THE EFFECT OF AN INSULIN-LIKE COMPOUND UPON THE AMOUNT AND DISTRIBUTION OF PRENATAL LOSS IN THE NEW ZEALAND WHITE RABBIT

by

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INTRODUCTION

In the livestock industry today there is no place for inefficiency. Emphasis is being placed on the size of animals, growth rates, feed conversion, etc., and with great success. Never before has the producer enjoyed larger, healthier animals of the high quality that he does today.

It is paradoxical, then, that the one phase of livestock production which could boost efficiency, in only one generation, by as much as 40% in some of our domestic animals has inadvertently been left behind.

The problem of low fertility, particularly in the female, has long plagued the livestock producer. This variation in female fertility can be divided into two main areas; failure in ovulation or fertilization, and failure to produce a live offspring. Some of the variation in fertility has been removed by improved nutritional status; by the addition of antibiotics to the ration; by a careful scientific approach in developing breeding schedules and in the final analysis, by culling a "problem" animal.

Admittedly, these procedures will remove some of the problem; but in addition, it also removes some otherwise desirable animals and in the case of the purebred breeder, some animals of considerable monetary value. By employing the above procedures, variations in ovulation rate and/or fertilization rate can be coped with. Ovulation and fertilization are basic to the problem and inherent to the parents,

but much of the problem does not lie solely in the sire or dam, as the case may be, but rather in the relationship between the dam and developing offspring.

Boyd (1965) lists two approaches to understanding the problem of prenatal survival; on the one hand, there are a large number of extrinsic factors which are known to affect the survival of the fetus, and on the other hand, there is a reasonable amount of information on the physiological mechanisms of maintenance of the fetus.

Physiologically, prenatal mortality implies a failure in one or more of the mechanisms maintaining the optimum relationship between the mother and fetus. Since adequate nutrition is basic to the well-being of life at any stage, prenatal death may well be the result of a poor nutritive exchange between the maternal and fetal circulations.

REVIEW OF LITERATURE

General Reproductive Characteristics of the Rabbit

The domestic breeds of rabbits commonly used in the research laboratory will breed more or less at any time of the year. Marshall (1956) reported that in the United States, July through September is the poorest period to attempt breeding. Asdell (1964) notes that during this "anestrus period" copulation does occur but fewer waves of follicles reach the rupturing state.

Asdell (1964) further reports that the doe is constantly in a state of estrus due to waves of follicles maturing in seven to ten day cycles. However, Pincus (1967) indicated that the copulation reflex may be most strongly exhibited by the doe shortly after partuition, i.e. 5-7 days.

From a comprehensive study on wild rabbits in Great Britain, Brambell (1948) observed that the doe will permit copulation most intensively from February to June. He also stated that if tame rabbits are kept under optimum conditions of temperature and nutrition this marked breeding season disappears.

Harris (1959) listed three major processes closely associated with ovulation; (a) the ripening of a follicle, (b) the rupture of a follicle and discharge of an ovum, and (c) the synchronization of the behavioral response of the organism. These three processes are related to the hypothalamus. The first two are regulated by the gonadotropic

secretion of the anterior pituitary gland, which is under the control of the hypothalamus, and the last appears to be evoked by a sufficient concentration of ovarian hormones in the blood acting on some neural mechanism in the posterior hypothalamus.

The process of ovulation seems to require, for its initiation, a sudden increase in the secretion of luteinizing hormone (LH) against a background of FSH secretion. In the rabbit, this pattern of pituitary secretion is triggered by a sensory stimulus usually supplied by the presence of the male and coitus (Harris, 1959). Brambell (1948) and Marshall (1956) also reported that the doe will ovulate only after coitus or strong sexual excitement. This ovulation after strong sexual excitement, even if coitus is not allowed, will result in pseudopregnancy which lasts for about 16 days (Asdell, 1964). Asdell further states that corpora lutea are formed and the mammary glands are ready for lactation during the pseudopregnant state.

Asdell (1964) cites investigations which have shown that there is a 10-hour interval between stimulation and ovulation of the matured follicles. He indicated that during the first hour of this interval sufficient FSH is released from the pituitary to cause the preovulatory ripening of the follicles. The newly ruptured follicles are readily recognizeable by the small papillae which form on the surface of the ovary at the points of rupture. Marshall (1956) and Brambell (1948) note that there is a set of mature follicles always present in the ovaries ready for stimulation and ovulation. Brambell (1948) found that if the mature follicles do not ovulate in 7-10 days they become atretic and are replaced immediately by another set.

After release, the ova pass through the fallopian tubes and into the uterine horns. Due to the presence of a cervix at the terminus of each uterine horn there can be no trans-uterine migration (Marshall, 1956). Brambell (1948), however, reported that there is some trans-peritoneal cavity migration.

Asdell (1964) states that both ovaries produce corpora lutea with approximately equal frequency--51.4% in the left. The corpora lutea develop gradually to a maximum size of 2.6mm at mid-pregnancy and are shrunken to 2mm at partuition. They disappear completely a few days post-partum (Brambell, 1948).

Chang (1959) has reported that a rabbit ejaculates on the average about 200 million spermatozoa at a time, but only about 2 million or 1% of the ejaculate moves into the uteri about 12 hours post-coitum. He stated that the cervices of the uteri act as barriers to prevent the entry of a large number of spermatozoa. From a study of fertilization in the rabbit in which he ligated a fallopian tube, Adams (1956), concluded that the tubo-uterine junction acts as an additional barrier against the passage of spermatozoa. He further concluded that although some spermatozoa reach the tubo-uterine junction soon after mating, 2 to 5 hours are required before the number of spermatozoa entering the fallopian tube is sufficiently high to produce maximum fertilization. Chang (1959) viewed this 2-5 hour time interval from a possibly different approach. He reported that 4-6 hours of capacitation are needed, whether in the uterus or fallopian tube, before the spermatozoa are rendered capable of penetrating through the mucoproteins of the zona pellucida.

Adams (1956) has shown that fertilization in the rabbit, even after superovulation, is an all or none effect. Excluding does with fewer than 5% of the eggs fertilized, he reported the mean proportion of eggs fertilized to exceed 95%.

Asdell (1964) allows six hours as the fertile life of the ova. They are capable of being fertilized only while in the extruded liquor folliculi at the top of the oviduct. In a study involving naturally mated rabbits, Chang (1959), found 100% fertilization of ova 24-28 hours post mating. Brambell (1948) has found that the fertilized egg cleaves in the ovarian end of the tube shortly after fertilization. The ovum then picks up an albuminoid envelope as it passes down the tube, and, at this stage, is no longer capable of fertilization.

The zygotes reach the uterine horns in three days as early blastocysts and remain free in the lumens for another three days (Asdell, 1964 and Brambell, 1948). Brambell (1948) recognized this as the time during which the zygotes become spaced out at approximately equal intervals.

