EFFECT OF EXOGENOUS HORMONES ON PREGNANCY MAINTENANCE IN THE PREPUBERAL RAT

by

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INTRODUCTION

The fecundity of a polytocous species depends not only on the average number of young born in each litter, but also the time required for the animal to reach sexual maturity, on the duration of pregnancy and lactation, and on the longevity of the reproductive period in the animal's life.

Numerous attempts have been made to increase the prolificacy of adult females by altering the endogenous hormone balance. The most common methods employed have been to increase the number of ova shed (superovulation) or to supplement the endogenous supply of the ovarian steroid hormones. Thus far, neither of these methods has substantially increased the number of young born.

Induced ovulation of the prepuberal animal has been used recently in an attempt to increase the fecundity of the animal by decreasing the time required from birth to sexual maturity. However, induced ovulation of the prepuberal animal has not increased the fecundity for even though the eggs of prepuberal animals are viable when transplanted into the adult, the eggs rarely develop in the reproductive tract of the prepuberal rat, mouse, rabbit, or hog.

Of the factors which have been postulated to cause this failure in embryonic development, an unfavorable uterine environment due to hormone deficiency appears to be the most likely. From the classical experiments of Allen and Corner (1929) and others, it has been fairly well demonstrated that the maintenance of a favorable uterine environment is partly dependent upon adequate quantities of estrogen and progesterone.

The prepuberal rat appears well suited for studying the effects of exogenous estrogen and progesterone on the reproductive process since the interaction of these steroids and the resulting synergistic or antagonistic effects can be observed by means of the fetal survival rates. It is, therefore, the purpose of this study to elucidate the role of estrogen and progesterone in inducing a uterine environment which is favorable to embryonic and fetal development in the prepuberal laboratory rat.

REVIEW OF LITERATURE

General Reproductive Characteristics

The laboratory rat (usually albino) is polyestrus all year round. The estrous cycle normally lasts 4 to 6 days. Ovulation occurs near the end of the 9 to 20 hour heat period; and, copulation occurs most frequently between 1 a.m. and 3 a.m. (Asdell, 1964).

The corpora lutea formed after rupture of the follicles are physiologically inactive unless the cervix is stimulated mechanically as occurs in coitus. Once the cervix is stimulated, prolactin is released from the anterior pituitary which causes the newly formed corpora lutea to secrete progesterone. If mating does not result in pregnancy, the rat becomes pseudopregnant for about 14 days (Asdell, 1964).

In the sexually immature rat, the vagina is a cord of cells without a lumen. As the first ovulation approaches, the central cells separate, forming a tube closed at the vaginal orifice by a thin membrane. This membrane ruptures at, or a little before, the first ovulation. Hence, the vaginal opening may be used as an indication of puberty (Asdell, 1964).

Vaginal changes in the rat are directly and promptly related to the stages of the ovarian cycle. Consequently, a vaginal smear is an excellent means of determining the stage of the estrous cycle. Young et al. (1941) described each type of vaginal smear as it is related to the stage of the estrous cycle. According to these workers, proestrus lasts from the time of only small round nucleated cells to 75% cornified cells. Heat begins when 25% of the cells are cornified and 75% are flat nucleated epithelial cells. During metestrus, there are only flat

nucleated epithelial cells. Diestrus is characterized by both nucleated epithelial cells and leucocytes.

Okigaki (1959) reported the length of proestrus, 13.5 hours; estrus, 25.3 hours; metestrus, 13.3 hours; and diestrus, 56.5 hours.

The average age and weight at puberty varies considerably with the strain of rat and rate of growth. Cole and Casady (1947) reported that the age at puberty may vary from 41 to 76 days and that the weight may range from 88 to 127 g. According to Flow Laboratories (1970), their Dublin Disease Resistant (DDR) rats are 54 to 62 days of age and weigh from 135 to 150 g at puberty.

Boyd and Hamilton (1952) gave the time of ovulation to be 8.5 hours after the onset of estrus. The maximum fertile life of the ovum was found to be 12 hours and that of the spermatozoa to be about 14 hours.

According to Willet (1953) 16 to 20 hours are needed from the time of human chorionic gonadotrophin (HCG) injection before ova may be found in the oviduct. Similar results have been reported by Caseda (1938), Pincus (1940), and Zarrow et al. (1958).

Branden and Austin (1954) reported that 47% of the ova are fertilized during the first hour after ovulation, 54% by the end of the second hour, and 90% by the end of the third hour. These authors felt that this progression indicated that not only do the sperm need capacitation for maximum fertilization (2 hours), but the ova as well (2 to 4 hours).

Blandau (1945) found that semen is present in the uterine segment of the oviduct 15 minutes after copulation and that after 1 hour it is found in all segments of the tract. He suggested that the copulation plug was necessary for the effective insemination of the

female. The copulation plug has two projections which fit into the cervices of the duplex uterus (Nalbandov, 1964).

Witschi (1962) described the early development of the rat embryo. The first cleavage occurs about 1.5 days after fertilization while the embryo is still in the oviduct. The conceptus enters the uterus on the third day and is in the morula form. By 4.5 days it forms a blastocyst which is approximately 0.12 mm in diameter. During the fifth day post coitus implantation begins and is completed by the eighth day. By the tenth day the placenta is established and exchange begins between the fetal and maternal circulations.

The gestation period is 22 days with very little variation. One trial, in which the time was accurately obtained by the use of obstetrical cages, gave 90% of the litters born from 21.5 to 22 days. The average of all gestations was 21.8 days (Asdell, 1964).

The mean litter size varies considerably with the strain. Asdell (1964) reported that a range from 6 to 9 young is representative. However, Buss (1970) found a mean litter size of 10.2 from mature DDR females. Battaglia (1969) reported an average litter size of 9.9 from DDR females.

Prenatal mortality reduces the average litter size of all species and the rat is no exception. Asdell (1964) stated that prenatal mortality from the loss of ova and fetal atrophy account for about one third of the eggs ovulated. Harper (1964) reported a 10.8% preimplantation loss and 8.7% postimplantation loss in the albino rat. McLaren and Michie (1959), working with the white mouse, found a total prenatal loss of 18.7%. Battaglia (1969), in working with DDR rats, found an average prenatal mortality of 20.8%.

In the past there have been questions raised about the validity of estimates of prenatal mortality, particularly concerning errors in counting and the effect of laparotomy upon pregnancy. Allen et al. (1946), in a study on prenatal mortality in wild rabbits, investigated the reliability of estimates based on the breeding-laparotomy-number born technique. They concluded that in a polytocous species: (a) the death of some of the embryos seldom terminates the entire pregnancy, as the dead embryos are resorbed and do not interfere with the course of development of the remaining young; (b) since the corpora lutea are persistent structures, the total mortality of ova throughout pregnancy up to examination can be determined from a comparison of corpora lutea to embryos; and, (c) a cumulative error of 6.4% in the original counts of corpora lutea does affect 25.6% of the experimental litters, but it is an unbiased error.

Hormones of Pregnancy

It is surprising how little is actually known and how much is assumed about the role of hormones during gestation. The requirements for estrogens and progestins in the development of the reproductive tract, cyclic function, estrus receptivity and all the events leading up to conception are well documented. However, the role of these hormones in further development and maintenance of pregnancy are by no means well understood.

Ovaries: It is generally assumed that where the length of gestation exceeds the luteal phase of the cycle, new endocrine mechanisms must be evoked to maintain pregnancy (Amoroso, 1960). In the case of the ovaries, the presence of a conceptus causes changes to occur which transform the

corpora lutea of the cycle into corpora lutea of pregnancy. The morphological and functional maintenance of the corpora lutea is affected through luteotropic factors, of either pituitary or extrapituitary origin, released as a result of a conceptus (Catchpole, 1969). In the rat, Riddle (1963) reported that prolactin appears to be luteotropic.

Estrogenic hormones, such as estradiol, estrone, and estriol, are the predominant hormones at the time of fertilization and are secreted by the ovaries throughout pregnancy. Estrogenic effects on the reproductive tract include increased cellular mitosis and hyperplasis of the endometrial cells and glands, hypertrophy of muscle cells and synthesis of actinomycin, collagen synthesis, glycogen deposition in the uterine musculature, and water uptake by the uterus, cervix, and vagina (Catchpole, 1969). Guyton (1967) reported that estrogens also increased the number of ciliated epithelial cells which line the oviducts. Since the activity of the cilia are involved in ova transport, it was suggested that the cilia help propel the ovum toward the uterus.

Progesterone adds a qualitative change to the development of the uterus which was already prepared by the action of estrogens. The uterine mucosa undergoes an enormous complication in glandular structure, the "swiss cheese" appearance of progestational proliferation, which is necessary for successful implantation (Catchpole, 1969).

Amoroso (1952) reported that progesterone promotes secretory changes in the mucosal lining of the oviducts. These secretions supply nutrition for the fertilized, dividing ovum as it passes through the oviduct.

Progesterone also acts to stimulate the secretory endometrium which furnishes nutrients for the embryo prior to implantation. The frequency and magnitude of uterine contractions are also decreased by the action of progesterone. This decrease in uterine contractions prevents expulsion of the implanting fetus (Csapo, 1956).

Progesterone exerts a mild catabolic effect on the body proteins. Although this effect may not be significant during the normal sexual cycle, it may be important during pregnancy when proteins are mobilized for use by the fetus (Guyton, 1967).

Ryan and Ainsworth (1966) concluded that the corpora lutea seem to be the main, if not the only, source of progesterone in the rat since they could not isolate progesterone from the placenta.

Placenta: The placenta of the rat reportedly produces less than 1 mcg of estrogen per day and little, if any, progesterone (Canivenc and Mayer, 1951). However, the placenta does secrete large quantities of a luteotropic substance which is able to maintain the corpora lutea (Catchpole, 1969).

Ray et al. (1955) reported that the rat placenta luteotropin is probably prolactin since extracts of the placenta are both luteotropic and lactogenic, and stimulate weak crop sac activity.

The mouse placenta has been shown to secrete progesterone (Forbes, 1957). However, Forbes (1957) found that the amount of progesterone secreted was insufficient to maintain pregnancy after ovariotomy.

Pituitary: Lyons (1943) has shown that pregnancy is quickly terminated if the rat is hypophysectomized prior to day 11 of gestation. But, pregnancy is not interrupted if the operation is performed after

this time. On the basis of these findings, it was suggested that the rat pituitary is indispensable in the first half of pregnancy because of its follicle and luteal cell stimulating hormones which, in the proper ratio and dose, will cause estrogen secretion by the ovaries. The lactogenic hormone, LTH, which stimulates the luteal tissue to secrete progesterone, is also required.

Hormone Levels During Gestation

Wiest (1958) found that for pregnancy to be maintained the rat corpora lutea must produce at least 0.5 mg of progesterone daily to neutralize the normally occurring estrogens of pregnancy. He also reported that during the last third of gestation the rat ovary contains 7.9 mcg progesterone per g of ovarian tissue. The 4-pregnen-20-3-one content of the ovary was reported as 10.1 mcg/g of ovarian tissue.

Ryan and Ainsworth (1966) found that the peripheral blood level of progesterone was 7 mcg/100 ml in the pregnant rat. They also reported that 20α hydroxy Δ^4 pregnen-3-one is found in the peripheral circulation in concentrations around 3.0 mcg/100 ml.

In contrast to Canivenc and Mayer (1951), who found that the placenta secretes about 1 mcg of estrogen daily, Ryan and Ainsworth (1966) could not detect estrogenic secretions from this tissue.

Effect of Ovariotomy During Pregnancy

Several investigators have reported that pregnancy is not maintained in the rat after ovariotomy. Common characteristics to all animals in which the ovary is necessary for the maintenance of pregnancy are a short gestation period (Zarrow, 1961), a prolonged life of the corpora lutea until parturition (Amoroso and Finn, 1962), and a placenta in which

progesterone production has not been demonstrated (Ryan and Ainsworth, 1966).

Ovariotomy at almost any time terminates pregnancy, by abortion or resorption of fetuses, in the rat (Klein, 1935), mouse, rabbit, and cow (Ryan and Ainsworth, 1966). However, pregnancy may be maintained by the injection of estrogen or estrogen and progesterone in the proper amounts, varying with the species (Catchpole, 1969). If rats are ovariotomized from 1 to 4 days post coitus, implantation is inhibited (Nutting and Meyer, 1963; Buchanon, 1969; Mayer, 1963). However, neither cell division nor oviduct transport of the conceptus is affected, and the blastocysts enter the uterine horns at the expected time (Psychoyos, 1966).

Although the blastocysts enter the uterine horns after ovariotomy, Buchanon (1969) found that they usually die unless exogenous hormones are administered before the eighth day of gestation.

In addition to supplementing estrogen and progesterone, certain additional operative procedures permit pregnancy to persist in the absence of the ovaries. Reduction of the fetuses to one in the rat, leaving all placental sites intact, allows pregnancy to continue, possibly by allowing a pooling of the placental hormones (Haterius, 1936). Also, in the rat and rabbit, release of the fetuses into the intraperitoneal cavity circumvents fetal death, implying that one effect of castration is to cause loss of uterine resilience (Corrier and Colonge, 1950).

Courrier (1952) concluded that the essential function of the corpora lutea secretions, during the postimplantation period, is their effect on the uterine musculature. Zeiner (1943) observed that embryo death after

ovariotomy appears to be the result of pressure from the uterine wall.

Kelsey and Meyer (1960) removed all but one or two of the corpora lutea on the eighth day of pregnancy. They found that two corpora lutea maintained gestation in most rats. With less luteal tissue, gestation was not sustained.

