length of copulatory pseudo-pregnancy. Several control animals were continually mated sterilely and several were kept pregnant to test the possible seasonal sexual variance that has been reported in the normal habitat. No evidence of seasonal cyclic variation was observed during the duration of the experiment, nor has such been reported under laboratory conditions (Orsini, 1961).

Many methods have been utilized for determining the estrous cycle of hamsters (Short and Woodnot, 1961), but the technique of Black et al. (1965) was most convenient and reliable. It is based on the characteristic pugnacity of the female hamster towards males placed in her cage at any time other than the evening of estrus. There is ample evidence that the change in behavior on this day is hormonally related and that high titers of circulating estrogens are responsible (Zarrow, 1964). If a male is placed in the female's cage during estrus, she will show the unmistakably characteristic behavior and stance of this stage in the cycle.

As a standard procedure every morning, a male was placed into each female's cage while observing the behavior of both. On day one of estrus, the male, normally on the defense, assumes an entirely different character. Stimulated, most probably by olfaction, he senses the receptivity of the female which is the sign taken to indicate the initiation of a new estrous cycle, and thus the end of each copulatory pseudopregnancy. Attempts to induce pseudopregnancy by means other than sterile copulation were less successful and abandoned.

Animals coming into heat following a long pseudopregnancy were usually less receptive to males, and generally had one normal four day cycle before going into another pseudopregnancy.

Females weighing between 55 and 85 grams were selected for experimentation. This weight variation is fairly consistent with ages 40 - 60 days. Sexual maturity in this species is achieved about 35 days after birth. All experimental animals were examined ten days after any surgery and selected for further investigation on the basis of weight and health. Each of these subjects had at least one, and most often two, normal post-surgical estrous cycles before continuing to the next procedure. The animals showing rapid weight loss or decreased activity were discarded and appropriate ones examined histologically as controls.

As an anesthetic, Nembutal at a dosage of 8 mg/100 gms. body weight, was injected intraperitoneally. This anesthetic was found to be ideal, since injections as often as twice

daily for three days had no observable harmful effects on the animal. However, Nembutal given on the morning of day three post-estrous, was shown to retard ovulation in 14% of the animals tested (Black, 1965). This exaggerated dosage regimen was used in isolated cases only and was not a normal part of the procedure.

Repeated observations of the pouch implant was easily accomplished with little or no effect on the host or donor tissue, following a reasonably sterile technique. Although it was virtually impossible to maintain aseptic conditions, every attempt was made to insure sterility where practical. All operating instruments were steam-sterilized and care was taken to guard against infection of the pouch by swabbing the area, using 70% ethanol as an antiseptic. Incidence of death through operative procedures was very low; however, a number of animals was lost during the investigations due to a rapidly dissipative illness affecting experimental and control animals alike. As a preventative, cleanliness and frequent cage changes were generally successful.

No attempt was made to establish a pure strain, and breeding in the laboratory provided a constant supply of young animals for investigation. There does not appear to be any significant difference with respect to estrous or pseudopregnant cycles among different strains (Black, 1965).

B. Removal of Ovaries and Uterus:

Five minutes after Nembutal administration, the animals were ready for surgery. In cases of insufficient anesthesia,

additional drug was given in .03 ml increments. The abdominal surface was clipped relatively free of hair and swabbed with 70% alcohol. A single median incision was made starting about 1 inch from the anus and proceeding cranial for approximately 1 inch. Care was taken to avoid cutting small vessels readily visible beneath the skin. A similar incision was then made through the body wall into the coelomic cavity. The uterus was located by probing gently beneath the bladder, hooking the cervix, and pulling upward to reveal the junction of both horns. The major vessels supplying the uterus were clamped with 5" Halstead mosquito forceps and ligated. A steady pull on the uterus brought the ovaries into view, and these also were clamped and ligated unless the surgical procedure dictated their removal. A major portion of the hamster ovary may lie buried within a fat pad so that especial care was taken in performing ovariectomy to insure that all portions were removed to guard against ovarian regeneration.

The organs to be transplanted were placed in sterile Hanks Balanced Solution containing glucose. The peritoneum and body wall were sutured, care being taken to cleanse the wound area with 70% ethanol. A clean operation site diminished the occurrence of reopening of the sutures by the animal and helped prevent infection. All animals were placed in clean cages following operative procedures. The elapsed time from incision to suture was approximately fifteen minutes, leaving about the same amount of time for completion of the next phase, the transplantation of selected tissues.

C. Preparation of Hamster for Transplantation:

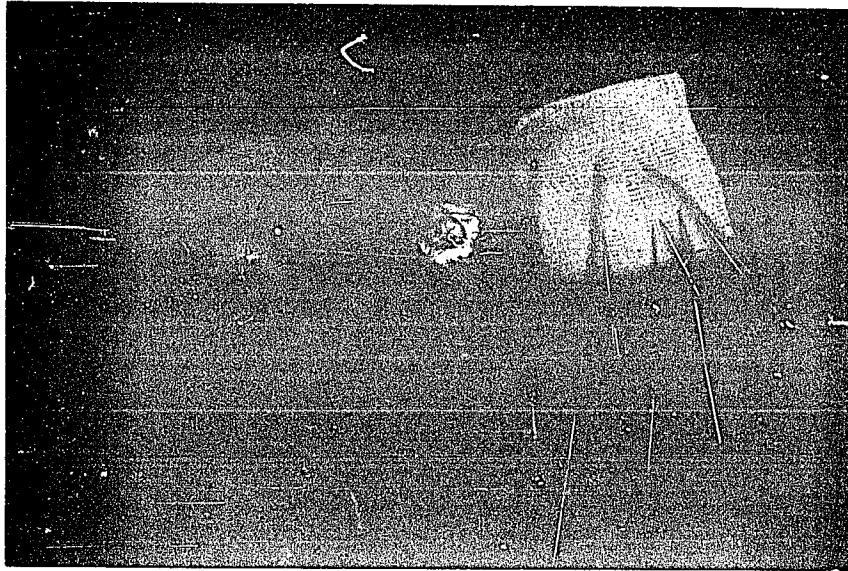
The pouch was everted by careful insertion of five inch, closed, blunt forceps into the side of the buccal cavity and slowly pushed caudad, lateral to the maxillae. At the furthest possible point, at which a strong resistance to continued insertion was met, the forceps were opened, pushed slightly, and reclosed. Eversion of the pouch was now accomplished by a firm yet steady pulling of the forceps and enclosed contents. The peripheral portions of the pouch were pinned onto a cork covered table using as few sterile insect pins as possible. The pouch was swabbed with 70% alcohol and wiped clean of food.

A small incision was made in the most distal portions of the epidermis of the pouch using iris scissors. A blunt probe was inserted (glass or metal) through the slit and a separation of the loose connective tissue that makes up the major portion of the intermembranous layer was made. a space large enough to accommodate the tissue to be transplanted was probed at the point where the major vessel that supplies the pouch bifurcates, thus assuring maximum blood supply for rapid vascularization. This junction was usually about one-half inch from the body in the fully everted pouch. The pouch was moistened from time to time with Hanks Balanced Solution. The hamster was now ready for transplantation (Figure 1).

D. Ovarian Transplants:

Ovaries to be transplanted were removed from the Hanks Balanced Solution, cleansed of adhering fatty tissue, and

Figure 1. Technique for Tissue Transplantation



Successful ovarian homotransplant showing proper position of the implanted tissue and instruments used in the procedure. Note vascularity, tissue size and location.

This transplant re-established normal estrous and pseudopregnant cycles in an ovariectomized animal. See text for further description.

placed at the slit opening in the pouch epithelium using microforceps to insure minimum damage of protruding follicles. The implant was then guided into its proper site using the blunt probe, care being taken not to rupture any of the many small vessels supplying the area. Usually, one suture was sufficient to close the slit, a procedure found necessary to guard against extrusion of the tissue by muscular action of the pouch or by food manipulation. It also insured protection against infection (rare but usually fatal when occurring in the pouch). A minor inflammation at the site of the incision was often noted, though swabbing with 70% ethanol prior to re-insertion of the pouch helped prevent further involvement.

E. Uterine Transplants:

It was determined that the handling of the uterine tissue was an important factor in providing maximum opportunity for a successful transplant. The method offering the best results was slitting the uterine horns longitudinally, and placing the tissue into the pouch in three or four sections. More rapid vascularization was noted using chunks rather than entire uterine horns. Care was taken to insure that the endometrium came in direct contact with the inner epidermal layers of the pouch providing the greatest vascular supply to this portion of the uterus. In many cases the unfolded uterus was found to re-assume a tubular orientation after a few days in the pouch. This problem could be counteracted by using smaller portions of uterine tissue, which more often remained flat. The single stitch used to close the site of insertion often

included the distal end of the transplant, apparently aiding in maintaining the proper orientation of the tissue.

When uteri from heterologous hosts were transplanted, the same procedure was followed with no pre- or post-operative conditioning of the animal attempted. For one experiment human endometrium was obtained, at the time of the operation, from a young woman undergoing unilateral ovariectomy. The endometrium was washed of adhering clotted blood using Hanks Balanced Solution.

Uteri taken from rabbits had arborized endometrium due to induced pseudopregnancy brought about by injection of 100 IU of Human Choriogonadotrophic Hormone (Follutein, Squibb).

In one series of experiments, endometrial scrapings were inserted with the thought that a more specific localization of the proposed antiluteal factor might thereby be made. Scraping the endometrium from the myometrium was a very difficult procedure, and it was practically impossible to assure that only endometrial cells were transplanted in these tests. Histologic studies indicated that endometrial scrapings were predominantly endometrial, but very small myometrial portions were found to be present in many cases. Scrapings approximately equivalent to one uterine horn were inserted using a #15 spinal trochar into which the small clumps were drawn. The same procedure was used for transplanting all heterologous endometrium. In all cases an attempt was made to place about the same amount of tissue as was inserted in

homologous transplantations. Constant re-examination of the implants showed that vascularization in successful cases was usually complete by ten to twelve days post-insertion.

Histologic verification that transplanted tissue had retained its original morphological characteristics was carried out wherever applicable. The explanted tissue was placed in Boiun's fluid, embedded in paraffin, sectioned serially at 8-10 micra, and stained in hematoxylin and eosin. Normal tissues from their original location were also treated in like manner to serve as comparative controls.

RESULTS

A. General

Experimental animals were placed in groups based on the presence or absence, and the location of, the ovary and uterus (Table I).

Table I

Grouping of Experimental Animals

Group	Ovary	Uterus	Tissue Transplanted to Hamster Pouch	Animal Donor
A	N	N	None	None
B	N	X	Uterus	Hamster
C	N	X	None	None
D	X	X	Ovary	Hamster
E	X	X	Ovary-Uterus Same Pouch	Hamster
F	X	X	Ovary-Uterus Different Pouch	Hamster
G	X	N	Ovary	Hamster
H	N	X	Uterus	Rat
I	N	X	Uterus	Rabbit
J	N	X	Uterus	Human
K	N	N	Uterus	Hamster
L	N	X	Endometrium	Hamster
M	N	X	Myometrium	Hamster

N Normal position in body

X Removed from normal position

B. Ovary Transplantation

Removal of the ovary from its normal site and subsequent transplantation to the cheek pouch was successfully accomplished in 65% of the attempts (Table II). Figure 2 shows such a transplant.



Figure 2. Successful Ovarian Homotransplant.

See text Note position, size, and vascular pattern of the transplant.

Counted as unsuccessful were all animals dying for any reason or not showing estrus after surgery. Of the animals living fifteen days post-surgery, the rate of success was found to be 72% (Table II). Table II also indicates that ovaries transplanted into the same pouch with uterine tissue were functional in only 33% of the attempts. Whether this

difference is a function of the size of the transplanted tissue, or whether it may represent a significant interaction of cells to alter the viability of the ovarian grafts is unknown. One possible explanation is that the increase in total tissue transplanted to the pouch might be responsible, in that vascularization of a larger mass of tissue might not be accomplished as readily as that of a smaller mass.

Table II
Results of Homotransplanted Tissues

Transplanted Tissue	Transplants Attempted	Successful Number	Successful 15 Days Post-Surgery
Ovary	48	31 (65%)	72%
Uterus	60	24 (40%)	42%
Ovary Uterus	30	13 (33%)	39%
Endometrium	14	5 (35%)	35%

As a criteria for success, the re-establishment of estrus was taken as the only positive sign. Although many animals showed altered behavior patterns following ovarian transplantation, only the return of the characteristic mating behavior was accepted as being indicative of ovarian function. The normalcy of the cycle was determined by observing at least two four day estrous cycles prior to sterile mating. Any animal not coming into estrous after twenty-five days post-surgery was eliminated. These were examined for possible ovarian regeneration in the body cavity, and the contents of the pouch removed and examined microscopically. It was noted

that if vascularization similar to that shown in figure 2 had not occurred in ten to twelve days, the tissue was usually rejected.

Unsuccessful transplants were used as controls for this part of the investigation. They demonstrated that ovarian removal brought about cessation of cyclic activity, and that successful transplantation of ovarian tissue was sufficient to return this pattern to normal.

All animals were sterile mated and the length of the induced pseudopregnancy was measured (Table III). The normal duration of pseudopregnancy was found to be 9.24 days (Group A). This figure agrees with that determined in other laboratories (Black, 1965; Orsini, 1961). When compared with the corresponding figure for Group G animals whose only ovarian tissue is in the pouch, the difference is found to be statistically insignificant using the Students t test. The value, 9.85 days, determined for this Group indicates that the position of the ovary does not impair its ability to regulate cyclic behavior. Further substantiation of normal function by the transplanted ovary is shown when Group C animals are compared with those of Group D. The respective figures, 18.50 days and 17.50 days, indicate a similar elongation of pseudopregnancy and return to heat when the animals are subjected to hysterectomy. In the first case, animals return to estrus through the normally positioned ovary, and in the second, through an ovary transplanted to the cheek pouch.