Implantation

Phelps (1946) has demonstrated that there is an increased blood supply just below the uterine epithelium at the time the blastocyst is about to implant. Boyd and Hamilton (1952) indicate that nutritive materials, in the form of glycogen, lipids, and other substances accumulate in the endometrium and will provide nourishment for the embryo in the event of pregnancy.

In the rabbit, during the sixth and seventh days after copulation, the blastocyst expands to fill the antimesometrial part of the uterine lumen. The trophoblast becomes attached to and eventually invades and destroys the epithelial lining coming into relation with the subepithelial tissues and blood vessels. The vessels themselves are invaded by the ninth day so that maternal blood is in contact with the trophoblast (Boyd and Hamilton, 1952).

Boving (1959) reported that implantation is not a single event but a sequence of integrated mechanisms. For simplicity he grouped the total mechanisms into muscular, adhesive and invasive actions. According to Boving, the muscular mechanisms transport, then space, then immobilize the blastocysts with respect to the length of the uterine horn; adhesive mechanisms attach first the noncellular membranes of the blastocyst to the endometrium and then they attach the trophoblast itself; invasive mechanisms result in the trophoblast penetrating the uterine epithelium. Brambell (1948) reported that this central type of implantation begins as early as seven days and by day fourteen the cavity of the yolk sac is opened to the uterine lumen.

Placentation

The mammalian ovum is small and does not contain a sufficient supply of nutriment for the developing embryo. It is retained for a period in the uterus, where, by special modifications of the uterine mucosa and a part of the ovum, a placenta is formed, and the transmission of nutriment from mother to embryo is made possible (Mossman, 1937).

According to Amoroso (1952), the nature and purpose of the placenta is to mediate the transference of metabolic materials between the mother and her offspring; this always involves the uterus (maternal placenta) and the chorion (fetal placenta). The chorion is the outermost of the fetal membranes which envelop the fetus.

Since, as Amoroso (1952) has indicated, it is the maternal blood that contains the nutritive materials required for metabolism of the fetus, any classification of placental types must be based on the number of membranes which intervene between the two circulations. Grosser (1909) classified the rabbit placenta as being "haemochorial;" i.e., the maternal endothelium has disappeared and the maternal blood directly reached the chorionic epithelium. Mossman (1926) rejected Grosser's view and indicated that the membrane between the two blood streams in the late rabbit placenta consists of only the fetal endothelium. Accordingly, he suggested that this type of placenta be classified as "haemoendothelial." Brambell (1948) indicated that this haemoendothelial type placenta is fully established by day 15.

Hammond (1937) has graphed the growth rates of the uterine contents in the rabbit during pregnancy. He reported that the fetal placenta developed early in pregnancy, for by its growth, it prepares the way for the future nutrition of the embryo. See Figure 1.

In the rabbit, up to about the 18th day, the growth made by the fetal placenta is greater than that made by 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 partuition (Hammond, 1937).

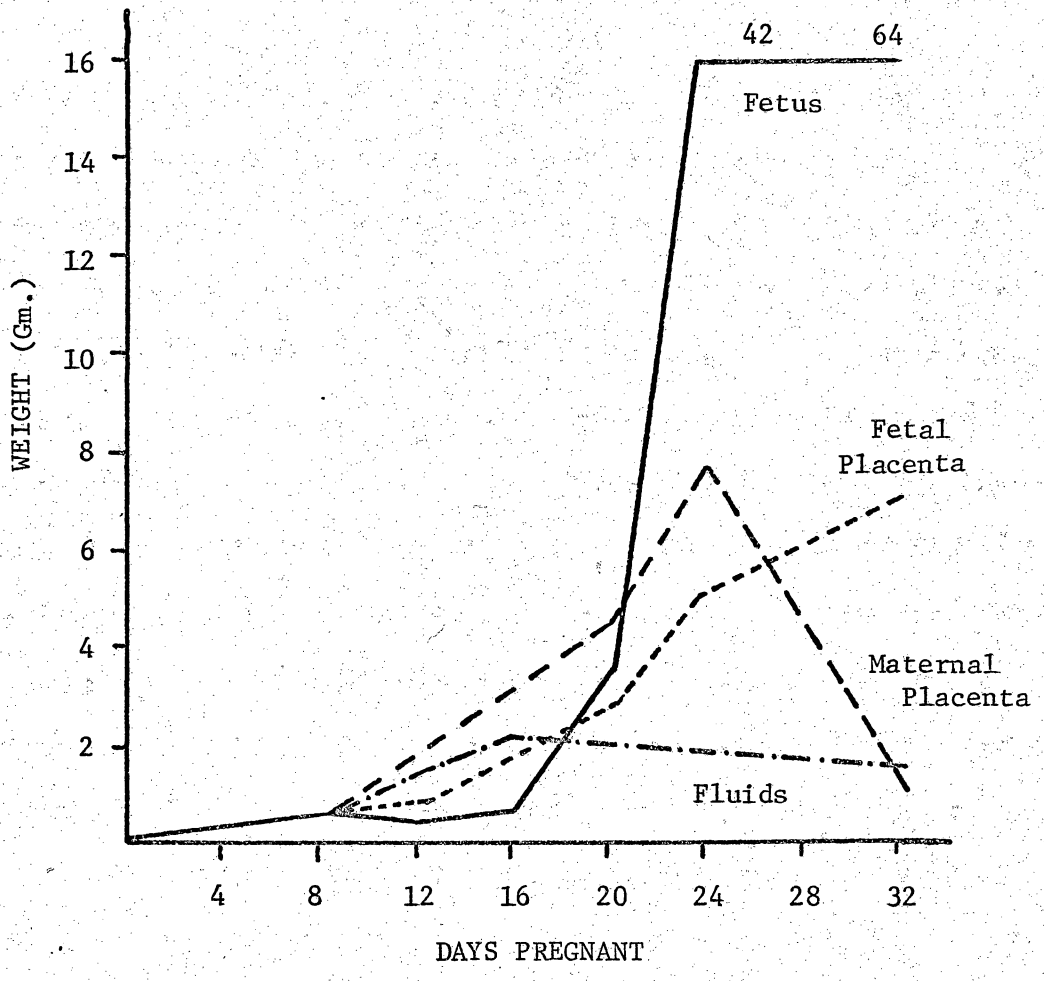

Figure 1. Growth of the uterine contents in the rabbit during pregnancy. (From Hammond, 1937.)

Fetal Nutrition

Amoroso (1952) states that the nutritive materials supplied to the fetus reach it direct from the circulating maternal blood in the placenta, "haemotrophe," or through the absorption of products of the endometrium itself, "histotrophe." The combination of these two elements of the fetal nutrition is known as "embryotrophe."

Histotrophe may be of two kinds, according to Amoroso; (1) histopoietic materials, i.e., secreted constituents of the uterine milk, and (2) histolytic materials such as disintegrating endometrial tissues and extravasated blood.

Amoroso (1952) points out that in the haemochorial type placenta, e.g., the rabbit, the histotrophic nutrition is insignificant after the early stages of development and nourishment of the fetus becomes possible largely by direct haemotrophic nutrition from the maternal blood.