Kraicer (1969) reduced the corpora lutea to three or less on the fifth day post coitus. In 17 of 18 rats with one corpus luteum, there was no ova implantation. Normal ovum implantation was obtained in 11 of 15 rats with two intact corpora lutea; however, all rats had signs of resorption in later gestation. All 11 rats with three corpora lutea had normal implantation, and 9 of the rats had at least one live fetus at 20 days of gestation. In the 11 rats with three corpora lutea, there were 55 live fetuses and 43 resorbing fetuses at term.

Pulkkinen and Csapo (1969) reported that bilateral ovariotomy performed on the 13th or 14th days of gestation result in the abortion of the fetuses since the placentae can not effectively compensate for the sudden loss of luteal secretion. The fetuses of animals ovariotomized on the 13th to 14th days of gestation die within 48 hours unless the mother is given progesterone. This "decemental" progesterone substitution therapy needs to be employed for 4 to 6 days as a temporary aid to maintaining pregnancy.

In contrast, Csapo (1969) found that ovariotomy, if performed on the 16th to 17th days of gestation, resulted in over 60% fetal survival at day 21 of pregnancy. The success of the ovariotomized animals in maintaining pregnancy was distinctly dependent on placental hypertrophy. If this placental hypertrophy did not occur, premature delivery started

on the 19th or 20th day. In those fetuses which went to term, the incidence of fetal defects was greatly increased over non-ovariotomized controls. Deformative hemorrhage, missing extremities and hydramnios were frequent, especially in the two fetuses located at the cervical end of the horn.

According to Csapo (1969) and Pulkkinen and Csapo (1969), the development of a "hostile" uterine environment after ovariotomy was readily prevented by "sustaining" progesterone replacement therapy with the minimum effective dose being 2 mg/day. The results of Csapo (1969) support the work of Zeiner (1943) who indicated that the detrimental effects of ovariotomy appear to be the pressure of the contracting uterine wall.

Induction of Ovulation and Pregnancy in Prepuberal Laboratory Animals

Smith and Engle (1927) were the first to show that superovulation could be induced in adult rats. They used daily implants of anterior pituitary tissue to achieve 20 to 48 ova per animal and from 19 to 29 implantations.

Cole (1937) induced mating and pregnancy in 26 to 31 day old Long-Evans rats (maturity reached from 33 to 55 days of age) by using 8 rat units of pregnant mares serum. The average litter size was 12.6.

Evans and Simpson (1940) used 10 and 15 rat units of FSH to induce ovulation and pregnancy in 26 to 34 day old rats. Of 45 rats treated with 15 rat units of FSH, 14 mated and 10 had litters at term, with an average litter size of 5. Of 15 rats injected with 10 rat units of FSH, 8 had litters at term, with an average litter size of 8. Evan and Simpson (1940) attributed the low birth rate to high levels of fetal reabsorption

after implantation.

Rowlands (1944) treated 40 to 50 g rats with varying levels of PMS. Injection of from 2 to 60 IU resulted in the ovulation of only 0 to 5 ova. However, injection of 30 IU of PMS followed 56 hours later by 20 IU of HCG produced an average ovulation of 26 ova. Doses of PMS in excess of 30 IU were found to be detrimental to follicular development.

Austin (1949) studied the preimplantation stages of embryonic development in sexually immature rats. Prepuberal rats 30 to 45 days of age (40 to 50 g) were treated with 20 IU of PMS, followed 56 hours later by 20 IU of HCG. Austin (1949) concluded that the poor reproductive performance of immature rats was due to the incomplete state of estrus (34% of the treated rats mated), low number of fertilized ova (16.4 ova fertilized from an average of 37.2 ovulations), and a high rate of fragmentation (of 16.4 ova fertilized, only 5.2 were normal 48 hours post coitus). By day 9 post coitus, the mean number of implantations was only 4.1.

Zarrow and Wilson (1961) studied the effect of age on the ability to induce superovulation by use of PMS and HCG. The method of inducing ovulation was held constant by using 30 IU of PMS followed 56 hours later by 10 IU of HCG. Using Westar strain rats, they reported that the average number of ova released showed an increase from one ovum per rat at 17 to 18 days of age to a maximum of 55 ova per rat at 23 to 32 days of age. After 32 days the count fell to 18 ova on 39 to 40 days of age. In contrast to rats treated at an earlier age, all rats 39 to 40 days of age ovulated as compared to 75% of the rats ovulating at 19 to 20 days of age.

Zarrow and Quinn (1963) studied the effect of using only PMS in contrast to using PMS and HCG to cause ovulation. They found that 30 IU of PMS and 10 IU of HCG was effective on 20 to 40 day old rats. But, PMS alone was effective starting on the 26th day of age. Treatment with PMS and HCG resulted in an initial average response of.03 ova at 18 days of age and an average maximum response of 61 ova at 22 days of age. PMS alone caused an initial average response of 2.8 ova at 20 days and a maximum response of 70.8 ova at 28 days of age. By 45 days of age, a drop to 8 to 10 ova was observed in both treatments.

Starkey (1969) used DDR rats 35 and 45 days of age (puberty reached 54 to 62 days of age) to study the reproductive performance of immature rats. He reported average ovulation rats of 26.5 and 14.2 in 35 and 45 day old rats, respectively. Thirty eight percent of the 35 day old and 48% of the 45 day old rats mated. In 22 rats 35 days of age, 1 had implantations at 10 days of gestation but resorbed the fetuses prior to term. Of 30 rats 45 days old, 1 had implantations and later gave birth to 9 living pups.

Sato (1962) treated immature rats with 20 IU of PMS and HCG. Eight of 35 females receiving the injections showed vaginal openings 40 to 60 hours after the injection of PMS. However, no matings occurred in these prepuberal animals.

In prepuberal mice, Runner and Gates (1954) and Smithberg and Runner (1956, 1957) have induced estrus and ovulation with PMS and HCG. Although several mice mated and possessed fertilized ova, all litters were lost by the eighth day post coitus.

Superovulation and Pregnancy in Prepuberal Hogs

Recent attempts have also been made to induce pregnancy in prepuberal gilts by use of PMS and HCG. Dziuk and Gehlbach (1966) induced ovulation in 95 to 130 day old gilts with 500 to 1250 IU of PMS and 500 IU of HCG. Fifty to 80 hours after artificial insemination, 81 ova were recovered from 18 gilts; of the 81 ova, 55 (68%) were fertilized. Twenty three days after insemination, 3 of 18 gilts had implanting fetuses as determined by laparotomy. However, these 3 animals did not maintain pregnancy to term. Similar results were reported by Baker and Coggins (1967).

Baker and Coggins (1966) treated 8 gilts (100 to 180 days old) with 500 IU of PMS and 500 IU of HCG. Three of the 8 had litters at 60 days of gestation with an average of 4.3 fetuses per litter.

Ellocott et al. (1969) induced ovulation in 3 to 4 month old gilts but found that pregnancy could not be maintained unless exogenous hormones were administered.

Effect of Exogenous Hormones on Pregnancy

The effect of exogenous hormones on establishing and maintaining pregnancy has been studied most frequently in either animals which are physiologically unable to secrete estrogen and progesterone (Fowler and Edwards, 1960; Smithburg and Runner, 1956, 1957), or in animals which have been ovariotomized (Kim and Foreman, 1968; Mayer, 1963; Nutting and Meyer, 1964; Cochrane and Meyer, 1957; Rubenstein and Forbes, 1963; Buchanon, 1969).

Smithburg and Runner (1956) induced and maintained pregnancy in prepuberal mice by daily injections of progesterone. In the intact group receiving 2 mg progesterone per day, 88.8% of the treated mice had full

term fetuses with an average of 3.7 fetuses per pregnant animal. In those mice castrated and maintained on 2 mg progesterone per day, 76% of the treated animals had full term fetuses with an average litter size of 2.6. According to the authors, the prepuberal mouse ovary, although capable of responding to gonadotrophins, does not possess the ability to maintain pregnancy due to incomplete differentiation of the corpora lutea.

Fowler and Edwards (1960) treated superovulated adult mice with estrogen and progesterone to study the effect on embryonic mortality. They found that short term treatment with progesterone during early pregnancy did not influence implantation or fetal mortality. However, 2 mg of progesterone daily from day 2 to 12 reduced the number of embryos which implanted and increased fetal mortality after implantation. Estrogen benzoate, given after mating, reduced fertility by decreasing the proportion of mice with implanting embryos. The adverse effects of the estrogen could not be alleviated by administration of progesterone.

Mayer (1963) ovariotomized pregnant rats on the fourth day post coitus and maintained viable embryos with from 1 to 10 mg of progesterone per day. Implantation was induced by a single injection of 1 mcg of estradiol. Psychoyos (1961) has shown that as little as 0.1 mcg estradiol will cause implantation in ovariotomized rats if the embryos are kept viable by daily injections of progesterone.

If ovariotomy is performed after noon on the fourth day post coitus, progesterone alone is sufficient to cause implantation and maintain pregnancy. Apparently there is an "estrogen surge" on day four which is necessary for nidation to occur (Mayer, 1963; Nutting and Meyer, 1963; Psychoyos, 1966; Cochrane and Meyer, 1957).

Saunders and Elton (1959) ovariotomized rats on the eighth day post coitus and found that a minimum of 10 mg progesterone per day per kg of body weight was necessary before any live fetuses could be found on the 20th day of gestation. In order to maintain pregnancy in all ovariotomized rats, 50 mg progesterone per kg of body weight per day was needed.

Saunders and Elton (1959) also found that pregnancy could be maintained in ovariotomized rats by either 20 mg per day per kg body weight of 17 α ethyl-19-nortestosterone, or 5 mg per day per kg body weight of 17 α (2 methallyl)-19-nortestosterone or 1 mg per day per kg body weight of 6α methyl-17 α acetoxyprogesterone.

Yochim and Zarrow (1961) ovariotomized mature rats on the 12th day of gestation and maintained pregnancy by exogenous estradiol and progesterone. Maximal postimplantation survival was obtained with a ratio of 1:50,000 (estradiol:progesterone). With 0.04 mcg estradiol and 2 mg progesterone daily, 87% of the implanted fetuses survived as compared to 73% fetal survival in rats ovariotomized and maintained only on 2 mg progesterone daily.

Lerner et al. (1962) ovariotomized rats on the eighth day post coitus and attempted to maintain pregnancy with varying levels of progesterone and with a combination of estrogen and progesterone. Rats maintained on 20 mg of progesterone per day had an average litter size of 7.9 with an average of 10.7 implantation sites. All rats maintained on 20 mg progesterone daily had litters at 20 days post coitus. Of those rats maintained on 10 mg progesterone daily, 71% had live pups with an average litter size of 5.9. On 5 mg progesterone daily, the average litter size dropped to 4.2. If 1 mcg of estrone was supplemented with

the varying levels of progesterone, it was found that 10 mg progesterone daily was sufficient to maintain pregnancy in all rats, with the average litter size being 9.6.

Kim and Foreman (1968) also ovariotomized rats on the eighth day post coitus and maintained pregnancy with progesterone. In addition, they histologically examined fetuses from each of the treated females on day 12 post coitus. Of 18 embryos examined from females maintained on 20 mg progesterone daily, 17 were normal and one was degenerating. Of those females maintained on 10 mg progesterone daily, 15 of the fetuses were normal and 4 were degenerating. When 1 mcg estrone was supplemented to the 10 mg progesterone, 15 fetuses were found to be degenerating and 5 were normal. Of 20 fetuses examined from females maintained on 1 mcg estrone, all were degenerating.

Csapo and Pulkkinen (1969) and Csapo (1969) reported that female rats ovariotomized on day 13 or 14 of gestation must be maintained on 2 to 4 mg progesterone daily in order to prevent abortion. If ovariotomy was performed on the 16th or 17th day of pregnancy, progesterone treatment was not necessary due to "placental hypertrophy".

Lyons (1943) maintained pregnancy in mature rats which were hypophysectomized and ovariotomized on the seventh to ninth days of gestation. By administration of estrone and progesterone 45 living (range 3 to 9) and 18 resorbing (range 1 to 6) fetuses were found in a group of seven animals, maintained on 1 mcg estrone and 4 mg progesterone. On the basis of these results, Lyons (1943) concluded that 1 mcg estrone and 4 mg progesterone can substitute for the ovaries during pregnancy.

Allen and Heckel (1937) demonstrated that pregnancy can be maintained to term in rabbits castrated on the 11th day post coitus if 2 mg progesterone were given from 11 to 15 days and 4 mg from days 15 to 28 of gestation.

Rubinstein and Forbes (1963) used subcutaneous implants of crystalline progesterone and 16-hydroxyprogesterone to maintain pregnancy in ovariotomized mice. Pregnancy was sustained with crystalline progesterone but not with 16-hydroxyprogesterone. The exogenous amount of progesterone needed deminishing as pregnancy progressed. A fetal survival rate of 90% was associated with an average estimated daily absorption rate of at least 0.91 mg of progesterone.

Zarrow et al. (1969) have reported that pregnancy is prolonged in sexually immature rats which mate after PMSG treatment. The average length of gestation for mature control, immature receiving 15 IU of PMSG, and 30 IU of PMSG was 21.4 days, 22.9 days and 24.8 days, respectively.

Day et al. (1963) implanted exogenous progesterone and estrogen into 132 intact gilts to study the effect on embryonic mortality. They administered 100 mg of progesterone caproate and 50 mcg of estradiol benzoate per 100 lb body weight on the 11th day of gestation. Although the treatment failed to statistically improve embryonic survival, a definite trend toward increased litter size was observed.

Ellicott et al. (1969) attempted to maintain pregnancy in prepuberal gilts 3 to 4 months of age after ovulation was induced by PMS and HCG. One group was fed ethynyl estradiol and 6 methyl-17-acetoxy progesterone

(MAP) or melengestrol acetate (MGA). Group 2 was implanted subcutaneously with a silicone capsule containing estrogen and a progestin. Group 3 served as controls. At 30 days post coitus, the following proportion of treated animals were pregnant: group 1, 8/24; group 2, 5/24; group 3, 0/5. In 5 gilts in group 1 which were continued on treatment, 2 farrowed litters of 5 and 6.

