A STUDY OF THE COMPARATIVE EFFECTIVENESS OF DIFFERENT METHODS FOR THE INDUCTION OF PSEUDOPREGNANCY IN THE MOUSE

A Thesis

Presented to

the Faculty of the School of Sciences and Mathematics

Morehead State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

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by

Ricky E. Collins

July, 1979

Accepted by the faculty of the School of Sciences and Mathematics, in partial fulfillment of the requirements for the Master of Science degree.

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ABSTRACT

A STUDY OF THE COMPARATIVE EFFECTIVENESS
OF DIFFERENT METHODS FOR THE INDUCTION
OF PSEUDOPREGNANCY IN THE MOUSE

Ricky E. Collins, M.S.
Morehead State University, 1979

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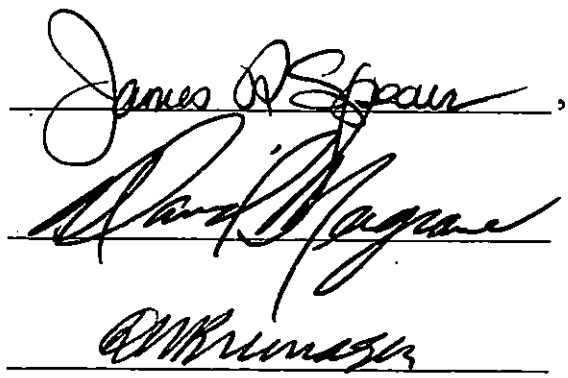
Pseudopregnant rats and mice have been used extensively in reproductive physiology research. Researchers have experimented with several methods for inducing pseudopregnancy by cervical stimulation. Most of the work to this date has been performed on rats, however, due to the disadvantages of using rats in some instances, there is a need for a method to induce pseudopregnancy in mice. There are numerous methods of cervical stimulation for inducing pseudopregnancy that have been employed successfully in rats, however, four distinct methods are used widely. These four are: vasectomized males, stimulation by electrical shock, stimulation by electrical vibration, and stimulation with a glass rod.

These four methods were tested with totally negative results being determined. The reasons for the total negativity of the results can not be determined, however, it is possible that the mechanisms of pseudopregnancy differ

between the mouse and other rodents or some portion or portions of the procedures used in this research were in error.

In conclusion, it is recommended, from the results of this study, that more research into the physiology of pseudopregnancy in the mouse is needed. It can also be concluded that this research indicates none of the methods tested as reliable for the induction of pseudopregnancy in the mouse.

Accepted by:



, Chairman

ACKNOWLEDGEMENTS

I would like to extend my sincere appreciation to the members of my graduate committee, Dr. James R. Spears, Dr. David Magrane, and Dr. David Brumagen for their assistance in the completion of this thesis as well as their guidance during my entire graduate curriculum. I would also like to thank them for the graudate assistantship which they awarded me during my studies. I would like to extend my appreciation to the other graduate students who afforded encouragement during this research.

A very special and sincere thanks to Dr. Madison E. Pryor who proofread and corrected grammatical errors and who took time out from his schedule to be of assistance whenever it was needed.

I would also like to extend appreciation to Janie Strunk for her time and effort in completing the typing of this thesis.

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INTRODUCTION

Pseudopregnant rodents have been used extensively in the past two decades in reproductive physiology research. Both rats and mice have been used for physiological studies of pregnancy, histological and morphological studies of the uterine environment during pregnancy and as recipients in ovum transfers.

Pseudopregnancy is defined as a condition in which the female reproductive system undergoes a series of changes similar to those of a normal pregnancy without the existence of a developing embryo. This phenomenen can occur in all mammals and can be induced in most lower mammals for research purposes. Researchers have experimented with rodents, ovines, bovines, and many other animals to determine the extent of the changes in the reproductive system as well as the characteristics of pseudopregnancy compared with normal pregnancy. The major research involving pseudopregnancy has been conducted with rodents.

The study reported in this paper deals specifically with testing the success rate of different methods of inducing pseudopregnancy in the mouse. Most of the work conducted has dealt with rats, therefore this research was conducted to the test the same methods on female

mice. In the mouse, successful induction of pseudopregnancy is dependent upon a stimulus applied to the cervix of the uterus. In order to determine the existence of a pseudopregnancy, it is also necessary to initiate a decidual response known as decidualization. Decidualization is the process a normal pregnancy goes through coincident to implantation of the blastocyst. A decidual response must be induced in pseudopregnant mice by subjecting the uterus to trauma. Both cervical stimulation and trauma are essential to the study of pseudopregnancy and must be induced separately.

