THE EFFECT OF A PROGESTERONE:ESTROGEN COMPOUND AND A HYPOGLYCEMIC AGENT ON PRENATAL MORTALITY IN THE NEW ZEALAND RABBIT

by

Gary L. Brubaker

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in partial fulfillment for the degree of

MASTER OF SCIENCE

in

Animal Science

APPROVED:

T. N. Meacham
Chairman, T. N. Meacham

G. W. Litton

R. G. Saacke

G. Colmano

April, 1969

Blacksburg, Virginia

TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	2
General Reproductive Characteristics of the Domestic Rabbit	2
Seasonal Variation in Reproductive Traits of Domestic Rabbits	4
Gestation	5
Prenatal Mortality	8
Causes of Prenatal Mortality	10
Hormonal Influences on Prenatal Mortality	12
The Hormones of Pregnancy	14
Exogenous Progesterone and Estrogen and Their Effects on Pregnancy	16
The Effects of Chlorpropamide on Pregnancy	18
The Action of Provest	20
OBJECTIVES	22
MATERIALS AND METHODS	23
Animals	23
Housing	23
Feeding	23
Breeding	25
Determination of Preimplantation and Postimplantation Losses	26
Methods of Computation	27
RESULTS	28
DISCUSSION	41

Page

SUMMARY 48

LITERATURE CITED 50

VITA 56

LIST OF TABLES

Table		Page
I	Reproductive performance of the control does	29
II	Reproductive performance of the chlorpropamide treated does	30
III	Reproductive performance of the low level Provest + chlorpropamide treated does	31
IV	Reproductive performance of low level Provest treated does	32
V	Reproductive performance of the high level Provest + chlorpropamide treated does	33
VI	Reproductive performance of the high level Provest treated does	34
VII	Mean reproductive performance of the control and treated groups	35
VIII	Means and standard errors of reproductive performances for all control and treated does	36
IX	Analysis of variance for prenatal losses, number born, and ovulation rates	37
X	Mean reproductive performance differences among the control and treated groups	38

ACKNOWLEDGEMENTS

The author would first like to extend his most sincere thanks and appreciation to his major professor, Dr. T. N. Meacham, for his invaluable assistance and extreme patience in helping to make this thesis possible; his expert guidance both in research and course work are deeply appreciated.

The author would also like to thank Dr. D. F. Watson and Mrs. Violet Drake for their vital assistance in performing the laparotomies and for the use of the Veterinarian Science Department's facilities.

He would like to sincerely thank Dr. G. Colmano, a committee member whose guidance and advice are greatly appreciated in the preparation of this thesis. Special thanks also are extended to committee members Dr. R. G. Saacke and Professor Litton for making this thesis possible.

The author would especially like to thank his wife and typist, Donna, without whose encouragement and patience this thesis would not have been possible.

Other thanks are extended to Dr. J. A. Lineweaver for his time and advice and to Richard Battaglia and other graduate students who have all supplied knowledge and assistance in the preparation of this thesis.

INTRODUCTION

Today, as in the past, the livestock industry is making a concerted effort to improve animals for the production of meat, milk, wool, and other purposes. Increased emphasis has been placed on producing larger numbers of more vigorous offspring per animal in an effort to increase reproductive efficiency. Man has dealt with the problems of inefficiency among his livestock for many centuries, but only in the last few years has he been able to cope with these problems in a more logical and scientific manner.

A major limiting factor to reproductive efficiency is the problem of embryonic or prenatal mortality. Embryonic mortality in mammals represents a considerable source of economic loss. In cattle and horses, which normally bear only one offspring at a time, it is often hard to distinguish between embryonic mortality and infertility since the proportion of matings not followed by the birth of living offspring is all one observes. This is commonly as high as 33% in cattle. In polytocous species, the loss of an entire litter sometimes occurs; more often, however, only a few individuals in a litter die, and therefore, the effect of prenatal mortality is to lower the average litter size.

Prenatal mortality in part seems to be related to a failure or failures in the synchronization of physiological reactions between the maternal and fetal environment. Since many of the reactions occurring between parent and offspring during pregnancy are directly or indirectly affected by hormonal balances, any additional knowledge gained from experimentation regarding hormonal influences on prenatal mortality will prove to be of value.

REVIEW OF LITERATURE

General Reproductive Characteristics of the Domestic Rabbit

The domestic rabbit, Oryctolagus cuniculus, does not seem to have a definite breeding season if kept under normal conditions and provided with shelter and feed (Worden and Leaky, 1962). Rabbits are induced ovulators and some dispute has arisen as to whether a true estrous cycle actually exists. Suggestions concerning possible cycle lengths have been 4 to 6 days (Myers and Poole, 1962), and in other cases, 7 days or multiples thereof (Asdell, 1964).

Other reports indicate a 14 to 16 day cycle and this is based on the pseudopregnant period; that is, if the female is mated or sexually stimulated and conception does not take place, the corpus luteum will last for about 16 days.

In the United States there seems to be an optimum season for reproduction which occurs from May to July (Hammond and Marshall, 1925). During this time, in the absence of the male, the doe remains in heat almost indefinitely and waves of follicles mature, lasting from 7 to 10 days, arise and then atrophy. This atrophy may be due to degeneration and phagocytosis of the granulosa, with fibrotic ingrowth of the theca interna, or to extravasation of blood and the formation of blood follicles (Marshall, 1956). If coitus is permitted, the mature follicles grow and ovulation occurs approximately 10 hours later (Asdell, 1964).

This follicular growth is apparently sparked by a neural stimulus since intense sexual excitement, such as the act of mounting or being mounted by another buck or doe, evokes it. The follicular growth is

due to the release of FSH from the anterior pituitary and this is thought to occur in about an hour or more after coitus (Asdell, 1964).

The developing follicle takes about 18 days to mature and in the nonbred rabbit, this development is followed by a phase of atrophy lasting for about 9.5 days (Asdell, 1964).

The time of ovulation in the rabbit can be predicted with greater accuracy than is possible with other species. Asdell (1964) has found that, after allowing for the time taken by the spermatozoa to reach the top of the oviduct, the eggs have a fertile life of not more than 6 to 7 hours after ovulation. Asdell (1964) also indicates that they are capable of being fertilized only while they are in, or as they leave, the plug of extruded liquor folliculi at the top of the oviduct. As the ova move down the tube and acquire a layer of albumin, they are no longer fertilizable (van Tienhoven, 1968). The percentage of successful inseminations and the litter size decrease at a constant rate with inseminations from 5 hours before to 2 hours after ovulation. Asdell (1964) indicates that the ova retain their capacity for fertilization for a maximum of 8 hours after being shed from the ovary; however, Chang (1951) has shown that fertilization is usually reduced after 4 hours post ovulation. Ova just liberated from the follicles are surrounded by cumulus cells of the stratum granulosum. These cells are dispersed by the penetration of spermatozoa into the mass. This happens normally within 1 to 1.5 hours after ovulation (Asdell, 1964). If spermatozoa are not present, it takes about 17 hours for these cells to disperse. Passage of ova through the oviduct requires about 62 to 82 hours (Pincus, 1930).

The duration of pregnancy is approximately 32 days in domestic rabbits (Hammond and Marshall, 1925).

In a doe that conceives immediately after parturition and is nursing a large litter (above three to four), implantation is not delayed but the embryos die in the blastocyst stage. If smaller litters are nursed, the pregnancy continues normally. A nursing doe goes out of heat in about a week because of follicular atresia with the loss of about half the normal weight of the ovaries. The uteri also atrophy and almost resemble the state found in an ovariectomized doe; new follicles mature rapidly after weaning (Hammond and Marshall, 1925).

Seasonal Variation in Reproductive Traits of Domestic Rabbits. Wild rabbits experience definite seasonal variation in reproductive performance (Bullough, 1961). Spring and summer seem to be the natural breeding season and during the remainder of the year the does are in a state of anestrus. Some researchers indicate that domestic rabbits also manifest seasonal patterns in reproductive performance (Hammond and Marshall, 1925; Ross et al., 1961; Clegg and Ganong, 1959).

In a study conducted in southern California, Sittmann et al. (1964) found a highly significant seasonal trend in reproductive activity of the domestic rabbit. Services leading to conception, expressed as monthly percentages of all services, indicate that September matings result in the lowest conception rates.

Other workers have also demonstrated seasonal variations in reproductive performance in both wild and domestic rabbits (Kihlstrom, 1958; Sawyer, 1959). Experimental studies have suggested that these variations or seasonal changes are dependent on changes in temperature and possibly light.

Gestation. Before pregnancy can occur in the female reproductive tract, certain preparative hormone-regulated changes must occur. These include increased vascularization of the endometrial tissue, growth and coiling of the uterine glands, and perivascular leukocyte infiltration (Hafez, 1968). If pregnancy is to be maintained, these changes must occur at the proper time. This has been emphasized in attempts to transfer fertilized ova, which have failed when the estrous cycles of the donor and recipient were not synchronized (Willet, 1953).

During early pregnancy the uterine glands, which are abundant in the intercotyledonary zones of the endometrium, lie in the midst of numerous blood and lymph vessels. At this stage the glands appear to be secreting uterine milk (Amoroso, 1952).