Zimbelman and Smith (1965) ovariotomized dairy heifers 56 days post coitus and maintained pregnancy in 7 of 9 heifers treated with 4 mg of melengestrol acetate (MGA) per day. Daily doses of less than 4 mg resulted in abortion or fetal mummification.

Day et al. (1959) ovariotomized gilts on the 15th day after mating and slaughtered on the 25th day of gestation. Thirteen of 17 ovariotomized gilts injected daily with 100 mg progesterone and 50 mcg of estradiol benzoate per 100 lb body weight were pregnant when slaughtered. The average embryonic mortality in the 13 ovariotomized gilts at 25 days of pregnancy was 22% in comparison to 33% in the intact gilts slaughtered at the same stage of pregnancy.

Effect of Exogenous Hormones on Histology of the Female Tract

Kim and Foreman (1968) analyzed the uterine epithelial height of mature rats which were ovariotomized on day 8 post coitus and maintained pregnant by exogenous hormones until day 12. The mean epithelial height for normal controls, 20 mg progesterone daily, 10 mg progesterone daily, 10 mg progesterone with 1 mcg estrone daily, and 1 mcg estrone daily was 20.8 μ, 19.4 μ, 18.8 μ, 18.1 μ and 33.9 μ, respectively. These epithelial height differences were all significantly different.

Wilson and Zarrow (1962) histologically examined the ovaries of immature rats which were superovulated by high doses of PMS. Results showed that doses of PMS in excess of 75 IU resulted in low levels of superovulation due to the formation of large cystic follicles. In the large cystic follicles, the granulosa cells appeared to degenerate and the follicles were enlarged and filled with fluid. There was also a considerable amount of luteinization present in the thecal cell layer.

Green (1960) found that in mice high doses of PMS will cause premature luteinization of certain follicles. These luteinized follicles were characterized by intense luteinization of the granulosa cell layer. The entrapped ova were usually necrotic and floated free within the follicle. He reported that there appeared to be a proliferation of the interstitual tissue.

Howe et al. (1964) histologically examined the endometrium of prepuberal Holstein heifers treated with estrogen, progesterone, PMS and HCG. Group 1 served as controls, group 2 received PMS and HCG, group 3 received 50 to 100 mg of progesterone for 5 days followed by PMS and HCG: and, group 4 was treated the same as group 3 except that 10 to 25 mg of estradiol-17 was administered. Epithelial cell height from the uterine mucosa for groups 1, 2, 3,and 4 were 15.0 μ, 28.1 μ, 23.5 μ, 29.3 μ, respectively.

Smallwood and Sorensen (1969) fed feedlot heifers Repromix, containing the synthetic progestin medroxyprogesterone acetate, for 18 days at a rate of 180 mg per head per day. Animals were killed 2, 3, 4, and 5 days after the drug was stopped; estrus and ovulation occurred on day 3 or 4. The day means of the epithelial height increased from 18.4 μ on day 2 to 23.4 μ on day 3 and finally to 39 μ on day 4. On day 5, the

epithelial height dropped to 19.7 μ. These results are in close agreement with the work of Johnson (1965).

Schultz et al. (1969) reported the results of a karyometric study on the epithelial cells of the bovine endometrium. Cows were ovariotomized on the 10th day of the estrus cycle and then treated for three days with various hormones. The mean nuclear size for the group receiving 100 mg of progesterone per day, 3 mg of estradiol, and the control group were 33.58 μ, 31.19 μ and 29.19 μ, respectively. Statistical analysis showed that there was a highly significant difference in nuclear size between all groups. These researchers concluded that the nuclei of the epithelial cells lining the endometrium respond directly or indirectly to hormone influence by an increase in area which can be detected by karyometry examination. They further suggested that the increase in nuclear area may be associated with an increased cell secretion rate.

OBJECTIVES

1) To determine if the prepuberal DDR rat is able to maintain pregnancy after induced ovulation.
2) To elucidate the progesterone requirements so as to maintain pregnancy in the prepuberal DDR rat.
3) To histologically compare the uteri of prepuberal and mature DDR rats.

MATERIALS AND METHODS

Animals

Seventy-five mature female Dublin Disease Resistant (DDR), 30 mature male DDR, and 520 prepuberal female DDR rats constituted the experimental animals used in this study. The 30 mature males and 375 prepuberal rats were purchased from Flow Laboratories, Inc., Dublin, Virginia. At the time of purchase, the male rats weighed 175 to 200 g and were approximately 90 days of age. The prepuberal female rats were 23 to 33 days old at the time of purchase. The remaining 145 prepuberal females and the 75 mature females were raised from the purchased stock.

Feeding

All rats were provided with water and feed ad libitum. The feed was a commercial ration[1] containing a guaranteed composition of 24% minimum crude protein, 4% minimum crude fat, and 4.5% maximum crude fiber.

Housing

Animals were housed in a building 11.58 m x 6.09 m x 2.43 m (L x W x H) with illumination controlled at 16 hr light and 8 hr dark. Prepuberal rats were kept in large cages, 25.4 cm x 54.4 cm x 20.3 cm, with 10 to 15 females per cage. Mature females were housed, five per cage, in cages 25.4 cm x 36.6 cm x 20.3 cm. The breeding males were housed, one to a cage, in cages 25.4 cm x 17.8 cm x 20.3 cm. After surgery, gestating females were kept, two in a cage, in cages 25.4 cm x 17.8 cm x 20.3 cm.

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[1]Wayne Lab Blox, a commercial rat, mouse, and hamster feed manufactured by Allied Mills, Wayne Laboratory Diet Division, Fort Wayne, Ind.

Gonadotrophin Administration

Prepuberal female DDR rats, 45 days of age, were subcutaneously injected with 20 IU of pregnant mare serum (PMS)[2] between 10 and 11 pm. Fifty-six hours after treatment with PMS, the rats were subcutaneously injected with 20 IU of human chorionic gonadotrophin (HCG)[3]. The stock solution of HCG was diluted, at the time of use, with physiological saline so that 20 IU of HCG was carried in 0.4 cc of diluent.

Breeding

One mature female was placed with a male and checked daily for copulation plugs. Each of the prepuberal females was placed with a male 4 to 8 hours after HCG treatment and examined the following morning for copulation plugs or sperm deposits. The prepuberal females were left with the males only for the night succeeding HCG administration. Mature females were left with the males until bred.

Hormone Administration

Progesterone[4] was dissolved in peanut oil so that 0.4 cc contained the appropriate quantity of the hormone. Prepuberal mated rats received 0.4 cc of the peanut oil with 1.5, 2.5, 3.5, 5.0, or 10.0 mg progesterone daily from day 2 to day 20 post coitus. Prepuberal control rats received only the peanut oil from day 2 to 20 post coitus.

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[2]Gonadin, a pregnant mare serum in a concentration of 50 IU per cc. Manufactured by Cutter Laboratories, Berkeley, California.

[3]Chorionic Gonadotrophin, a human chorionic gonadotrophin containing 250 IU per cc. Manufactured by Haver-Lockhart Laboratories, Kansas City, Missouri.

[4]Progesterone (Δ^4-pregnen-3,20-dione). Distributed by Sigma Chemical Company, St.Louis, Missouri.

Estrogen[5] was dissolved in peanut oil so that 0.4 cc contained 0.2 mcg of estrogen. Rats treated with estrogen received the compound subcutaneously on the third day post coitus.

Laparotomy and Ovariotomy

Laparotomies were preformed on the eighth or ninth day post coitus, just after implantation occurs in the rat. Laparotomies were performed while the rat was under ether anaesthesia. After appropriate asceptic preparation of the abdomen, a midventral incision was made approximately 1 to 2 cm below the xiphoid process and extending caudally for approximately 3 to 4 cm. Through this incision, the uterine horns were exposed and counts made of the number of normally developing embryos. At this time, any abnormalities or resorptions were noted and recorded. Corpora lutea counts were not made during laparotomy because it would have necessitated subjecting the rat to excessive trama, since the ovaries are difficult to locate and expose without hemorrhage, and since the ovarian bursa must be removed to obtain accurate counts.

For those females which were to be ovariotomized, the same surgical procedure was followed. Through the incision, the ovaries were exposed, tied off, and removed. After recording implantations, the uterine horns were replaced, the incision sutured, and the area treated with scarlet oil.

Laparotomy, for the most part, had little effect on the gestating rat. However, any laparotomized or ovariotomized rat which showed

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[5]16-Epiestriol. A 16β Hydroxy-17β -estradiol compound. Distributed by Sigma Chemical Company, St.Louis, Missouri.

signs of abdominal infection was removed from the trial.

Determination of Embryonic Survival

Ovulation rates were determined by the number of corpora lutea observed at necropsy on the 20th day of gestation. Preimplantation survival rates were determined by comparing the number of corpora lutea with the number of uterine bulges counted at laparotomy. Postimplantation survival was determined by comparing the number of developing fetuses at laparotomy with the number of fetuses observed at necropsy. Total embryonic survival was determined by a comparison of the number of corpora lutea with the number of fetuses at necropsy.

Histological Procedures

To histologically compare the effect of various hormones and gonadotrophins on the reproductive tract of the rat, three animals were killed at the time periods listed below. The treatments were as follows: (1) prepuberal mated rats killed at 3, 5, 7 and 9 days post coitus; (2) mature mated rats killed at 3, 5, 7 and 9 days post coitus; (3) prepuberal rats induced to ovulate and receiving 3.5 mg progesterone per day and killed at 3, 5, 7 and 9 days post ovulation; (4) prepuberal, untreated; (5) PMS + 56 hours; (6) PMS-HCG + 24 hours; (7) PMS-HCG + 48 hours; (8) PMS-HCG + 72 hours; (9) mature rats in estrus; and, (10) mature rats in diestrus.

After the rats were killed by cervical dislocation, the reproductive tracts were removed and fixed for 48 to 72 hours in neutral buffered formalin. After fixing, the tissues were washed and then stored in 70% alcohol until weighed. After weighing, a 1 cm section of the uterine horn was removed approximately 1 cm posterior from the bifurcation of

the cervices. The tissue was embedded in paraffin and cross sections of the horn cut at 6 microns. After mounting, the tissue was deparaffinized and stained with Harris' hematoxylin and eosin, using routine histological procedures.

Photomicrographs were taken of the uterine endometrial area using a 100X oil immersion objective and a 10X ocular. Kodak Panatomic X film was used for all photographing. A series of 3 to 5 photomicrographs was taken from each of 3 slides from each rat with a total of 9 to 15 per animal. Enlargements were made from the negatives so as to give a final magnification of 2500X. The outlines of 10 to 15 "normal" nuclei per animal were measured using a compensatory polar planimeter. Two measurements were taken from each nucleus and the average used for analysis. Normal nuclei were defined, for this study, as those which were well in focus and did not appear pyknotic. In addition, 10 to 15 cells per animal were measured to determine endometrial cell height. Measurements were made from the basement membrane to the free surface of the cell using a calibrated 12X ocular and a 100X oil immersion objective.

Statistical Procedures

Analyses of variance, with one-way classifications, were used to determine the effect of gonadotrophin administration and exogenous hormones upon ovulation rate, number of rats maintaining pregnancy, average litter size,percent prenatal survival, and the effect of ovariotomy on litter size and prenatal survival. The analysis of variance was modified to accommodate the unequal sample size as described by Li (1964).

All percentage data was transformed to arc-sin equivalents prior to analysis.

Analysis of variance, with equal sample size, was used to determine differences in reproductive tract weight, endometrial cell height, and endometrial cell nuclear area. A randomized block analysis was used to compare prepuberal control and progesterone treated rats for differences in reproductive tract weight, endometrial cell height, and endometrial cell nuclear area.

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

RESULTS

Induction of Vaginal Opening, Estrus and Mating

Subcutaneous administration of PMS and HCG appears to be quite efficient for inducing vaginal opening and estrus in the prepuberal rat. Vaginal orifices were found to be patent in 412 of 420 rats (98.0%) on the second day after PMS administration. Although the vaginal smears were sometimes difficult to judge, approximately 75% of 120 rats examined 18 to 24 hours after HCG treatment provided smears consisting purely of cornified cells.

Despite the fact that approximately 75% of the rats were in estrus as indicated by the vaginal smear, only 170 of 420 (40.4%) of the PMS-HCG treated rats mated during their 24 hours of exposure to a male.

Reproductive Performance of Control and Progesterone Treated Rats

Table I contains the means, standard errors and statistical analysis for the number of rats with fetuses and average litter size per mated rat in the prepuberal progesterone, mature control, and prepuberal control groups.

At 8 days post coitus, there was no significant difference ($P>.05$) between the mature control and the 3.5 mg progesterone group in the number of mated animals with implanting fetuses. The difference in the number of animals with fetuses was significant ($P<.05$) between these two groups and the groups receiving 1.5, 2.5, 5 and 10 mg progesterone daily. The number of mated animals with fetuses at 8 days post coitus was significantly ($P<.05$) smaller in the prepuberal control as compared to all other groups.