DeFeo listed seven methods for cervical stimulation to induce pseudopregnancy (DeFeo, 1966). Three of the methods are seldom used and could possibly include undersirable effects, therefore only the four widely used methods will be tested. The four methods to be tested are as follows: (1) mating the females with vasectomized males (Allen, 1922); (2) stimulating the cervix with a glass rod (Long and Evans, 1922); (3) stimulation of the cervix with electrical shock (Shelesnyak, 1931 and Staples, 1965); and (4) stimulating the cervix with electrical vibration (DeFeo, 1966).

DeFeo also reported several methods for the production of deciduomata by traumatization to the endometrium of the uterus (DeFeo, 1962). This study will employ one of these methods, the technique of introduction of a silk suture through the lumen of the left horn of the uterus.

The purpose of this research is to determine the most effective method for inducing pseudopregnancy in the mouse. This method should be reliable and easily applied to large numbers of females in those research areas requiring pseudopregnant subjects.

MATERIALS AND METHODS

Mice of the ICR strain (Harlan Industries) were maintained in an environment of 27 C. and were subjected to ten hours of light and fourteen hours of darkness per day. They were fed Purina Lab Chow (Purina Co.) and supplied with water ad libitum. Males and females were housed separately at a density of ten per cage except when paired for test purposes.

Four methods of inducing pseudopregnancy through cervical stimulation were tested. In testing the effectiveness of these four methods of cervical stimulation (Group A) five females approximately sixteen weeks of age, were subjected to each test method for inducing pseudopregnancy. Two types of controls were employed, each using five females. The first of the controls (Group B) included mice that were cervically stimulated, but were not traumatized for decidual response. Females in the second control group (Group C) were not subjected to cervical stimulation, but trauma surgery was performed. Group C served as the control for all subsequent experimentation. Each of the four sets of test females (Group A) received cervical stimulation by one of the four methods tested, and were subjected to decidual inducing trauma by utilizing the

thread method of trauma induction.

Mice to be tested and control animals were first treated with Follicle Stimulating Hormone (FSH) and Human Chorionic Gonadotrophin (HCG) (U.S. Biohcemical) to initiate estrus. Mice of both groups were given five International Units of FSH interperitoneally, diluted in physiological saline, to initiate follicle growth. Five International Units of HCG in saline dilutant were injected; 45-48 hours later. The HCG was administered as a source of lutenizing hormone to promote ovulation and decidual response.

Immature male mice were vasectomized at approximately nine weeks of age, anesthetized using 0.1 mL. of Sodium Pentobarbitol at a concentration of 2.5 mg/mL. (Wyeth Co. 50 mg/mL.) An incision was made in the lower abdomen, the vas deferentia were isolated, crushed with a hemostat, and clipped. The wound was then closed, using Autoclips (Clay Adams Co.). The mice were frequently observed to assure proper healing. The mice were used in experimentation when they were sixteen weeks of age.

Females previously cervically stimulated were subjected to traumatization of the left uterine horn by using the method of inserting a suture thread through the uterine lumen (Long and Evans, 1922). This operation

was performed the fourth day after cervical stimulation, following procedures recorded in a publication on decidual response by Long and Evans (1922). Thread was passed through the lumen using a standard sewing needle approximately 1 cm. in length and 0.5 mm. in diameter. Experimental animals were anesthetized with Sodium Pentobarbitol in a concentration of 0.1 mL./2 mL. of saline. The abdomen was opened; the left side of the uterus was exposed and the thread was passed through the uterine horn at two points. One thread was passed through the horn near the ovary; another thread was passed through the horn near the cervix. The threads were passed through the horn laterally and approximately 2 cm. of thread were left to float freely in the abdominal cavity. The wound was closed using Autoclips and the females were observed frequently to assure proper healing. Group B female mice were subjected to sham operations in which the left horn was exposed, but was not traumatized with a thread.

Following the preparation of vasectomized males, traumatized females, and the establishment of control groups, testing of the four methods of inducing pseudopregnancy was initiated.