In early pregnancy, when the tract is under the influence of progesterone and estrogen levels are low, the mucous secretions of the cervix and anterior vagina thicken and become sticky. During most of the gestation period a thick mucous plug seals the cervix and prevents the entrance of bacteria or other contaminants from the vagina. Late in pregnancy, when the estrogen levels are increased, the mucous-secreting cells are activated again. This is evident in dairy cows when strings of mucus are frequently seen hanging from the vulva near parturition (Salisbury and Van Demark, 1961).

For an interval of about 3 days after fertilization of the rabbit ovum, the zygote remains in the oviduct. The zygote then passes into the uterus where it floats freely for another 3 days (Gregory, 1930). Transportation of the fertilized ovum may occur at different rates in different species; the ovum arrives when the uterus is in the appropriate condition (Chang, 1951).

Harper (1966) conducted a study concerning the effects of progesterone and estrogen on ovum transport through the oviduct in rabbits. His data indicate that estrogen stimulates ovum transport and progesterone depresses it and that the rate of transport in intact animals depends upon the relative amounts of these two hormones. Both hormones act on the ampullar musculature, and in this way, affect the speed of travel of the ova.

Around the fourth day the rabbit blastocyst enters the uterus. The blastocysts become distributed down the length of the uterine horns as the result of the muscular churning movements. Progesterone is involved in blastocyst transport by decreasing the irritability of the myometrium and by reducing the spread of response to stimuli (Boving, 1959).

Phelps (1946) has indicated that there is an increased blood supply just below the uterine epithelium at the time the blastocyst is about to implant.

During the period the blastocyst is forming, the zona pellucida becomes thinned and is eventually dissolved so that the trophoblastic cells can come into direct contact with the maternal endometrium (Amoroso, 1952).

In the rabbit the blastocyst expands to fill the shallow depressions that form the antimesometrial part of the uterine lumen. The uterus then becomes edematous (probably a result of estrogen secretion) and clasps the blastocyst (Enders and Schalfke, 1967). The trophoblast eventually invades and destroys the epithelial lining and comes into direct contact with the subepithelial tissues and blood vessels. The

maternal vessels themselves are in direct contact with the trophoblast by day 9 post coitum (Boyd and Hamilton, 1952). Implantation is completed by day 12.

The attachment of the blastocyst to the uterine wall and the invasion of the latter by the former leads to formation of the placenta, which enables an exchange of nutrients and gas between embryo and mother. Rabbits have a hemoendothelial type placenta which has a discoid, cup-shaped or spheroidal shape (Grosser, 1909). The nutritive materials absorbed by the placenta or fetal membranes directly from the circulating blood are called the hemotrophe, while the histotrophe refers to secretions and degradation of the endometrium and also extravasated maternal blood which is absorbed.

The placenta contains two separate blood streams which are separated by a number of layers. In the rabbit the maternal and fetal blood flow in different directions, thus creating a counter current exchange for transfer of substances from maternal to fetal circulation. This counter current arrangement has the greatest advantage when the diffusion capacity is high and the perfusion rate of the blood is low (van Tienhoven, 1968).

The transfer of nutrients across the placenta takes place largely by active transport. In addition to the transfer functions of the placenta, there is evidence of active metabolism of a number of nutrients. Glucose can be oxidized to lactic acid; the placenta can synthesize glycogen and can convert pyruvate and other intermediates of the tricarboxylic acid cycle to carbon dioxide and water. Neutral lipids and phospholipids are synthesized in the placenta (Hagerman, 1964). These lipids may later be used for fetal nutrition.

In the rabbit, up to about the 18th day, the growth of the fetal placenta is greater than that of the fetus itself. The maximum rate of growth occurs in the fetal placenta about day 22 and thereafter continues to fall gradually until the end of pregnancy, while the growth rate of the fetus continues to parturition (Hammond, 1937).

Prenatal Mortality

Prenatal mortality seems to occur in all species of animals and, depending upon the time or stage of gestation in which the loss occurs, it can be divided into preimplantation and postimplantation losses. Preimplantation includes the time from ovulation until the blastocyst attaches to the uterine wall; postimplantation includes the time from attachment until parturition (Healy et al., 1961).

Adams (1960b) classified mortality in the rabbit as: preimplantation, days 2.5 to 7; postimplantation, days 7 to 10 (early deaths), days 10 to 17 (middle deaths), and days 17 to 24 (late deaths).

Allen et al. (1946), in a study with wild rabbits, investigated the reliability of estimates of prenatal mortality based on counts of corpora lutea, implantation sites, and embryos. From this study the authors concluded: (a) death of part of the embryos in a polytocous mammal rarely terminates pregnancy as resorptions take place and do not interfere with the development of the remaining litter; (b) the corpora lutea on the ovaries are very persistent and remain identifiable until parturition; and (c) a total error of 6.4% in the original counts of corpora lutea affected 25.6% of the litters, but was distributed evenly throughout gestation.

In estimating preimplantation and postimplantation losses, laparotomies are performed shortly after implantation occurs. This operation, involving an abdominal incision and observation of ovaries and uterine horns, does not significantly affect the embryos so as to increase embryonic death (Brambell, 1948).

In domestic animals, most of the prenatal mortality occurs in the first 4 weeks of gestation. Hanly (1961), working with gilts and sows, found that most of the embryonic deaths were observed to occur by day 25 and the loss varied from 23 to 50%.

In cattle the majority of embryonic deaths occur between the 16th and 25th day (Hawk et al., 1955). This is the period of rapid growth and differentiation of the embryo and the extra-embryonic membranes. The embryo is dependent for its survival on the absorption of nutrients contained in the uterine milk from about the 16th day of gestation (Greenstein and Foley, 1958) until the fetal-maternal union is established at about 36 days of pregnancy. The death of the embryo during this period may be due to an unfavorable uterine environment (Hafez, 1962).

In polytocous species, fertilization rate is almost 100%, but the number of implantations and the number of young carried to term is less than the number of eggs fertilized. The discrepancy between the number of eggs fertilized and the number of fetuses in the uterus varies greatly among the individuals of one species, but is similar for most polytocous species (Hafez, 1962).

In the rabbit, contrary to the pig and other domestic species (Hanly, 1961), the greatest prenatal loss occurs during the post-

implantation period. Brambell (1948) estimated that in the wild rabbit at least 43.3% of the ova were lost between ovulation and term with the peak mortality occurring about 12 days post coitum. He established that 10.2 to 13% of the ova were lost before implantation and 18% after implantation. The embryonic loss in the domestic rabbit amounts to 10% before implantation and 18% after implantation. Most of the postimplantation mortality occurs immediately following implantation (Adams, 1960a).

Estimates of prenatal loss are usually based on comparisons between the number of corpora lutea and the number of embryos surviving to a later stage, by obtaining data at two or more intervals during early pregnancy. The rate of embryonic mortality is calculated as the number of non-viable embryos expressed as a percentage of the total number of corpora lutea. The loss occurring shortly before implantation is probably caused by the failure of synchronization between the transport of the morula through the uterotubal junction and the progestational development of the endometrium (Noyes and Dickman, 1960). The marked prenatal mortality which occurs following implantation may be due to a deficiency in placental development or inadequacy of the endometrial vascular supply (Hafez and Tsutsumi, 1966).

Causes of Prenatal Mortality. Prenatal mortality can be due to maternal, embryonic, or fetal-maternal failures. Maternal failure tends to fall on all of the embryos as a whole, resulting in complete loss of pregnancy; whereas, embryonic failure tends to affect individual embryos of a given gestation, resulting in partial loss of pregnancies. In other cases, the maternal environment is unfavorable and not all implanted embryos can be supported (Hafez, 1962).

Runner (1951) divided prenatal mortality in polytocous animals into two categories: those which are intrinsic to the developing embryo and those due to maternal limitations. Maternal limitations presumably will tend to be felt by all members of a litter. Intrinsic factors, on the other hand, will tend to be expressed upon individual embryos within a litter.

The genetic make-up and nutrition of the dam, over crowding in the uterus, hormonal imbalance, thermal stress, and many other environmental factors all contribute to prenatal mortality.

It is known that prenatal mortality is influenced by age and parity of the dam (Finn and Keen, 1962). In cattle (Erb and Holts, 1958) and sheep (Edgar, 1962), embryonic loss is higher in young animals than in old ones. In swine it is higher in sows than in gilts (Perry, 1956).

Caloric intake and specific nutritional deficiencies affect ovulation and fertilization rates, as well as prenatal death. In swine high caloric intake or continuous unlimited feeding increases ovulation rate but also increases the embryonic mortality up to implantation time (Haines et al., 1959).

The frequency and repeatability of embryonic loss is determined by the genetic make-up of the sire and dam, as well as the breeding system. In cattle prenatal death in the first half of pregnancy is 27% in inbred dams and 11% in outbred ones (Hawke et al., 1955).

With advancing pregnancy, the embryo becomes increasingly dependent upon the placenta for its survival. The degree of placental development is primarily influenced by the availability of space and vascular supply within the uterus. As the number of implantations increase,

the vascular supply to each site is reduced; this restricts placental development and results in embryonic and fetal death (Eckstein et al., 1955; McLaren and Michie, 1960; Hafez, 1964). A high percentage of fused placentae occurs in over crowded uteri in the rabbit (Hafez, 1964).

McLaren and Michie (1960) suggested a limited nutrient pool theory as a cause of prenatal mortality. They indicated that the fetal blood furnished adequate amounts of nutrients to provide for maximum embryonic survival, but if the supply was inadequate, one or more fetuses would die.