Table I

Means, Standard Errors and Differences in Conception Rate and Litter Size Per Mated Rat in Prepuberal Progesterone Treated and Mature Control Rats

Treatment	No. of Mated Rats	No. of Rats with Fetuses[a] 8 days p.c.	No. of Rats with Fetuses[a] 20 days p.c.	Average Litter Size Per Mated Rat[b] 8 days p.c.	Average Litter Size Per Mated Rat[b] 20 days p.c.
Prepuberal					
Control	30	2 (6.6)[c]	2 (6.6)[c]	0.56 ± 0.3[e]	0.53 ± 0.5[c]
1.5 mg	15	5 (33.3)[d]	3 (20.0)[c]	2.8 ± 1.0[d,e]	1.4 ± 0.8[c]
2.5 mg	15	7 (46.6)[d]	6 (40.0)[c]	3.3 ± 1.1[d,e]	2.8 ± 1.0[d]
3.5 mg	15	12 (80.0)[e]	11 (73.3)[d]	6.0 ± 1.3[c]	5.2 ± 1.1[d,e]
5.0 mg	15	5 (33.3)[d]	4 (26.6)[c]	1.9 ± 0.8[d,e]	1.5 ± 0.6[c]
10.0 mg	15	7 (46.6)[d]	4 (26.6)[c]	1.8 ± 0.7[d,e]	1.2 ± 0.7[c]
Mature					
Control	15	13 (86.6)[e]	13 (86.6)[d]	9.1 ± 0.9[c]	7.7 ± 1.1[e]

a Percent of mated animals with fetuses in ( ).

b Total number fetuses divided by the number of animals mated.

c,d,e Values within columns with same superscript not significantly different (P>.05).

The number of mated rats with fetuses at 20 days post coitus in the mature controls and the 3.5 mg progesterone group was not significantly different. However, there was a significant difference ($P < .05$) between these two groups and the prepuberal control, 1.5, 2.5, 5, and 10 mg progesterone groups. At 20 days post coitus, the differences between the prepuberal control group and the 1.5, 2.5, 5, or 10 mg progesterone groups was not significant.

At 8 days post coitus, there was no significant difference in litter size per mated rat between the mature control and 3.5 mg progesterone groups. However, there was a significant difference ($P < .05$) in litter size per mated rat in the mature control and 3.5 mg progesterone groups as compared to the 1.5, 2.5, 5, 10 mg progesterone groups and the prepuberal control group.

At 20 days post coitus, the average litter per mated rat was significantly greater ($P < .05$) in the 3.5 mg progesterone group as compared to the 1.5, 2.5, 5, and 10 mg progesterone groups. There was no significant difference between the mature control and 3.5 mg progesterone groups in litter size per mated rat at 20 days post coitus.

Table II contains the means, standard errors, and differences in the reproductive performance of the control and progesterone treated rats which maintained pregnancy.

The ovulation rates for pregnant rats were 11.3, 12.0, 12.6, 11.4, 13.7, 12.0, and 14.0 for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5, and 10 mg progesterone groups, respectively. There were no

Table II

Means, Standard Errors and Differences in Corpora Lutea Counts, Litter Size Per Pregnant Rat, and Number of Resorbing Fetuses in Prepuberal Progesterone Treated and Mature Rats

Treatment	No. Rats Mated	No. Rats Pregnant[a]	No. of C.L.[b]	Average Litter Size Per Pregnant Rat		Average No. Resorbing Fetuses at 20 days p.c.
				8 days p.c.	20 days p.c.	
Prepuberal						
Control	30	2[c]	12.0 ± 0.7[c]	8.5 ± 0.3[c,d,e]	8.0 ± 0 [c,d]	0.50 ± 0.3[c]
1.5 mg	15	5[d]	12.6 ± 0.6[c]	8.4 ± 1.0[c,d,e]	4.2 ± 0.2[d,e]	4.20 ± 1.3[c]
2.5 mg	15	7[d]	11.4 ± 0.9[c]	7.1 ± 1.4[c,d,e]	6.0 ± 1.4[c,d,e]	1.14 ± 0.9[c]
3.5 mg	15	12[e]	13.7 ± 0.8[c]	9.0 ± 1.0[c,d]	7.8 ± 1.4[c,d]	1.16 ± 0.6[c]
5.0 mg	15	5[d]	12.0 ± 0.6[c]	5.8 ± 1.3[d,e]	4.6 ± 1.2[d,e]	1.20 ± 0.3[c]
10.0 mg	15	7[d]	14 ± 1.6[c]	3.8 ± 1.1[e]	2.7 ± 1.3[e]	1.14 ± 0.3[c]
Mature						
Control	15	13[e]	11.3 ± 0.3[c]	10.5 ± 0.3[c]	8.9 ± 0.7[c]	1.61 ± 0.6[c]

a Rat considered pregnant if implanting fetuses found at laparotomy on day 8.

b Total number of C.L. on both ovaries 20 days post coitus.

c,d,e Values within columns with same superscript not significantly different (P>.05).

significant differences ($P > .05$) among these groups in ovulation rate.

The average litter size at 8 days post coitus in rats which maintained pregnancy was 10.5, 8.5, 8.4, 7.1, 9.0, 5.8 and 3.8 for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5 and 10 mg progesterone groups, respectively. The average litter size per pregnant rat was significantly ($P < .05$) reduced, at 8 days, in the 5 and 10 mg progesterone groups as compared to all other treatments.

At 20 days post coitus, the average litter size per pregnant rat was 8.9, 8.0, 4.2, 6.0, 7.8, 4.6, and 2.7 for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5 and 10 mg progesterone groups, respectively. The average litter size per pregnant rat at 20 days post coitus was significantly ($P < .05$) reduced in the 1.5, 5 and 10 mg progesterone groups as compared to the other treatments.

The average number of resorbing fetuses at 20 days post coitus, calculated from the number of viable fetuses on day 8 post coitus, was 1.4,0.5, 4.2, 1.1, 1.2, 1.2 and 1.1 for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5 and 10 mg progesterone groups, respectively. The differences between groups in the number of resorbing fetuses at 20 days post coitus was not significant.

Table III contains the means and standard errors for preimplantation, postimplantation, and total prenatal survival rates of the progesterone and control pregnant rats. In table IV, the arc sin transformed values for the same parameters are given along with the statistical analysis.

The 78.4% preimplantation survival rate for the mature pregnant control group was significantly different ($R < .05$) from the values of 55.4%, 54.6%, 53.4%, 52.6%, 50.1% and 54.0% for the prepuberal control,

Table III

Means and Standard Errors for
Preimplantation, Postimplantation, and Total Survival Rates
of Pregnant and Progesterone Treated Prepuberal Rats

Treatment	No. Rats Mated	No. Rats Pregnant[a]	% Preimplantation Survival[b]	% Postimplantation Survival[c]	% Total Survival[d]
Prepuberal					
Control	30	2	71.2 ± 1.0	94.4 ± 5.2	67.1 ± 6.3
1.5 mg	15	5	65.7 ± 6.1	41.3 ± 18.3	30.8 ± 14.7
2.5 mg	15	7	64.2 ± 16.8	76.2 ± 16.1	50.8 ± 12.0
3.5 mg	15	12	58.2 ± 7.6	75.3 ± 8.4	55.3 ± 7.6
5.0 mg	15	5	59.0 ± 5.6	64.5 ± 15.6	38.1 ± 9.7
10.0 mg	15	7	50.1 ± 13.6	40.8 ± 15.9	23.7 ± 12.4
Mature					
Control	15	13	92.6 ± 2.4	84.8 ± 6.2	78.4 ± 5.8

[a] Rat considered pregnant if implanting fetuses found at laparotomy on day 8 post coitus.
[b] (Number C.L. divided by the number implanted fetuses on day 8) x 100.
[c] (Number fetuses on day 8 divided by the number fetuses on day 20) x 100.
[d] (Number fetuses on day 20 divided by the number C.L.) x 100.

Table IV
Differences in Prenatal Survival Rates
Between Control and Progesterone Treated Rats (Arc-Sin Transformed)

Treatment	No. of Mated Rats	No. of Pregnant Rats[a]	% Preimplantation Survival[b]	% Postimplantation Survival[c]	% Total Survival[d]
Prepuberal					
Control	30	2	55.4[e]	90.0[e]	58.5[e,f]
1.5 mg	15	5	54.6[e]	36.8[e]	27.3[f,g]
2.5 mg	15	7	53.4[e]	65.1[e]	42.2[e,f,g]
3.5 mg	15	12	52.6[e]	63.8[e]	47.4[e,f]
5.0 mg	15	5	50.1[e]	54.0[e]	34.7[g]
10.0 mg	15	7	54.0[e]	37.2[e]	21.7[g]
Mature					
Control	15	13	78.4[f]	72.0[e]	63.9[e]

[a] Rat considered pregnant if implanting fetuses found at laparotomy on day 8 post coitus.
[b] (Number C.L. divided by the number implanting fetuses on day 8) x 100.
[c] (Number fetuses on day 8 divided by the number fetuses on day 20) x 100.
e,f,g Values within columns with same superscript not significantly different ($P > .05$).

1.5, 2.5, 3.5, 5, and 10 mg progesterone groups.

There were no significant differences ($P > .05$) in the postimplantation survival rates of 72.0%, 90.0%, 36.8%, 65.1%, 63.8%, 54.0%, and 37.2% for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5, and 10 mg progesterone groups, respectively.

Total embryonic survival rates were 63.9%, 58.5%, 27.3%, 42.2%, 47.4%, 34.7% and 21.7% for the mature control, prepuberal control, 1.5, 2.5, 3.5, 5, and 10 mg progesterone groups, respectively. There was no significant difference ($P > .05$) between the mature control, prepuberal control, 2.5, and 3.5 mg progesterone groups. Total survival rates were significantly reduced ($P < .05$) in the 1.5, 5, and 10 mg progesterone groups as compared to the other treatments.

The analysis of variance for ovulation rate, number of animals with fetuses at 8 days post coitus, number of animals with fetuses at 20 days post coitus, average litter size at 8 days post coitus, average litter size at 20 days post coitus, preimplantation fetal survival, postimplantation fetal survival, and total fetal survival rates are presented in appendix table IX.

The reproductive performance of individual rats in the control, prepuberal control, 1.5, 2.5, 3.5, 5, and 10 mg progesterone groups are presented in appendix tables XII, XIII and XIV.

Effect of Ovariotomy and Progesterone Replacement in Prepuberal Rats

Table V presents the means, standard errors, and differences in the implantation rate, number of fetuses at 20 days post coitus, and postimplantation survival rates for prepuberal rats ovariotomized on day 8 post coitus and maintained on 3.5 or 5 mg progesterone.

Table V
Means, Standard Errors and Differences in
Implantation Rate, Number of Fetuses and Postimplantation
Survival of Prepuberal Rats Ovariotomized and Maintained
on 3.5 or 5.0 mg Progesterone Daily

Progesterone Level (Day 8 to 20 p.c.)	No. of Rats	No. of Implantations on day 8 p.c.	No. of Fetuses on day 20 p.c.	Postimplantation Survival[a,b]
0 mg/day	5	9.6 ± 0.9[c]	0 ± 0 [c]	0 ± 0 (0)[c]
3.5 mg/day	8	9.5 ± 1.1[c]	6.0 ± 1.9[d]	63.2 ± 13.8 (62.8)[d]
5.0 mg/day	9	8.6 ± 1.1[c]	4.7 ± 1.4[d]	55.1 ± 11.0 (58.0)[d]

[a] Arc-Sin Transformation Values.
[b] (Number fetuses at 8 day p.c. divided by the number fetuses at 20 days p.c.) x 100.
[c,d] Values within columns with same superscript not significantly different ($P > .05$).

On day 8 post coitus, the average number of fetuses was 9.6, 9.5, and 8.6 for the groups maintained on 0, 3.5, and 5 mg progesterone, respectively. There was no significant difference in litter size on day 8 post coitus.

The average number of fetuses on day 20 post coitus was 0, 6.0, and 4.7 for the 0, 3.5, and 5 mg progesterone groups, respectively. The average litter size at 20 days was significantly ($P<.05$) reduced in the control group as compared to the 3.5 and 5 mg progesterone groups. There was no significant difference between the 3.5 and 5 mg groups.

Postimplantation survival rates were 0, 62.8, and 58.0% for the control, 3.5, and 5 mg groups, respectively. There was no significant difference between the 3.5 and 5 mg progesterone groups in postimplantation survival. There was, however, a significant difference ($P<.05$) between the control and the progesterone treated groups. The analysis of variance may be found in appendix table X and the reproductive performance of individual rats in appendix table XV.

Effect of Estradiol on Pregnancy in the Prepuberal Rat

Estradiol had a detrimental effect on pregnancy in prepuberal rats. Mated prepuberal rats were treated with 0.2 mcg of estradiol on day 4 post coitus. In addition, these rats received 0, 1.5, 2.5, or 3.5 mg progesterone daily from day 3 to day 20 of pregnancy. No implanting embryos were found in any of these treatment groups when the rats were laparotomized on day 9 post coitus. Several of the rats on 3.5 mg progesterone were maintained on this level until day 20 post coitus, but no fetuses were found on day 20.

Effect of Ovariotomy on Gestating Mature Rats

Table VI contains the means, standard errors, and statistical differences of implantation rates, litter size, and prenatal survival rates of mature rats ovariotomized during gestation.

Implantation rates, recorded at ovariotomy, were 10.5, 9.9, 10.3, and 9.8 for the sham operated, ovariotomized on day 8, ovariotomized on day 14, and ovariotomized on day 17 post coitus, respectively. There was no significant difference ($P > .05$) in implantation rates.

The average number of fetuses at 20 days was 8.9, 0, 0, and 6.7 for the sham operated, ovariotomized on day 8, ovariotomized on day 14, and ovariotomized on day 17 post coitus, respectively. There was a significant difference ($P < .05$) between the sham operated and the ovariotomized on day 17 post coitus groups. There was also a significant difference ($P < .05$) between these two groups and the groups ovariotomized on day 8 or 14 post coitus.

Percent survival after ovariotomy was 67.0, 0, 0, and 58.8 for the sham operated, ovariotomized on day 8, ovariotomized on day 14, and ovariotomized on day 17, respectively. There was a significant difference ($P < .05$) between the sham operated and ovariotomized on day 17 as compared to the rats ovariotomized on day 8 or 14 post coitus. There was no significant difference ($P > .05$) between the sham operated and ovariotomized on day 17 groups.