Vasectomized Male Method of Inducing Pseudopregnancy

Five vasectomized males were paired with five virgin females, and allowed to remain together for 24 hours. The males were then collected and the females were placed together in one cage. Females were traumatized on day four.

Glass Rod Method of Inducing Pseudopregnancy

Five females were subjected to cervical stimulation using a glass rod constructed from a Pasteur pipette. The pipette was modified by heating the small end to form a bead; the bead served as the stimulator. The rod was inserted into the vagina until resistance was encountered; resistance being accepted as evidence that contact with the cervix had been made. The cervix was then tapped gently fifty to sixty times initiating lordosis (Long and Evans, 1922). Each female was then placed in a separate cage and traumatized on the fourth day following cervical stimulation.

Electrical Shock Method of Inducing Pseudopregnancy

Five females were subjected to cervical stimulation using an electrical probe designed from a diagram produced by Staples (1965). Several modifications in the

probe were made to effect responses restricted to the cervix. The probe was attached to a Biological Stimulator (ImpScope, Heath Co.) which delivered fifty volts at approximately 200 pulses per second (pps). The probe was inserted into the vagina until cervical resistance was encountered. An electrical current was administered for seven seconds (Staples, 1965). Each female, was placed in a separate cage.

Electrical Vibration Method of Inducing Pseudopregnancy

Five females were subjected to cervical stimulation using a method developed by DeFeo (1965). A probe, similar to the one developed by DeFeo was used. The probe, a small copper wire with a copper bead soldered to the tip, was inserted into the vagina until cervical resistance was encountered. The source of vibration was an etching tool to which a copper tip had been attached. After insertion, the vibrator was turned on for sixty seconds and then removed. Each female was placed in a separate cage and traumatized on the fourth day following cervical stimulation.

To compare the effectiveness of the four procedures for inducing pseudopregnancy, females of both Groups A and B were sacrificed on the ninth day after cervical stimulation. Sacrifice was effected by the

REVIEW OF LITERATURE

Studies involving the induction of pseudopregnancy began with the work of Edgar Allen in 1917. Allen made major contributions by determining the length of the estrus cycle in mice. He also induced pseudopregnancy by using vasectomized males for cervical stimulation. Long and Evans (1922), working with rats, reported research work similar to that of Allen. Long and Evans speculated about other processes of inducing pseudopregnancy, and successfully induced pseudopregnancy in rats by using a glass rod to stimulate the cervix. Their attempts to stimulate the cervix of rats in any phase of the estrus cycle other than proestrus proved unsuccessful for the induction of pseudopregnancy in all cases (Long and Evans, 1922). Long and Evans indicated that the appearance of decidual tumors (deciduoma) was an indication for pseudopregnancy. They obtained a seventy percent effectiveness using the glass rod method of cervical stimulation (Long and Evans, 1922). The induction of deciduoma was affected by inserting a silk suture into the uterine lumen. Long and Evans were also careful to note that the insertion of the thread did not produce sufficient trauma to cause deciduoma formation, and that threads inserted during one cycle did not initiate

the severing of the spinal cord after Spears (1978). The abdomen was opened and the entire uterus, from the cervix to the oviducts, was removed intact. The uteri were then examined for decidual tumors. The presence of tumors was accepted as being indicative of a positive test for pseudopregnancy; the absence of tumors was accepted as a negative test. Group C (control) were also examined for decidual tumors.

Restraint of the mouse for the cervical stimulation techniques was effected using a small restraint box (Sargent Welch Scientific Co.) which was closed off on one end. The mouse was allowed to enter the box and a removable panel was placed behind the mouse. A slit in the panel provided the access for the stimulation.

REVIEW OF LITERATURE

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decidual response in subsequent cycles. This lack of response in subsequent cycles was attributed to the growth of epithelial cells which adhere to the threads at the conclusion of one estrus cycle (Long and Evans, 1922).