Bishop (1964) suggested that a considerable part of prenatal mortality is unavoidable and, in fact, should be regarded as a normal way of eliminating unfit genotypes from the population.

Other environmental factors influencing reproduction are ambient temperature (Ulberg, 1958), season and light (Dutt, 1960). Many of these environmental factors affect the nervous and/or endocrine systems which cause hormone imbalances that may directly account for much of the reproductive failure observed in domestic animals (Hafez, 1968).

Hormonal Influences on Prenatal Mortality. Since the synchronization of events between maternal and fetal environment are directly or indirectly controlled by hormonal balances, hormone inadequacies may contribute significantly to prenatal mortality.

The most critical period of survival for the embryo is during implantation (Adams, 1960b). Normally, the developing corpus luteum secretes progesterone, the action of which on the female tract is thus correlated with the development of the embryos. Even under natural conditions, the failure of the embryos to implant may often be due to the

absence of receptivity in the uterine mucosa at the appropriate time (Adams, 1960b).

Although the mechanism involved in maintaining the corpora lutea in pregnant females remains obscure, the general role of progesterone during gestation is somewhat better understood. An important function of progesterone is to promote changes in the endometrium, thus preparing the uterus for implantation of the fertilized ova. Progesterone induces the formation of a secretory endometrium in a uterus previously sensitized by estrogens; the endometrium is characterized by an increase in mucosal thickness, increased coiling of the glands, edema of the stroma, and presence of glycogen droplets in the glandular cells. Progesterone also inhibits spontaneous uterine motility and keeps it in a state of quiescence, thus preventing expulsion of the embryo. Progesterone also is important in the mechanisms of implantation (Hafez, 1968).

Boving (1959) hypothesized that progesterone influences implantation through a number of mechanisms. These may be grouped as muscular, adhesive, and invasive mechanisms.

Of the muscular mechanisms, blastocyst spacing is progesterone-regulated. Progesterone decreases the contractibility of uterine muscle and aids in expansion of the blastocyst.

Among the adhesive mechanisms, progesterone has a preparatory function in promoting the tubal deposition of the mucolemma or mucin coat on the embryo. This coat, in conjunction with the gloiolemma from the uterus, provides the first adhesive attachment. Probably during that attachment, as during the adhesive attachment of the trophoblast

to the endometrium, progesterone helps by increasing endometrial carbonic anhydrase. This facilitates the transfer of bicarbonate from the blastocyst to the endometrium by speeding the conversion of carbonic acid to carbon dioxide (which is then removed by the maternal circulation). The attendant local liberation of alkalinity makes both membranes and trophoblast sticky.

Among the mechanisms of invasion, the same progesterone-regulated reaction is responsible for local alkali-induced dissociation of uterine epithelium which clears the path, as it were, for the intrusion of the trophoblast (which remains undissociated because it is syncytial) (Boving, 1959).

Estrogen is important, acting along with progesterone, in maintaining pregnancy. Estrogens stimulate marked growth of the uterus resulting in an increase in the mass of both the endometrium and myometrium. Estrogen is also essential in causing uterine contractions which are important in spacing of the embryos (Boving, 1959).

The Hormones of Pregnancy

The ovary possesses two functions: the production of ova and the production and release of three types of hormones -- estrogens, progestogens, and relaxin. The first two are commonly referred to as the female sex hormones and are steroids with the typical cyclopentanoperhydrophenanthrene nucleus. Relaxin is a polypeptide with a molecular weight of about 10,000 and is active during the latter part of gestation (Hafez, 1962).

In the normal, non-pregnant female, extrogens are secreted in significant quantities only by the ovaries, though some is secreted by the adrenal cortex. During pregnancy in some species,

quantities are also secreted by the placenta; as much as 300 times the amount secreted by the ovaries during an estrous cycle may be produced by the placenta during pregnancy (Guyton, 1967).

The primary function of the estrogens is to cause cellular proliferation and growth of the tissues of the sexual organs and of other tissues related to reproduction (Hafez, 1968).

The estrogens have a marked effect on the fallopian tubes; they cause the glandular cells to proliferate and the number of ciliated epithelium cells that line the fallopian tubes to increase. The activity of the cilia is also stepped-up and this undoubtedly helps to propel the fertilized ovum toward the uterus (Guyton, 1967).

Estrogens, in general, increase the metabolic rate to a slight extent by causing increased quantities of fat in the subcutaneous tissues. Estrogens also stimulate retension of water, sodium, calcium, nitrogen, and phosphorus (Guyton, 1967).

An important function of progesterone is to promote secretory changes in the endometrium, thus preparing the uterus for implantation of the fertilized ovum. Progesterone decreases the frequency of uterine contractions, thus helping to prevent expulsion of the implanted ovum (Csapo, 1956).

Progesterone promotes secretory changes in the mucosal lining of the fallopian tubes. These secretions are necessary for nutrition of the fertilized, dividing ovum as it passes through the fallopian tube prior to implantation (Amoroso, 1952).

Progesterone also exerts a mild catabolic effect on the body's protein similar to that of the gluco-corticoids. An important point

here is the fact that although this effect may not be significant through the normal sexual cycle, it possibly is quite significant during pregnancy, when proteins must be mobilized for use by the fetus (Guyton, 1967).

Exogenous Progesterone and Estrogen and Their Effects on Pregnancy.

Corner and Allen (1929) described a method for the preparation of corpora lutea extracts which were therapeutically substituted for the intact corpora lutea. The extracts produced progestational changes in the endometrium of mature rabbits and when castration was performed 18 hours post-mating, pregnancy was maintained to term.

Allen and Heckel (1937) demonstrated that pregnancy can be maintained to term in rabbits castrated on the 11th day after mating, providing 2.0 mg. of progesterone were given daily from the 11th to 15th days and 4.0 mg. of progesterone daily from the 16th to 28th days of gestation. Under these circumstances the same changes occur in the uterus and mammary gland as was observed in normal pregnancy.

Courier and Kehl (1938) pointed out that if progesterone therapy is to be given to the ovariectomized pregnant rabbit, the injections should be given on the day before the operation so as to eliminate the possibility of even a brief interruption in the availability of the hormone.

Chang (1951) maintained pregnancy in recipient intact rabbits with injections of exogenous progesterone. Both 1-day and 6-day ova were transferred to opposite horns of recipient rabbits. Chang found that large initial doses are important for the reception and transportation of ova from the tube to the uterus and the maintenance of pregnancy after implantation.

Smithberg and Runner (1956) induced and maintained pregnancy in prepuberal mice using gonadotrophins and progesterone. Implantation was induced in both intact and castrated mice at a dosage of .125 to .25 mg. of progesterone daily. Maintenance of pregnancy to term by larger dosages of progesterone (1 to 2 mg.) were used; castrated mice carried slightly fewer live young, however.

Rubinstein and Forbes (1963) studied the effects of subcutaneous implants on maintenance of pregnancy in ovariectomized mice using crystalline progesterone and 17 α-hydroxyprogesterone. Seventeen α-hydroxyprogesterone failed to maintain pregnancy, but crystalline progesterone did maintain pregnancy; the exogenous amount needed diminished as pregnancy progressed. A fetal survival rate of 90% or more was associated with an average estimated daily absorption of at least .91 mg. of progesterone when ovariectomy had been performed on day 11.

Although progesterone undoubtedly aids in maintenance of pregnancy, a combination of progesterone and estrogen has been reported to be more effective in some cases. There seems to be a very critical ratio between progesterone and estrogen that will maintain pregnancy in mammals.

Courier and Kehl (1938) have shown that a progesterone:estrogen mixture in a ratio of 750:1 will successfully bring about implantation in ovariectomized rabbits. They indicated that narrower ratios of progesterone:estrogen (lower than 200:1) had no effect in lowering implantation losses.

Hafez and Pincus (1956), studying the hormonal requirements for implantation, indicated that when narrower ratios (250:1 to 20:1) were used, the ova were blocked in the tube and degenerated before reaching the uterus.

Lerner et al. (1962) maintained pregnancy in rats ovariectomized on the eighth day of pregnancy using 17 α-dihydroxyprogesterone derivatives as well as other progestogens. They found that the addition of estrogen enhanced the maintenance of pregnancy, probably by augmenting the progesterone.

Day et al. (1959) demonstrated the use of a progesterone:estrogen combination to maintain pregnancy in ovariectomized gilts. Day et al. (1963), in a study involving 132 gilts, evaluated the effects of exogenous progesterone and estrogen when administered as an implant on the rate of embryonic mortality. They administered 100 mg. of progesterone caproate and 50 mcg. of estradiol benzoate per 100 lb. of body weight (2000:1) on the 11th day after mating. Although the treatment failed to statistically improve embryonic survival, a definite trend toward increased litter size was observed.

The Effects of Chlorpropamide on Pregnancy

Diabinese (chlorpropamide) is an oral hypoglycemic agent. Its mechanism of action is not completely understood but it is not an oral insulin.

Diabinese was discovered and synthesized by researchers of the Chas. Pfizer and Co., Inc. It was evaluated in a broad clinical program to obtain clinical and pharmacological data. According to Pfizer laboratories, these studies have shown it to be a potent,

active, oral hypoglycemic agent, valuable in the treatment of selected diabetic patients. It is generally used alone to control the mild to moderately severe adult, stable diabetic, and occasionally combined with lowered amounts of insulin to control adult diabetes with "brittle" characteristics.