Body and Tract Weights

Table VII contains the means and standard errors for body and reproductive tract weights of animals used for histological examination. Appendix table XVI shows the actual body weight and reproductive tract

Table VI
Means, Standard Errors and Differences in
Implantation Rate, Litter Size, and Prenatal Survival of Mature
Rats Ovariotomized at Varying Times After Mating

Treatment	No. Rats	No. Implantations at Time of Ovariotomy	No. of Fetuses 20 days p.c.	% Survival After Ovariotomy[a]
Sham operated on day 8 p.c.	13	10.5 ± .3[b]	8.9 ± .7[b]	84.8 ± 6.2 (67.0)[b]
Ovariotomy on day 8 p.c.	6	9.9 ± .4[b]	0[c]	0[c]
Ovariotomy on day 14 p.c.	10	10.3 ± .5[b]	0[c]	0[c]
Ovariotomy on day 17 p.c.	15	9.8 ± .5[b]	6.7 ± .9[d]	68.4 ± 2.5 (58.8)[b]

[a] Arc-Sin Transformed Values in ( ).

[b,c,d] Values within columns with same superscript not significantly different ($P > .05$).

Table VII
Means, Standard Errors and Differences of Reproductive Tract Weight Per 100 g Body Weight, Endometrial Cell Height and Endometrial Cell Area from Treated and Control Rats

Treatment	No. Rats	Tract Weight per 100 g Body Weight	Endometrial Height (microns)	Nuclear Area (microns)
Prepuberal				
No treatment	3	114.6 ± 17.8[a]	11.2 ± .19[d]	25.5 ± .11[f]
PMS 56 hours	3	328.4 ± 19.5[b]	28.8 ± 1.31[a,c]	34.7 ± .46[a,e]
PMS, HCG 24 hr.	3	298.3 ± 23.8[b]	37.9 ± 1.80[a]	37.7 ± .46[b]
PMS, HCG 48 hr.	3	248.2 ± 12.4[c]	23.0 ± .82[b,c]	35.4 ± .38[c,e]
PMS, HCG 72 hr.	3	267.6 ± 3.6[c]	19.6 ± .44[b,c]	29.3 ± .27[d]
3 day, mated	3	213.0 ± 18.2[a]	21.9 ± .46[a]	28.1 ± .35[a]
5 day, mated	3	199.5 ± 6.4[a]	15.6 ± .28[b]	25.7 ± .85[a]
7 day, mated	3	159.3 ± 8.8[b,d]	19.8 ± .90[c,e]	25.6 ± .35[a]
9 day, mated	3	144.1 ± 4.0[c,d]	19.1 ± .44[d,e]	25.1 ± .27[a]
3 day progest.	3	212.2 ± 3.2[a]	27.3 ± .50[a]	32.3 ± .43[a]
5 day progest.	3	221.6 ± 2.2[a]	27.3 ± .54[a]	32.6 ± .34[a]
7 day progest.	3	189.5 ± 6.8[a]	24.5 ± .57[b]	31.6 ± .31[a]
9 day progest.	3	200.9 ± 4.9[a]	23.9 ± .46[b]	31.9 ± .51[a]
Mature				
Estrus	3	261.7 ± 3.1[a]	16.6 ± .31[a]	12.2 ± .24[a]
Anestrus	3	127.6 ± 5.2[b]	12.6 ± .71[b]	11.0 ± .22[b]
3 day preg.	3	145.2 ± 10.4[a]	14.8 ± .45[a]	11.3 ± .19[a]
5 day preg.	3	215.8 ± 3.0[b]	19.1 ± .29[b]	14.9 ± .14[b]
7 day preg.	3	279.3 ± 12.6[c]	17.3 ± .98[c]	18.5 ± .33[c]
9 day preg.	3	767.0 ± 69.5[d]	15.6 ± .33[d]	17.4 ± .43[d]

a,b,c,d,e,f Values within each column within perimeter with same superscript not significant difference ($P \geq .05$).

weight for each animal used for histological analysis.

Tract weight per 100 g body weight increased significantly ($P<.05$) over non-treated controls as a result of PMS treatment, and then gradually decreased as the time post PMS and HCG injection increased. Prepuberal animals not receiving gonadotrophins had tract weights averaging 114.6 mg per 100 g body weight. Twenty IU of PMS resulted in an increase to 328.4 mg per 100 g body weight. Tract weight 24 hours after PMS-HCG treatment was 298.3 mg per 100 g body weight. By 72 hours post HCG injection, tract weights decreased to 267.6 mg per 100 g body weight.

In those prepuberal animals mated after PMS and HCG treatment, tract weights decreased significantly ($P<.05$) as time post coitus increased. At 3 days post coitus tract weights were 213.0 mg per 100 g body weight. By 9 days post coitus tract weights had decreased to 144.1 mg per 100 g body weight.

Progesterone administration to prepuberal rats after PMS and HCG treatment[6] caused an increase in weight over prepuberal mated females. However, there was no significant ($P>.05$) change in uterine weight regardless of the number of injections. At 3 days post ovulation, tract weight averaged 212.2 mg per 100 g body weight. At 9 days post ovulation tract weight still averaged 200.9 mg per 100 g body weight.

When mature animals in estrus were compared with mature rats in diestrus, there was a significant difference ($P<.05$) in tract weight.

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[6]Prepuberal progesterone treated rats were not mated so as to permit a comparison with the prepuberal mated rats which do not usually conceive after mating (see reproductive trials).

Estrus tracts averaged 261.7 mg per 100 g body weight while diestrus tracts weighed 127.6 mg per 100 g body weight.

In mature pregnant rats, reproductive tract weight increased significantly ($P < .05$) from 3 days post coitus to day 9 post coitus. Reproductive tract weight averaged 145.2, 215.8, 279.3, and 767.0 mg per 100 g body weight at 3, 5, 7, and 9 days post coitus, respectively. The rapid increase in weight from day 7 to day 9 post coitus appeared to be due to the rapidly increasing weight of the fetuses.

Table VIII shows a comparison in the reproductive tract weight between prepuberal mated rats, and those rats which were ovulated and received progesterone. At 3 days post coitus, there was no significant difference ($P > .05$) between the two groups in tract weight. However, at 5, 7, and 9 days post coitus, tract weight per 100 g body weight was significantly ($P < .05$) increased in those rats receiving progesterone.

Uterine Endometrial Cell Height and Nuclear Area

Table VII contains the means and standard errors for endometrial cell height and nuclear area from prepuberal and mature rats. In general, nuclear size and cell height appeared to be positively correlated, with the cell height showing a greater response to the treatments imposed.

Prepuberal rats receiving no treatment had endometrial cells averaging 11.2 μ in height and nuclear area of 25.5 μ . These figures rose to 28.8 μ for cell height and 34.7 μ for nuclear area when rats were treated with 20 IU of PMS. Administration of PMS and HCG increased cell height and nuclear area to 37.9 μ and 37.7 μ , respectively. From 24 to 72 hours post HCG injection there was a steady decrease in cell height and

Table VIII
Comparison of Tract Weight, Endometrial Cell Height and
Endometrial Nuclear Area Between Immature Control and Progesterone Treated Rats

Treatment	Time			
	3 days	5 days	7 days	9 days
Tract wt./100 g body wt.				
Immature mated	213.0[a]	199.5[a]	159.3[a]	144.1[a]
Immature 3.5 mg progesterone	212.2[a]	221.6[b]	189.5[b]	200.9[b]
Endometrial cell height (μ)				
Immature mated	21.9[a]	15.6[a]	19.8[a]	19.1[a]
Immature 3.5 mg progesterone	27.3[b]	27.3[b]	24.5[b]	23.9[b]
Endometrial nuclear area (μ)				
Immature mated	28.1[a]	25.7[a]	25.6[a]	25.1[a]
Immature 3.5 mg progesterone	32.3[b]	32.6[b]	31.6[b]	31.9[b]

a,b Values within each column within perimeter with same superscript not significant difference ($P > .05$).

nuclear area.

In comparing the PMS treated group with the tracts examined 24, 48, and 72 hours post PMS-HCG injection, there were significant differences ($P < .05$) in both cell height and nuclear area. Cell height for the PMS, PMS-HCG + 24 hours, PMS-HCG + 48 hours, and PMS-HCG + 72 hours were 28.8 μ, 37.9 μ, 23.0 μ, and 19.6 μ, respectively. Nuclear area measurements were 34.7 μ, 37.7 μ, 37.7 μ, 35.4 μ, and 29.3 μ for the PMS, HCG + 24 hours, HCG + 48 hours and HCG + 72 hours, respectively.

In prepuberal mated rats examined 3, 5, 7, and 9 days post coitus there were no significant differences ($P > .05$) in nuclear area. However, when the same uteri were compared in endometrial cell height, there was a significant decrease ($P < .05$) in cell height from day 3 to day 9 post coitus. The significant ($P < .05$) decreases in cell height occurred from day 3 to 5 post coitus and from day 5 to 7 post coitus. Cell height was 21.9 μ, 15.6 μ, 19.8 μ, and 19.1 μ at 3, 5, 7 and 9 days post coitus.

Progesterone treatment of prepuberal rats resulted in a significant decrease ($P < .05$) in cell height from day 3 to day 9 post ovulation. However, there was no significant difference ($P > .05$) in nuclear size from day 3 to day 9 post coitus. Cell height averaged 27.3μ, 27.3μ, 24.5μ, and 23.9μ on day 3, 5, 7 and 9 post ovulation, respectively. The significant ($P < .05$) decrease in cell height occurred between 5 and 7 days post coitus. Nuclear area measured 32.3μ, 32.6μ, 31.6 μ, and 31.9μ on day 3, 5, 7 and 9 days post ovulation, respectively.

Endometrial cell height and nuclear area were significantly different ($P < .05$) between mature rats in estrus and diestrus. Estrus cell height and nuclear area was 16.6μ and 12.2μ, respectively. Diestrus

cell height and nuclear area averaged 12.6μ and 11.0μ , respectively.

In mature pregnant rats, cell height and nuclear area at 3 days post coitus was 14.8μ and 11.3μ , respectively. At 9 days post coitus, the measurements for endometrial cell height and nuclear area were 15.6μ and 17.4μ , respectively. The mature pregnant group was the only treatment in which nuclear area did not appear to be correlated with cell height. There was a significant ($P<.05$) increase in nuclear area as pregnancy progressed. At 3 days post coitus nuclear area averaged 11.3μ and rose to 19.1μ on day 5 post coitus. After 5 days post coitus, endometrial cell height slowly declined from 19.1μ to 15.6μ on day 9 post coitus. The difference in endometrial cell height was significant ($P<.05$).

Table VIII shows a comparison between the prepuberal rats receiving 3.5 mg progesterone (ovulation induced but rats not mated) and the prepuberal rats which were mated (but not maintained on progesterone). In comparing the two groups at the same time after ovulation, there were significant differences ($P<.05$) in both endometrial cell height and endometrial nuclear area.

Plates 1, 2, 3, 4, and 5 are examples of uterine cross sections obtained from 45 day old prepuberal rats receiving various hormone treatments.

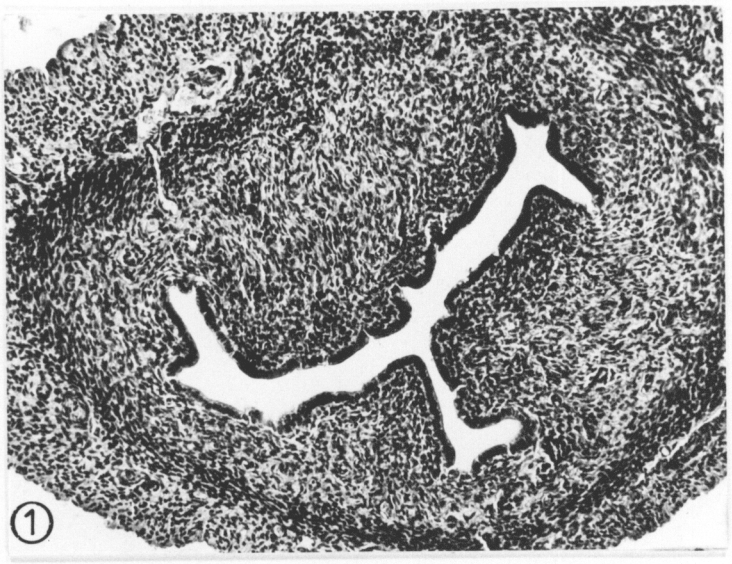
PLATE 1

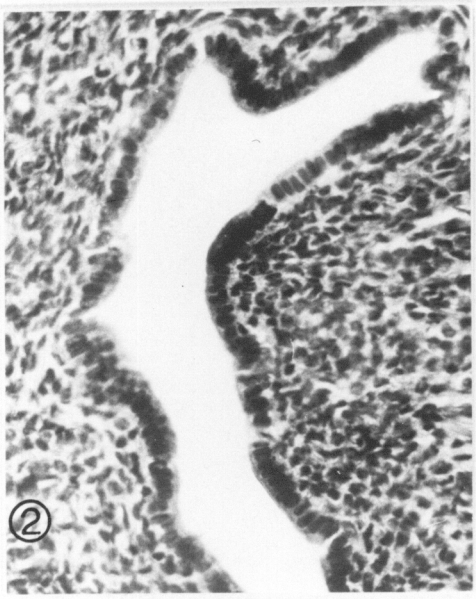
Cross section of uterine endometrium from 45 day old untreated prepuberal rat

(1) 130 X
(2) 285 X
(3) 1300 X

Note the lack of endometrial folding and the low columnar cells which form the endometrial lining