A comparison of pseudopregnancy lengths when the ovary

Table III

Results of Measured Pseudopregnancies

Group	Animals #	Cycles #	Average Duration Pseudopregnancy (Min-Max)	Normal	Deviation from Normal
A	10	37	9.24 (8-11)	9.24	0.0
B	15	60	13.25 (8-20)	9.24	4.04
C	20	67	18.49 (15-25)	9.24	9.25
D	16	54	17.50 (16-22)	9.24	8.26
E	13	43	12.27 (9-17)	9.24	3.03
F	9	29	13.65 (9-17)	9.24	4.41
G	8	28	9.85 (9-12)	9.24	0.61
H	8	14	14.75 (9-17)	9.24	5.51
I	6	18	15.45 (9-18)	9.24	6.24
J	2	10	17.50 (13-19)	9.24	8.26
K	6	14	9.25 (8-10)	9.24	0.01
L	8	30	13.00 (9-15)	9.24	3.76
M	8	12	17.00 (15-19)	9.24	7.76

and uterus are placed in the same or different pouches (Group E and F respectively) shows that the proximity of the two tissues may be of some importance in determining ovarian periodicity. This matter will be more fully discussed later in the results. It should be noted, however, that an ovary transplanted within uterine tissue is able to regulate cyclic behavior.

Re-establishment of the estrous cycle in an ovariectomized animal was the primary evidence that the transplanted tissue was similar to a normal ovary. Histological examination of successful homotransplants gives further evidence, showing that the ovary retains both its normal morphological and physiological characteristics.

The normal ovary (Figure 3) showing primordial follicles and surrounding interstitial tissue can be compared with the same cell types in the transplanted ovary (Figure 4). The respective nuclei and nucleoli of the various ova are clearly visible in both cases. Follicular cells surrounding the ovum appear similar in each ovary with some multilayer follicles evident. The germinal epithelium and a few blood vessels are similar, further attesting to the comparability of the two tissues. The transplanted ovary shown in Figure 4 was removed after having brought an ovariectomized animal into estrus and pseudopregnancy of normal duration. This position of the ovary was selected because of its similarities to the normal; however, it should be reported that some portions had undergone fatty degeneration and tissue necrosis was evident.

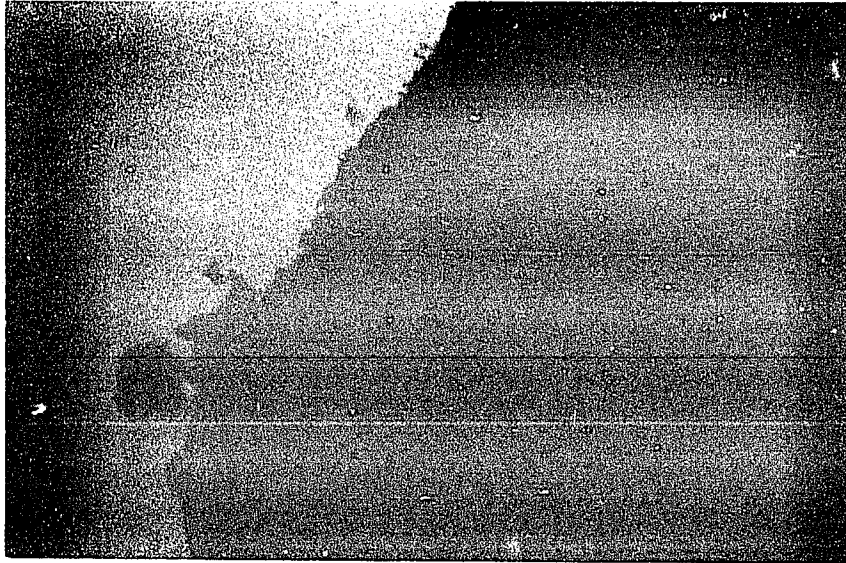


Figure 3. Normal Ovary. Primordial follicles
100X See text

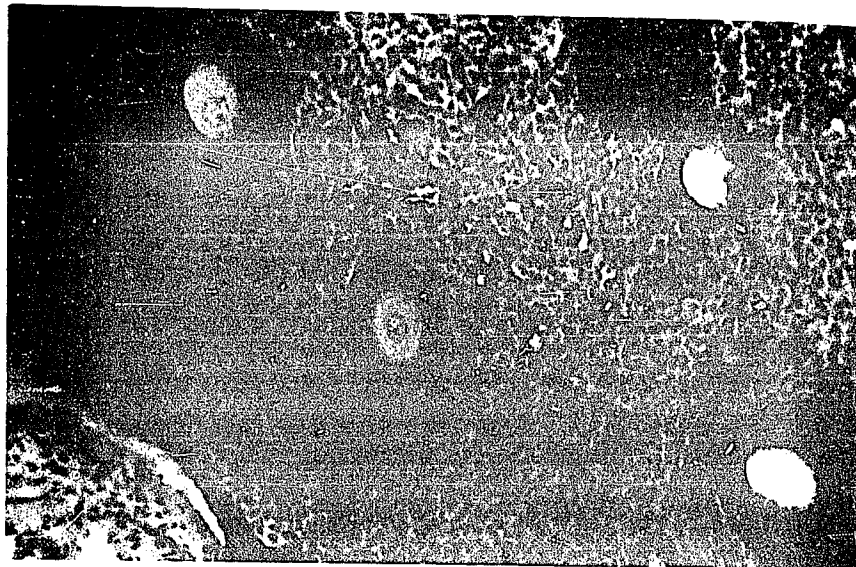


Figure 4. Transplanted Ovary. Primordial
follicles 100X See text

An examination of the cell types found in developing follicles of the transplanted ovary reveals that the process of follicular maturation was similar to that observed in the normal ovary. Follicular atresia, present in both tissues, did not appear to be more prevalent in either. A normal ovary with a large developing follicle and many primordial follicles surrounded by interstitial tissue is shown in Figure 5. A comparison of the cells found in this photomicrograph with those found in the transplanted ovary show definite similarities of the corresponding cell types (Figure 6). The antrum, though much larger in the normal ovary due to the plane through which the section was cut, is surrounded by follicular cells which are nearly identical to those seen in the transplanted ovary. The germinal epithelium is barely distinguishable in both figures, as is the membrane granulosa in certain areas. Although thecal layers are not obvious in these illustrations, they were visible under higher power. The transplanted ovary (Figure 6) was removed after two estrous cycles of normal duration and was examined specifically for signs of ovulation. No success in observing ovulation in a transplanted ovary can be reported, although corpora lutea at various stages of regression have been identified.

A follicle from a transplanted ovary is shown undergoing atresia in Figure 7. The antrum is filled with debris from degenerating follicular cells. A large, multilayered, primordial follicle with a nucleated ovum is also visible.

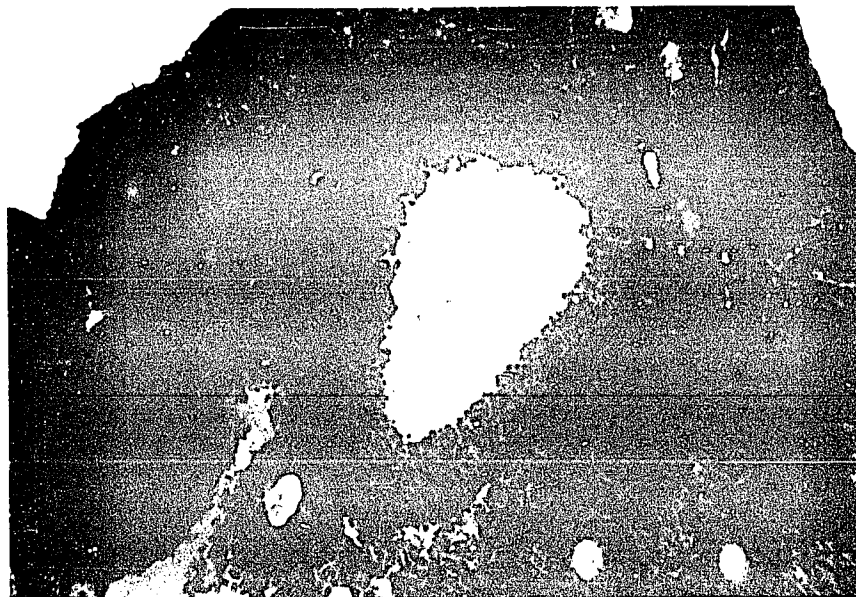


Figure 5. Normal Ovary. Developing follicle
100X See text

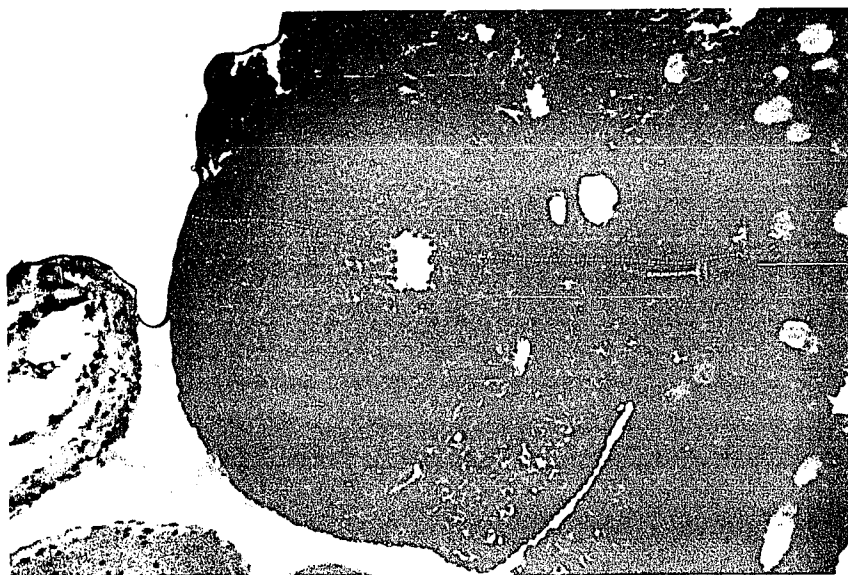


Figure 6. Transplanted Ovary. Developing
follicle 100X See text

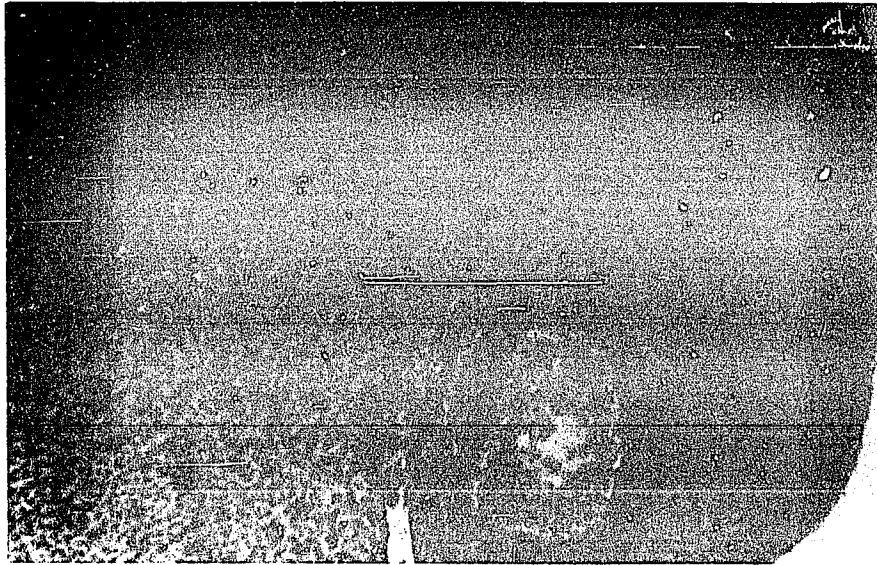


Figure 7. Transplanted Ovary. Atretic
follicle 100X See text

The transplanted ovary is capable of developing fully-mature Graafian follicles. This is clearly demonstrated in the following photomicrographs (Figures 8 and 9). A section of a transplant shows two large follicles and many primordial and atretic follicles (Figure 8). The medulla of the ovary is distinguishable from the cortex and closer observation of the structures at the arrows shows them to be degenerating corpora lutea.

The Graafian follicle in the lower portion of the photomicrograph was examined at higher power (Figure 9). The follicular cells forming the intact cumulus oophorus, the ovum with nucleus and nucleolus, the zona pelucida, and in some portions, the theca interna are all visible. The antrum, filled with follicular liquid is large, indicating the maturity of the follicle.



Figure 8. Transplanted Ovary. Graafian
follicles 45X See text

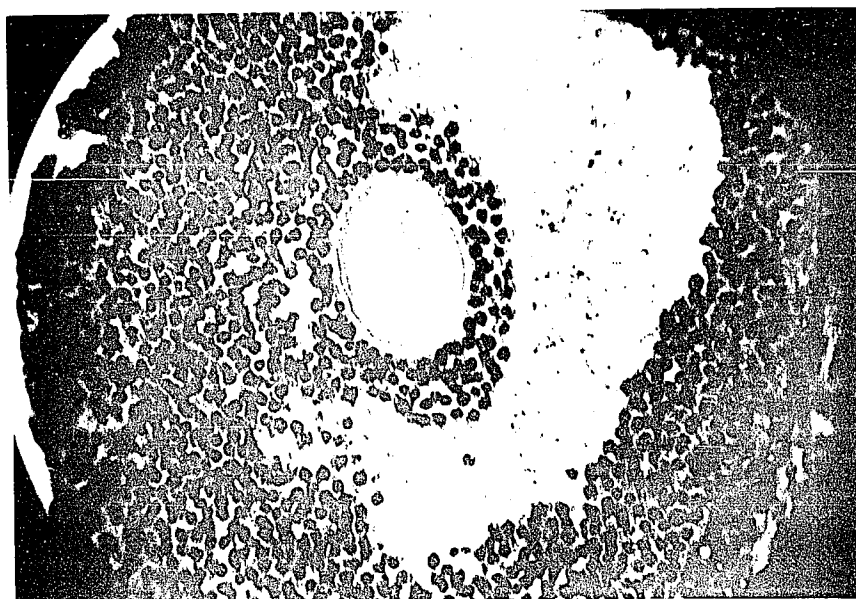


Figure 9. Transplanted Ovary. Graafian
follicle 440X See text

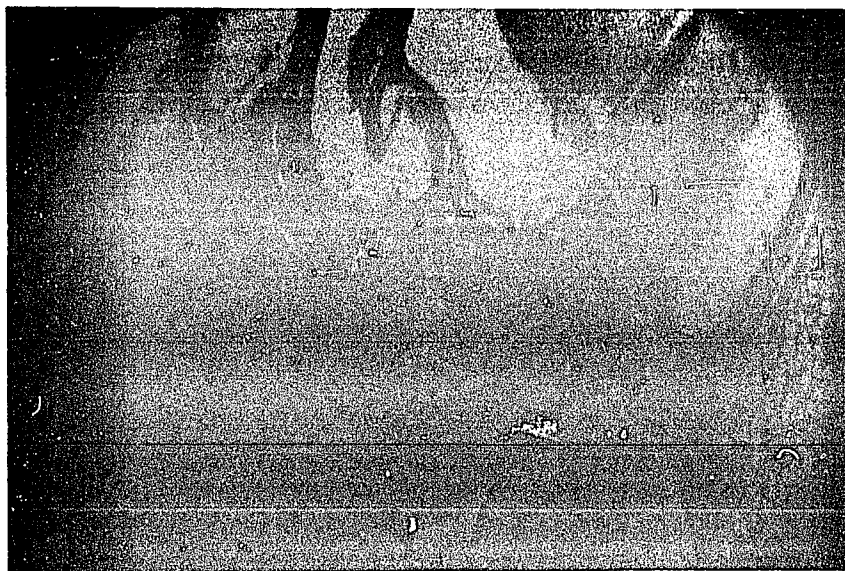


Figure 10. Transplanted Ovary. "Cystic"
follicles 45X See text

In a number of cases the transplanted ovary showed marked changes in morphology and function (Figure 10). This ovary was excised after 8 weeks in the pouch during which time it grew to unusual size yet did not re-establish estrus behavior. Where this "cystic" like condition was observed, the animals did not show normal estrous behavior. In a few cases transplanted ovaries were successful in re-establishing estrus for one or two cycles, then enlarged considerably and ceased to function normally. The above ovary has little or no interstitial tissue, few primordial follicles, and a large number of fluid-filled, enlarged follicles. The ovum is not clearly visible in any of the follicles although there may be one shown at the arrow. Perhaps the slight difference in temperature between the body cavity and the pouch could be responsible for this phenomenon.