Chlorpropamide is classified as an arylsulfonylurea. It is 1-(p-chlorobenzenesulfonyl)-3-propylurea. It has a molecular weight of 276.76 and an empirical formula of $C_{10}H_{18}O_8N_2SCl$. It is a white, odorless, crystalline powder soluble in alcohol, moderately soluble in chloroform, sparingly soluble in benzene and ether, and insoluble in water. While chlorpropamide is a sulfonamide derivative, it is devoid of antibacterial activity.

According to Chas. Pfizer and Co., Inc., acute and chronic studies demonstrate a very low order of toxicity in mice, rats, and dogs. Doses more than 20 times larger (in proportion to body weight) than are recommended therapeutically were well tolerated for periods of up to 20 months.

It is rapidly absorbed from the gastro-intestinal tract and exerts a hypoglycemic effect within 1 hour; this effect becomes maximal in 3 to 6 hours and persists for at least 24 hours (Pfizer Laboratory Division, 1963).

Chlorpropamide, as well as other hypoglycemic sulfonamide derivatives, acts almost exclusively in the mature-onset type of diabetes, in which there is available, but non-useful, insulin emanating from the pancreatic islets. The mechanisms whereby chlorpropamide act have not been established with certainty (Pfizer Laboratory Division, 1963).

There is considerable evidence that such compounds act primarily on the β-cells to promote the production of endogenous insulin; thus their effectiveness depends upon the number of β-cells remaining in the islets or the number that can be regenerated (West et al., 1966).

Since the effectiveness of exogenous insulin is potentiated by the sulfonamides, there is the possibility that they prevent insulin degradation by inhibiting the insulinase action of the liver under certain conditions; sulfonylurea-like drugs may suppress the production of glucose by the liver. Also destruction of the α-cells, with a decrease in glucagon production, has been suggested as a possible mechanism of action of chlorpropamide. Thus, it appears that no single action can or does explain the antidiabetic effects of chlorpropamide in man and animals (Turner, 1966).

The use of chlorpropamide administered during pregnancy has been investigated. Battaglia and Meacham (1969), in a study involving New Zealand white rabbits, determined the effects of chlorpropamide upon prenatal survival. They found that feeding this hypoglycemic agent for a period of 5 days immediately after mating resulted in significantly ($P<.01$) smaller postimplantation losses when compared to the control does. Although the mechanism of action remains unknown, it was suggested that chlorpropamide, through its insulin-like action, made more nutrients available to the fetal circulation.

The Action of Provest

Provest, a compound manufactured by the Upjohn Company, is presently being sold on the market as an oral contraceptive agent for

birth control in women. Each tablet of this compound contains 10 mg. of medroxyprogesterone acetate and 0.05 mg. of ethinyl estradiol. The chemical name is 17-Hydroxy-6α -methylpregn-4-ene-3-, 20-dione 17 Acetate, 19-Nor-17α-pregna-1, 3, 5 (10)-trien-20 yne-3, 17-diol.

According to the Upjohn Company, Provest is virtually 100% effective in preventing conception when taken from the fifth through the 24th day of the cycle. The contraceptive action of Provest is thought to be due to the inhibition of ovulation resulting from gonadotrophin suppression. It is also possible that mechanisms other than the inhibition of ovulation are active. These mechanisms include the production of a resting endometrium which is unfavorable for implantation similar to that seen during pregnancy. Also the possibility exists that there is a production of a viscid cervical mucus which may contribute to the prevention of conception.

Provest induces both an estrogenic and a progestational uterine response in rats and dogs and causes minimal increase in the male accessory gland weights of rats. Gonadotrophin-inhibiting activity is reflected by suppression of testicular, ventral prostrate, and seminal vesicle weights in the immature rat; and suppression of estrous cycles in the mature rat. In rats, Provest, like progesterone, has glucocorticoid activity (Upjohn Company, 1968).

OBJECTIVES

1. To determine the effects, if any, of a compound containing a specific ratio of progesterone to estrogen on prenatal mortality in the rabbit.
2. To determine the dosage of this progesterone:estrogen compound which will have the most effect on prenatal mortality.
3. To determine the effects, if any, of an interaction between a hypoglycemic agent and various dosages of the progesterone: estrogen compound on prenatal mortality.

MATERIALS AND METHODS

Animals

Thirty female and six male New Zealand rabbits were used to initiate this study. All rabbits were mature, weighing between 3.18 and 4.54 kg. All rabbits were bought from local dealers whose management practices involved the use of various degrees of inbreeding. After receiving these rabbits, all females were isolated for 16 days in case of pseudopregnancy.

Housing

All animals were kept in individual wire cages measuring 30" x 18" x 24" (L x W x H). The floors of the cages were of heavy gage mesh, measuring 3/8" x 1". The wire floor greatly facilitated cleaning and aided in minimization of disease.

The animals were housed in a large animal room 30' x 20' x 20'. This room was not air conditioned, and although it remained cooler than the outside temperature, temperatures inside during the summer were somewhat higher than what could be considered comfortable.

Feeding

Each animal in this study was fed approximately at the same time each day and received about 4 oz. of commercial rabbit pellets. This feed was a Wayne Rabbit Ration containing: 16.7% protein, 4.1% fat, 12.5% crude fiber, 8.3% ash, 7.7% moisture, 50.7% nitrogen-free extract, and 89.2% TDN.

This level of nutrition furnishes approximately .22 lb. of TDN and .042 lb. of total protein daily. These requirements are in

accordance with the requirements for pregnant does as listed by the National Research Council (1966).

One week prior to the feeding of chlorpropamide, the females were conditioned to consume the ration in ground form. It was necessary to feed a ground ration due to the fact that the chlorpropamide is water-insoluble and had to be mixed in the feed.

The chlorpropamide feed was prepared in 50 lb. batches. To exactly 2 lb. of the ground ration, 1.14 gm. of chlorpropamide were added for premixing. This premix then was added to an additional 48 lb. of the ground ration.

The groups received chlorpropamide at the rate of 1.5 mg. per ounce of feed and were on the ration for an average of 14 days. The treatments began on day 2 after breeding and were discontinued on the 15th day of gestation.

The source of progesterone and estrogen was Provest; it contains medroxyprogesterone acetate and ethinyl estradiol.

From the dosage prescribed for the use of Provest, it was determined that 127.6 mg. per day was recommended for a 60 kg.individual. Guyton (1967) indicates that a 60 kg. female has approximately 5400 ml. of blood. According to Asdell (1964), the rabbit has 6.2 ml. of blood per 100 gm. of body weight. From this an equivalent daily dosage of Provest was calculated for the rabbit. Two such calculations were made throughout the experiment to adjust for any change in body weight.

From these calculations an initial dosage of 5.4 mg. per day was thought to be at or near the physiological level of progesterone:

estrogen in the domestic rabbit. This 5.4 mg. per day is termed the low level Provest, and this dosage squared, or 29.16 mg. per day, was used as the high level Provest treatment.

After the dosage per day had been determined, the pills were ground into a fine powder and the calculated amounts were put into definite volumes of ion-free water. Water was used as a carrying medium because it would not affect the steroids present and gave more uniform suspension in comparison with previously tried oil. This suspension was injected into gelatin capsules which were administered to each rabbit orally, using an apparatus similar to a bolus gun.

The capsules were fed starting 2 to 3 days after breeding and continued until the 15th day when laparotomies were performed. The schedule for capsule administration followed the regular feeding routine.

Each individual cage had its own watering bottles and fresh water was provided daily.

Breeding

All females were double and usually triple mated using three different bucks. This random triple breeding was arbitrarily employed to guard against any failure of semen quality. The females were always taken to the buck's cage and the pairs were watched closely during the breeding process to insure that mating occurred. If intromission was not allowed by the doe, she was force-bred; this was accomplished by holding her and allowing the buck to mount. All females were immediately given an intravenous injection of 50 I. U.

of Chorionic Gonadotrophin[1] to insure ovulation. These injections should have no effect on ovulation rates. Stapes and Bialy (1967) have demonstrated that the average number of ovulation points per doe did not differ significantly among rabbits ovulated by LH, HCG, or natural matings.

If the female became pregnant, she was carried through gestation on her appropriate feeding and hormonal regime and rebred no earlier than 7 days post partum for her next treatment regime.

Determination of Preimplantation and Postimplantation Losses

Pregnancy was determined in each doe at 9 days post coitum by abdominal palpation and if she was found to be pregnant, laparotomy was scheduled 5 to 6 days later. The laparotomy was performed 14 to 15 days after breeding, just after implantation is known to occur in the domestic rabbit. The laparotomy was performed while the rabbit was under ether anaesthesia. After appropriate aseptic preparation of the abdomen, a mid-ventral incision was made approximately four inches below the xiphoid process and extending caudally for approximately two to three inches. Through this opening the uterine horns up to the ovaries were exposed. Counts were then made of the number of corpora lutea with ovulatory points and the number of normal developing embryos; any resorptions and abnormalities were noted and recorded.

The uterine horns and ovaries were then replaced, the incision sutured and treated with scarlet oil, and the rabbits allowed to recover.

The anaesthesia and laparotomies, for the most part, had little effect on the gestating does; but those animals which did not return

---

[1] Haver-Lockhart Laboratories, Kansas City, Missouri.

to feed within 1 to 2 days or showed uterine or abdominal infections were immediately removed from the study.