Huggett and Hammond (1952) visualize the supply of nutrients to the fetus as being supplied in three stages and report the following: first, the period when the blastocyst absorbs foodstuffs from the surrounding fluid commonly called the uterine milk; next, the period of absorption by the vitelline circulation in the yolk-sac, and, lastly, the period of absorption by the allantoic circulation in the placenta.

In the earliest stage the blastocyst absorbs directly from cell to cell. This method is replaced gradually by a vascular route in the yolk-sac and allantoic systems. The outer absorbing layer of the blastocyst (trophoblast) is the essential layer leading to attachment. The

quality of the trophoblast, which enables the blastocyst to absorb foodstuffs, also enables it to attach more efficiently to the uterine epithelium.

According to Huggett and Hammond (1952), the main function of the placenta is the transmission of materials from the mother to the fetus and vice versa. This transmission, according to these workers, depends upon: (a) the substance transmitted; (b) the placental structure of the animal concerned; (c) the structure of the placenta at the stage of pregnancy in question.

Hammond (1937) put forward the theory of Partition of Nutrients to explain the distribution of foodstuff in the maternal blood stream between the fetus and maternal tissue. In applying the theory, Hammond found the fetal red blood cells to have a greater affinity for oxygen than the red blood cells of the mother; this fact led him to conclude that the cells of the fetus and the fetal placenta have a higher metabolic rate than the cells of the maternal tissue. Hammond therefore stated that there is a growth gradient between the fetus and mother. He postulated that this difference in metabolic rate may be a result of the varying maturities of the respective cells.

Huggett and Hammond (1952) reported that the blood sugar concentration is higher in the maternal than in the fetal blood of all mammals except the Ungulates. Snyder and Hoskins (1928) have reported that the fetal blood sugar concentration in the human steadily increased toward term but at all points is lower than the mother's. These workers indicate that the balance of the evidence suggests that sugar passes

from the mother to the fetus by a diffusion mechanism, but the case is not clear cut for all mammals. This doubt that it is something more than a mere physical process is furthered by Huggett and Hammond's (1952) reference to the work of Claude Bernard (1859), in which he demonstrated by iodine staining the presence of deposits of glycogen in the rabbit's placenta. Huggett and Hammond (1952) state that this placental glycogen clearly has no relation to the transfusion of sugar nor to the levels of sugar in the mother and fetus, which fluctuate rapidly in parallel and in no way affect the placental glycogen. This glycogen is considered to be an endogenous metabolic product associated with the growth and structure of the placenta.

Davies (1955) also reported a positive gradient for glucose from the maternal to the fetal side of the placenta in the normal rabbit. His values were 110-125 mg% for the maternal blood and 20-68% for the fetus. In addition he postulated that it is an "active" mechanism causing the transfer. The exact mechanism, he reports, is unknown but a "simple diffusion" mechanism theory is inadequate. He felt it necessary to propose some "active" mechanism theory perhaps involving a carrier system. The basis for repudiating a simple diffusion mechanism stems from the fact that the blood sugar of the sheep fetus consists predominantly of fructose while the maternal blood sugar is glucose (Huggett and Hammond, 1952).

Prenatal Mortality

Healy et al. (1961) have divided prenatal mortality into pre-implantation and postimplantation losses, depending on the period of gestation during which the loss occurs. Preimplantation loss includes the losses from ovulation until the time when the embryos should have attached to the uterine wall. Postimplantation loss includes the death loss from the time of attachment to the uterine wall until birth. Amoroso (1952) gives the interval between seven and twelve days as the implantation period for the rabbit.

Allen et al. (1946), in a study on sterility and prenatal mortality in wild rabbits, investigated the reliability of estimates of prenatal mortality based on counts of corpora lutea, implantation sites and embryos. They concluded the following: (a) the death of some of the embryos in a polytocous species seldom terminates pregnancy as the dead embryos are resorbed in situ and do not interfere seriously with the course of development of the remainder; (b) since the corpora lutea in the ovaries are remarkably persistent structures, remaining identifiable until partuition, the total mortality of ova throughout pregnancy upto the examination time can be determined from comparison of corpora lutea counts and embryos; (c) a total error of 6.4% in the original counts of the corpora lutea affected 25.6% of the litters but was distributed evenly throughout gestation.

Adams (1960) found, in 120 rabbit pregnancies, that 11.4% of the ova were lost before implantation and 18.3% were lost after implantation. In the wild rabbit, Brambell (1948), gave preimplantation estimates of 10.2% - 13.0% and a total loss figure of 43.3%.

The estimates of preimplantation and postimplantation losses, by necessity, involve a laparotomy usually performed shortly after implantation. The work of Parkes (1943) has shown that this operation does not significantly affect embryonic death subsequent to it.

From his work on the domestic rabbit Adams (1960), also reported that there appears to be two critical periods for the developing embryo. The first of these is during the switch over from the bilaminar omphalopleur (yolk sac) nutrition to the chorio-allantoic placental nutrition. Amoroso (1952) stated that this occurs from day seven to day fifteen. The second peak of mortality, according to Adams (1960), occurs between days 17 and 23. This period coincides with the period of uterine enlargement when the tension on the spherical conceptus is at a maximum.

Prenatal mortality occurs in all species of mammals to a greater or lesser degree. Harper (1964) reported 10.8% preimplantation loss and 8.7% postimplantation loss in the albino rat. Hanly (1961) reported on the amount of and stage of pregnancy at which embryonic death occured in farm animals. In gilts and sows, he found most of the loss to occur by day 25 and the loss varied from 23-50%. In fertile heifers and cows, 15-21% of the embryos are lost by day 34. For heifers and cows with a history of breeding problems, he reported that these figures rose to 39-59%. His report on sheep gives values ranging from 20-30% by day 40 to 48% at birth. Hulet et al. (1956) found essentially the same figures for sheep.

Hanly (1961) reported on a 10-year program involving 200 mares. He noted the difference between positive pregnancy diagnosis @ 40 days

and subsequent foaling's to be on the order of 11%. No mention was made of loss prior to 40 days.

Factors Affecting Prenatal Growth and Intrauterine Survival

Many researchers have tried to determine the basic causes of prenatal death and the list of possible causative agents is lengthy. Hanly (1961) listed several of these. Of most importance, he considered aging of the gametes, genetic abnormalities, age and parity of the dam, immuno-incompatibilities, environmental temperature and nutritional factors.

Runner (1951) tried to differentiate the intrinsic and maternal factors governing intrauterine survival. In this study, the experimental animals received transplants of genetically tagged eggs into the right ovarian capsule. At 18 days these females were examined for the numbers, types and distribution of offspring. He reported that the distribution of young in the uterine horns of control animals demonstrated an inverse relationship between numbers in opposite sides. Observations on the experimental animals which received transplanted eggs indicated a direct proportion between the numbers of young in opposite horns. This indicated that maternal factors effectively restricted the numbers of young in certain females. In certain other females, survival was more dependent upon factors intrinsic to the embryo. Corner (1923), unlike other workers, could not believe that faulty implantation was a prime source of embryonic death. He felt that internal (intrinsic) defects in the embryo were a more important cause. He felt a dead embryo was the end

result of a failing in any one of the intricate mechanisms inherent to the growing embryo.