C. Uterus Transplantation

Homologous transplantation of uterine tissue was successuilly accomplished in 42% of the attempts, significantly lower than that recorded for ovarian homotransplants (Table II). The criteria for measuring the success of uterine grafts was shortening of the hysterectomized pseudopregnant cycle. It was difficult, therefore, to determine a precise measure for establishing whether a transplant was successful or not. An arbitrary value of 14 days for pseudopregnancy length was considered evidence that the uterine tissue was functional in affecting luteolysis. The basis for setting 14 days as the criteria can be seen in a comparison of Group B and Group C animals (Figures 21 and 22). No hysterectomized pseudopregnant cycle was less than 15 days, (Group C) and when the uterus was transplanted to the pouch, 60% of the cycles were found to be less than 15 days. A full discussion of uterine transplants as affecting luteolysis will be found in the following section. Complete vascularization of the implant by day 10 post-surgery was found to be critical in determining the relative success of the procedure.

A transplant, successful in shortening hysterectomized pseudopregnancy by the above standards for three cycles, is shown in Figure 11. Vascularization of this tissue was good, and the implant, about three times normal as pictured here, occupied nearly one-fourth the total pouch area. It was inserted in the tubular confirmation, a procedure abandoned for more successful approaches.

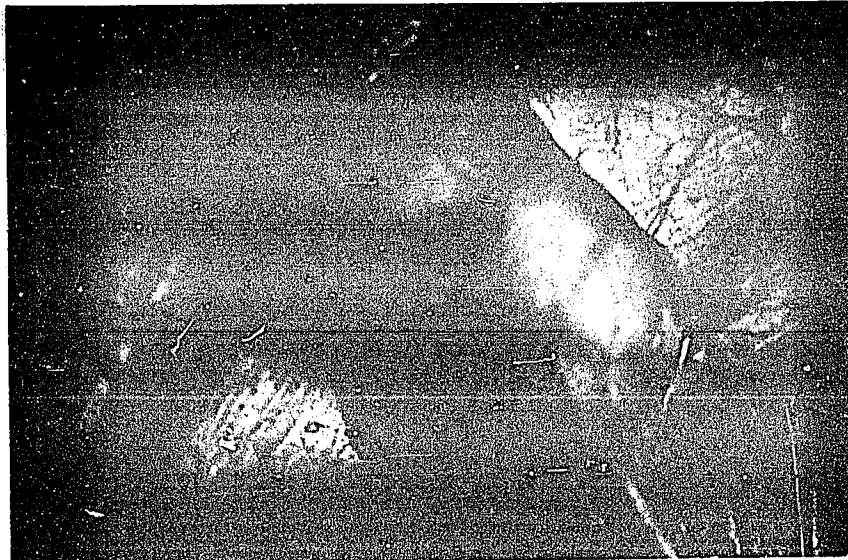


Figure 11. Transplanted Uterus in Cheek Pouch (approximately) 3X normal size.

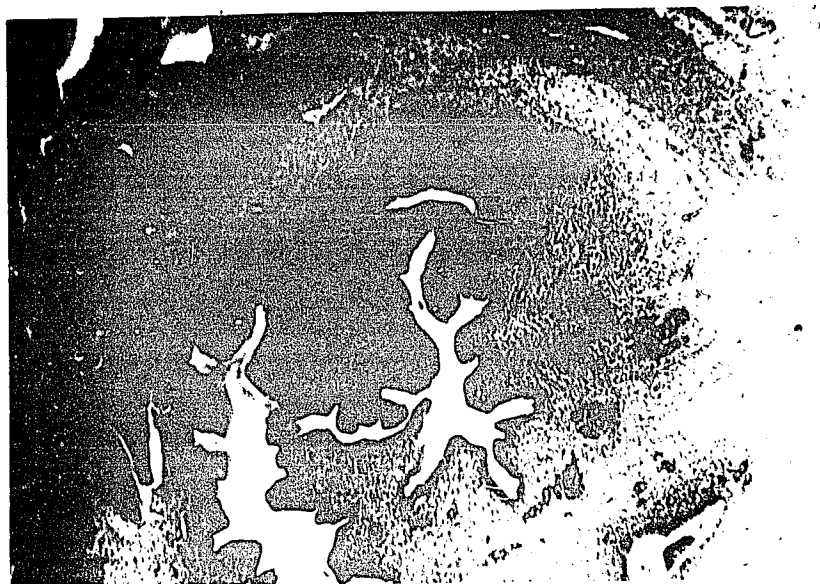


Figure 12. Normal Uterus. Cross section
45X See text

One of the possible explanations for the lack of greater success when whole uterine horns were transplanted can be seen in a comparison of the normal uterus with one transplanted for seven weeks. Figure 12 shows the normal confirmation of the hamster uterus with characteristic endometrium, myometrium and endometrial glands obvious. The pattern and size of the endometrium and its gland should be compared with the same tissue as seen in the transplanted uterus (Figure 13). The latter graft was successful in shortening hysterectomized pseudopregnancy for two cycles, and then did not function in this manner for twenty days. Excised to determine the reason for this change in function, the resulting examination showed a marked enlargement of the endometrial glands and a fusing of the endometrium in many areas. Such a drastic alteration in morphology may be the cause of the altered physiology of the tissue. A possible explanation for this fused condition of the endometrium may be the constant constricting pressure of the pouch membranes between which the transplant was placed. Also, normal muscle tonus could not be expected without innervation, perhaps contributing to the collapse of the lumen. Examination of more transplanted uteri has not been attempted due to our reluctance to interrupt successful cycling. It cannot be assumed, therefore, that all uteri transplanted in the tubular confirmation have the same fate. This was the major reason, however, that a more suitable method for accomplishing uterine transplantation was adopted.

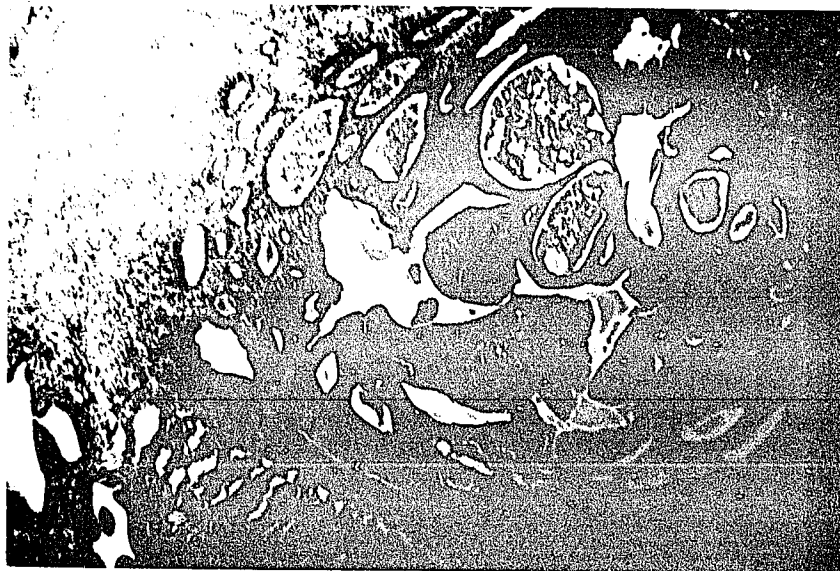


Figure 13. Transplanted Uterus. Cross section 450X See text

Greater success in achieving shortening of pseudopregnancy was obtained when the uterine tissue was slit longitudinally and unfolded prior to insertion into the pouch. Figure 14 shows a section of a transplant treated in that manner. Normal size and confirmation of the tissue should be noted when compared to Figure 12. Unlike Figure 13, no enlargement of endometrial glands nor fusing of tissue can be detected. A closer examination of the endometrial folds from the above section (at arrow) shows the endometrium to be normal. No tissue necrosis is evident and the columnar epithelium lining what used to be the lumen appear indistinguishable from those of the normal uterus. The tissue was effective in shortening the cycle length of pseudopregnant-hysterectomized animals.

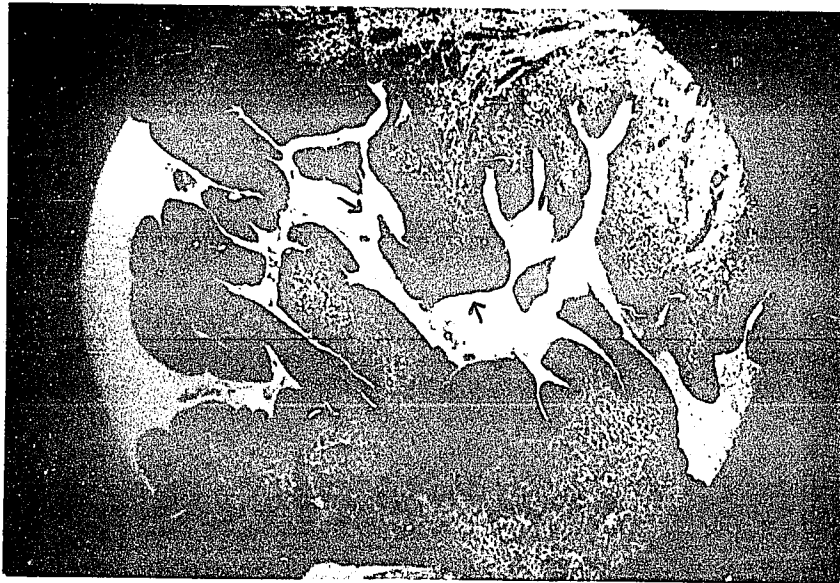


Figure 14. Transplanted Uterus. Unfolded
45X See text

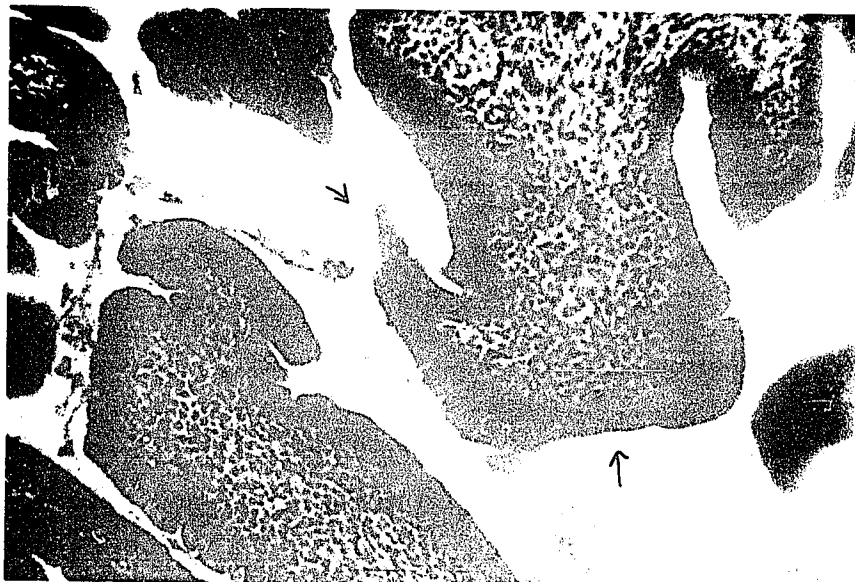


Figure 15. Transplanted Uterus. Unfolded
100X See text

Endometrial scrapings and fragments cut from this portion of the uterus were transplanted to the pouch and examined histologically after removal. For comparison, a fragment fixed in Boiun's fluid at the time similar pieces were transplanted, is shown in Figure 16. When the two tissues were compared, there was a definite similarity of tissue types, although some aberrations were found in the transplanted endometrium (Figures 17 and 18). The most obvious difference is once again to be found in the endometrial glands. Though not as large as those found in the whole uterus, they nevertheless approach cystic size. This fragment, and others like it, were effective in shortening the hysterectomized pseudopregnant cycle in the hamster from which they were removed.