At 14 to 15 days the corpora lutea and developing embryos were quite obvious, the uterine swellings being about 12 to 15 mm. across.

Preimplantation losses were determined as a result of comparison of the number of corpora lutea and uterine swellings at 15 days post coitum. Postimplantation losses represent the comparison of developing fetuses at 15 days with either the number born or the number observed at necropsy at 25 to 27 days of gestation. Those females with unusually large numbers of embryos at 15 days were sacrificed at 25 days to insure accurate counts of the young.

All does were divided into one control and five treated groups. The treated does were divided into chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest groups.

Methods of Computation

Prenatal mortality was divided into preimplantation and postimplantation losses; in addition, total losses were calculated according to the method of Brambell (1948).

The mean percentage data were transformed to their arc-sin equivalents for statistical analysis. The arc-sin transformations were made of all percentage data to bring the figures toward the boundaries of the normal distribution curve. The procedure of analysis of variance for unequal sample sizes was used (Li, 1966).

Significant differences among the means were located using Duncan's New Multiple Range test with the modification method for unequal sample size as developed by Kramer (1956).

RESULTS

Ovulation Rates

Individual ovulation rates for the does in each of the six experimental groups are shown in tables I, II, III, IV, V, and VI. The mean ovulation rates for each of these groups are summarized in table VII.

The mean ovulation rates of 11.22, 11.33, 10.88, 10.85, 9.25, and 10.25 for the control, chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest groups, respectively, are not significantly different. This would be expected owing to the random assignment of all experimental animals to the control and treated groups.

Prenatal Losses

Prenatal mortality data are presented in tables I, II, III, IV, V, and VI for individual litters in each of the six experimental groups. Table VII summarizes the mean mortality figures for all six groups. In addition, mean figures for each criteria of the various treatments are tabulated in table VIII along with their respective standard errors; all percentage figures are transformed to their respective arc-sin equivalents. The results of the analysis of variance are found in table IX. Table X shows the mean reproductive performance of all groups and indicates which are significantly different from each other.

Preimplantation Losses. The mean preimplantation losses in the present experiment of 22.90, 14.46, 7.50, 10.13, 22.50, and 49.80% for the control, chlorpropamide, low level Provest + chlorpropamide, low

Table I. Reproductive Performance of the Control Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
21	9	7	-- [f]	22.22	-- [f]	-- [f]
31	20	18	8	10.00	55.55	60.00
34	14	12	3	14.28	75.00	78.57
15	8	7	5	12.50	28.57	37.50
16	6	3	0	66.66	100.00	100.00
18	10	8	6	20.00	25.00	40.00
22	13	12	10	7.69	16.66	23.07
24	12	11	-- [f]	8.33	-- [f]	-- [f]
29	9	5	4	44.44	20.00	55.55
Average	11.22	9.22	5.14	22.90	45.82	56.38

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.
[f] Animals removed from study due to infection or sickness after laparotomy.

Table II. Reproductive Performance of the Chlorpropamide Treated Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
28	9	8	7	11.11	12.50	22.22
32	10	10	8	0.00	20.00	20.00
36	10	9	7	10.00	22.22	30.00
17	12	10	8	16.66	20.00	33.33
7	10	8	--[f]	20.00	-- [f]	-- [f]
35	14	11	8	21.42	27.27	42.85
5	13	8	6	38.46	25.00	53.84
20	8	7	--[f]	12.50	-- [f]	-- [f]
23	16	16	--[f]	0.00	-- [f]	-- [f]
Average	11.33	9.67	7.33	14.46	21.16	33.70

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.
[f] Animals removed from study due to infection or sickness after laparotomy.

Table III. Reproductive Performance of the Low Level Provest + Chlorpropamide Treated Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
30	7	5	4	28.57	20.00	42.85
27	11	10	7	9.09	30.00	36.36
23	11	12	7	0.00	41.66	36.36
14	9	7	5	22.22	28.57	44.44
25	14	15	8	0.00	46.66	42.85
26	10	11	9	0.00	18.18	10.00
32	13	12	8	7.69	33.33	38.46
34	10	12	9	0.00	25.00	10.00
4	13	13	9	0.00	30.76	30.76
Average	10.88	10.78	7.33	7.50	27.46	32.45

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.

Table IV. Reproductive Performance of Low Level Provest Treated Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
25	12	12	11	0.00	8.33	8.33
26	12	7	6	41.66	14.28	50.00
15	8	8	6	0.00	25.00	25.00
19	9	8	6	11.11	25.00	33.33
31	10	10	0	0.00	100.00	100.00
27	14	14	11	0.00	21.42	21.42
28	11	9	6	18.18	33.33	45.45
Average	10.85	9.71	6.57	10.13	32.48	40.50

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.

Table V. Reproductive Performance of the High Level Provest + Chlorpropamide Treated Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
19	8	5	0	37.50	100.00	100.00
24	10	6	3	40.00	50.00	70.00
29	8	7	5	12.50	28.57	37.50
35	11	11	5	0.00	54.54	54.54
Average	9.25	7.25	3.25	22.50	58.27	65.51

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.

Table VI. Reproductive Performance of the High Level Provest Treated Does

Doe number	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
20	10	5	0	50.00	100.00	100.00
33	10	7	5	30.00	28.57	50.00
14	9	2	0	77.77	100.00	100.00
18	12	7	5	41.66	28.57	53.33
Average	10.25	5.25	2.50	49.80	64.28	77.08

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.

Table VII. Mean Reproductive Performance of the Control and Treated Groups

Treatments	Total number corpora lutea [a]	Total number embryos [b]	Number born	% Preimplantation loss [c]	% Postimplantation loss [d]	% Total loss [e]
Control	11.22	9.22	5.14	22.90	45.82	56.38
Chlorpropamide	11.33	9.67	7.33	14.46	21.16	33.70
Low level Provest + chlorpropamide	10.88	10.78	7.33	7.50	27.46	32.45
Low level Provest	10.85	9.71	6.57	10.13	32.48	40.50
High level Provest + chlorpropamide	9.25	7.25	3.25	22.50	58.27	65.51
High level Provest	10.25	5.25	2.50	49.80	64.28	77.08

[a] Number of C. L. on both ovaries counted during laparotomy at 15 days.
[b] Number of uterine swellings in both uterine horns counted during laparotomy at 15 days.
[c] (Number of uterine swellings ÷ number of C. L.) x 100 counted during laparotomy at 15 days.
[d] (Number born ÷ number of uterine swellings counted during laparotomy at 15 days) x 100.
[e] (Number born ÷ number of C. L. counted during laparotomy at 15 days) x 100.

Table VIII. Means and Standard Errors of Reproductive Performance for all Control and Treated Does

Treatment group	Number C. L.	Number born	% Preimplantation loss [a,d]	% Postimplantation loss [b,d]	% Total loss [c,d]
Control	11.22 ± .91	5.14 ± 1.01	27.29 ± 3.82	44.45 ± 7.62	51.01 ± 6.84
Chlorpropamide	11.33 ± .91	7.33 ± 1.09	21.28 ± 3.82	27.24 ± 8.23	35.21 ± 7.39
Low level Provest + chlorpropamide	10.88 ± .91	7.33 ± .89	15.20 ± 3.82	33.28 ± 6.72	34.07 ± 6.03
Low level Provest	10.85 ± 1.03	6.57 ± 1.01	17.02 ± 4.32	35.97 ± 7.62	41.00 ± 6.84
High level Provest + chlorpropamide	9.25 ± 1.32	3.25 ± 1.33	26.61 ± 5.47	53.73 ± 10.08	58.04 ± 9.05
High level Provest	10.25 ± 1.32	2.50 ± 1.33	45.07 ± 5.47	61.16 ± 10.08	68.70 ± 9.05

[a] (Mean number of uterine swellings ÷ mean number of C. L.) x 100; counted at 15 days.
[b] (Mean number born ÷ mean number of uterine swellings counted at 15 days) x 100.
[c] (Mean number born ÷ mean number of C. L. counted at 15 days) x 100.
[d] Arc-sin transformation and its respective standard error.

Table IX. Analysis of Variance for Prenatal Losses, Number Born and Ovulation Rates

Source	SS	d.f.	Ms	F
Preimplantation losses				
Treatment	2,981.37	5	596.27	4.53 **
Error	4,738.22	36	131.62	
Postimplantation losses				
Treatment	4,178.92	5	835.78	2.06
Error	12,597.08	31	406.36	
Total prenatal losses				
Treatment	4,941.73	5	988.35	3.02 *
Error	10,162.82	31	327.83	
Number born				
Treatment	112.03	5	22.41	3.16 *
Error	219.65	31	7.09	
Ovulation rate				
Treatment	15.02	5	3.00	.42
Error	266.81	36	7.41	

* Significantly different ($P<.05$).
** Significantly different ($P<.01$).