Other workers, (McLaren and Michie, 1960), suggested a limited nutrient pool theory. They felt that the fetal blood furnished inadequate amounts of nutrients. If the supply was sorely inadequate one or more fetuses would die or at least be retarded. Healy et al. (1961) proposed an alternative theory for growth and subsequent survivability in the mouse. They felt the problem could be reduced to one of hydrodynamics, i.e., fetal size can be related to the pressure at which maternal blood is delivered to each implantation site down the corresponding offshoot of the uterine artery. This is called the hemodynamic theory and it is based upon the arterialization of the uterus.

Brambell and Mills (1947) observed that the yolk-sacs of 7-12 day wild rabbit embryos were gelatinized. They related this abnormality to the preimplantation mortality which reaches a maximum at 12 days. They sectioned clots of fibrin prepared from rabbit-blood plasma and obtained slides with a similar histological appearance to the gelatinized clots found in the yolk sacs. A similar reticulum was reported in the tame rabbit though not so frequently and was developed to a lesser extent.

Bishop (1964) suggested that a considerable part of embryonic death is unavoidable and should not be regarded as pathological in any way. In fact, he felt that it should be regarded as a normal way of eliminating unfit genotypes in each generation. He further suggests that a considerable part of embryonic death is attributable to the male or to the mating system.

Effect of Energy

On Ovulation

Bellows et al. (1963) reported that an increase in the level of feeding for the ewe a few weeks prior to mating is an excellent way to increase the ovulation rate. They postulated that the addition of grain to the maintenance diet of hay increased the pituitary weight and hence the total FSH potency and pituitary LH potency in spite of unaffected gonadotropin concentration in the anterior pituitary.

Rigor et al. (1963) found the same beneficial effect of flushing upon ovulation in gilts. They interpreted the results as indicative of a pituitary-ovarian feedback relationship functioning so that at a given level of corpus luteum activity a higher FSH secretion exists in the high energy gilts. They concluded that there would be greater follicular development occuring before corpora lutea regression during the cycle of high energy gilts and more follicles matured at the ensuing estrus.

El-Sheikh et al. (1955) fed eight ewes on hay alone plus an additional group on hay + 2# grain for nine months. The grain feeding significantly increased the ovulation rate but also significantly increased the prenatal mortality measured at 40 days.

On Prenatal Mortality

Hoxsey et al. (1960) fed a protein supplement to range ewes for two weeks prebreeding with various feeding systems after breeding. They reported the prenatal death to be 12.5% in the supplemented as compared to 7.6% in the non-supplemented group.

Gossett and Sorensen (1959) fed two unrestricted diets with different energy contents per cwt to two groups of swine. At 25 days the embryonic death varied little between the high energy and low energy groups, but at 40 days the low energy group suffered 25.5% embryonic death while the high energy group suffered a 42.3% death loss.

Self et al. (1955) working with swine, tried to combine the known advantage of high energy feeding (H) on ovulation rate with the apparent advantage to embryonic survival of low energy feeding (L). They distributed the feeding regime into three phases: prepuberty to first estrus; first estrus to service at second estrus, and lastly, service to slaughter at 25 days. They reported a significantly better embryo survival rate in gilts on LLL regime as compared to HHH feeding.

Haines et al. (1958) fed pigs at two energy levels subsequent to breeding. The high energy feeding was associated with a non-significantly greater survival rate based on the percentage of corpora lutea represented by embryos at 25 and 40 days.

Haines et al. (1959) fed two energy levels prior to breeding. They reported earlier puberty and higher ovulation rate in the high energy, as well as, a considerably but still insignificantly less embryonic death loss.

Action of Diabinese

According to Pfizer laboratories (1963), Diabinese is an oral hypoglycemic agent. Its mechanism of action is not completely understood but it is not an oral insulin. Diabinese (chlorpropamide) is classified as an arylsulfonylurea. It is 1-(p-chlorobenzene-sulfonyl)-3-

propylurea having a molecular weight of 276.76 and an empirical formula of $C_{10}H_{13}O_3N_2SCl$. Pfizer reports that it is absorbed rapidly from the gastrointestinal tract. They report that within one hour after a single dose, it is readily detectable in the blood and the level reaches a maximum within two to four hours.

Genes and Charnaya (1965) after studying reactions of organs and tissues of healthy dogs to chlorpropamide and of the organs and tissues of pancreatectomized dogs to chlorpropamide reported that the drug produces blood glucose-reducing effect predominantly by the protentiation of the insulin effect and to a smaller degree by increased secretion of insulin by the pancreas. They felt that this explains why antidiabetic sulfonamides do not bring about, even after long years of treatment, exhaustion of the beta cells of the pancreatic gland.

Anton and Gonzales-Rodriguiz (1964) injected chlorpropamide at the rate of 250mm/day intravenously to normal and pancreatectomized dogs. They reported that the drug exhibited its maximum effect in 30 minutes and concluded that it acts on the beta-cells of the pancreas.

Turner (1966) also reported its influence on the beta-cells of the pancreas; but he also proposed that sulfonamides in general may prevent the degredation of endogenous insulin by inhibiting the insulinase action of the liver. This hypothesis is shared by Duncan and Clarke (1965). From a direct analysis of the hypoglycemic action of the sulfonamides in animals, Loubatieres (1957), concluded that the pancreas is indispensable to the hypoglycemic action. In a totally depancreatized animal the effect does not occur; however, one needs to leave only a fragment of the pancreas for the action to become manifest. Loubatieres also found the

other endocrine glands to be non-essential for the hypoglycemic effect. In fact, adrenalectomy and hypophysectomy increase the manifestation. He also reported that the liver itself is not absolutely necessary.

Duncan and Clarke (1965) agree that the action of the sulfonylureas depends upon the presence of the pancreas and the ability of the beta-cells to secrete a sufficiency of endogenous insulin.

OBJECTIVES

1. To determine the amount and distribution of embryonic death in a group of New Zealand White rabbits.
2. To determine what effect, if any, the feeding of a hypoglycemic agent would have upon this amount and distribution of embryonic death.
3. To determine the hypoglycemic effect of chlorpropamide on the blood sugar level in the gestating rabbit.

MATERIALS AND METHODS

Animals

Twenty female and five male New Zealand White rabbits were used to initiate this study. These rabbits, utilized in a previous reproduction study, plus ten additional rabbits--nine female and one male--purchased from Dublin Laboratory Animals, Inc., Dublin, Virginia, constituted the experimental subjects for this experiment.

The average age and weight of the females was twenty-two months and 4.2 kg. respectively.