Figure 16. Endometrial Fragment. Similar to those actually transplanted 100X
See text

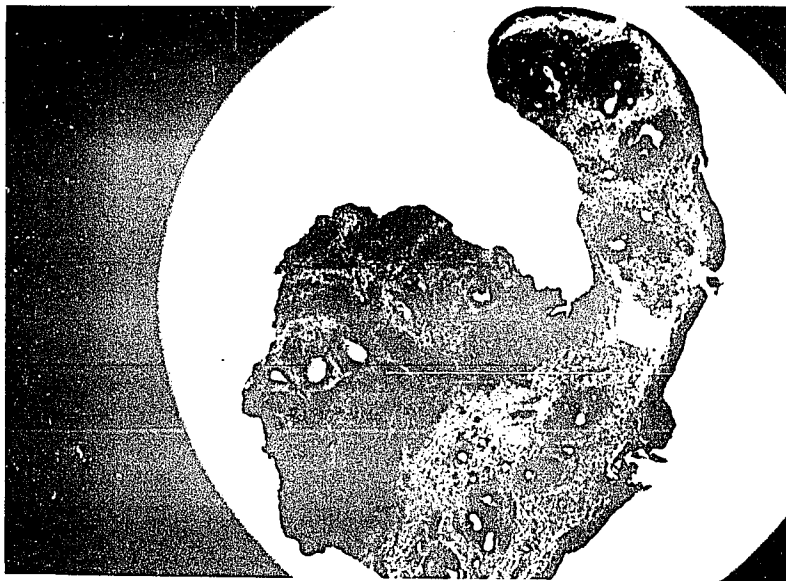


Figure 17. Endometrial Fragment. After
transplantation 45X See text

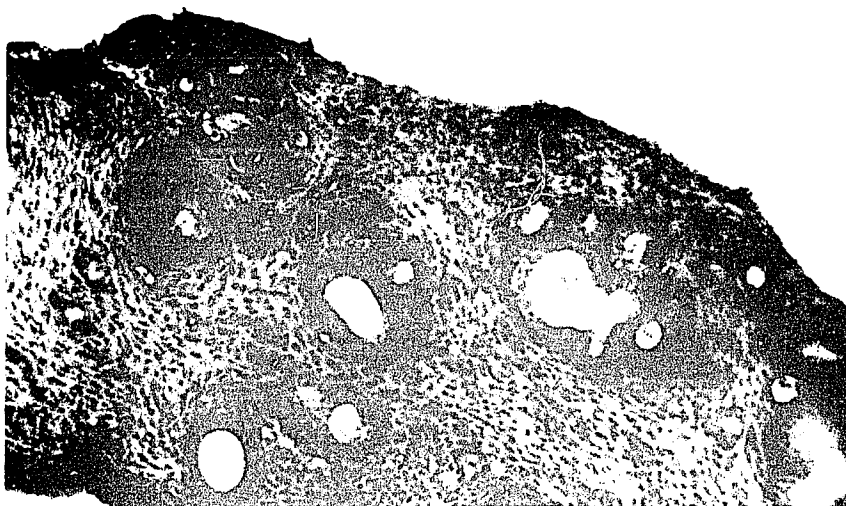


Figure 18. Endometrial Fragment. After
transplantation 100X See text

D. Homologous Uterine Transplantation and Luteolysis

Figures 20 through 26 show the results of experiments designed to demonstrate the relationship existing between the ovary and uterus of the Syrian hamster. Table I explains the basis for grouping of the experimental animals and Table III presents results of measured pseudopregnancies.

Normal duration of pseudopregnancy was found to be 9.24 days (Group A, Figure 20). When the uterus was removed and transplanted to the cheek pouch, this period was lengthened by 4 days (Group B, Table III and Figure 21). However, when compared to the length of pseudopregnancy measured for hysterectomized animals (Group C, Figure 22), which was determined to be 18.49 days (Table III), this represents a statistically significant shortening of the hysterectomized pseudopregnant cycle.

In an effort to determine what spatial relationship, if any, might be important in influencing luteolysis, a series of operations was performed which altered the normal anatomical relationship of the ovary and uterus. When the ovary was removed from its normal position in the coelomic cavity and placed in the pouch, the length of pseudopregnancy was not significantly different from that observed in normal animals (Group G, Figure 26). To further test the contention that the proximity of the uterus to the ovary could be important in luteolysis, ovaries and uteri were transplanted in the same pouch (Group E, Figure 24), and in different pouches in the same animal (Group F, Figure 25). Pseudopregnancy in

Group E was found to be significantly shorter than the same period in Group F, although the difference was only 1.38 days.

When the ovary and uterus were both removed and only the ovary transplanted to the pouch (Group D, Figure 23), the length of pseudopregnancy was not significantly altered from that observed in normal hysterectomized animals (Group C, Figure 22).

Ten hysterectomized animals from Group C, after each had exhibited three pseudopregnant cycles of at least 16 days' duration, had uterine tissue transplanted to their cheek pouch. Although the uterus was successfully transplanted in only four of these animals, in each of these cases the length of pseudopregnancy was significantly shortened (Table IV).

Table IV

A Comparison of Hysterectomized Pseudopregnant Cycle Length, before and after Uterine Transplants

Group	Animals #	Cycles #	Average Duration Pseudopregnancy	Normal
C	20	67	18.50	9.24
C (a)	4	9	13.00	9.24
B	15	60	13.25	9.24

C and B See Table I

C (a) Uterus successfully transplanted after three hysterectomized pseudopregnant cycles of at least 16 days' duration.

Figure 20. Length of Copulatory Pseudopregnancy in Normal
Animals. (Group A)

Figure 21. Length of Copulatory Pseudopregnancy in Hyster-
ectomized-uterus-transplanted Animals. (Group B)

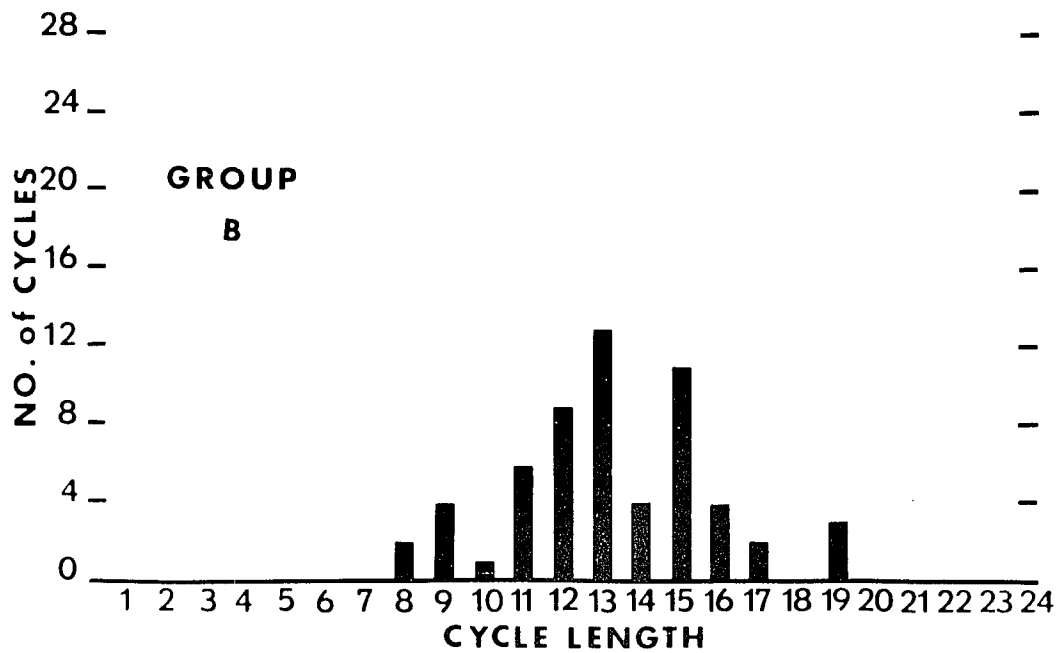
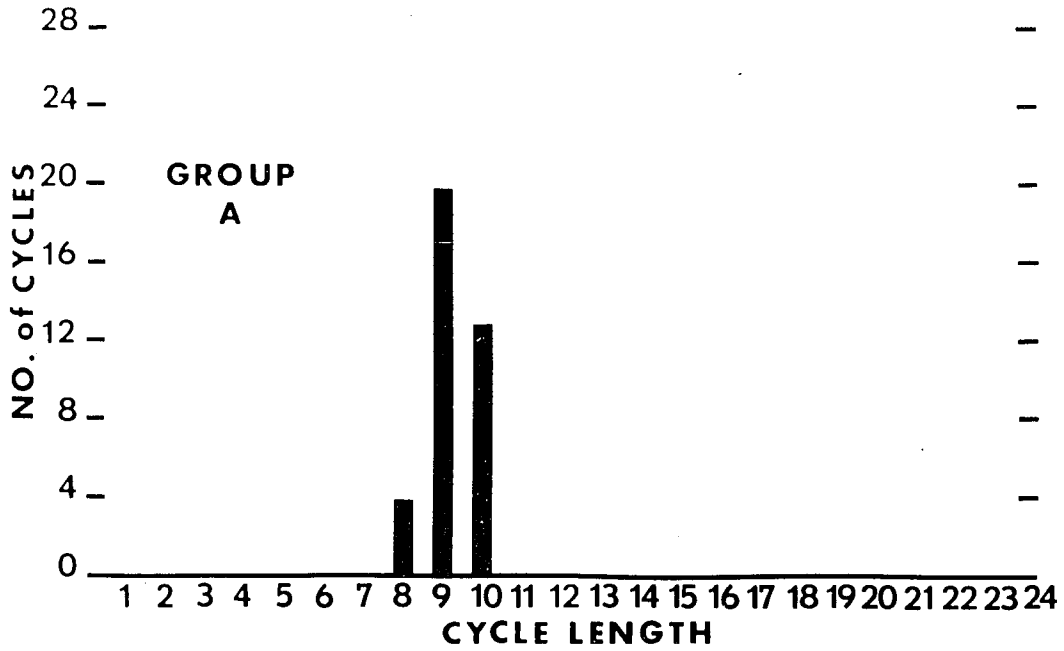


Figure 22. Copulatory Pseudopregnancy in Hysterectomized
Animals. (Group C)

Figure 23. Copulatory Pseudopregnancy in Ovariectomized-
hysterectomized-ovary-transplanted Animals.
(Group D)

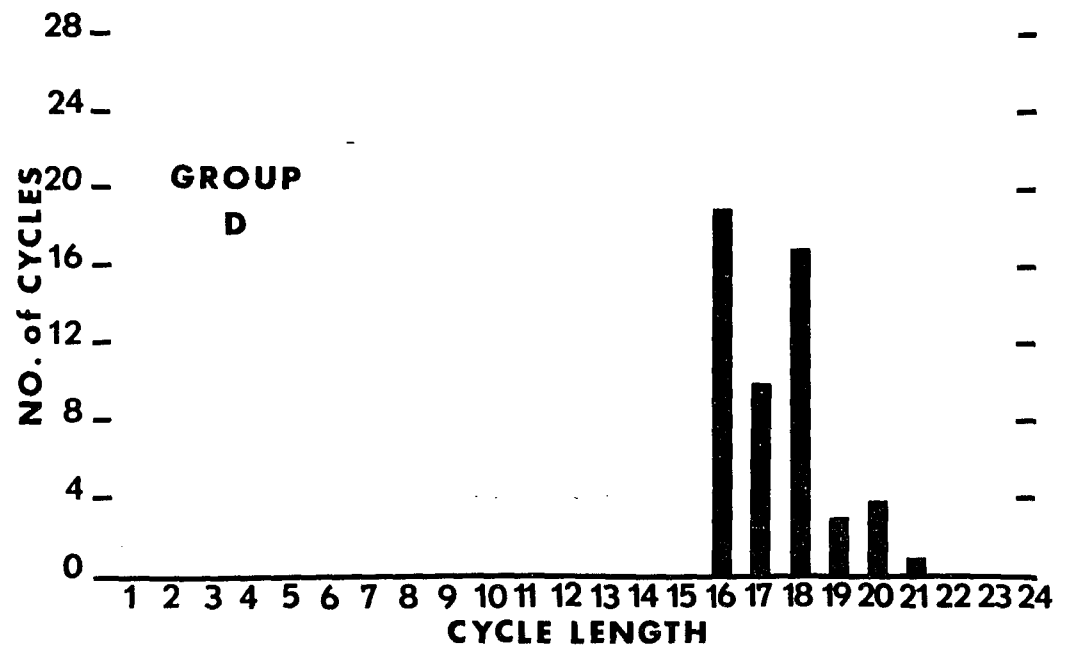
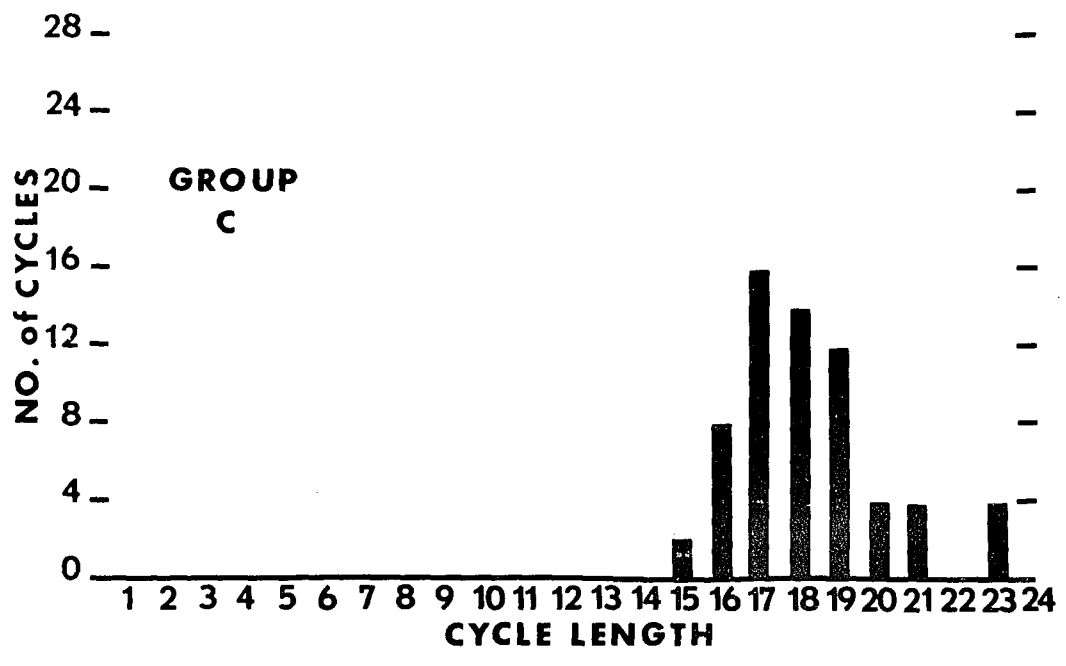