Slonaker (1929) was also successful in inducing pseudopregnancy in the rat using vasectomized males; his success rate was approximately equal to that obtained by Long and Evans (1922). Slonaker also obtained a seventy percent success rate in using the glass rod technique to induce pseudopregnancy. Meyer, Leonard, and Hisaw (1929) also induced pseudopregnancy in rats using the glass rod method. This response was noted while studying the effect of anesthesia in the induction of pseudopregnancy. Meyer, Leonard, and Hisaw observed that anesthesia effectively reduced the number of pseudopregnancies. Their conclusions suggested that nervous influence, rather than hormonal, caused pseudopregnancy. Haterius (1932) published a paper in which he discussed the effects of partial sympathectomy and parasymphathectomy of pseudopregnancy in the rat. Rats were then subjected to the three methods for induction of pseudopregnancy: (1) cervical stimuli using a glass rod (after Long and Evans, 1922); (2) mating with vasectomized males (after Allen, 1922); and (3) electrical shock (after Shelesynak, 1931).

Although Haterius did not specify the type of decidual response trauma that he used, he obtained 100 percent pseudopregnancy success in the animals which had abdominal sympathectomy and cervical ganglionectomy using vasectomized males. However, mechanical and electrical stimulation produced poor results. Controls for animals subjected to mechanical and electrical stimulation produced lower pseudopregnancy rates of 46.8 percent and 91.6 percent, respectively. Haterius concluded that nervous mechanisms were operative in the artificial induction of pseudopregnancy and that such nervous mechanisms appear to be located in the sympathetic chains.

Shelesnayak (1931) developed a method for cervical stimulation in which he utilized an electrical shock to induce pseudopregnancy. A speculum was used to visualize the cervix to which a slight shock was applied by using a small copper electrode connected to an induction coil. The shock was concentrated on the cervix and no attempt was made to stimulate the vagina. Employing the shock method, Shelesnyak obtained 82.1 percent indidence of pseudopregnancy. Shelesnyak also showed, that the presence of the electrode in the vagina alone did not induce pseudopregnancy. He also observed that the average duration for pseudopregnancy

was fourteen days. Greep and Hisaw (1938) also employed the electrical shock method for inducing pseudopregnancy. They used two electrodes, spaced two millimeters apart, and an electrical current was passed through the cervix for a period of five to ten seconds. Greep and Hisaw obtained a 76 percent success rate in their experimentation. The duration of pseudopregnancy was observed to be from twelve to fourteen days.

Barraclough and Sawyer (1959) obtained 50 to 60% effectiveness in inducing pseudopregnancy in rats by using two drugs, reserpine and choropromazine, which were administered during the poestrus or estrus phase of the estrus cycle. Barraclough and Sawyer obtained a 100 percent success rate in effecting pseudopregnancy when either drug was administered during the diestrus phase. Reserpine and chloropromazine were administered to rats at concentrations of 1 mg/kg and 50 mg/kg of body weight respectively. The uterine horns were traumatized by inserting a thread through the lumen (after Long and Evans, 1922), and the rats were sacrificed on day three after trauma surgery. Deciduoma formation was used as an indication of pseudopregnancy.

In 1965, Staples reviewed the works of Shelesnyak (1931) and Greep and Hisaw (1938) and employing their methods, developed a probe for electrical shock stimu-

lation of the cervix. The probe developed by Staples did not require visualization of the cervix and thus made the stimulation process easier. Staples probe consisted of a tubular rod with electrodes attached at two points. These electrodes permitted the conveyance of electrical current through the rod to induce pseudopregnancy. Staples inserted the probe into the vagina and stimulated the cervix with a current of fifty volts for five seconds. Staples also tested the effect of stimulating the vagina and/or the cervix by varying the position of the electrodes on the rod. Staples performed stimulation of the cervix and vagina during estrus and recorded results comparable with those obtained with stimulation of the cervix only.

V.J. DeFeo (1965) developed the most recent method for the induction of pseudopregnancy in the rat. DeFeo's technique utilized a vibrator which consisted of a modification of an etching tool. He fitted a brass tip to the end of the etching tool and inserted the tip to make contact with the cervix. The vibrator was activated for sixty seconds, and then removed. DeFeo obtained 100 percent induction of pseudopregnancy with this method and his method has been used extensively since 1965.

Induction of pseudopregnancy in rodents requires the presence of a second force in addition to that

involving cervical stimulation. Traumatization of the uterus must be affected to cause a decidual response. The rodent experiences the eccentric type of implantation, in that the endometrium extends out over the blastocyst to cause implantation. In pseudopregnancy, the decidual response is characterized by the development of distinct nodules representive of the attempt to implant a blastocyst. There are many methods, and variations of methods, for inducing decidual response. Decidual response was first observed by Loeb (1908); however, Corner and Warren (1919) and Long and Evans (1922) were the first to study and observe deciduoma in the rat. Parkes (1929) was first to observe decidual response in the mouse.