Housing

The animals were kept in individual wire cages measuring 30 x 18 x 24 (l x w x h). The floors of the enclosures were of a heavy gauge mesh measuring 5/8" x 1". The complete wire floor was chosen to facilitate cleaning and to hold disease to a minimum. No undue stress resulting from the mesh was experienced by the rabbit.

The rabbits were housed in a large animal room 30 x 20 x 20. This room remained considerably cooler than the outside temperature but was not air conditioned; therefore, the animals were subjected to changes in temperature over a short period of time but never experienced the extremes.

Feeding

Each animal in the study received four ounces of Wayne Rabbit Ration daily. This pelleted ration contains 16.7% protein, 4.1% fat,

12.5% crude fiber, 8.3% ash, 7.7% moisture, 50.7% nitrogen-free extract, and 89.2% T.D.N. as determined by a proximate analysis.

This level of nutrition furnishes .22 pounds of T.D.N. and .042 pounds of total protein daily. These quantities are in accord with the requirements for pregnant does as listed by the National Research Council (1966). For one week, prior to the initiation of this study, the females were conditioned to consume the ration in a ground form. This was necessitated by the fact that the chlorpropamide is water-insoluble and had to be mixed in the feed. Chlorpropamide, an arylsulfonylurea, is 1-(p-chlorobenzene-sulfonyl)-3-propylurea. It has a molecular weight of 276.76 and an empirical formula of $C_{10}H_{13}O_3N_2SCl$. The chlorpropamide feed was prepared in 50 lb. batches. 1.142 gm of chlorpropamide was added to exactly 2 lbs. of the ground ration for premixing. This premix was then added to an additional 48 lbs. of ration and allowed to mix for one hour.

The animals were randomly assigned to one of four experimental groups: a control group, receiving no chlorpropamide; a five-day group, receiving the chlorpropamide feed for five days starting the day after breeding; a 15-day group, and a group receiving the drug for the entire 32 days of gestation. To equalize the inherent variation within the rabbits the does were switched to the subsequent feeding regime following each gestation period. The does which had been on the five-day regime received the drug for 15 days during the next gestation and so on.

Breeding

The females were naturally mated to one of the six bucks and then remated immediately to one of the remaining five. This double

breeding was employed to guard against any failure of semen quality. The pairs were watched closely for signs of the buck ejaculating. If intromission was not allowed either of the bucks the doe was deemed pseudopregnant and scheduled for rebreeding 17-19 days later.

If the female became pregnant, she was carried through gestation on her appropriate feeding regime and rebred five days post-partum. The young were removed immediately after partuition, usually within four to six hours. Using this breeding schedule the author experienced no difficulty in keeping approximately two-thirds of the females bred. The remaining one-third would not permit copulation and were consistently in a state of pseudopregnancy.

Ovulation and Implantation Counts

Pregnancy was determined at nine days by abdominal palpation. If the doe was pronounced pregnant she was scheduled for laparotomy at 13-15 days, or just subsequent to implantation. The laparotomy was performed under ether anaesthesis after injecting each doe with two cc. of atropine. After the appropriate preparation of the abdomen a mid-ventral incision was made approximately three inches below the xiphoid process and extending caudally for approximately four inches. Through this opening the uterine horns up to the ovaries were exposed. Counts were made of the corpora lutea and developing embryos; any abnormalities were noted at this time. See Figures 2 and 3.

The uteri were carefully replaced, the incisions suctured and treated with scarlet oil, the abdomens wrapped with gauze and the rabbits were allowed to recover. The gauze wrapping was removed the following day.

Figure 2. The uterine horns showing fetal development at 14 days.

Figure 3. The left ovary showing corpora lutea development at 14 days.

The anaesthesia and postoperative trauma apparently had little effect on the animals. If it was deemed necessary, an additional coating of scarlet oil was applied one week later.

The corpora lutea and developing embryos were quite obvious at 14 days. The corpora lutea measured 2-3 mm and the uterine bulges 10-15 mm. On every operation, the corpora lutea and developing embryos were counted by no less than three observers.

To arrive at preimplantation losses the numbers of developing embryo sites were compared with the number of corpora lutea. Post-implantation loss represents the comparison of developing fetuses at laparotomy with the number born. Computation of the total losses involved a comparison of corpora lutea and live fetuses at partuition.

Birth weights were recorded for each individual rabbit and rounded to the nearest whole gram.

Blood Glucose

Blood was collected from two rabbits from each of the three experimental groups and from two representing the control group. The heart puncture technique was employed using a twenty gauge needle one and one-half inches long, attached to a five cc. glass syringe.

Two or three cc. of blood were collected on four occasions; two days before breeding, and on the fifth, fifteenth and twenty-second days following breeding. The samples were taken in the morning before feeding.

Upon extraction, the blood was deposited in a tube containing 10% potassium oxalate solution to await processing. All samples were collected, and processed to the filtrate stage and stored under toulene in a 0°C freezer. Glucose standards were run and all filtrates processed at one time.

The glucose concentrations were determined by the Nelson-Somogyi method (Somogyi, 1945), using an Evelyn colorimeter.

RESULTS AND DISCUSSION

Ovulation

Individual ovulation rates for the does in each of the four experimental groups, indirectly arrived at through corpora lutea counts made at the laparotomies, are listed in Tables 1, 2, 3, and 4. The mean ovulation rates for each of these groups are summarized in Table 5.

The means of 8.3, 8.1, 8.3, and 8.1 for the control, 5-day, 15-day and 32-day Diabinese groups respectively, are quite similar. This is to be expected due to the random assignment of does to the various treatment regimes.

The ovulation rates observed are slightly higher than those reported by Asdell (1964) and Brambell (1944-45). They report means of 7 for the domestic strains and 5 for the wild type. Both of these researchers report wide breed differences. The individual variation within the groups, 2-11 in the control group, is in agreement with the above researchers. Asdell (1964) reports a range of 1 to 12.

Implantation

The total number of embryos implanted and surviving at 12 to 14 days for each litter in the experiment is listed in Tables 1, 2, 3, and 4. In addition a summary of the mean number of embryos for each of the four groups is found in Table 5.

The control, 5-day, 15-day and 32-day Diabinese-fed groups had mean values of 8.2, 7.8, 8.1 and 7.5 respectively. An analysis of

Table 1. Reproductive Performance of the Control Group of Does.

Litter	Total number corpora lutea[a]	Total number embryos[b]	Number born	% Preimplantation loss[c]	% Postimplantation loss[d]	% Total loss[e]	Average birth weight
1	11	11	6	00.0	45.5	45.5	55.2
2	9	7	6	22.2	14.3	33.3	50.2
3	9	9	5	00.0	44.4	44.4	35.6
4	8	8	5	00.0	37.5	37.5	64.4
5	9	9	6	00.0	33.3	33.3	47.8
6	7	7	3	00.0	57.1	57.1	86.3
7	2	9	3	25.0	66.7	75.0	51.0
8	8	8	5	00.0	37.5	37.5	58.6
9	2	5	3	00.0	40.0	40.0	60.2
Average	8.3	8.2	4.7	5.2	41.8	44.8	56.6

[a] Number of C.L. on both ovaries counted during laparotomy at 13 to 15 days.