Figure 24. Copulatory Pseudopregnancy in Ovariectomized-hysterectomized Animals with Ovary and Uterus Transplanted to the Same Pouch. (Group E)

Figure 25. Copulatory Pseudopregnancy in Ovariectomized-hysterectomized Animals with ovary and Uterus Transplanted to Different Pouches. (Group F)

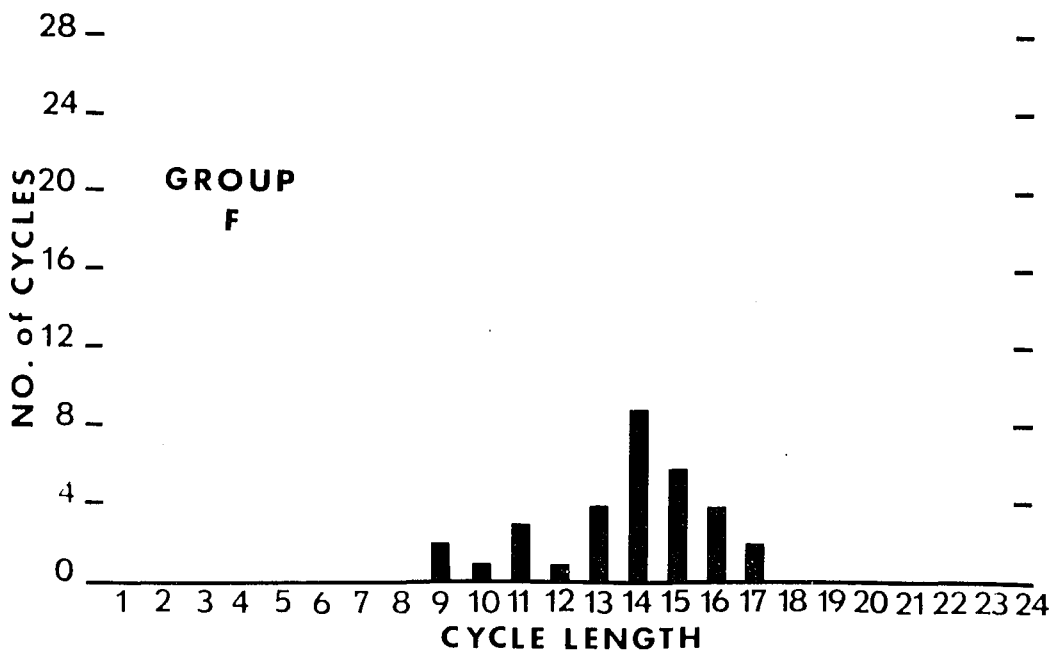
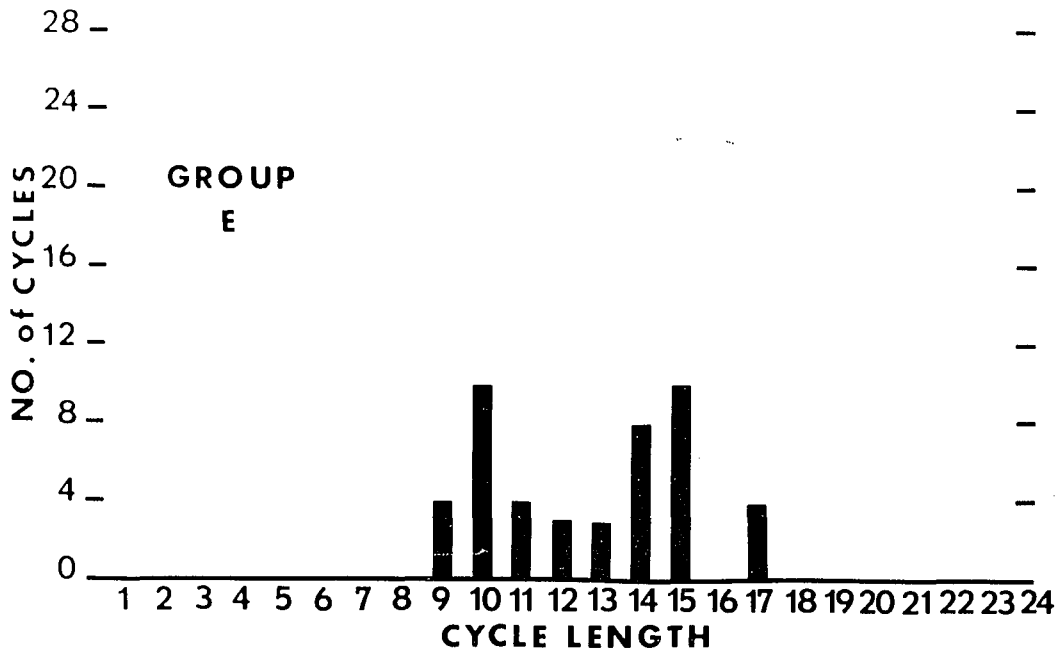
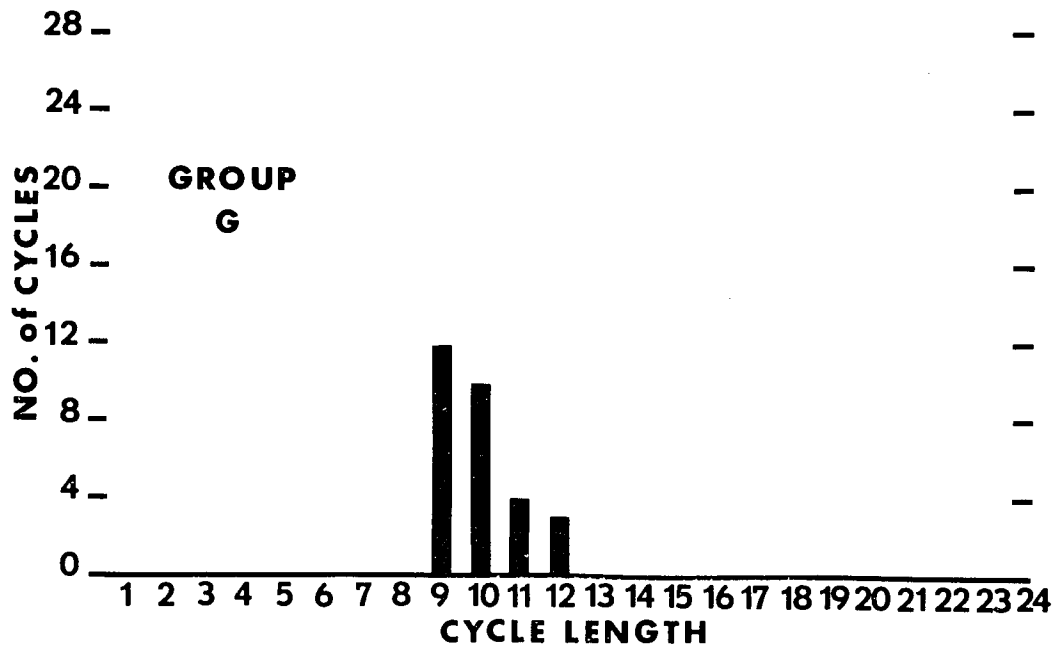


Figure 26. Copulatory Pseudopregnancy in Ovariectomized
Animals with Ovary Transplanted to Pouch.
Group G)



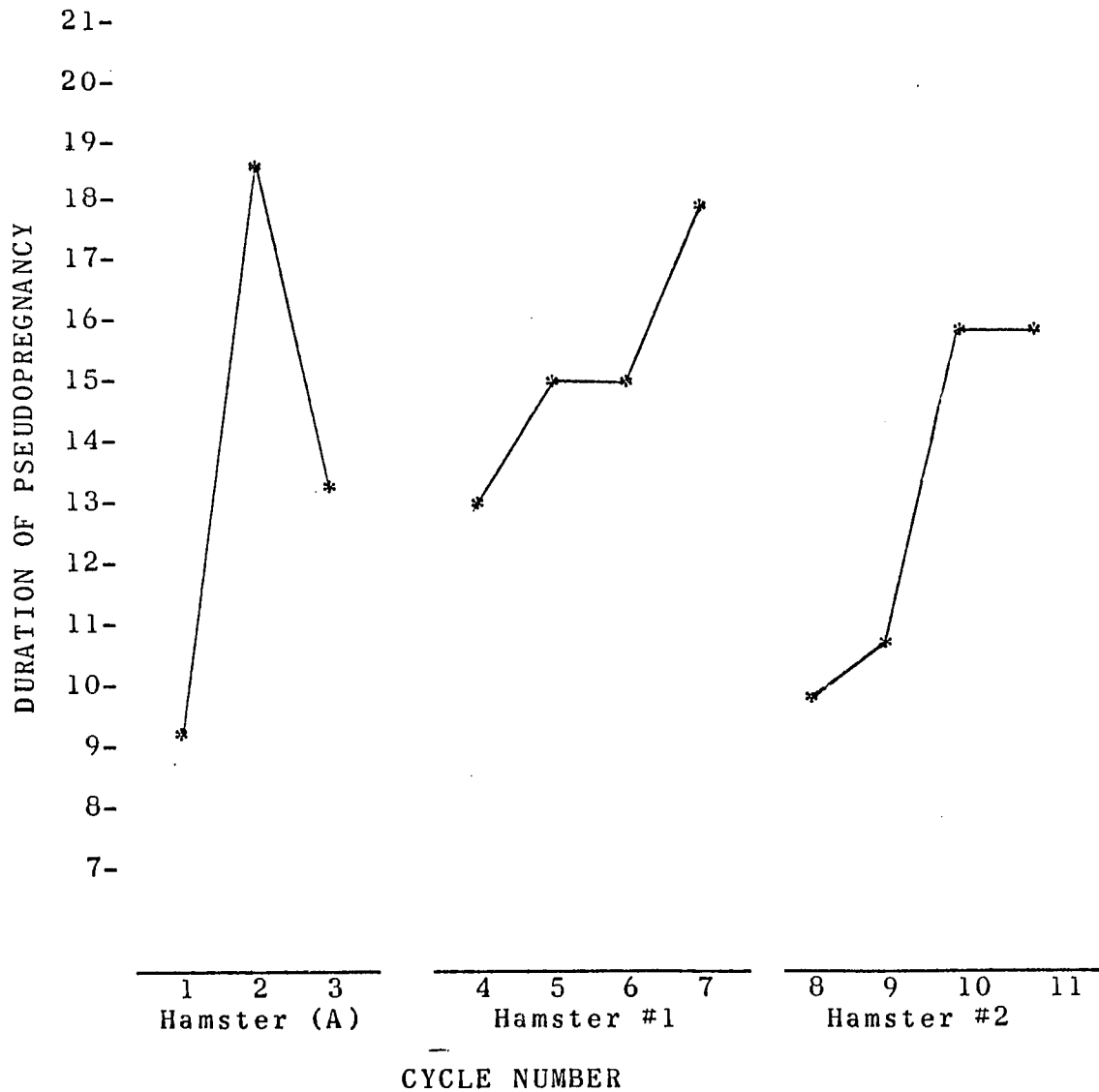
In order to determine whether it was endometrium, myometrium, or a combination of these two tissues that was effective in inducing luteolysis, endometrium was separated from the myometrium and portions of one or the other tissue type placed in separate animals. The results of these experiments are shown in Figures 27 - 29. Eight animals were used in each Group, and the length of hysterectomized pseudopregnancy before and after insertion of the specific uterine tissue was measured.

Of the eight animals receiving endometrium (Group L), five showed at least one shortened hysterectomized pseudopregnant cycle, while none of the eight animals that received myometrium (Group M) showed any evidence of cycle shortening. It should be noted, however, that in very few cases did this endometrial effect of shortened cycle continue for more than one pseudopregnancy. Possible explanations for this observation will be considered in the discussion.

Histologic examination of myometrium transplants showed four to be adequately vascularized after 12 days. As can be seen in Figure 29, however, the length of hysterectomized pseudopregnancy remained virtually unaltered.

Since a reduced amount of uterine tissue tends to cause an elongation of pseudopregnancy, an attempt was made to determine what effect an excess of uterine tissue might have on this cycle. A shortening of pseudopregnancy in the normal animal with one extra uterine horn was not observed in any of six subjects.

Figure 27. Effect of Transplanted Endometrium on Hysterectomized Pseudopregnancy

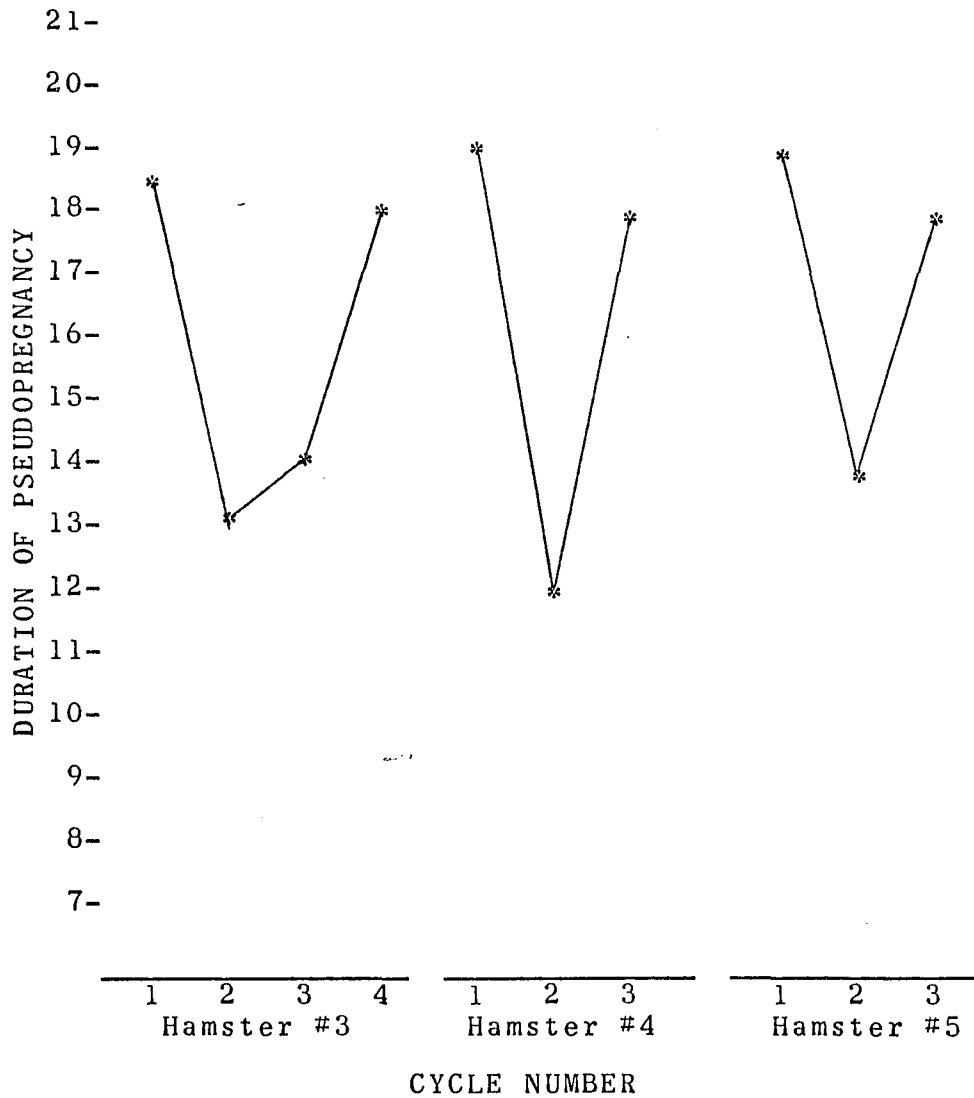


Cycle Number:

- 1 = Normal pseudopregnancy (Average)
- 2 = Hysterectomized pseudopregnancy (Average)
- 3 = Hysterectomized-uterine-transplant pseudopregnancy (Average)
- 4-11 = Hysterectomized-endometrium-transplanted pseudopregnancy

(A) = Control results

Figure 28. Effects of Transplanted Endometrium on
Hysterectomized Pseudopregnancy (Cont.)