PLATE 1





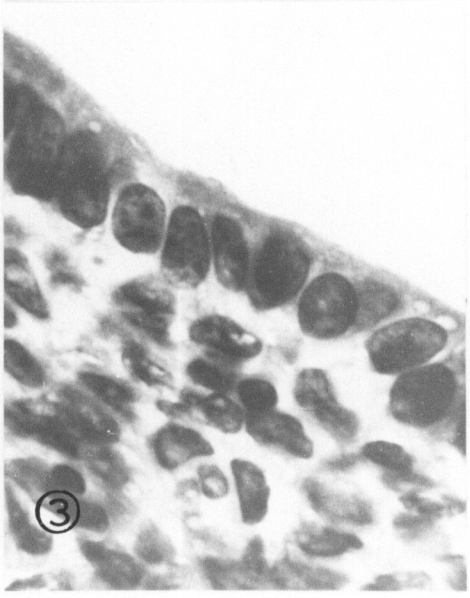


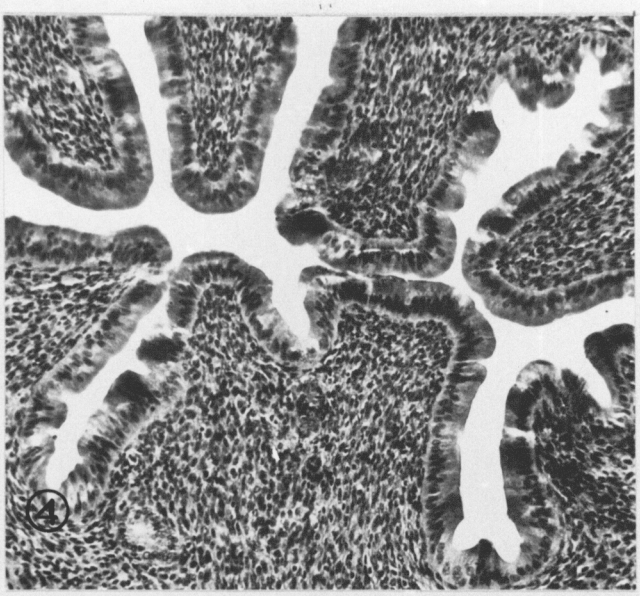
PLATE 2

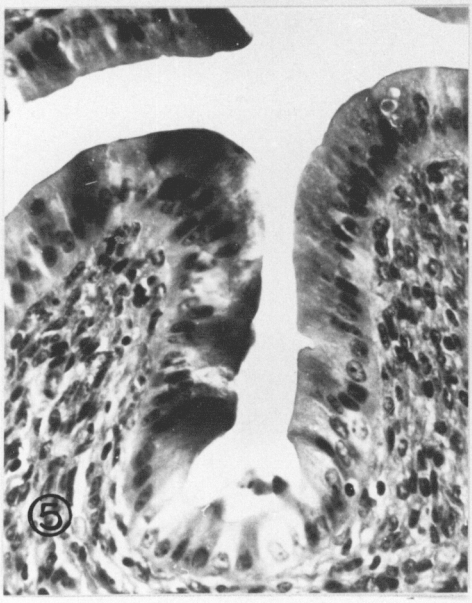
Cross section of uterine endometrium from prepuberal rat killed 56 hours after treatment with 20 IU PMS

(4) 130 X
(5) 285 X
(6) 1300 X

Note the effect of PMS on increasing the endometrial cell height and the extensive folding of the endometrium as compared to the untreated uterus (plate 1)

PLATE 2





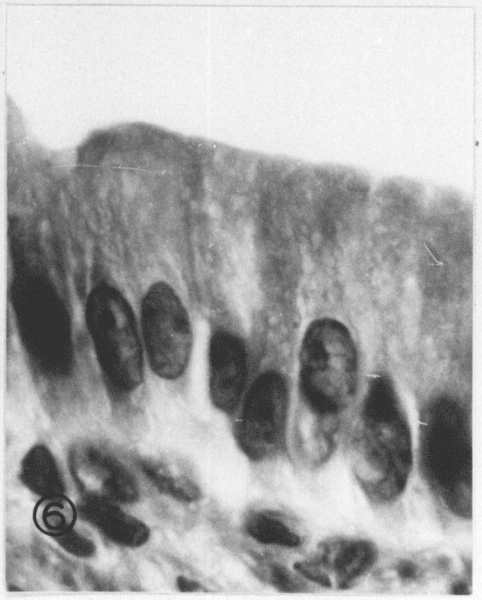


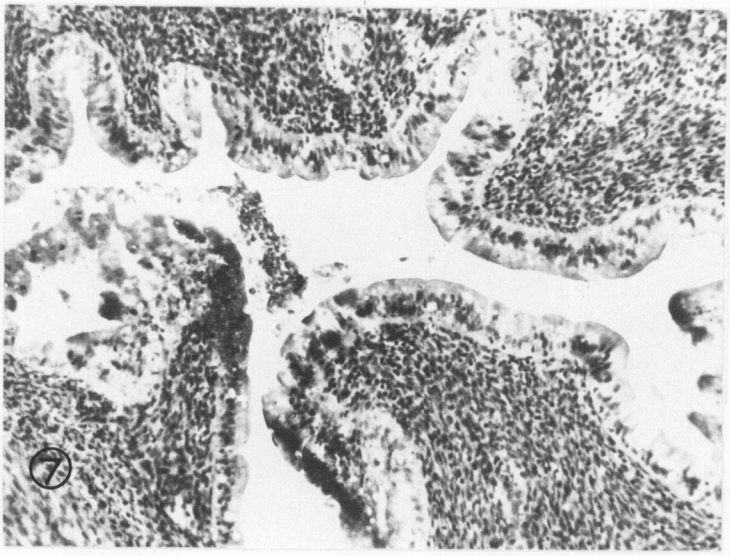
PLATE 3

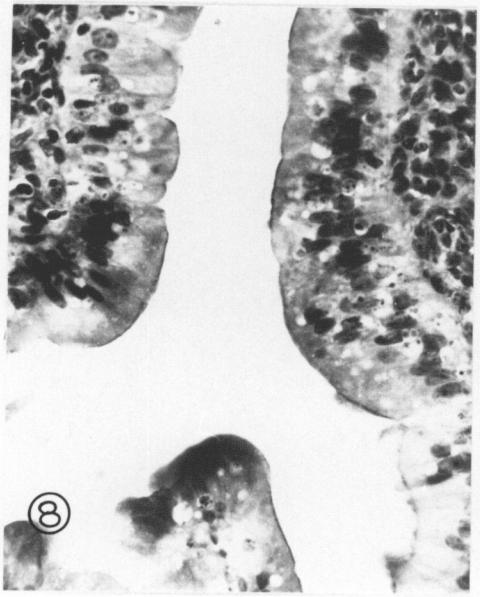
Cross section of uterine endometrium from prepuberal rat killed 24 hours after ovulation was induced by 20 IU PMS and 20 IU HCG

(7) 130 X
(8) 285 X
(9) 1300 X

Note the similarity of the endometrial folding in this plate as compared to PSM treatment alone. However, extensive vacuolation can now be noted in the endometrial cells as a result of the HCG. This vacuolation is not present with only PMS treatment (plate 2).

PLATE 3





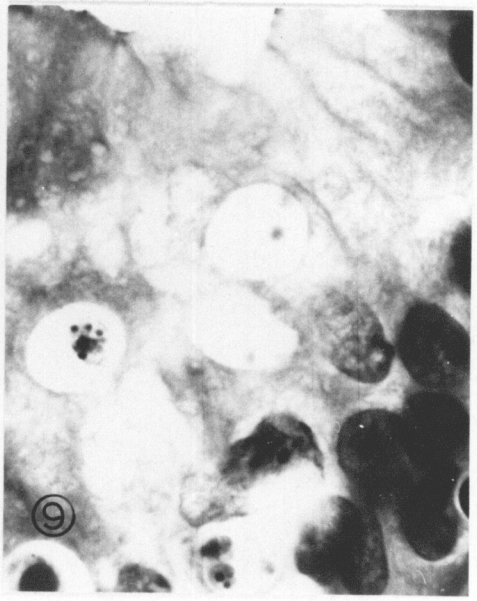


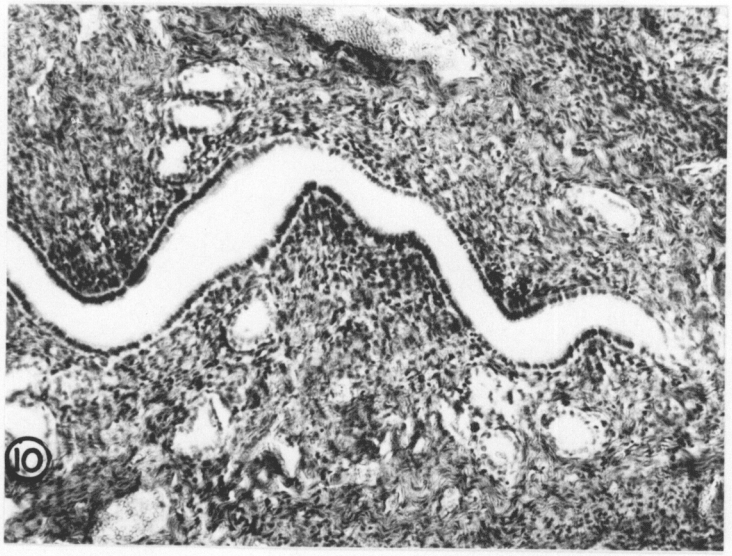
PLATE 4

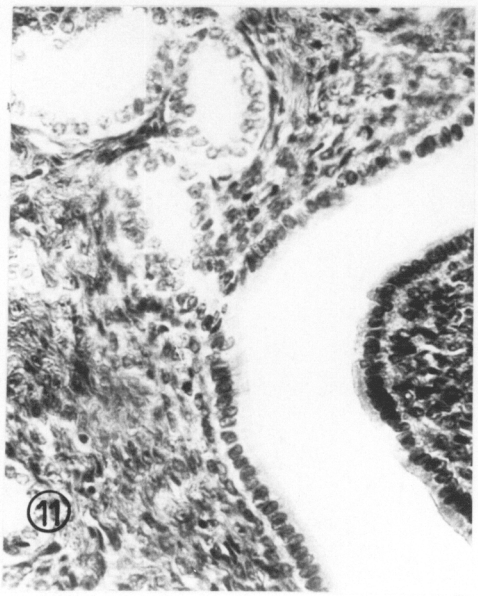
Cross section of uterine endometrium from prepuberal rat 7 days after ovulation and mating were induced by 20 IU PMS and 20 IU HCG

(10) 130 X
(11) 285 X
(12) 1300 X

Although this rat was mated, it should be noted that the lack of endometrial folding and the low columnar cells are very similar to the state found in the untreated prepuberal rat (plate 1).

PLATE 4





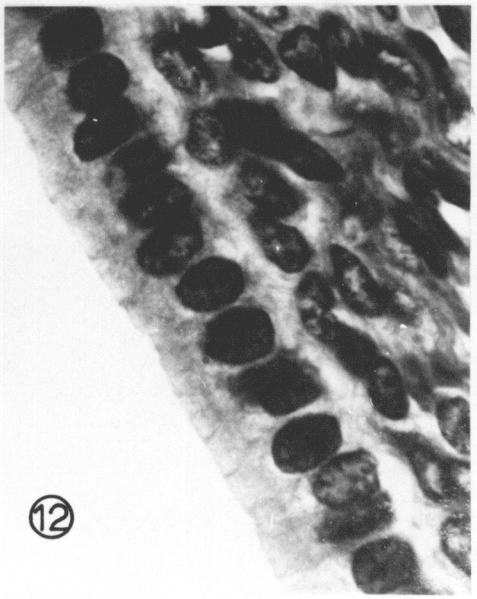


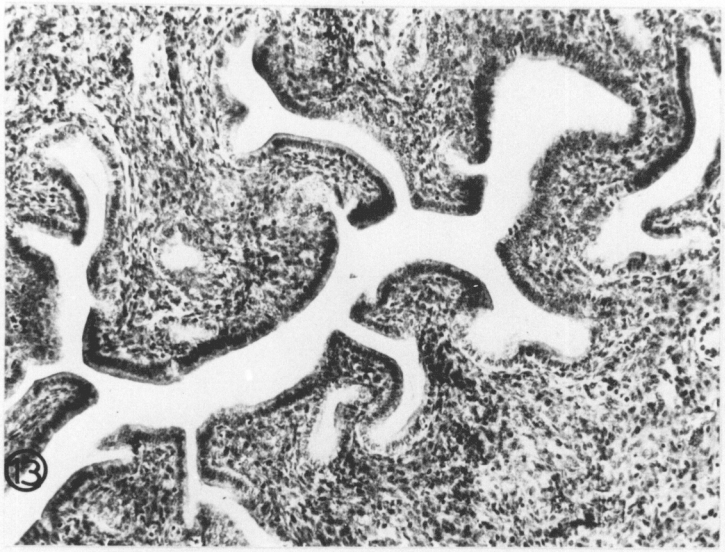
PLATE 5

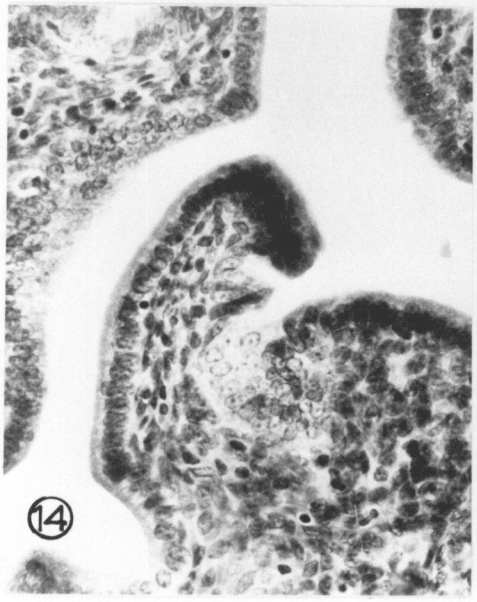
Cross section of uterine endometrium from prepuberal rat treated with 3.5 mg progesterone daily from day 2 to day 7 post ovulation. Ovulation was induced with 20 IU PMS and 20 IU HCG.