Long and Evans (1922) reviewed the work of Robert Frank on deciduomata, and succeeded in producing decidual tumors in rats by inserting suture thread into the uterine lumen. Maximum results were obtained when the threads were inserted on days three or four following cervical stimulation. Long and Evans neglected to publish the source of this idea for the insertion of some type of a foreigh body into the uterus. Long and Evans did conclude that a foreign body in the uterus during pseudopregnancy initiated a response similar to that produced by the presence of a blastocyst in a

normal pregnancy. This decidual response was attributed to the presence of healthy, active corpora lutea at least three days of age. These same corpora lutea were responsible for the proliferation of the uterine epithelium in preparation for the implantation of the blastocyst in a normal pregnancy.

Krehbiel (1937) published a paper in which he reviewed the steps involved in deciduomata formation in rats. He successfully induced a decidual response by applying electrical stimulation to the mesometrial and entimesometrial surfaces of the uterine horn. Krehbiel experimented with several mechanical methods of inducing a decidual response before employing electrical stimulation. Electrical stimulation proved to introduce fewer variables into the experimentation. Krehbiel used electrical shock to induce decidual response in one pseudopregnant horn, while allowing the other horn to become pregnant. He examined the implantation sites of the normal pregnancy and compared them with decidual tissue collected from the pseudopregnancy. Krehbiel found that deciduoma produced by electrical stimulation resembled the normal implantation sites more than deciduoma produced by mechanical stimulation. The electrically stimulated deciduoma were compared daily with the normal pregnant horns, beginning with day six

and continuing through day seven. Krehbiel discovered from these daily observations, the close proximity between normal implantation sites and deciduoma tumors produced by electrical stimulation. The major differences Krehbiel observed between decidual tumors and normal implantation sites are as follows: (1) the absence of an enlarging cavity, (2) no distinct implantation zone, (3) smaller mesometrial areas, (4) an earlier climax of growth and glycogen distribution, and (5) a loss of surface epithelium. Krehbiel also observed that mechanically stimulated deciduoma contained small clots, whereas electrical stimuli did not produce such clots.

In 1962, DeFeo published a paper in which he detailed the duration of sensitivity of the uterus to a decidual response. DeFeo experimented with mature rats, in which he induced pseudopregnancy by tapping the cervix with a glass rod (a modification of the procedure developed by Long and Evans, 1922). He induced a decidual response by scratching the uterine lumen endothelium with a knife-edged needle. DeFeo determined the day of maximal sensitivity, by removing the uterine horns on day five after traumatization and weighing them. He determined that day four after cervical stimulation was maximal for the production of deciduoma tissue. DeFeo also determined that maximal sensitivity lasted approximately 24 hours,

and regressed rapidly. DeFeo employed several methods in an attempt to prolong sensitivity until day five, but he was not successful. He concluded that once maximal sensitivity had been reached, the uterus lost the ability for another response until a subsequent cycle.

C.A. Finn and P.M. Keen (1962) reviewed the work of Shelesynak (1952) and tested Shelesynak's results for deciduoma formation in rats, in which he used histamine as an inducing agent. Finn and Keen also tested several other substances to ascertain their ability to induce deciduoma. They tested organic solvents (benzene, glycerol, and citrimide), and found them to be deciduoma depressing agents rather than inducing agents. They also tested oils, e.g. olive oil, and arachis oil, and found that both oils induced massive deciduoma. Finn and Keen concluded that these deciduomal effects were responses to the oiliness of the substances tested. Carrageenin was also tested and massive decidual tumors were observed. Other polysaccarhides were tested, but only agar produced a significant response. Finn and Keen compared their results with those obtained using different concentrations of saline. They also tested some other natural occuring body substances, with varying results. Heparin was the most effective of these substances used for inducing a decidual response. Finn and Keen concluded from the

above studies that deciduoma formation in normal pregnancy is a response by the uterus to a foreign body, the implanting blastocyst (Turner, 1976). This response can be compared to granulation tissue formation by heparin and carrageenin (Finn and Keen, 1962).