Table X. Mean Reproductive Performance Differences Among the Control and Treated Groups

Treatments	Number C. L.	Number born	% Preimplantation loss [a,d]	% Postimplantation loss [b,d]	% Total loss [c,d]
Control	11.22 [e]	5.14 [e,f,g]	27.29 [e,g]	44.45 [e]	51.01 [e,f,g]
Chlorpropamide	11.33 [e]	7.33 [e]	21.28 [f,g]	27.24 [e]	35.21 [f,g]
Low level Provest + chlorpropamide	10.88 [e]	7.33 [e]	15.20 [f]	33.28 [e]	34.07 [f,g]
Low level Provest	10.85 [e]	6.57 [e,f]	17.02 [f,g]	35.97 [e]	41.00 [f,g]
High level Provest + chlorpropamide	9.25 [e]	3.25 [f,g]	26.61 [e,f,g]	53.73 [e]	58.04 [e,f]
High level Provest	10.25 [e]	2.50 [g]	45.07 [e]	61.16 [e]	68.70 [e]

[a] (Mean number of uterine swellings ÷ mean number of C. L.) x 100; counted at 15 days.
[b] (Mean number born ÷ mean number of uterine swellings counted at 15 days) x 100.
[c] (Mean number born ÷ mean number of C. L. counted at 15 days) x 100.
[d] Arc-sin transformed data.
[e,f,g] All data with the same subscripts are not significantly different ($P<.05$).

level Provest, high level Provest + chlorpropamide, and high level Provest, respectively, are shown in tables I, II, III, IV, V, and VI for the individual litters. Mean preimplantation losses are summarized for all six groups in table VII. Statistical analysis (table X) indicated that preimplantation loss in the low level Provest + chlorpropamide group was significantly lower ($P<.05$) than that in both the control and the high level Provest groups. The loss in the low level Provest group is significantly lower ($P<.05$) than the loss in the high level Provest group and tended to be lower than the control.

Postimplantation Losses. Postimplantation losses are tabulated in tables I, II, III, IV, V, and VI. Table VII presents the mean postimplantation data for each of the six groups. The mean losses were 45.82, 21.16, 27.46, 32.48, 58.27, and 64.28 for the control, chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest groups, respectively. The differences among the means were not statistically significant.

Total Prenatal Losses. Mean total losses amounted to 56.38, 33.70, 32.45, 40.50, 65.51, and 77.08% for the control, chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest, respectively (table VII). Total losses for individual litters are presented in tables I, II, III, IV, V, and VI. Table X indicates that there was no significant difference between the control and treated groups; there was, however, a significant difference ($P<.05$) between the low level Provest + chlorpropamide and the high level Provest. There was also a significant

difference between the high and low level Provest groups. The difference between the high level Provest and the chlorpropamide group was also significant ($P<.05$).

Number Born

The mean litter size was 5.14, 7.33, 7.33, 6.57, 3.25, and 2.50 rabbits for the control, chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest groups, respectively (table VII). Individual litter data are presented in tables I, II, III, IV, V, and VI. Table X contains the mean number born of all six groups and locates differences among the experimental groups.

There was a significant difference ($P<.05$) between the high and low levels of Provest, both with and without chlorpropamide, but no difference between any of the treated groups and the control. The high level of Provest and the high level of Provest + chlorpropamide are also significantly different ($P<.05$) from the chlorpropamide.

DISCUSSION

Ovulation Rate

The mean ovulation rates in this study, ranging from 9.25 to 11.33, are comparable to those found by Adams (1960b) and Hafez and Pincus (1956) of 11.4 and 10.31, respectively.

As mentioned in the Materials and Methods, warm temperatures were experienced throughout the late summer months. A fan was in use, but had a limited capacity in cooling the room to the more comfortable temperatures. During this period the laparotomized does were not pregnant and had ovaries lacking mature follicles, indicating a state of anestrus. Seasonal variation and its effects on reproduction in the rabbit has been observed by other researchers. Sittmann et al. (1964) indicated very low ovulation rates and a general anestrus in many rabbits during September, especially during periods of high temperature.

Following these warm months, cooler temperatures prevailed and normal breeding patterns were observed. Since many rabbits still had to be force-bred, it was decided that all rabbits should receive injections of chorionic gonadotrophin immediately after copulation to insure ovulation.

Preimplantation Losses

The mean preimplantation losses for the controls are somewhat higher than has been reported elsewhere. Preimplantation losses usually amount to 10%+ (Hafez, 1968) in New Zealand rabbits. Adams (1960a) reported that in does in which implantation occurred, the loss amounted to 9.7% of the ova being lost before implantation. However, Adams also observed that approximately 5% of the untreated does suffered a total

loss of their embryos before implantation which he does not include in the 9.7% figure. As mentioned in the Materials and Methods, the strains of rabbits used in the present experiment are the result of inbreeding. Although little work has been done with strain differences in rabbits, researchers have found wide variation in different strains of mice. Fekete (1947) found differences in two strains of mice concerning the degree of prenatal mortality. In addition, Braden (1958) has indicated variation between strains of mice in respect to other phenomena of reproduction such as: time of ovulation; ovulation rate; the incidence of abnormalities of egg maturation and fertilization; and the length of the gestation period. It is possible, then, that the somewhat higher incidence of preimplantation losses in the present study could be partially attributed to the inbred strain of rabbits used.

Groups receiving low levels of Provest + chlorpropamide were found to have a significantly lower ($P<.05$) preimplantation loss than the control animals. The effects of progesterone and estrogen in combination with chlorpropamide suggests an augmentation effect.

The possibility that chlorpropamide could augment the progesterone treated rabbits is demonstrated by work done by Rennie et al. (1965). Their work indicates that the luteal cells of the corpora lutea increase by some 34,000 cells from 24 hours to 192 hours after ovulation. At the same time, the glycogen content within this tissue increases from a low of 1.39 mg./gm. at 24 hours to a high of 2.63 mg./gm. at 120 hours. Chang (1951) indicates that it is not the total dose, but the initial high dose of progesterone that contributes most to the maintenance of pregnancy in non-ovulated intact rabbits. This initial

increased progesterone requirement, then, could be satisfied by the increased glycogen content of the highly proliferating luteal cells due to the action of chlorpropamide.

Although little information is available on the subject, the possibility exists that chlorpropamide may stimulate increased glycogen storage in the endometrium and be used in the histotrophe (uterine milk) for nourishment of the embryos prior to implantation. Amoroso (1952) has indicated a histotrophe made of histolytic materials, or materials derived from the breaking down of endometrial tissue. If the action of chlorpropamide is to increase glycogen stores in the endometrium, histolytic secretions would be rich in glycogen. This glycogen-rich histotrophe, then, could provide the blastocyst with additional energy needed for survival and implantation and thus aid in lowering preimplantation losses.

Postimplantation Losses

The control group suffered a postimplantation loss of 45.82%. This is in accord with other researchers. Battaglia and Meacham (1969) reported a mean postimplantation loss of 41.8% for the control group.

The chlorpropamide group tended to have the lowest postimplantation loss. The data in table VII indicate a lowering effect of death loss when chlorpropamide is administered in combination with Provest; however, this effect is not significant and can be regarded only as a trend.

Contrary to these results, Battaglia and Meacham (1969) found that feeding chlorpropamide considerably lowered postimplantation losses in New Zealand white rabbits. The mechanism of action of this insulin-

like compound, however, remains unknown. Turner (1966) suggests that insulin may act directly upon intracellular enzyme systems, may control cell membrane permeability, may act through an energy transfer with vasopressin, or it may act directly on the gene loci to increase the formation of m-RNA in muscle, which in turn may promote the production of enzymes and structural proteins.

Regardless of the mechanism of action, it seems likely that the chlorpropamide may increase the permeability of the maternal placenta in order that greater amounts of glycogen, lipids, and other metabolites could be built up in this tissue. Thus, the fetal circulation would have more nutrients made available to it.

Total Prenatal Losses

The total prenatal loss of 56.38% was observed for the control does. Brambell (1948) reported a total loss of 43.3% in the wild rabbit and Hafez (1968) indicates 30%+ for domestic rabbits in general. Battaglia and Meacham (1969) reported a total loss of 44.8% for New Zealand white does.

Examination of the total prenatal losses in the present study indicates no difference between any of the treated groups and the control group. There was, however, a difference between the high level Provest group and the other treatment groups. The high level Provest group suffered a very high loss of 77.08%. This group was receiving 29.16 mg. of progesterone:estrogen daily; a level greater than 5 times that calculated to be near the physiological level.

According to earlier studies, 0.5 to 2 mg. is required for the maintenance of pregnancy in the rabbit (Corner and Allen, 1929; Pincus

and Werthessen, 1938). Other investigators, however, have determined that while in the early stages of pregnancy about 1 mg. per day is sufficient, in later stages up to 5 mg. of progesterone daily is necessary (Courier and Kehl, 1938). Chang (1951) indicated that a total dose of 50 to 75 mg. is sufficient to maintain pregnancy throughout the 32-day period in the intact non-ovulated pregnant rabbit. In the present study, approximately 450 mg. of a progesterone:estrogen mixture were given over a 15-day period; a total dose approximately 6 to 9 times that observed by Chang to maintain pregnancy.

It is likely that such large doses of the progesterone:estrogen mixture might block oviduct rhythmic contractions necessary for retaining the ova in the oviduct. This would allow the oviduct fluids to transverse the oviduct at a faster rate and wash some of the ova into the uterus with it. Csapo (1956) reported results of experiments showing the action of estrogen and progesterone on the uterine myometrium. From these observations, he proposed the concept of "progesterone block". Csapo and Takeda (1965) studied "progesterone block" on single muscle fibers from rabbit uteri and suggested that progesterone suppresses electrical activity of the muscle fibers and restricts it to the point of origin.