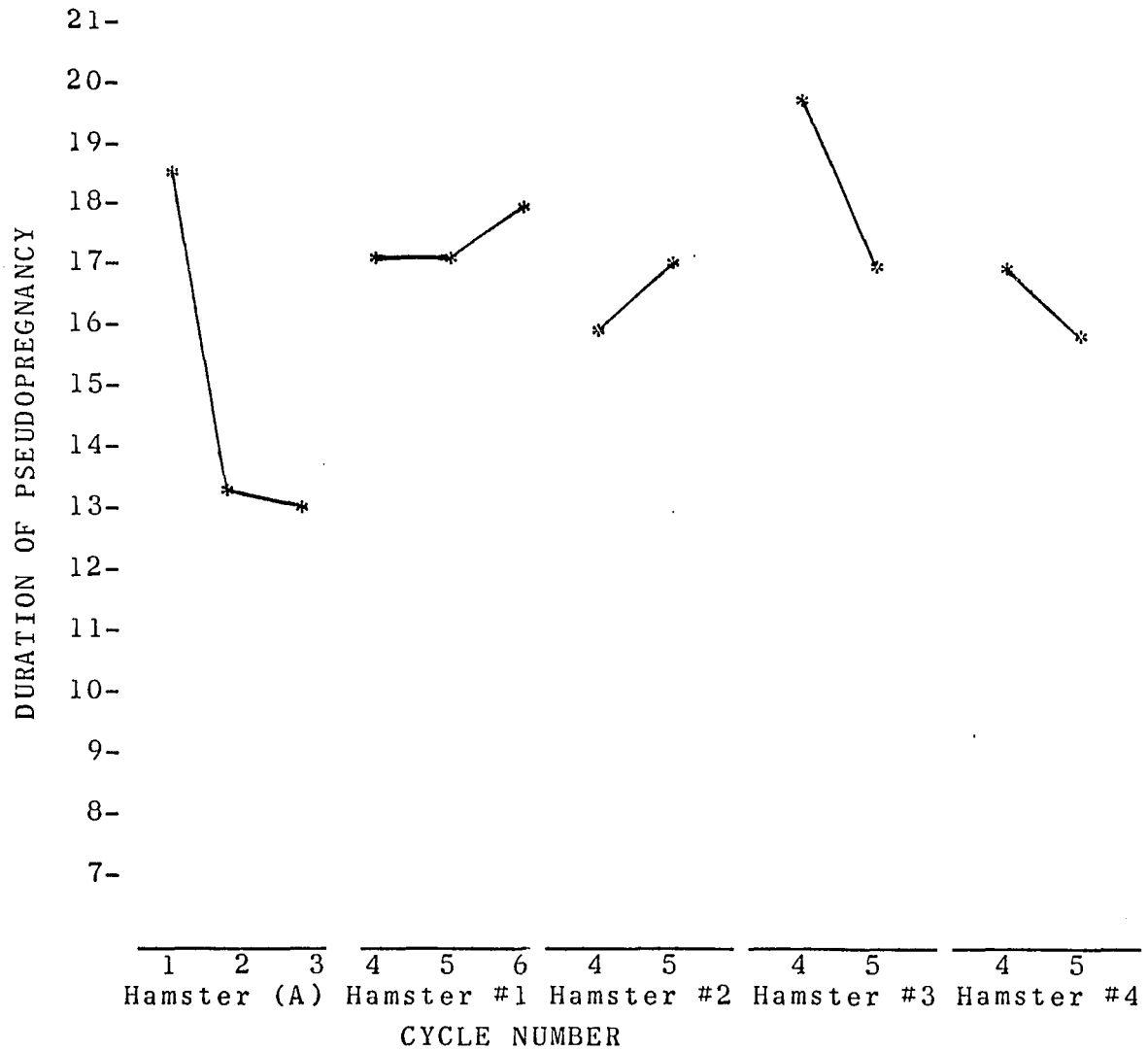


Cycle Number:

1 = Hysterectomized pseudopregnancy (Average)

2-4 = Hysterectomized-endometrium-transplanted pseudopregnancy

Figure 29. Effects of Transplanted Myometrium on Hysterectomized Pseudopregnancy



Cycle Number:

- 1 = Hysterectomized pseudopregnancy (Average)
- 2 = Hysterectomized-uterine transplant
- 3 = Hysterectomized-endometrium-transplanted pseudopregnancy
- 4-6 = Hysterectomized-myometrium-transplanted pseudopregnancy

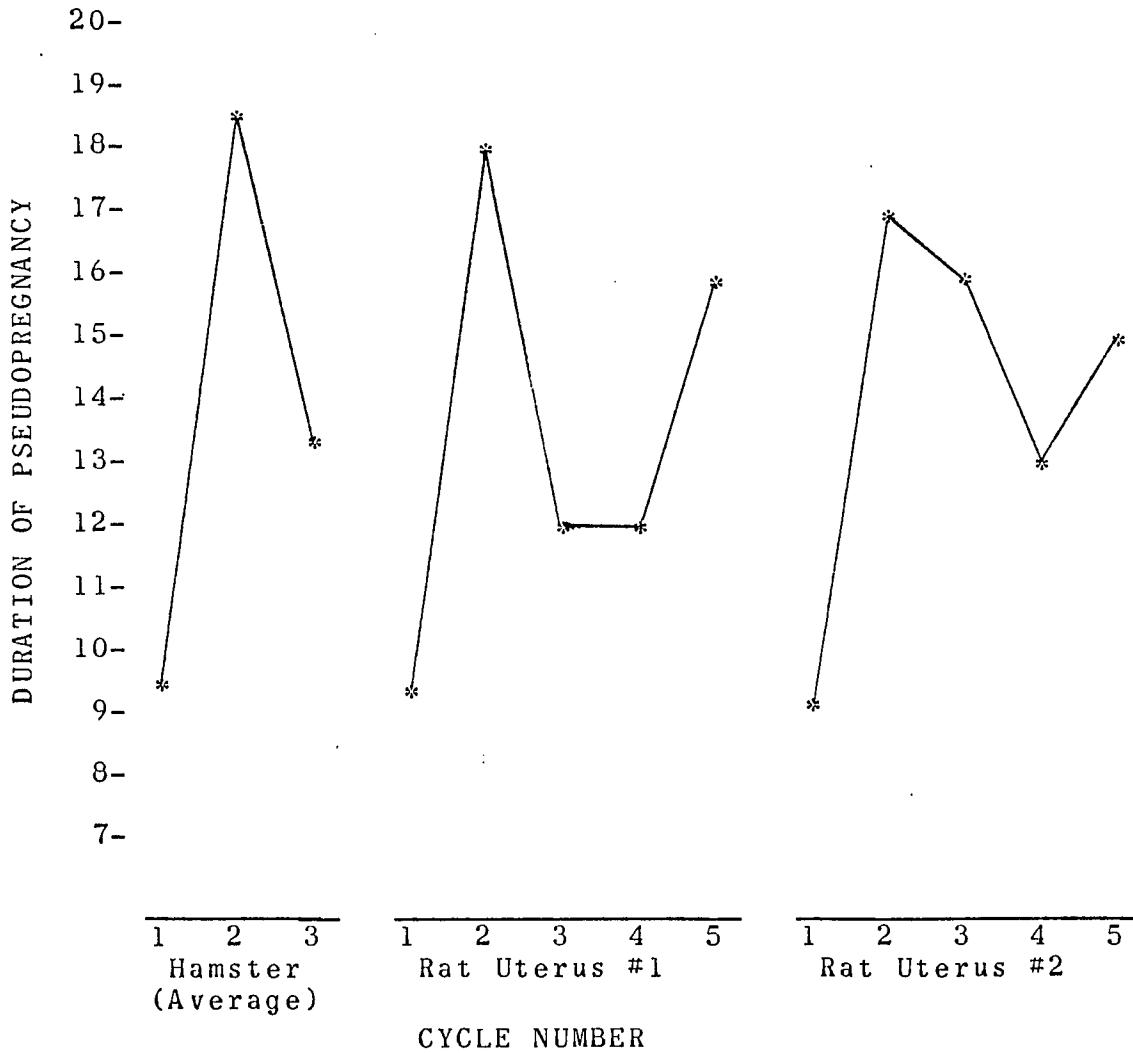
E. Heterologous Transplantation and Luteolysis

Transplantation of estrous rat uteri into the cheek pouch of hamsters after they have been hysterectomized and observed for at least one pseudopregnant cycle, significantly reduces the cycle elongation characteristic of uterine removal (Group H, Table III, Figures 30 and 31). Of eight animals tested, only three did not show shortening of hysterectomized pseudopregnancy following uterine transplantation. It was noted that each of these three unsuccessful attempts could have resulted from using non-estrous uteri. All of the successful transplants were of rat uteri in which care was taken to insure that the animal was in estrous prior to hysterectomy.

The results for transplanted rabbit uteri were less definite. Although the average duration of hysterectomized pseudopregnancy following transplantation of rabbit uteri was found to be 14.45 days (Group I, Table III), the number of animals showing significant shortening was less than that for the rat. Considerable more evidence must be accumulated before any definite conclusions can be made. Figures 32 and 33 present the measured pseudopregnancies in six animals tested with rabbit uteri. Shortening below thirteen days was found in only one animal, although shortening below fifteen days was found in six of the eighteen cycles measured.

Human endometrium, transplanted on day six into two hamsters previously made pseudopregnant, produced inconclusive results (Group I, Table III and Figure 34).

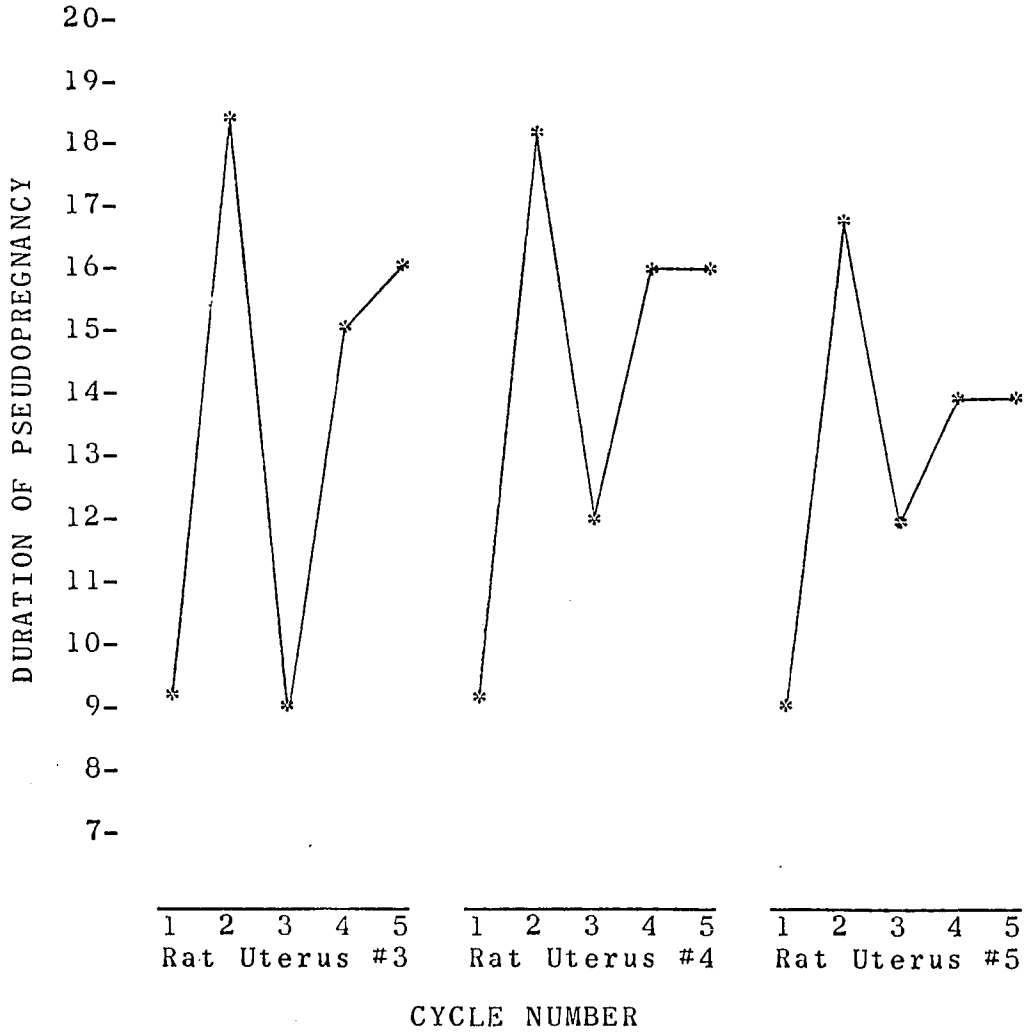
Figure 30 Effects of Transplanted Rat Uterus on Hysterectomized Pseudopregnancy in the Hamster