Greef, Dullaart, and Zeilmaker (1976) speculated about the idea of luteotrophic action for decidual tissue. They performed a series of experiments designed to determine the ability of the decidual tissue to produce progesterone. This experimentation was carried out on three groups of test rats. Females were either hypophysectomized, hysterectomized, or both hypophysectomized and hysterectomized, and each group was traumatized for decidual response. The trauma was performed by crushing the uterine horns with forceps. Control females were sham operated. Greef, Dullaart, and Zeilmaker measured the blood level progesterone; they also measured the weight of the corpora lutea in all groups. It was observed that progesterone levels increased in deciduoma bearing females, which lengthened the period of pseudopregnancy to approximately 22 days; the duration of control pseudopregnancy was fourteen days. After the operations outlined above, Greef, Dullaart, and Zeilmaker observed that those females with hypophysectomy and decidual tissue had significantly higher levels of

progesterone than those females with hypophysectomy and an absence of decidual tissue. Females with hysterectomy and decidual tissue showed a decrease in progesterone levels, but no significant decline was noted in those females with hysterectomy and no decidual tissue. In females with both hysterectomy and hypophysectomy and decidual tissue, the progesterone levels were similar to control hypophysectomized animals. Greef, Dullaart, and Zeilmaker concluded that females with decidual tissue produced progesterone or a luteotrophic hormone in the corpora lutea.

The work of Greef, Dullaart, and Zeilmaker (1976) prompted Armstrong et. al.(1976) and Talley et. al. (1976) to search for progesterone and estrogen receptor mechanisms associated with the decidual tissue cells. Armstrong was able to prove the existence of high affinity, low capacity, receptors for progesterone while Talley (1976) determined the presence of estrogen receptors. Armstrong used sucrose density contrifugation of decidual tissue from pseudopregnant rats. He observed that progesterone binding occured between day three and day five. He also noted that binding decreased between day five and day seven. Armstrong concluded from these observations that (1) the number of progesterone receptors is a constant, and after all the

receptors are utilized, there are no further produced during that particular cycle; (2) progesterone levels enhance proliferation of decidual cells for a given period, then regression occurs; (3) a priming does of estrogen is required before progesterone will initiate the decidual response. Talley et. al. (1976) made a follow-up study on the presence of estrogen receptors. She used sucrose density centrifugation of decidual tissue and observed that low levels of estrogen were required to insure proper proliferation of the decidual cells. She also observed that deciduomal regression was similar to programmed cell death, and that increased levels of estrogen after day seven did not prevent regression from occuring. Talley et. al. concluded that the number of estrogen receptors was approximately 30,000/cell, and no further production of receptors could be initiated during that cycle.

RESULTS

To determine the most effective method of inducing pseudopregnancy in the mouse, four methods of cervical stimulation for the induction of pseudopregnancy were tested. Each experiment consisted of two groups of five females each, Group A (test females) and Group B (control females). Another group labeled Group C, also containing five females, was used as a control for the entire series of experiments and will be discussed later. The results of each of the A and B groups are summarized in the following pages.

Vasectomized Male Method:

All four of the left uterine horns of Group A that were examined showed characteristic accumulation of fat tissue (one of the females of Group A died during the trauma surgery). The fat tissue was primarily associated with the threads that were inserted for trauma induction. All of the uteri were highly vascularized, and one contained a yellowish fluid. However, no decidual nodules or tumors were noted and a negative determination for pseudopregnancy was made. Group B females showed no vascularization or nodules in the lumen. They also showed no massive accumulation of fat tissue, and the horns were small with no distentions on amy part of the

horn. There was also no difference noted between the left and right uterine horns.

Glass Rod Method:

Group A females showed, as in the previous method, a characteristic accumulation of fat tissue around the left uterine horn. There was some vascularization of the uterine horns, but this was not as pronounced as in the vasectomized male method. There was no indication of decidual nodules or tumors in the uterine lumen. Group B females showed small, novascularized uteri which were very similar to the Group B of the previous method. There were no decidual nodules or tumors noted.