Rowson (1951), working with cows, found that out of 357 ova shed in the presence of a large corpus luteum, or after progesterone injections prior to breeding, not a single ovum was found which was fertilized, while in most cases the ova had descended into the uterus prematurely. He went on to add further proof to this increased rate of flow theory by administering estrogen. He found that estrogen inhibited the effects

of progesterone by restoring the oviduct contractions and permitted the fertilized ova to remain in the oviduct the proper length of time.

In the present study, the high levels of Provest were administered 48 to 72 hours post coitum. Since in the rabbit the fertilized ova must remain in the oviduct for 72 hours, it is entirely conceivable that the high progesterone:estrogen levels inhibited oviduct contractions, thus increasing the flow rate and permitting many ova to enter the uterus too soon. Chang (1951) has shown that in order to obtain successful transplantation of the ovum in the rabbit, the timing of its arrival in the uterus is very important and if there is much variation from the normal, then the percentage of successful implantations decreases.

Hafez (1962) indicates that if the stage of development of the embryo and endometrium is slightly asynchronous, implantation may take place, but if asynchronization is greater (24 to 48 hours), implantation is severely inhibited and usually fails to occur altogether.

In the present experiment the ratio of progesterone to estrogen was 200:1 (10 mg./.05 mg.). It is believed that at low doses, estrogen acts synergistically with progesterone. Reddy et al. (1958) indicated that progesterone maintains a physiological balance of estrogens during pregnancy by promoting their excretion through the kidneys when present in excessive amounts. As gestation advances, the amount of estrogen increases, reaching a maximum at term. In this way the balance between the estrogens and progesterone is controlled. This is also supported by Lerner et al. (1962) who found that estrogen enhances the pregnancy maintaining activity of progesterone in the rat when progesterone and estrogen are present in ratios between 5,000 and 10,000 to 1.

Courier (1950) indicates that an estrogen:progesterone ratio may be synergistic at low doses, but antagonistic at higher doses because part of the progesterone may not affect the tissue and, therefore the actual ratio would be considerably narrowed. Thus, the Provest group, by receiving the high dosage, was actually receiving a narrower ratio of progesterone:estrogen or a greater level of estrogen. The effects of high levels of estrogen are detrimental to both implantation and pregnancy (Lerner et al., 1962).

Number Born

In the present study, the mean number born for the control does was 5.14. This is in accord with Battaglia and Meacham (1969) who observed a mean litter size of 4.7 for New Zealand white rabbits. Adams (1960a) reported a mean number of living fetuses at 28 days of 7.5 with a range of 1 to 12 for New Zealand does.

Since the number born is a function of all losses occurring between fertilization and parturition, it is an important consideration, especially from a practical point of view. While the data show a trend toward increased number born in certain of the treated groups, statistical analysis indicates that there is no significant difference between treated and control animals. Does with the greatest number born are from the chlorpropamide and low level Provest + chlorpropamide groups.

SUMMARY

Forty-two New Zealand rabbit pregnancies were used to determine the effects of Provest, a compound containing 10 mg. of progesterone and .05 mg. of estrogen; chlorpropamide, a hypoglycemic agent; and any interaction between these compounds on prenatal mortality. The effect of the compounds were measured on two stages of pregnancy, preimplantation and postimplantation periods; in addition, ovulation rates and litter size were considered.

The treated groups received chlorpropamide, low level Provest + chlorpropamide, low level Provest, high level Provest + chlorpropamide, and high level Provest.

Statistical analysis indicates a significant difference ($P<.05$) between the control and the low level Provest + chlorpropamide groups concerning preimplantation losses. The effect of the combination of Provest and chlorpropamide are thought to be due to maintenance of a pregnant endometrium, supplementation of the histotrophe, and increased availability of glucose to fetal tissues. There was no differences between the treated and control does concerning postimplantation and total losses, although chlorpropamide does showed a tendency toward lower losses.

It was found that high levels of Provest were detrimental in both preimplantation and postimplantation periods; the reasons likely due to very high levels or an imbalance between progesterone and estrogen.

There was no statistical difference between any of the treated and the control does concerning the number of fetuses at birth, although the low level Provest and low level Provest + chlorpropamide

treated does had the largest mean litters. The high level Provest group had a significantly lower ($P<.05$) number of fetuses at birth in comparison with the chlorpropamide, low level Provest, and low level Provest + chlorpropamide groups.

LITERATURE CITED

Adams, C. E. 1960a. Prenatal mortality in the rabbit, Oryctolagus cuniculus. J. Reprod. Fert. 1:36.

Adams, C. E. 1960b. Studies on prenatal mortality in the rabbit, Oryctolagus cuniculus. The amount and distribution of loss before and after implantation. J. Endocr. 19:325.

Allen, P., F. W. R. Brambell and I. H. Mills. 1946. Studies on sterility and prenatal mortality in wild rabbits. I. The reliability of estimates of prenatal mortality based on counts of corpora lutea, implantation sites and embryos. J. Exp. Biol. 23:312.

Allen, W. M. and G. P. Heckel. 1937. Maintenance of pregnancy by progesterone in castrated rabbits on the 11th day. Am. J. of Physiol. 125:31.

Amoroso, E. C. 1952. Placentation. In: Marshall's Physiology of Reproduction, Vol. II (Third ed.) (Ed. A. S. Parkes). Longmans, Green and Co., London, New York and Toronto pp. 127-311.

Asdell, S. A. 1964. Patterns of Mammalian Reproduction. (Second ed.) Cornell University Press, Ithaca, New York.

Battaglia, R. A. and T. N. Meacham. 1969. Effect of chlorpropamide upon prenatal losses in rabbits. J. Animal Sci. 28:1. (Abstr.)

Bishop, M. W. H. 1964. Paternal contribution to embryonic death. J. Reprod. Fert. 7:383.

Boving, B. G. 1959. Endocrine influence on implantation. In: Recent Progress in the Physiology of Reproduction. (Ed. C. W. Lloyd) Academic Press, Inc., New York and London pp. 205-225.

Boyd, J. D. and W. J. Hamilton. 1952. Cleavage, early development and implantation of the egg. In: Marshall's Physiology of Reproduction. (Ed. A. S. Parkes) Longmans, Green and Co., Ltd., London 2:1.

Braden, A. W. H. 1958. Strain differences in the incidence of polyspermia in rats after delayed mating. Fert. and Ster. 3:243.

Brambell, F. W. R. 1948. Prenatal mortality in mammals. Cambridge Philosophical Soc., Biol. Rev. 23:370.

Bullough, W. S. 1961. Vertebrae Reproductive Cycles. Methuen, London.

Chang, M. C. 1951. Maintenance of pregnancy in intact rabbits in the absence of corpora lutea. Endocr. 48:17.

Clegg, M. G. and W. F. Ganong. 1959. Environmental factors other than nutrition affecting reproduction. Reprod. in Domestic Animals, Vol. II. (Ed. H. H. Cole and P. T. Cupps) Academic Press, New York.

Corner, G. and W. M. Allen. 1929. Effects of corpora lutea extracts on maintenance of pregnancy in castrated rabbits. Am. J. of Physiol. 88:326.

Courier, R. 1950. Interactions between estrogens and progesterone. Vitamins and Hormones 8:179.

Courier, R. and R. Kehl. 1938. The effects of exogenous progesterone and estrogen on implantation in ovariectomized rabbits. C. R. Soc. de Biol. 127:529.

Csapo, A. I. 1956. The mechanism of effect of the ovarian steroids. Recent Progr. Hormones Res. 12:405.

Csapo, A. I. and H. Takeda. 1965. Effect of progesterone on the electric activity and intrauterine pressure of pregnant and parturient rabbits. Am. J. Obst. and Gyn. 91:221.

Day, B. N., L. L. Anderson, M. A. Emmerson, L. N. Hazel and R. M. Melampy. 1959. Effects of estrogen and progesterone on early embryonic mortality in ovariectomized gilts. J. Animal Sci. 17:879.

Day, B. N., F. E. Romach and J. F. Lasley. 1963. Influence of progesterone and estrogen on early embryonic mortality in swine. J. Animal Sci. 22:637.

Dutt, R. H. 1960. Temperature and light as factors in reproduction among farm animals. J. Dairy Sci. 43. (Suppl. no. 123.)

Eckstein, P., T. McKeown and R. G. Record. 1955. Variation in placenta weight according to litter size in the guinea pig. J. Endocr. 12:108.

Edgar, D. G. 1962. Studies on infertility in ewes. J. Reprod. Fert. 3:50.

Enders, A. C. and S. Schalfke. 1967. A morphological analysis of the early implantation stages in the rat. Am. J. Anat. 120:185.

Erb, R. E. and E. W. Holts. 1958. Factors associated with estimated fertilization and service efficiency of cows. J. Dairy Sci. 41:1541.

Fekete, E. 1947. Differences in the effect of uterine environment upon development in DBA and C57 Black strains of mice. Anat. Rec. 98:409.

Finn, C. A. and P. M. Keen. 1962. Failure of histamine to induce deciduomata in the rat. Nature (London) 194:602.

Gregory, P. 1930. The early embryology of the rabbit. Carnegie Institute of Washington, Contributions to Embryology 125:141.

Greenstein, J. S. and R. C. Foley. 1958. Early embryology of the cow. I. Gastrula and primative streak stages. J. Dairy Sci. 41:409.