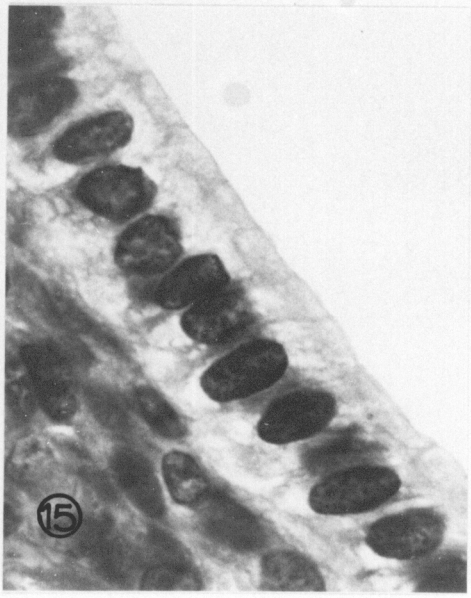
(13) 130 X
(14) 285 X
(15) 1300 X

Note the increase in endometrial folding and the taller columnar cells as a result of progesterone treatment. The increase in endometrial folding may have accounted for the fact that progesterone treated rats implanted fetuses but the controls (plate 4) did not.

PLATE 5







DISCUSSION

Induction of Vaginal Opening, Estrus, and Mating

It is difficult to compare the incidence of vaginal opening, estrus, and mating between prepuberal rats studies due to the differences in age and strain of rats, and the type and levels of gonadotrophins employed.

The 98% vaginal opening following PMS treatment reported here is in general agreement with the results of Austin (1949) and in contrast to the findings of Sato (1962). Austin (1949) reported that the vaginal orifices of all rats examined were patent 48 to 60 hours after treatment with 20 IU of PMS. However, Sato (1962) reported that only 8 of 35 prepuberal rats showed vaginal opening 40 to 60 hours after treatment with 20 IU of PMS.

Seventy-five percent of the treated prepuberal rats were in estrus after PMS-HCG treatment as indicated by vaginal smears. These findings are essentially the same as reported by Austin (1949) who found that 80% of the rats showed vaginal estrus after PMS-HCG administration.

Acceptance of coitus following induced estrus averaged 40.4% in 420 prepuberal DDR rats. Although the rats averaged 40.4% acceptance, it should be noted that the acceptance of mating varied from a low of 23.3% to a high of 70.0% on different nights during the course of the experiment. Previous workers have shown that mating is extremely variable and reportedly ranges from 0% (Sato, 1962) to 92% (Wu and Meyer, 1966). Between these extremes, figures of 34% (Austin, 1949), 38% (Starkey, 1969), and 63% (Evans and Simpson, 1940) have been reported for the acceptance of coitus after induced ovulation with PMS and HCG.

Ovulation Rate, Number Pregnant, and Litter Size

The ovulatory response of the prepuberal rat to gonadotrophins was relatively low with the mean group ovulation rates, based on corpora lutea counts, averaging 12 to 14. Corpora lutea counts from individual rats ranged from 9 to 20. The low response to gonadotrophins, as compared to the ovulatory responses obtained by Austin (1949), Grayburn and Brown-Grant (1968), and Rowlands (1944), proved to be quite beneficial since a direct comparison could be made with the mature rat in litter size. The variable ovulatory response to 20 IU PMS and 20 IU HCG becomes apparent when the average ovulation rate of 12.4, found in this study is compared to the previously reported ovulation rates of 37.2 (Austin, 1949), 45.0 (Grayburn and Brown-Grant, 1968), and 14.0 (Rowlands, 1948).

In prepuberal control rats, pregnancy was established in only 2 of the 30 mated animals as determined by implantations on day 8 post coitus. This pregnancy rate of 6.6% is lower than those reported by Cole (1937) and Austin (1949) in rats, but is similar to the findings in prepuberal mice by Engle (1927), Runner and Gates (1954), and Smithberg and Runner (1956, 1957). Cole (1937), using a crude serum extract of mare gonadotropic hormone, induced pregnancy in 30 of 41 mated prepuberal rats. Austin (1949) reported that pregnancy was established in 12 of 45 prepuberal rats mated after PMS and HCG administration. Smithberg and Runner (1956) reported that although ovulation and receptivity to the male may be induced in prepuberal mice, pregnancy is established in less than 10% of the mated mice.

The quantity of gonadotrophins employed to induce ovulation and mating does not appear to be responsible for the low pregnancy rate since Austin

(1949) used the same levels of PMS and HCG. However, since the pregnancy rate at 8 days in the DDR prepuberal rat closely parallels the results reported in prepuberal mice (Smithberg and Runner, 1956), it is quite possible that a similar physiological state of an unfavorable uterine environment may exist in both cases.

From an endocrine standpoint, optimum fetal survival is obtained due to the response of the tissue to adequate doses of a synergistic combination of estrogen and progesterone (Courrier, 1950). Conversely, according to Yochim and Zarrow (1961), suboptimal maintenance of pregnancy may be due to one or more of the following: hormone deficiency (decreased absolute levels), progesterone deficiency (estrogen antagonism), estrogen deficiency (suboptimal synergism), or hormone excess (increased absolute levels). On the bases of these antagonistic and synergistic effects of estrogen and progesterone, one might postulate why suboptimal pregnancy maintenance was observed in several of the progesterone groups. However, since the necessity of estrogen in the process of pregnancy maintenance has been repeatedly demonstrated in the ovariotomized rat, (Zeiner, 1943; Yochim and Zarrow, 1961) it must be assumed that estrogen is produced by the prepuberal rat. If endogenous estrogen were not produced, implantation and pregnancy maintenance could not occur (Nutting and Meyer, 1963).

At 8 days post coitus, the number of pregnant rats and the average litter size indicated that levels of progesterone below 3.5 mg resulted in estrogen antagonism. The physiological effect of this antagonism being "tube locking" with the fertilized ova remaining in the oviduct for an extended period of time (Turner, 1966). In the case of the 5 and 10 mg progesterone levels, it is possible that suboptimal

synergism (estrogen deficiency) may have resulted. Since a significant increase in estrogen is necessary for nidation to occur (Nutting and Meyer, 1963 and Mayer, 1963), it seems possible that the estrogen deficiency caused by 5 to 10 mg of progesterone may have inhibited implantation of some, or all, of the embryos.

At 20 days post coitus, the suboptimal levels of progesterone, particularly in the 1.5 mg group, appeared to be most detrimental to fetal development. The adverse effects of 1.5 mg progesterone are shown most dramatically in the average of 4.2 resorbing fetuses per litter in this group as compared to 1.1 resorbing fetuses per litter in the 3.5 mg group. This extremely high resorption rate indicates a very marked progesterone deficiency.

As indicated by the average number of resorbing fetuses at 20 days post coitus, excessive amounts of progesterone (5 to 10 mg daily) do not appear to be detrimental after implantation is complete. The author feels this may be due to the increased endogenous estrogen production by the newly formed placenta which prevents suboptimal synergism.

Yochim and Zarrow (1961) found excessive quantities of progesterone to be detrimental to fetal survival in ovariomized mature rats. As they increased progesterone levels from 1.5 to 2.0 mg, the average litter size increased from 4.5 to 7.5. As the level was further increased to 3.0 mg, the average litter size again decreased to 5.8.

Although there was no significant difference in total prenatal survival between the 3.5 mg progesterone group and the mature control group, it does appear that prenatal survival rates could be improved in prepuberal rats. Aside from the possibility that an optimum uterine

environment has not been established with progesterone alone, there is also the distinct possibility that fertilization failure has accounted for a substantial portion of this reduced litter size in the prepuberal animal. In 30 prepuberal mated rats, Austin (1949) observed that only 22 rats had fertilized ova 24 hours after mating. From these 22 mated rats, he recovered 787 eggs of which 548 were fertilized for a fertilization rate of 69.6%. If a similar fertilization rate occurred in this study, the possibility exists that total survival rates in excess of 70% may be impossible to obtain due to a depressed fertilization rate.

Effect of Ovariotomy and Progesterone Replacement in the Prepuberal Rat

To further examine the relationship of exogenous progesterone to postimplantation fetal survival, 27 prepuberal mated rats were bilaterally ovariotomized on the 8th day post coitus and 0, 3.5, or 5 mg of progesterone was administered daily until the 20th day of gestation. Although the difference between 63.2% and 55.1% postimplantation survival for the 3.5 and 5.0 mg groups, respectively, was not statistically significant, the trend did favor improved survival with the 3.5 mg level. Since the survival rate was slightly higher in the 3.5 mg group, it appears that the corpora lutea of the prepuberal rat produce a very small quantity of progesterone.

It is also of interest to compare the postimplantation survival rates of the intact and ovariotomized rats maintained on the same levels of progesterone. Survival rates for the intact prepuberal rats on 3.5 and 5.0 mg were 75.3% and 64.5%, respectively, while the survival rate of the ovariotomized rats on 3.5 and 5.0 mg levels were 63.2% and 55.15, respectively. The slightly depressed fetal survival in the ovariotomized

prepuberal rats appears to be due to estrogen deficiency (suboptimal synergism) resulting from the loss of ovarian estrogen.

Smithberg and Runner (1956) observed that ovariotomy in mice resulted in a decrease in the number of prepuberal mice with fetuses and a depression in the average litter size. In intact prepuberal mice receiving 2 mg progesterone per day, 88.8% of the mice were pregnant with an average litter size of 3.7 at 20 days of gestation. In contrast, 76% of the ovariotomized prepuberal mice were pregnant with an average litter size of 2.6 at 20 days of gestation.

Effect of Estrogen on Pregnancy in the Prepuberal Rat

One of the more surprising aspects of these experiments was that pregnancy in the intact and prepuberal ovariotomized rat was possible in the absence of exogenous estrogen; especially since exogenous estrogen has been considered necessary for nidation (Nutting and Meyer, 1963; Mayer, 1963) and maintenance of pregnancy (Lyons, 1943; Zeiner, 1943) in the mature ovariotomized rat.

Although exogenous estrogen was not needed for pregnancy maintenance in the prepuberal rat, previous pilot studies and the reports of Nutting and Meyer (1963) indicated that 0.2 mcg of estradiol might increase the number of rats which implanted fetuses. In the present study, however, it was found that a single injection of estradiol, alone or in combination with progesterone, totally inhibited implantation. Smithberg and Runner (1956) also found that the administration of estrogen either alone or in combination with progesterone proved deleterious to pregnancy in prepuberal mice. The adverse effects became most apparent when concentrations of 0.1 to 1.0 mcg of estradiol alone or in combination with

2.5 mg of progesterone was administered prior to implantation.

Effect of Ovariotomy on Gestating Mature Rats

For many years, it has been assumed that ovariotomy, performed at any time during gestation, terminated pregnancy in the rat (Klein, 1935; Lerner et al., 1963; and, Nutting and Meyer, 1963). However, Van der Vies and Feenstra (1967), Csapo (1969), and Pulkkinen and Csapo (1969) have recently found that if ovariotomy is performed late in gestation, pregnancy is not routinely terminated. Pregnancy is maintained due to hypertrophy of the placenta and sufficient progesterone and estrogen secretion.

To further establish the existence of a luteo-placental shift, gestating rats were ovariotomized on the 8th, 14th, or 17th day post coitus. In those rats ovariotomized on the 8th or 14th day post coitus, pregnancy was terminated in all cases by abortion or resorption of the litter. In those rats ovariotomized on the 17th day post coitus, a 68.4% fetal survival rate was recorded from day 17 to 21 post coitus.

The 68.4% post operational survival rate in this study compares quite favorably with the 63% fetal survival reported by Csapo (1969) on rats ovariotomized at the same stage of gestation.

The success of the ovariotomized mature rat to maintain pregnancy appears to be distinctly dependent on placental hypertrophy for the production of sufficient quantities of progesterone to inhibit abortion. If sufficient quantities of progesterone were not produced, premature delivery started on the 18th or 19th days post coitus (Csapo, 1969). The author of this study noted that rats delivering prematurely only expelled a limited number of fetuses and that their placentae were retained.

However, extra ovarian production of progesterone did not appear to provide complete protection to the fetuses which reached term for the incidence of fetal defects and hemorrhage was frequent; especially in those 2 fetuses located in the cervical ends of the uterine horns.

These experiments tend to indicate that in pregnant rats the corpora lutea are not essential toward the end of gestation providing that sufficient quantities of extra-ovarian progesterone can be produced by the placentae. This apparent luteo-placental shift results in a localization of myometrial regulation and allows the uterine horns to function independently and deliver their contents at different times. This was observed in several rats which aborted all the fetuses in one horn but delivered those in the other horn at term.

Body and Reproductive Tract Weight

Reproductive tract weight and tract weight per 100 g body weight appeared to be correlated with the treatment imposed on the animal. Obvious differences were evident in comparing the tract weight per 100 g body weight in prepuberal mated control rats and those induced to ovulate and maintained on 3.5 mg progesterone. In prepuberal mated rats, the tract weight per 100 g body weight decreased from 213 mg to 144 mg from day 3 to 9 post coitus. In the progesterone group, the tract weight per 100 g body weight remained relatively constant, declining from 212 mg to 201 mg from day 3 to 9 post ovulation.