Cycle Number:

- 1 = Normal pseudopregnancy (Average)
- 2 = Hysterectomized pseudopregnancy
- 3-5 = Hysterectomized pseudopregnancy following uterine transplantation

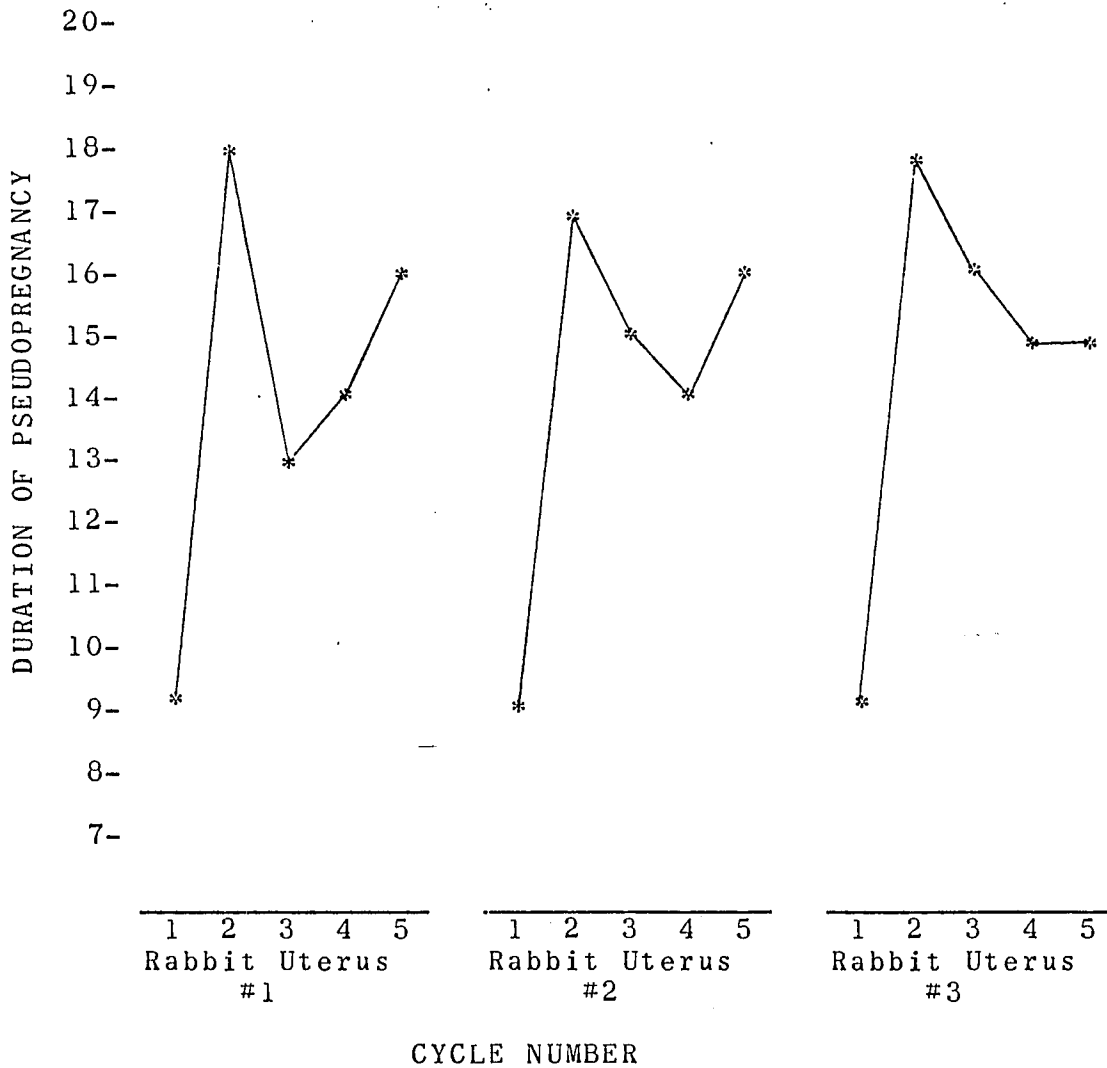
Figure 31. Effects of Transplanted Rat Uterus on Hysterectomized Pseudopregnancy in the Hamster



Cycle Number:

- 1 = Normal pseudopregnancy (Average)
- 2 = Hysterectomized pseudopregnancy
- 3-5 = Hysterectomized pseudopregnancy following uterine transplantation

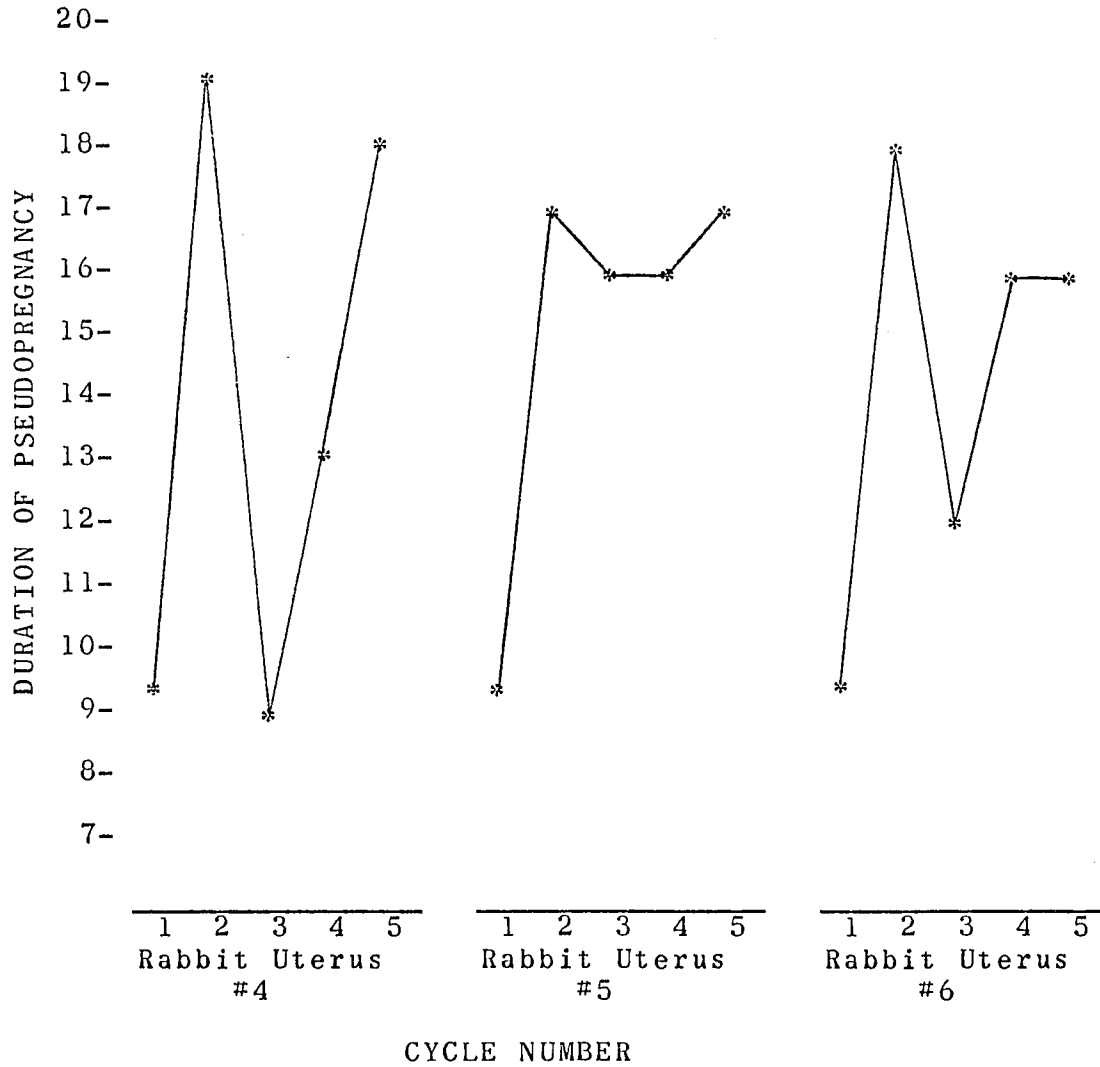
Figure 32. Effects of Transplanted Rabbit Uterus on Hysterectomized Pseudopregnancy in the Hamster



Cycle Number:

- 1 = Normal pseudopregnancy (Average)
- 2 = Hysterectomized pseudopregnancy
- 3-5 = Hysterectomized pseudopregnancy following uterine transplantation

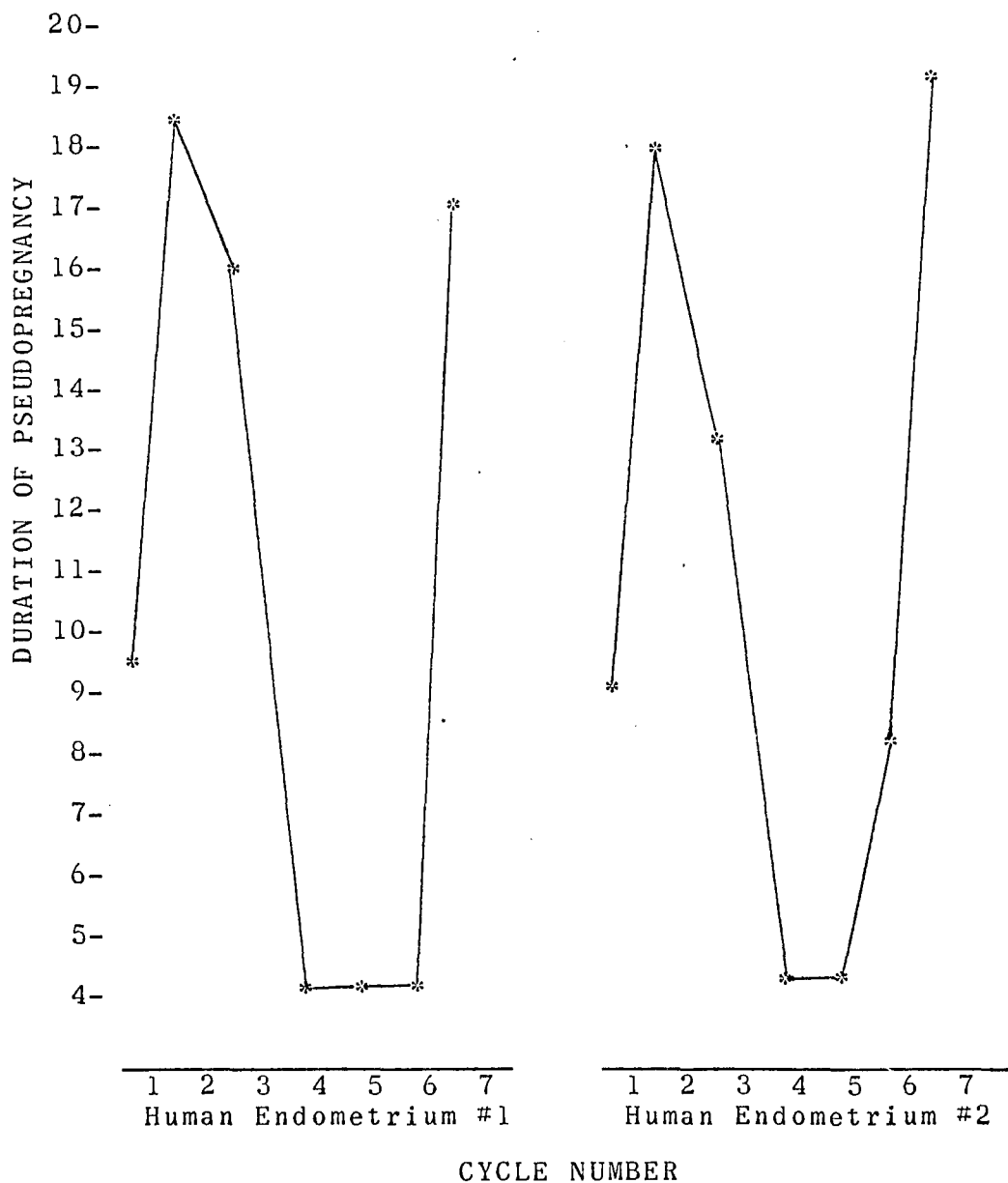
Figure 33. Effects of Transplanted Rabbit Uterus on Hysterectomized Pseudopregnancy in the Hamster



Cycle Number:

- 1 = Normal pseudopregnancy (Average)
- 2 = Hysterectomized pseudopregnancy
- 3-5 = Hysterectomized pseudopregnancy following uterine transplantation

Figure 34. Effects of Transplanted Human Endometrium on Hysterectomized Pseudopregnancy in Hamsters



Cycle Number:

- 1 = Normal pseudopregnancy (Average)
 - 2 = Hysterectomized pseudopregnancy
 - 3-7 = Length of cycles following transplantation of human endometrium
- Normal estrous cycle is four days

DISCUSSION

The importance of elucidating the mechanism, or mechanisms, by which the uterus may control luteal life span is clear since proper implantation of a fertilized ovum is impossible without the presence of a functional corpus luteum. It is, therefore, not only for academic interest that this complex inter-relationship should be clarified, but also because of its extreme practical importance. A thorough understanding of uterine control over ovarian periodicity could lead to a safer, more effective contraceptive device and aid in the treatment of spontaneous abortion due to progesterone deficiency, a common cause of interrupted pregnancy.

While attempting to find a method to demonstrate this regulatory ability of the uterus, a technique evolved, an outgrowth of that described by Duby and McDaniel (1965), which may find future use as a tool for investigating various aspects of ovarian periodicity. A short discussion of this method, its advantages and shortcomings, is essential to a thorough understanding of the results that have been presented. A proper evaluation of the findings would be impossible without first recognizing some of the inherent limitations of this method as compared to some of those previously utilized to demonstrate uterine control over corpora lutea life span.