[b] Number of bulges in uterine horns counted during laparotomy at 13 to 15 days.

[c] (Number of uterine bulges ÷ number of C.L.) x 100 at 13 to 15 days.

[d] (Number born ÷ number of uterine bulges at 13 to 15 days) x 100.

[e] (Number born ÷ number of C.L. at 13-15 days) x 100.

Table 2. Reproductive Performance of the 5-day Diabinese-Treated Group of Does.

Litter	Total number corpora lutea[a]	Total number embryos[b]	Number born	% Preimplantation loss[c]	% Postimplantation loss[d]	% Total loss[e]	Average birth weight
6	9	9	8	00.0	11.1	11.1	56.5
2	9	9	8	00.0	11.1	11.1	52.8
3	8	8	7	00.0	12.5	12.5	76.9
4	5	4	4	20.0	00.0	20.0	74.0
5	10	9	8	10.0	11.1	20.0	61.4
6	7	7	7	00.0	00.0	00.0	57.1
7	5	4	4	20.0	00.0	20.0	70.3
8	11	11	9	00.0	18.2	18.2	54.7
9	7	7	6	00.0	14.3	14.3	74.2
10	10	10	9	00.0	10.0	10.0	56.1
Average	8.1	7.8	7.0	5.0	8.8	13.7	63.4

[a] Number of C.L. on both ovaries counted during laparotomy at 13 to 15 days.

[b] Number of bulges in uterine horns counted during laparotomy at 13 to 15 days.

[c] (Number of uterine bulges ÷ number of C.L.) x 100 at 13 to 15 days.

[d] (Number born ÷ number of uterine bulges at 13 to 15 days) x 100.

[e] (Number born ÷ number of C.L. at 13-15 days) x 100.

Table 3. Reproductive Performance of the 15-day Diabinese-Treated Group of Does.

Litter	Total number corpora lutea[a]	Total number embryos[b]	Number born	% Preimplantation loss[c]	% Postimplantation loss[d]	% Total loss[e]	Average birth weight
1	9	9	9	00.0	00.0	00.0	68.3
2	9	9	9	00.0	00.0	00.0	65.3
3	7	7	7	00.0	00.0	00.0	58.5
4	7	5	4	28.6	20.0	42.9	83.5
5	9	9	9	00.0	00.0	00.0	69.3
6	8	7	7	12.5	00.0	12.5	79.1
7	7	7	7	00.0	00.0	00.0	61.3
8	12	12	12	00.0	00.0	00.0	40.0
9	9	10	6	00.0	40.0	40.0	63.0
10	6	6	4	00.0	33.0	33.0	60.0
Average	8.3	8.1	7.4	4.1	9.3	12.8	64.8

[a] Number of C.L. on both ovaries counted during laparotomy at 13 to 15 days.

[b] Number of bulges in uterine horns counted during laparotomy at 13 to 15 days.

[c] (Number of uterine bulges - number of C.L.) x 100 at 13 to 15 days.

[d] (Number born - number of uterine bulges at 13 to 15 days) x 100.

[e] (Number born - number of C.L. at 13-15 days) x 100.

Table 4. Reproductive Performance of the 32-day Diabinese-Treated Group of Does.

Litter	Total number corpora lutea[a]	Total number embryos[b]	Number born	% Preimplantation loss[c]	% Postimplantation loss[d]	% Total loss[e]	Average birth weight
1	9	8	7	11.1	12.5	22.2	60.1
2	7	7	7	00.0	00.0	00.0	67.6
3	8	7	7	12.5	00.0	12.5	65.6
4	8	8	6	00.0	25.0	25.0	53.2
5	9	8	8	11.1	00.0	11.1	51.0
6	10	8	8	20.0	00.0	20.0	47.9
7	10	10	9	00.0	10.0	10.0	52.3
8	8	8	6	00.0	25.0	25.0	63.0
9	7	7	7	00.0	00.0	00.0	61.9
10	5	4	4	20.0	00.0	20.0	62.0
Average	8.1	7.5	6.9	7.5	7.3	14.6	58.5

[a] Number of C.L. on both ovaries counted during laparotomy at 13 to 15 days.

[b] Number of bulges in uterine horns counted during laparotomy at 13 to 15 days.

[c] (Number of uterine bulges ÷ number of C.L.) x 100 at 13 to 15 days.

[d] (Number born ÷ number of uterine bulges at 13 to 15 days) x 100.

[e] (Number born ÷ number of C.L. at 13-15 days) x 100.

Table 5. Mean Reproductive Performance of the Control and Diabinese-Treated Groups of Does.

Treatments	Total number corpora lutea[a]	Total number embryos[b]	Number born	% Preimplantation loss[c]	% Postimplantation loss[d]	% Total loss[e]	Average birth weight
Control	8.3	8.2	4.7	5.2	41.8**	44.8**	56.6
5-day Diabinese	8.1	7.8	7.0	5.0	8.8	13.7	63.4
15-day Diabinese	8.3	8.1	7.4	4.1	9.3	12.8	64.8
32-day Diabinese	8.1	7.5	6.9	7.5	7.3	14.6	58.5

[a] Number of C.L. on both ovaries counted during laparotomy at 13 to 15 days.

[b] Number of bulges in uterine horns counted during laparotomy at 13 to 15 days.

[c] (Number of uterine bulges ÷ number of C.L.) x 100 at 13 to 15 days.

[d] (Number born ÷ number of uterine bulges at 13 to 15 days) x 100.

[e] (Number born ÷ number of C.L. at 13-15 days) x 100.

** Significantly ($P < .01$) greater than the treated groups.

variance of the preimplantation losses indicated that there were no significant differences among the groups regarding this fraction of prenatal loss. The author feels that an extrapolation from the preimplantation loss analysis to the mean number of embryos is valid and meaningful because preimplantation loss is a function of the corpora lutea number and the number of surviving embryos at implantation (12 to 14 days).

Prenatal Mortality

In keeping with the convention of Healy et al. (1961), the data for the prenatal mortality are divided into and listed as a preimplantation loss and a postimplantation loss. A total loss figure was also computed according to Brambell (1948). Tabulations of these data are found in Tables 1, 2, 3, and 4, by individual litter, in each of the experimental groups. In addition, mean figures for these various losses by treatment are listed in Table 5. Table 6 contains the same means for prenatal losses with the addition of their arc-sin transformations and standard errors. The transformation of these percentage figures was necessary to bring the skewed distribution towards normality.

The mean preimplantation losses in this experiment, i.e., 5.2, 5.0, 4.1 and 7.5% for the control, 5-day, 15-day and 32-day Diabinese groups respectively, are somewhat lower than those found by other researchers. Adams (1960) reported 11.4% while Brambell (1948) reported 10.2 to 13.0%. No explanation for this is available other than the possibility that conditions may have been near optimum for the rabbits in this study.