Electrical Shock Method:

Group A females showed fat tissue accumulation in four of the five females, with fluid accumulation in one of the four. The fifth female showed no tissue accumulation or fluid accumulation. There was no appearance of decidual tumors or nodules in any of the Group A females. Four of the Group B females were clear of fat, and the horns appeared nonvascularized with no presence of decidual tissue. The fifth female had tissue accumulation around both uterine horns, but no decidual tissue was noted.

Vibrator Method:

Group A females showed accumulation of fat tissue in all cases, and one of the females showed accumulations of fluid within the left uterine horn. None of the Group A females contained any decidual nodules or tumors. Some distention of the left horn was noted in the female with the fluid, but this was attributed to the presence of the fluid. Group B females showed small, nonvascularized uteri with no apparent decidual tissue being forned.

Group C females were used only one time and they served as controls for the trauma induction surgery for the entire series of experimentation. The females of Group C were subjected to trauma surgery and sacrificed five days later. This was done to correspond to the day nine of pseudopregnancy in the test animals since the test animals were traumatized on day four of pseudopregnancy and sacrificed five day later. None of the Group C females contained any nodules or tumors which could be determined as decidual tissue. Three of the mice of this group contained massive accumulation of fat tissue at the site of introduction of the thread. The remaining two mice showed fat tissue accumulation, but in much smaller amounts. The presence of fat tissue around all of the A groups, as well as in Group C, indicates that the tissue formation was in response to

the placement of the threads.

DISCUSSION

The purpose of the experiments was to determine the most effective method for inducing pseudopregnancy in the mouse by cervical stimulation. Most of the past research concerned with induction of pseudopregnancy has been performed on rats. Long and Evans (1922), Allen (1922), Shelesnyak (1931), Staples (1965), and DeFeo (1965) all used cervical stimulating methods to induce pseudopregnancy in rats and obtained high success rates. Edgar Allen (1917) and Parkes (1928) experimented with mice and were moderately successful in inducing pseudopregnancy using the vasectomized males.

In these experiments, successful induction of pseudopregnancy was determined by observing the presence of decidual tissue in the traumatized left uterine horns of experimental mice of the strain ICR. An attempt was made to initiate the development of nodules by the insertion of a suture thread into the uterine lumen. Decidual tumors appear as small distentions or masses of tissue which give the uterus an irregular configuration when the exterior surface is examined. The right horn of each animals' uterus was left intact in all the mice used in this experiment. The mice were examined on day nine of pseudopregnancy because Loeb (1908) determined

day nine as the day of peak growth of decidual tissue. The results for each of the methods tested in the experiment were completely negative. There was no formation of decidual nodules or tumores in any of the experimental animals. Control mice were also entirely devoid of decidual tissue.

Each test method is discussed below, and some of the advantages and disadvantages are noted:

Vasectomized Male Method:

The use of vasectomized males for cervical stimulation incurs two problems: (1) the receptivity of the females to mating, and (2) the response of the males to the receptivity of the females.

All female mammals except primates undergo cyclic changes in the reproductive system. This cycle is characterized by four distinct phases (proestrus, estrus, diestrus, metestrus) and is known as the estrus cycle (Long and Evans, 1922). These phases are linked to the precise release of hormones (Talley et. al., 1976 and Armstrong et. al., 1976). The receptivity of the female to mating depends on which phase of the cycle the mouse is in at the time mating is attempted. The optimum phase for receptivity is the estrus phase. The estrus cycle in the mouse is approximately four days in length, therefore, the female becomes receptive for mating every fourth day.

It is possible, as was done in this research, to induce receptivity by timed injections of gonadotropins. This procedure is reliable in producing females in the estrus phase of the estrus cycle. This technique allows for experimentation on all females at one time. Without use of this technique, it would be necessary to wait for each female to approach the estrus phase individually. The problem with this technique is that there is no established method that can be used to determine which phase of the cycle the female is in. The use of vaginal smears is undesirable in pseudopregnancy studies because pseudopregnancy may be induced as a result of obtaining the vaginal smear (Long and Evans, 1922).

The response of the male to the females receptivity is also difficult to determine. Males may or may not choose to mate. The use of vasectomized males may add another variable to the results. Although there is no evidence to support this assumption, it is possible that due to the surgical trauma on the reproductive system the procedure for vasectomizing the male may interfere with the mating response or the ability of the male to deposit a vaginal plug in the female. It must also be noted that the vaginal plug remains intact for approximately six hours before it disintegrates. In this research, there was no indication of a vaginal plug in

any of the five test animals, or the five controls. Because of the absence of plugs, it was impossible to determine if mating had occured. Despite of the problems indicated, this is the only method that has even been proved successful for induction of pseudopregnancy in the mouse.