Homologous ovarian transplants ~~re-~~established the normal estrous and pseudopregnant cycles in a high percentage of animals in which they were the only ovarian tissue present (Table II). The first few cycles under the control of these ovaries in the pouch were generally of normal duration. In many cases, however, cycle lengths tended to increase as the transplants grew to abnormal size. Where this growth preceded to a "cystic-like" condition (Figure 10) all cycling stopped. As an explanation for the occurrence of these non-functioning ovaries, it is suggested that the temperature of the pouch may be slightly lower than that of the body cavity. It has been well established (Zarrow, 1964) that a small change in temperature can seriously affect the ovary, as transplants to the ear and tail of rabbits have shown. In these cases there seems to be an increase in the amount of androgens produced by the ovary, and an enlargement of follicles has been noted similar to those described above (Hill, 1961). It is difficult to accept this as a full explanation, however, since follicular enlargement occurred in only ten percent of the animals used in this study. If temperature change alone could be responsible for producing a non-functioning graft, then a larger percentage of such ovaries would be expected.

A second possibility is that the constant constricting pressure exerted by the pouch membranes that completely encircle the transplanted tissue may prevent ovulation, possibly increasing the number and size of Graafian follicles present.

No evidence to substantiate or refute this possibility can be presented, since all attempts to observe ovulation occurring in the pouch were unsuccessful.

For purposes of this investigation, only the first three or four cycles following ovarian transplantation were recorded. Control animals indicated that these were generally of normal duration, and little error is anticipated with respect to the abnormal growth observed in transplants two to three months old. Some ovarian grafts remain functional in our laboratory after eight months in the pouch, arguing against the contention that temperature change or constricting pressure may be the sole causes of abnormalities.

The use of ovarian transplantation was primarily to show that the spacial relationship between ovary and uterus was not an extremely important factor in the regulation of corpus luteum life span. It is impossible to say, however, that the ovary in the cheek pouch is identical in all respects to the ovary in the body cavity.

Homologous uterine transplantation was more difficult to accomplish successfully than was ovarian transplantation, although continued modification of the original technique provided an increasing rate of success in this venture. Attempts to transplant the entire uterine horn intact were rarely successful. Slitting the uterine tube and placing the endometrium against the inner epidermal membranes enhanced the chances of shortening hysterectomized pseudopregnancy. The most suitable method, however, was cutting the slit uterus

into small pieces prior to insertion, assuring the greatest degree of success in reversing the elongated pseudopregnant cycle.

Transplantation of the entire uterine horn, intact, may have been unsatisfactory due to the supposed lack of movement of fluids through the normally open lumen. In the pouch, perhaps under the influence of the constant constriction previously mentioned, the lumen closes, the endometrium fuses, and the endometrial glands enlarge to the cystic stage. These conditions could easily contribute to the generally atrophied state of the transplant and possibly account for the loss of luteolytic activity. Slitting and cutting the uterus into pieces alleviated some of these difficulties, or at least made the procedure more effective.

When endometrium alone was placed in the pouch, shortening of the hysterectomized pseudopregnant cycle generally lasted for only one or two cycles. It may be that one or two cycles is the functional limit of the endometrium, at least with respect to its luteolytic capabilities. Examination of the pouches in which endometrium was placed revealed that by twenty-six to thirty days after insertion, the tissue was no longer visible. This was not true, however, for animals in which chunks of slit uterine tissue were placed, indicating the possibility of a necessary relationship existing between the endometrium and myometrium. Myometrium alone failed to shorten hysterectomized pseudopregnancy. This was taken as further proof of the proposed anti-luteal factor's endometrial

origin. However, it should be made clear that although myometrial transplants were virtually free of connecting endometrium, small portions of muscle cells from the myometrium were generally found in the endometrial transplants.

The results of these investigations, confirming as they do the work of Duby and McDaniel (1965), dictate the placing of the Syrian hamster among those animals in which a definite uterine anti-luteal effect has been demonstrated. As mentioned earlier, the other species so included are: the guinea pig, rabbit, laboratory rat, gilt, cow, sheep, and mouse. Similar investigations on the ferret, opossum, macaque, and human have provided no evidence for the existence of a regulatory relationship between the uterus and ovary.

The only previous work concerning luteolysis in the Syrian hamster is that reported by Duby and McDaniel (1965). Their investigations showed that uterine transplantation to the cheek pouch significantly shortened the length of hysterectomized pseudopregnancy. Their results, however, although showing a statistical significance, were based on the measurement of the first pseudopregnant cycle following hysterectomy and uterus transplantation. Using this method, the effects of Nembutal as an anesthetic, and the operation itself, might have influenced the length of pseudopregnancy. They reported in their work that Nembutal was capable of elongating the normal four day estrous cycle to five and sometimes six days, and reasoned that this effect was brought about by the ability of Nembutal to block ovulation. There

is no reliable information regarding what possible effects this drug might have on the pseudopregnant cycle. The method used in the present investigation measures the length of pseudopregnancy only after the animal has survived ten days post-surgery, and has exhibited normal estrous periods. This eliminates the possibility that the operative procedure had any affect on the length of pseudopregnancy induced by sterile copulation ten to fourteen days following hysterectomy and transplantation.

Duby and McDaniel reported that transplantation of the uterus to the cheek pouch shortened hysterectomized pseudopregnancy by 1.75 days. In the current investigation the cycle was found to be shortened by 5.25 days. The disparity between these two results may be accounted for by two factors. The best results in our investigation were recorded after slitting and cutting the uterus in small pieces, a procedure not used by Duby and McDaniel for the work they reported. In most cases, the third and fourth pseudopregnant cycles were found to be slightly shorter than the first and second cycles following uterine transplantation to hysterectomized animals. Since the figure reported in this investigation is an average of these three or four cycles, it is reasonable to assume that it would be smaller than that measured for the first cycle observed by Duby and McDaniel.

As further evidence that the figure 13.25 days (Table III) represents an accurate measurement of the effects of uterine transplantation on hysterectomized pseudopregnancy,

pieces of uterine tissue inserted in the pouch of animals having shown at least three elongated hysterectomized pseudopregnant cycles, significantly shortened this cycle length to 13.00 days (Table IV).

In addition to demonstrating that the uterus is capable of inducing luteolysis, attempt was made to determine what spatial relationship might exist between the ovary and uterus that could contribute to this process. By transplanting both ovary and uterus to the same pouch in one series of animals, and putting these two tissues into different pouches in another series of animals, the effect of completely separating the two could be determined by measuring the length of hysterectomized pseudopregnancy in both Groups (Groups E and F, Table III). Although not large, the difference in pseudopregnancy as measured for these Groups, shows a statistical significance. The animals receiving both tissue types in the same pouch had cycles that were 1.38 days shorter than the cycles measured for animals with the ovary and uterus separated. This spatial relationship would seem to be of secondary importance in the hamster, since luteolysis occurred when distance separating the ovary and uterus was almost as great as possible in terms of vascular connections. Inskeep (1965) reported retarded luteolysis in rabbits in which the ovary and uterus were not confluent, a relationship that does not hold true for this investigation. Further evidence against the importance of the two organs being in close proximity to one another will be shown when a discussion

of the effects of rabbit uterus transplanted to the hamster cheek pouch is considered in the next section.

No other study has reported the effects of transplanting endometrium alone to hysterectomized animals, although many investigators have hypothesized the localization of the proposed anti-luteal factor in this specific uterine tissue. The present investigation was able to demonstrate quite clearly that endometrium transplants were effective in regulating corpora lutea breakdown. As the results show (Group L, Table III), shortening of hysterectomized pseudopregnancy was observed with endometrium, but not when myometrium alone was transplanted to the cheek pouch (Group M, Table III). Convincing evidence has been presented for the existence of a specific anti-luteal factor of endometrium origin, circulated via the blood stream and effective in regulating the length of luteal function.

Duby and McDaniel (1965) presented evidence supporting the original work of Loeb which proposed an inverse relationship between the length of pseudopregnancy and the amount of uterine tissue left intact. Sub-total hysterectomy in the guinea pig had shown that one quarter of one uterine horn was sufficient to induce luteolysis. This threshold amount, according to Loeb, was essential for normal luteolytic activity by the uterus. Duby and McDaniel did not attempt a determination of threshold for the hamster, but were able to show that increased uterine removal resulted in elongation of pseudopregnancy.

To test the reciprocal assumption, i.e., that increased amounts of uterus transplanted to the cheek pouch of normal animals could shorten the length of pseudopregnancy below average, a series of intact animals received increasing amounts of uterus. No shortening of pseudopregnancy below eight days was observed (Group K, Table III). These results are not surprising, however, since it has been shown that as little as one uterine horn can maintain the normal duration of pseudopregnancy in the hamster (Duby and McDaniel, 1965). It would seem, therefore, that as much as one uterine horn could be considered an excess for inducing luteolysis, and amounts of uterine tissue above the normal would not be likely to show any increased shortening effect.

The results of homologous uterine transplantation as affecting hysterectomized pseudopregnancy are often spread over a wide duration of cycle lengths (Figures 20 - 26). A possible explanation for this observation might be that this spread represents degrees of transplantation success. Possible also is the fact that different methods used within the same Group of animals, some of which were shown to be better than others, could be reflected by increased shortening of the cycle length. Group A (Figure 20) presents a normal curve for pseudopregnancy in the intact hamster. Group C (Figure 22), although spread over a wider range, nevertheless shows a definite elongation of pseudopregnancy due to hysterectomy. Note that only three cycles were measured at less than sixteen days. Groups B, E and F

(Figures 21, 24 and 25), whose measured pseudopregnancies depend upon the relative success of the transplantation procedure and the amount of functional uterus successfully vascularized, show a wide range of values for this cycle length. Note that the majority of pseudopregnancies are below sixteen days in all of these Groups, with major concentrations at values of ten to thirteen days. These values reflect the most successful transplantation procedure. It is interesting to mention here that Duby and McDaniel (1965), when determining the effect of partial hysterectomy on pseudopregnancy length, found that the presence of one uterine horn resulted in the elongation of pseudopregnancy to 13.9 days. This value closely approximates those recorded in many animals in which this amount of tissue was transplanted to the cheek pouch.

Evidence from this part of the study strongly supported the existence of a luteolytic factor, most probably of endometrial origin. To ascertain what species specificity might be present if such a factor does regulate the life span of the corpus luteum, heterologous transplantation of uterine tissue into hysterectomized hamsters was performed, and the length of pseudopregnancy measured (Groups H, I and J, Table III).

As expected from previous reports (Duby et al., 1965), transplanation of heterologous tissue was considerably less successful than implanting homologous tissue. However, a series of animals (Group H, Table III) transplanted with rat

uteri following hysterectomy showed a significant shortening of pseudopregnancy. The same experiment with rabbit uteri was less conclusive, although four of the six animals receiving estrous uteri from rabbits showed at least one shortened pseudopregnant cycle. Nearly all traces of shortening were gone during the following cycles, however. Human endometrium transplanted to the cheek pouch failed to show any reproducible evidence of cycle shortening. The latter result is perhaps not surprising, however, since luteolytic activity in human uterus has not been demonstrated. Examination of the pouches eighteen days after transplantation failed to reveal any endometrium remaining. It is interesting to note, however, that the normal periodicity was greatly disturbed at first, the animals often showing estrus two days in a row. It was difficult, also, to induce pseudopregnancy in these animals. No definite conclusions can be made from the present experiments regarding the effects of human endometrium on hamster ovarian periodicity.

The results from these heterologous studies indicate that the effect of inducing luteolysis may not be species specific, and that uterine tissue, and more specifically endometrium, may be uniquely endowed with a mechanism for controlling luteal life span. When taken with the indications arising out of the homologous studies, this investigation supports either of two of the views presented in the introduction: (1.) There may be a specific anti-luteal factor, of endometrial origin, whose production at critical times in the ovarian

cycles regulates the life span of the corpus luteum. This factor is probably blood borne and not species specific, or (2.) Uterine tissue, primarily endometrium, may be able to metabolize a specific ovarian product. The removal of the uterus or inactivation by pregnancy may be sufficient to remove this ability resulting in high levels of the hormones (Estrogen or Progesterone) which might act through the hypothalamus in a manner already discussed to maintain luteal function.

There is no evidence from this investigation that could support or refute any indirect action of the uterus mediated through the hypothalamus to control luteolysis, although the weight of experimental evidence to date indicates that the ultimate mechanism may very well act in this way (Hansel, 1965 and Nalbandov, 1963).

Prior investigations indicating that the process of luteolysis is neurologically controlled through the uterine nerves cannot be supported by this work (Nalbandov, 1963). All evidence presented to date concerning the hamster points to a factor being carried by the circulatory system and capable of regulating corpora lutea function.

SUMMARY

1. Homologous ovarian transplantation into the cheek pouch of ovariectomized hamsters successfully re-established estrous and pseudopregnant cycles of normal lengths.

2. Total hysterectomy in the hamster prolongs corpora lutea life span as measured by the length of pseudopregnancy (18.49 days as opposed to 9.24 days).

3. Successful reversal of this elongation is accomplished by homologous uterine transplantation to the cheek pouch.

4. Ovaries and uteri transplanted to the same pouch establish pseudopregnant cycles consistently shorter than those observed in animals receiving ovaries and uteri in different pouches.

5. Transplantation of estrous rat uteri into the cheek pouch of hamsters significantly shortened hysterectomized pseudopregnant cycle length.

6. Transplantation of uteri removed from rabbits on day fourteen of pseudopregnancy produced shortened hysterectomized pseudopregnant cycles when transplanted to the cheek pouch of hamsters. The results were less conclusive, however, than those obtained with transplantation of rat uteri.

7. Transplantation of human endometrium in hysterectomized hamsters failed to show evidence of pseudopregnant cycle shortening.

8. Homologous transplantation of myometrium into the hamster cheek pouch did not shorten hysterectomized pseudo-pregnancy.

9. Homologous transplantation of endometrium into the cheek pouch of the hamster shortened hysterectomized pseudo-pregnancy equivalent to that observed for successful uterine transplants.

10. The mechanism for control of corpora lutea life span of the hamster appears to originate in the uterine endometrium. Proper regulation of ovarian periodicity may depend upon the presence of a threshold amount of uterine tissue which may produce an anti-luteal factor. This principle or factor may not be entirely species specific.

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