Table 6. Means and Standard Errors for Prenatal Losses in the Control and Diabinese-Treated Groups of Does.

Treatment group	Sample size	Mean preimplantation loss[a]	Mean postimplantation loss[b]	Mean total loss[c]
Control	9	5.2%(25.42 ± 4.83)[d]	41.6%(69.82 ± 5.28)**	44.8%(62.27 ± 7.61)**
5 day Diabinese	10	5.0%(24.64 ± 3.91)	8.8%(32.43 ± 2.23)	13.7%(37.71 ± 2.71)
15 day Diabinese	10	4.1%(23.34 ± 4.15)	9.3%(29.90 ± 6.51)	12.8%(36.50 ± 7.15)
32 day Diabinese	10	7.5%(28.71 ± 3.80)	7.3%(29.28 ± 4.32)	14.6%(38.69 ± 3.97)

a (Mean number of uterine bulges ÷ the mean number of C.L.) x 100; at 13 to 15 days.

b (Mean number born ÷ mean number of uterine bulges at 13 to 15 days) x 100.

c (Mean number born ÷ mean number C.L. at 13 to 15 days) x 100.

d The arc-sin transformation and its standard error are given in parenthesis with the actual mean.

** Significantly ($P < .01$) greater than the treated groups.

The mean postimplantation loss of 41.8% for the control group is in accord with the 30+% figure of Brambell (1948). However, the postimplantation estimates for the 5-day, 15-day and 32-day Diabinese-fed groups, bring 8.8, 9.3, and 7.3% respectively, are significantly lower ($P<.01$) than the control group.

The total loss figure of 44.8% for the control group is again in accord with other researchers. The 5-day, 15-day and 32-day treated groups' figures are again significantly lower than the control ($P<.01$).

The analysis of variance of these data appear in Table 7. Note that both the mean postimplantation losses and mean total losses of the Diabinese-fed groups differ significantly ($P<.01$) from the control group values. Examination of the standard errors indicated that the three Diabinese-fed groups were not significantly different.

The methods for computing the losses at the various stages were explained in the review of literature. The reliability of estimates based on these methods is inherently quite high (Allen et al., 1946). To heighten this accuracy, data for this trial were collected by three qualified observers.

Phelps (1946) demonstrated an increased vascularity just below the uterine epithilium prior to implantation. Boyd and Hamilton (1952) reported an accumulation of glycogen and lipids in the endometrium prior to pregnancy. The developing rabbit embryo, prior to implantation, is dependent primarily upon these maternal stores and other stores inherent to itself for survival. The data presented in this report on preimplantation loss tends to indicate that under normal conditions this

Table 7. Analysis of Variance for Prenatal Losses.

Source	Degrees of freedom	Mean Squares		
		Preimplantation loss	Postimplantation loss	Total loss
Treatment	3	52.3702	3580.1517**	1408.3329**
Error	35	168.5661	226.6506	309.8371

** Significantly ($P < .01$) greater than the treated groups.

nourishment is adequate to maintain the embryo through implantation. A graphic explanation of this phenomenom appears in Figure 1. Note that the embryo during the interval from day 7 to day 12 increases very little in weight while the other uterine contents are increasing rapidly. Nutrition for the preimplantation embryo is not demanding and one would not expect large losses due to nutritional deficiencies.

The distribution of the losses, during the prenatal period, from these rabbit data is reversed when compared to the distribution of losses in other species; i.e., Hanly (1961), working with gilts and sows, and Hulet et al. (1956), working with sheep, found the greatest prenatal loss to occur in the preimplantation stages.

The data for postimplantation losses in this study lead to some interesting hypotheses. Recall that the mean figures were 41.8% for the control group, 8.8% for the 5-day, 9.3% for the 15-day, and 7.3% for the 32-day groups. From even a cursory examination of these means, it appears obvious that the addition of the hypoglycemic insulin-like drug affects some mechanisms closely associated with the well being of the fetus. Since Diabinese is a sulfonamide and since it is the nature of this type compound to act primarily upon the beta-cells of the pancreatic islets to promote the production of endogenous insulin, the author feels it a valid assumption to explain the increased survivability through heightened insulin effects.

According to Turner (1966), insulin may act directly upon intracellular enzyme systems, may control the permeability of the cell membrane, may act through an energy transfer with vasopressin, or it may act

directly upon a gene loci to increase the formation of messenger RNA in muscle which would in turn, promote the synthesis of enzymes and structural proteins.

The initial hypothesis was that the Diabinese would move across the placenta and enter into the blood stream of the fetus. At that point, it could exert its insulin-like effects. If, in fact, Diabinese does cross the placenta, improved fetal nutrition could explain the increased survivability. McLaren and Michie (1960) reported that the fetal blood furnished inadequate amounts of nutrients to maintain optimum growth and survivability of large litters. The data presented in this experimented seem to agree with this for the control group. However, from a look at the treated groups' data, it is apparent that the fetal blood is quite capable of furnishing nutrients for very large litters, if in fact, these nutrients can gain access to the metabolic pathways of the fetus.

Since it is inconclusive whether Diabinese does cross the placenta, an alternative hypothesis may be employed to explain the increased survivability. It is a documented fact (Guyton, 1966) that during the early stages of pregnancy, a reservoir of metabolites, lipids, carbohydrates, and amino acids is built up in the maternal placenta. Presumably, this is to augment the somewhat static supply of nutrients from the maternal circulation after the fetus becomes implanted.

At any rate, the primary effect of Diabinese in this experiment may have been to increase the permeability of the maternal cells, including the cells of the maternal placenta, so that greater glycogen,

lipids and other metabolite stores could be built up. Then, when the fetal circulation came in contact with the placental tissue of the mother, more nutrients would be available to it.

In the last analysis, the most reasonable explanation may well be a combination of the two hypotheses.

The total loss figures are a function of the preimplantation losses plus the postimplantation losses. From the practical point of view the total loss is the important figure. However, if one is ever to control the total loss, he must know at what time to apply his remedy.

There is much literature today suggesting that high energy feeding is detrimental to prenatal survival. From an interpretation of the work of Davies (1955), who was also investigating placental transmission in the rabbit, one can assume that high energy feeding results in an elevated blood glucose level both for the dam and for the fetus, though the level for the fetus always remains lower than that of the dam in swine and other monogastric species. Guyton (1966) attributes this lower level of glucose in the fetal circulation to the rapid utilization of glucose by the fetus. This agrees with the work of Hammond (1937) who reported a higher metabolic rate in the fetal cells than in cells of the mother.

The elevated glucose levels following high energy feeding should evoke the appropriate insulin response; i.e., insulin production should increase and cell membranes should be made more permeable. It would appear as if the net physiological effect is not dissimilar to that of Diabinese feeding. Diabinese feeding may well be high energy feeding in reference to the individual cells. The striking differences

between survival rates of high energy-fed groups and Diabinese-fed groups may be due to the production of some toxic or near toxic by-products when high energy rations are fed.