Glass Rod Method:

The glass rod technique of cervical stimulation has also been used successfully in rats. This procedure was used by Long and Evans (1922), and resulted from the induction of pseudopregnancy while obtaining a vaginal smear with a syringe. The problem with glass rod stimulation is that it is difficult to apply to all recipients with the same frequency and pressure. It is also difficult to determine the exact site of stimulation, i.e. the researcher may stimulate the cervix in one female and only the rear portion of the vagina in another. This method is also time consuming; making it undersirable for use where large numbers of pseudopregnancies are required. In this research, the glass rod was introduced while holding the mouse by the tail. This was the only restraint required since the mice did not struggle after insertion of the glass rod. In stimulating the cervix, difficulty was noted in stimulating each of the females the same number of

times with the same rhythm and amount of pressure applied to the cervix.

Electrical Shock Method:

The use of electrical shock requires the use of sophisticated equipment, if the method is to be utilized properly. A thorough knowledge of electicity is essential to insure a high degree of success. A method of restraint is also required for this method, and the size of the mouse makes this technique especially difficult to effect. The small size of the mouse impedes the use of this method because the female must be kept still in order to insure a proper connection for the application of the shock to the cervix. In this research, a special biological stimulator was used to produce the shock. The mouse was restrained using a small plastic box. Since it was impossibel to restrain the mouse entiredly; this could have affected the connection produced.

Vibrator Method:

The use of the vibrator method seems to be the most efficient method for stimulating the cervix in rats. It is easy to apply and it has produced a high degree of success based on published experiments (DeFeo, 1966). However, in mice, it has proved totally ineffective for inducing pseudopregnancy (Carlson and DeFeo, 1963). In this research, the vibrator method required the least

amount of time, and restraint of the mouse presented only minor problems. Because of the past ineffectiveness of this method, it is not recommended for use in mice (Carlson and DeFeo, 1976).

The fact that all the results in this experiment were negative raised several questions about the validity of the procedures and techniques for use in mice. All of the procedures had formerly been used successfully in rats. The vasectomized male method has been previously used by researchers as the only method for successful induction of pseudopregnancy in the mouse. The other methods tested in this research were also tested in mice by Carlson and DeFeo (1963) and they also were unsuccessful in inducing pseudopregnancy in mice using any of these methods. Carlson and DeFeo (1963) failed to determine or speculate concerning the reasons for failure. All of the methods except the vasectomized male method require some type of mechanical stimulation. It is possible to assume from this that the female mouse may receive some other stimulus in addition to cervical stimulation. The assumption cannot be accepted without further research to determine the exact mechanism of pseudopregnancy in the mouse.

Another possibility for the negative results of this particular research may perhaps be attributed to the

hormones used (FSH and HCG). The normal cycling mouse becomes receptive for mating approximately every four days. This receptivity is linked to the hormones FSH and LH. Researchers in the past used Pregnant Mare Serum (PMS) as a source of FSH. Pregnant Mare Serum is no longer available and in this research, pure sheep FSH was used. The accurate dosage for pure FSH could not be found in any literature so a test was performed on three female mice using five international units as a dose. The females were then mated with fertile males and allowed to progress through pregnancy. The females were sacrificed on day nine of pregnancy and the uteri were examined for the presence of implanted blastocysts. Two of the three females were pregnant as indicated by the appearance of implantation sites. The dosage of five international units was accepted as being sufficient to cause follicle stimulation as evidenced from the above observations. Due to the small number of test animals in this dosage test, it is possible that pregnancy would have occured without the injection of the FSH. The dosage of HCG was adopted from previous experimentation by Kendall (1978). Kendall obtained varied results using a one-to-one ratio of FSH to HCG.

Several factors could be responsible for the negative results obtained. However, the probability that

all the pseudopregnancies would be negative does not correspond with the results. Mice become receptive for pregnancy every four days, and twenty mice were tested. This would seem to indicate that, even if the procedures or technique did not work, five of the mice should have become pseudopregnant due to random probability. Such was not the case, and no explanation for this phenomenon can be given.

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