Grosser, O. 1909. Eihaute und de placenta. From: Placentation. (E. C. Amoroso) In: Marshall's Physiology of Reproduction. Vol. II. (Third ed.) (Ed. A. S. Parkes) Longmans, Green and Co., London, New York and Toronto pp. 127-311.

Guyton, A. C. 1967. Textbook of Medical Physiology. (Third ed.) W. B. Saunders Co., Philadelphia, London and Toronto.

Hafez, E. S. E. 1962. Reproduction in Farm Animals. Lea and Febiger, Philadelphia.

Hafez, E. S. E. 1964. Effects of overcrowding in utero on implantation and fetal development in the rabbit. J. Exp. Zoo. 156:269.

Hafez, E. S. E. 1968. Reproduction in Farm Animals. (Second ed.) Lea and Febiger, Philadelphia.

Hafez, E. S. E. and G. Pincus. 1956. Hormonal requirements of implantation in the rabbit. Proc. Soc. Exp. Biol. Med. 91:531.

Hafez, E. S. E. and Y. Tsutsumi. 1966. Changes in endometrial vascularity during implantation and pregnancy in the rabbit. Am. J. Anat. 118:249.

Hagerman, D. D. 1964. Enzymatic capabilities of the placenta. Fed. Proc. 23:785.

Haines, C. E., A. C. Warnick and H. D. Wallace. 1959. The effects of two levels of energy intake on reproductive phenomena in Duroc Jersey gilts. J. Animal Sci. 18:347.

Hammond, J. 1937. Pregnancy and nutrition of the embryo in the rabbit. School Sci. Rev. 72:348.

Hammond, J. and F. H. A. Marshall. 1925. Reproduction in the Rabbit. Oliver and Boyd, London.

Hanly, S. 1961. Prenatal mortality in farm animals. J. Reprod. Fert. 2:182.

Harper, M. J. K. 1966. Hormonal control of transport of eggs in cumulus through the ampulla of the rabbit oviduct. Endocr. 78:568.

Hawk, H. W., W. J. Tayler and L. E. Casida. 1955. Effect of sire and system of mating on estimated embryonic loss. J. Dairy Sci. 38:420.

Healy, M. J. R., A. McLaren and D. Michie. 1961. Fetal growth in the mouse. Proc. Roy. Soc. (London) 153:367.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 9:307.

Kihlstrom, J. E. 1958. The effect of artificial light upon the production of male sperm antigglutin in rabbits. Int. Congr. An. Reprod. Papers 3:17.

Lerner, L. J., D. M. Brennan, E. Yiacas, M. Dephillipo and A. Borman. 1962. Pregnancy maintenance in ovariectomized rats with 16, 17, dehydroxyprogesterone derivatives and other progesterones. J. Endocr. 70:283.

Li, Jerome C. R. 1966. Statistical Inference I. Edwards Brothers, Inc., Ann Arbor, Michigan.

Marshall, F. H. A. 1956. The breeding season. In: Marshall's Physiology of Reproduction. Vol. I. (Third ed.) (Ed. A. S. Parkes) Longmans, Green and Co., London, New York and Toronto pp. 1-42.

McLaren, A. and D. Michie. 1960. Control of prenatal growth in mammals. Nature (London) 187:363.

Myers, K. and W. E. Poole. 1962. Oestrous cycle in the rabbit, Oryctolagus cuniculus. Nature (London) 195:358.

Noyes, R. W. and Z. Dickman. 1960. Relationship of ovular age to endometrial development. J. Reprod. Fert. 1:186.

N. R. C. 1966. Nutrient requirements of domestic animals. No. 9. Nutrient requirements of rabbits. National Research Council, Washington, D. C.

Perry, J. S. 1956. Observations on reproduction in a pedigree herd of large white pigs. J. Agr. Sci. 47:332.

Pfizer Laboratories Division, Charles Pfizer and Co., Inc. 1963. Scientific Circ. No. 60-0853-00-3.

Phelps, D. 1946. Endometrial vascular reactions and the mechanism of nidation. Am. J. Anat. 79:167.

Pincus, G. 1930. Observations of the living eggs of the rabbit. Proc. Roy. Soc. (London) 107B:132.

Pincus, G. and N. T. Werthessen. 1938. Maintenance of pregnancy in castrate rabbits with exogenous progesterone. Am. J. Physiol. 124:484.

Reddy, V. B., D. T. Mayer and J. F. Lasley. 1958. Hormonal modification of the intra-uterine environment in swine and its effect on embryonic viability. Research Bul. 667. Univ. of Mo. Agr. Exp. Sta., Columbia, Missouri.

Rennie, P., G. R. Davenport and W. Welborn. 1965. Cellular changes, protein and glycogen content in developing rabbit corpora lutea. Anat. Rec. 153:289.

Ross, S., P. B. Sawin, V. H. Denenberg and M. X. Zarro. 1961. Maternal behavior in the rabbit: Yearly and seasonal variation in nest-building. Behavior 18:454.

Rowson, L. E. 1951. Methods of producing multiple ovulation in cattle. J. Endocr. 9:260.

Rubenstein, L. and T. R. Forbes. 1963. Maintenance of pregnancy with subcutaneous pellets of progesterone in ovariectomized mice. Proc. Soc. Exp. Biol. Med. 113:1043.

Runner, M. N. 1951. Differentiation of intrinsic and maternal factors governing intrauterine survival of mammalian young. J. Exp. Zoo. 116:1.

Salisbury, G. W. and N. L. Van Demark. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman and Company, San Francisco and London.

Sawyer, C. H. 1959. Seasonal variation in the incidence of spontaneous ovulation in rabbits following estrogen treatment. Endocr. 65:523.

Sittmann, D. B., W. C. Rollins, K. Sittmann and R. B. Casady. 1964. Seasonal variation in reproductive traits of New Zealand white rabbits. J. Reprod. Fert. 8:29.

Smithberg, M. and M. N. Runner. 1956. The induction and maintenance of pregnancy in prepuberal mice. J. Exp. Zoo. 133:441.

Stapes, R. E. and G. Bialy. 1967. Rabbit zygote transport and development following various methods of ovulation induction. Endocr. 81:400.

Turner, C. D. 1966. General Endocrinology. (Fourth ed.) W. B. Saunders Co., Philadelphia, London and Toronto.

Ulberg, L. C. 1958. The influence of high temperature on reproduction. J. Heredity 49:62.

Upjohn Company. 1968. Scientific Circ. No. 6214.

van Tienhoven, A. 1968. Reproductive Physiology of Vertebrates. W. B. Saunders Co., Philadelphia, London and Toronto.

West, E. S., W. R. Todd, H. S. Mason and J. T. Van Bruggen. 1966. Textbook of Biochemistry. (Fourth ed.) Macmillan, New York and London.

Willett, E. L. 1953. Egg transfer and superovulation in farm animals. Iowa State College J. Sci. 28:83.

Wordon, A. N. and J. S. Leaky. 1962. The behavior of rabbits. The Behavior of Domestic Animals. (Ed. E. S. E. Hafez) Williams and Wilkins, Baltimore.

VITA

The author of this thesis, Gary L. Brubaker, was born on October 29, 1945, in DuBois, Pennsylvania. He is the son of John and Jean Brubaker of Brockway, Pennsylvania.

The author has a brother, Robby, who is presently in the military service, and a sister, Peggy, who is a senior at Brockway Area High School.

The author graduated from Brockway Area High School in 1963 and entered Delaware Valley College, Doylestown, Pennsylvania. On July 2, 1966, he married the former Donna Mowrey of Reynoldsville, Pennsylvania. In June of 1967 he graduated from Delaware Valley College with a B. S. Degree in Animal Science.

In April 1967, the author received a three year National Defense Education Act Fellowship to pursue both an M. S. and Ph. D. at Virginia Polytechnic Institute, Blacksburg, Virginia.

The author and his wife presently have one child, Jill Suzanne, born March 15, 1969.

Gary L. Brubaker

THE EFFECT OF A PROGESTERONE:ESTROGEN COMPOUND AND A HYPOGLYCEMIC AGENT ON PRENATAL MORTALITY IN THE NEW ZEALAND RABBIT

by

Gary L. Brubaker

ABSTRACT

Forty-two New Zealand rabbit pregnancies were used to determine the effects of Provest, a compound with progesterone activity; Diabinese (chlorpropamide), a hypoglycemic agent; and any interaction between these compounds on the amount and distribution of prenatal mortality. One control and five treated groups were observed during laparotomies at 15 days post coitum and again at term for determination of preimplantation and postimplantation losses. The treated groups received chlorpropamide, low levels of Provest + chlorpropamide, low levels of Provest, high levels of Provest + chlorpropamide, and high levels of Provest. The low levels of Provest were given at a rate of 5.4 mg. per day and the high level groups received 29.16 mg. daily. Treatment was from 2 to 3 days post coitum until the 15th day of gestation. Chlorpropamide was fed from day 2 until day 15 of gestation. Statistical analysis indicated a significantly lower ($P<.05$) preimplantation loss in the low level Provest + chlorpropamide treated group as compared to the control does. The high levels of Provest, either with or without chlorpropamide, tended to show higher prenatal losses than the control does. It was felt that the low level Provest + chlorpropamide reduced preimplantation loss by maintaining the pregnant condition of the endometrium, supplementation of the histotrophe, and by increasing

the availability of glucose to the fetal tissues. The detrimental effects of high levels of Provest are thought to be due to very high progesterone activity or an imbalance between progesterone and estrogen.