Birth Weights

Average birth weights for the individual litters are listed in Tables 1, 2, 3, and 4. Table 5 contains the mean birth weight for the control and each of the treated groups. An analysis of variance indicated that the means were not significantly different (see Table 8).

An examination of the control mean, 56.6 gm., and the average for the three treated groups, 62.3 gm., does, however, indicate a trend toward heavier birth weights in the treated groups.

This trend may be a result of either of the following mechanisms or of a combination of both. As explained earlier, the drug may have passed through the placenta and into the fetal blood stream causing a resultant increased permeability of the fetal cells to the nutrients normally available to them. This assumption is in accord with the work of Seller (1964), in which she demonstrated a decrease in fetal blood glucose levels after insulin injection in the mother. In addition the fetal portion of this drug may have entered the cells and augmented enzyme synthesis and the production of more glucose-6-phosphate or the protein anabolic mechanisms may have been heightened. These mechanisms would account for heavier birth weights by providing greater amounts of metabolites to the fetal cells and by enabling these cells to utilize them more rapidly.

Table 8. Analyses of Variance for Blood Glucose Levels and Average Birth Weights.

Source	Blood glucose levels		Average birth weights	
	Degrees of freedom	Mean Squares	Degrees of freedom	Mean Squares
Gestation effect	3	367.665**		
Treatment effect	3	27.093	3	148.24
Gestation x treatment interaction	9	93.338		
Error	16	61.254	35	115.38

** $P < .01$

An obvious weak point in this hypothesis stems from the fact that the fetus is not dependent upon the maternal circulation for nutrition until the fifteenth day of gestation. An explanation based on increased fetal uptake of nutrients made available to them by the maternal circulation implies that implantation has occurred. This begins on about the seventh day and is completed about day fourteen. Unless one assumes that Diabinese was ingested by the trophoblast from the histiotrophe, the increased birth weights of the 5-day treated group cannot be explained by this hypothesis.

A second hypothesis is that the permeability of the maternal placenta was increased and that this resulted in a heightened storage of lipids, carbohydrates and proteins in this organ. In the rabbit, the fetal circulation comes into direct contact with the maternal placenta. It is not unreasonable to postulate that if more nutrients are stored in this exchange area more nutrients will, in fact, be exchanged. This latter hypothesis does not depend on the transplacental migration of Diabinese, but would still result in more nutrients being made available to the fetal cells and would satisfactorily account for increased birth weights for all treated groups.

Effect of Diabinese on the Blood Glucose of the Gestating Rabbit

The analysis of variance for the mean blood glucose concentrations is found in Table 8. Table 9 contains the actual mean concentrations expressed in mg. %.

Note, in Table 8, that the effect on blood glucose due to the stage of gestation is highly significant ($P<.01$), while the effect

Table 9. Mean Blood Glucose Levels in the Control and Diabinese-Treated Does. (Mg. percent)

Treatment	Number of does	Mean blood glucose levels: Two days prior to breeding	Five days post-coitus	Fifteen days post-coitus	Twenty-two days post-coitus
Control	2	42.8	26.2	49.9	21.3
5-day Diabinese	2	43.5	40.1	37.0	30.9
15-day Diabinese	2	43.1	40.1	48.3	29.8
32-day Diabinese	2	30.5[a]	38.7	46.7	35.4

[a] One doe had blood glucose level of 20.0 mg. %.

on blood glucose due to the drug is not. It appears that, in the non-diabetic animal, Diabinese is unable to override the normal glucostatic mechanisms of the body.

The data indicate a marked drop in maternal blood glucose levels after 15 days for all groups including the control. After the fifteenth day, the endpoint of placentation, the fetus is completely dependent upon the maternal circulation for its nutrients. Apparently, during the last one-half of pregnancy, the drain of glucose from the maternal bloodstream is too rapid for the digestive glycolytic and gluconeogenic mechanisms to replace when food intake is limited.

SUMMARY

Thirty-nine New Zealand White rabbit pregnancies were used to determine the effects of Diabinese, a hypoglycemic drug, upon prenatal survival. Data were presented and discussed for a control group of nine litters, receiving none of the drug, and for three groups of 10 litters each, which were fed 100 mg. daily of the drug for either five, fifteen or thirty-two days post-coitus.

The analysis of variance indicated that there were no significant differences among the four experimental groups in preimplantation losses. The control group, however, suffered a significantly greater ($P < .01$) postimplantation loss than did the three Diabinese groups. Total loss figures were also analyzed and the highly significant ($P < .01$) difference between the control and treated groups again indicated that survivability of the fetus was enhanced by feeding Diabinese to the gestating rabbit. There were no significant differences among the three groups receiving Diabinese in prenatal losses.

Birth weights of all the young were recorded. While there appears to be a trend toward heavier birth weights in the Diabinese treated groups, a statistical analysis showed no significant differences.

Blood glucose levels were determined for maternal blood samples from each treatment group at various stages of pregnancy. The stage of gestation was found to exert a highly significant ($P < .01$) influence upon the levels of glucose in the blood. There were no significant differences in blood glucose levels among the experimental groups.

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THE EFFECT OF AN INSULIN-LIKE COMPOUND UPON THE AMOUNT AND DISTRIBUTION OF PRENATAL LOSS IN THE NEW ZEALAND WHITE RABBIT

by

Richard A. Battaglia

ABSTRACT

Thirty-nine rabbit pregnancies were divided into three treatment groups of ten each and a control group of nine. The primary objective of the experiment was to determine the amount and distribution of prenatal losses when the gestating does were fed 100 mg. daily of a hypoglycemic drug, Diabinese. Group I (control) received the same daily ration as the treatment groups with the exception of the drug. Group II received the drug for five days post-coitus. Groups III and IV received the drug for fifteen days and for the whole of gestation, respectively. Laparotomies were performed on all does twelve to fourteen days post-coitus. Corpora lutea and embryo counts were collected during laparotomy. After birth of the young estimates of preimplantation, postimplantation and total prenatal losses were calculated. Birth weights of the young and blood glucose levels of the does were collected. Means for Groups I, II, III and IV, respectively, were: 5.2, 5.0, 4.1, and 7.5% for preimplantation loss; 41.8, 8.8, 9.3, and 7.3% for postimplantation loss; 44.8, 13.7, 12.8, and 14.6% for total prenatal loss and 56.6, 63.4, 64.8, and 58.5 gm. for average birth weights. There were no significant differences in preimplantation losses, a highly significant difference ($P < .01$) in favor of the Diabinese groups for postimplantation losses,

and a highly significant difference ($P < .01$) in favor of Diabinese groups for total losses. Stage of gestation exerted a highly significant ($P < .01$) influence upon blood glucose levels. Young from Diabinese-treated does tended to have heavier birth weights.