From these results, in conjunction with the histological findings, it appears that the corpora lutea of the prepuberal mated rat does not produce sufficient quantities of progesterone to adequately prepare the uterus for implantation. These findings are reflected in the (1) decreased

uterine weight, (2) histological results showing no signs of the endometrial folding necessary for implantation (Plate 4 vs Plate 5), and (3) increased reproductive efficiency by the use of exogenous progesterone. In prepuberal mice, Smithberg and Runner (1956) also found a significant decrease in the uterine weight and histological and histochemical evidence of atrophy of the corpora lutea.

The difference in tract weight in the mature rat in estrus (261 mg per 100 g) as compared to anestrus (127 mg per 100 g) is probably due to the increase in water uptake and cell hypertrophy and hyperplasia induced by estrogen. As reported by Catchpole (1969), estrogen is the predominant hormone at the time of ovulation and fertilization and results in an increase in cell mitosis, hyperplasia of the endometrial cells and glands, and increases the water uptake by the uterus, cervix, and vagina.

Uterine Endometrial Cell Height and Nuclear Area

The histological comparison of endometrial cell height and nuclear area between the prepuberal mated control rats and those receiving progesterone provided a significant amount of information about the hormonal requirements necessary for implantation and pregnancy maintenance in the prepuberal DDR rat. In the present study, both endometrial cell height and nuclear area decreased significantly in the prepuberal mated controls as the time post mating increased. In contrast, endometrial cell height and nuclear area did not decrease significantly in the prepuberal rats which were maintained on 3.5 mg progesterone.

Plates 4 and 5 also show an obvious difference in the degree of folding in the endometrium. The lack of folding in the mated pre-

puberal rat (Plate 4) is very similar to the condition observed in the 45 day old rat prior to gonadotrophin or hormone treatment (Plate 1). Conversely, there was a significant increase in the folding of the endometrium as a result of progesterone treatment (Plate 5). As indicated by Hafez (1968), a folded endometrium is necessary for successful implantation of the free floating blastocyst.

On the basis of both endometrial folding, cell height, and nuclear area differences, it is postulated that implantation and pregnancy maintenance is not usually observed in the prepuberal DDR rat due to progesterone deficiency (estrogen antagonism) resulting from insufficient production, or release of prolactin from the hypophysis.

Several other workers have also reported an increase in endometrial cell height as a result of exogenous progesterone treatment. Howe et al. (1964) found that endometrial cell height in prepuberal calves increased from 15 μ to 23.5 μ after treatment with 100 mg progesterone for 5 days. Schultz et al. (1969) ovariotomized Holstein cows and found a significant increase, over controls, in nuclear area of the endometrial cells as a result of treatment with 100 mg progesterone for 3 days. Kim and Forman (1966) found the histological appearance of the endometrium in bilaterally ovariotomized rats treated with 20 mg progesterone to be similar to that of the nonovariotomized rats. Endometrial cell height in the controls averaged 20.8μ and in the ovariotomized rats receiving 20 mg progesterone per day cell height averaged 19.4μ .

SUMMARY

The reproductive performance of 45 day old prepuberal DDR rats was analyzed to determine the effect of daily treatment of varied levels of progesterone upon several reproductive phenomena. Daily treatment with 3.5 mg progesterone significantly increased ($P<.05$) the conception rate, litter size, and prenatal survival over the other progesterone levels. The reproductive efficiency increased as the quanitity of progesterone was increased to 3.5 mg per day and then declined as the level was further increased to 10.0 mg per day. The average litter size and prenatal survival was not significantly ($P>.05$) different between the prepuberal group receiving 3.5 mg progesterone and the mature control group. The average litter size for the 3.5 mg group was 7.8 and for the mature control group was 8.9. Total prenatal survival was 55.3% and 78.4% for the 3.5 mg progesterone and mature control groups, respectively.

A single injection of estradiol, either alone or in combination with daily progesterone treatment, proved to be detrimental to embryo survival by inhibiting implantation.

No significant difference ($P>.05$) was found in the average litter size or in the prenatal survival between rats bilaterally ovariotomized on the eighth day post coitus and treated with 3.5 or 5.0 mg progesterone daily. The average litter size at 20 days of gestation was 6.0 and 4.7 fetuses per rat maintained on 3.5 and 5.0 mg progesterone per day, respectively. Post ovariotomy fetal survival for rats maintained on 3.5 and 5.0 mg progesterone per day was 63.2% and 55.1%, respectively.

Histological examination of the uteri from prepuberal mated rats which were not pregnant and unmated progesterone treated prepuberal rats indicated that both the endometrial cell height and the endometrial cell nuclear area were significantly increased ($P<.05$) as a result of progesterone treatment. In addition, while the endometrial cell height and nuclear area remained relatively constant in those rats receiving exogenous progesterone, the cell height and endometrial nuclear area decreased significantly ($P<.05$) in the prepuberal mated rats as the time post mating increased.

The results of these trials tend to indicate that the 45 day old prepuberal DDR rat does not routinely maintain pregnancy as a result of corpora lutea failure and the resulting progesterone deficiency. It also appears that a uterine environment, favorable to embryonic and fetal survival, may be induced with daily treatment with 3.5 mg of progesterone administered subcutaneously.

Ovariotomy of the mature gestating rat resulted in resorption or abortion of all fetuses when the operation was performed prior to the 17th day of gestation. A 68.4% post ovariotomy fetal survival rate was obtained in those rats ovariotomized on the 17th day of gestation. From these results it appears that, in the rat, the ovaries are dispensable toward the end of gestation providing that sufficient quantities of progesterone are produced by the placentae.

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APPENDIX

Table IX. Analysis of Variance for the Reproductive Performance of Control and Progesterone Treated Rats.

Source	df	MS	F
Ovulation Rate			
Treatment	6	61.1	1.44
Error	37	42.2	
No. of rats with fetuses 8 days post coitus			
Treatment	6	1.34	7.17**
Error	113	.18	
No. of rats with fetuses 20 days post coitus			
Treatment	6	1.29	7.80**
Error	113	.16	
Average litter size per mated rat 8 days p.c.			
Treatment	6	133.1	8.80**
Error	113	15.5	
Average litter size per mated rat 20 days p.c.			
Treatment	6	114.3	9.60**
Error	113	11.9	
Average litter size per pregnant rat 8 days p.c.			
Treatment	6	38.6	3.37**
Error	44	11.4	
Average litter size per pregnant rat 20 days p.c.			
Treatment	6	36.6	2.73*
Error	44	13.4	
Preimplantation Survival			
Treatment	6	987.8	4.38**
Error	37	225.4	
Postimplantation Survival			
Treatment	6	1587.3	1.81
Error	44	874.4	

Table IX. (cont.)

Source	df	MS	F
Total Survival			
Treatment	6	1731.0	3.75**
Error	42	460.8	
Average no. resorbing fetuses at 20 days p.c.			
Treatment	6	7.0	1.99
Error	44	3.5	

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

Table X. Analysis of Variance for Reproductive Performance of Mature and Prepuberal Ovariotomized Pregnant Rats.

Source	df	MS	F
Mature- no. of fetuses at time of ovariotomy			
Treatment	3	245.4	2.17
Error	40	112.6	
Mature- average litter size 20 days p.c.			
Treatment	3	216.3	31.2**
Error	40	6.9	
Prepuberal- no. of fetuses at time of ovariotomy			
Treatment	1	2.0	.13
Error	16	14.6	
Prepuberal- no. of fetuses at 20 days p.c.			
Treatment	1	10.0	.37
Error	15	27.0	
Mature- postovariotomy fetal survival			
Treatment	3	14345.4	41.4**
Error	40	346.3	
Prepuberal- postovariotomy fetal survival			
Treatment	1	29.0	.028
Error	15	1029.0	

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

Table XI. Analysis of Variance for Reproductive Tract Weight and Histological Results.

Source	df	MS	F
Tract weight of 45 day old control and gonadotrophin treated rats			
Treatment	4	32992.0	32.18**
Error	14	1025.0	
Tract weight of mated prepuberal rats 3,5,7, and 9 days p.c.			
Treatment	3	3210.0	24.40**
Error	11	131.1	
Tract weight of prepuberal rats 3,5,7,and 9 days on 3.5 mg progesterone			
Treatment	3	192.3	2.69
Error	11	71.4	
Tract weight of mature rats 3,5,7, and 9 days p.c.			
Treatment	3	238827.0	57.0**
Error	11	4189.6	
Nuclear area- prepuberal 3,5,7, and 9 days p.c.			
Treatment	3	54.3	1.58
Error	116	34.2	
Nuclear area- prepuberal on 3.5 mg progesterone for 3,5,7, and 9 days			
Treatment	3	5.6	.10
Error	116	52.3	
Nuclear area- mature 3,5,7, and 9 days p.c.			
Treatment	3	309.0	126.1**
Error	116	2.4	

Table XI. (cont.)

Source	df	MS	F
Nuclear area- 45 day old control and gonadotrophin treated			
Treatment	4	508.0	78.1**
Error	155	6.5	
Cell height- prepuberal 3,5,7, and 9 days p.c.			
Treatment	3	200.0	14.4**
Error	116	13.8	
Cell height- prepuberal on 3.5 mg progesterone for 3,5,7, or 9 days			
Treatment	3	102.0	11.5**
Error	116	8.8	
Cell height- mature 3,5,7, and 9 days p.c.			
Treatment	3	87.6	9.3**
Error	116	9.3	
Cell height- 45 day old control and gonadotrophin treated			
Treatment	4	2580.0	4.4**
Error	155	586.3	

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

Table XII. Reproductive Performance of Individual Prepuberal and Mature Control Rats

Animal	Total No. C.L.[a]	Total No. Embryos[b]	Total No. Born	Preimpl. Survival[c]	Postimpl. Survival[d]	Total Survival[e]
Prepuberal						
1	13	9	8	69.6	88.8	61.5
2	11	8	8	72.8	100.0	72.8
3 to 30	-	0	0	0.0	-	0.0
Mean[f]	12	8.5	8	71.2	94.4	67.1
Mature						
1	13	11	11	84.7	100.0	84.7
2	13	11	11	84.7	100.0	84.7
3	10	10	8	100.0	80.0	80.0
4	11	11	9	100.0	81.9	81.9
5	10	7	5	70.0	71.5	50.0
6	12	12	11	100.0	91.7	91.7
7	12	12	11	100.0	91.7	91.7
8	11	10	10	90.9	100.0	90.9
9	11	11	11	100.0	100.0	100.0
10	12	12	2	100.0	16.7	16.7
11	12	11	9	90.7	81.9	75.0
12	12	11	9	90.7	81.9	75.0
13	11	10	9	90.9	90.0	81.8
14 to 15	-	0	0	0.0	-	0.0
Mean[f]	11.3	10.5	8.9	92.6	84.8	78.4

[a]Total number of corpora lutea on both ovaries 20 days post coitus.
[b]Total number of uterine swellings at laparotomy 8 days post coitus.
[c](No. uterine swellings on day 8 p.c. divided by no. C.L.) X 100.
[d](No. born divided by no. uterine swellings on day 8 p.c.) X 100.
[e](No. born divided by no. C.L.) X 100.
[f]Means are for pregnant rats only.

Table XIII. Reproductive Performance of Individual Prepuberal Rats Maintained on 1.5, 2.5, or 3.5 mg Progesterone Daily

Animal	Total No. C.L.[a]	Total No. Embryos[b]	Total No. Born	Preimpl. Survival[c]	Postimpl. Survival[d]	Total Survival[e]
1.5 mg						
1	13	7	2	53.9	28.6	15.4
2	11	5	0	45.5	0.0	0.0
3	12	9	7	75.0	77.8	59.4
4	15	12	12	80.0	100.0	80.0
5	12	9	0	75.0	0.0	0.0
6 to 15	-	0	0	0.0	-	0.0
Mean[f]	12.6	8.4	4.2	65.7	41.3	30.8
2.5 mg						
1	11	11	9	100.0	81.9	71.9
2	17	10	8	59.9	80.0	47.1
3	10	2	0	20.0	0.0	0.0
4	10	7	5	70.0	71.5	50.0
5	9	1	1	11.1	100.0	11.1
6	12	10	10	83.3	100.0	83.3
7	11	9	9	81.9	100.0	81.9
8 to 15	-	0	0	0.0	-	0.0
Mean[f]	11.4	7.1	6.0	64.2	76.2	50.8
3.5 mg						
1	12	8	7	66.6	87.5	59.4
2	-[g]	3	2	-	66.6	-
3	10	7	6	70.0	85.9	60.0
4	20	19	19	95.0	100.0	95.0
5	14	9	8	64.3	88.9	57.2
6	13	4	3	30.8	75.0	23.1
7	14	11	11	78.6	100.0	78.6
8	15	6	6	40.0	100.0	40.0
9	-[g]	2	0	-	0.0	0.0
10	10	9	7	90.0	87.8	70.0
11	14	3	1	21.5	33.3	7.1
12	15	9	3	60.0	88.9	54.4
13 to 15	-	0	0	0.0	-	0.0
Meanf	13.7	9	7.8	58.2	75.3	55.3

[a]Total number of corpora lutea on both ovaries 20 days post coitus.
[b]Total number of uterine swellings at laparotomy 8 days post coitus.
[c](No. uterine swellings on day 8 p.c. divided by no. C.L.) X 100.
[d](No. born divided by no. uterine swellings on day 8 p.c.) X 100.
[e](No. born divided by no. C.L.) X 100.
[f]Means are for pregnant rats only.
[g]Corpora lutea regressed prior to necropsy.

Table XIV. Reproductive Performance of Individual Prepuberal Rats Maintained on 5.0 or 10.0 mg Progesterone Daily

Animal	Total No. C.L.[a]	Total No. Embryos[b]	Total No. Born	Preimpl. Survival[c]	Postimpl. Survival[d]	Total Survival[e]
5.0 mg						
1	11	8	6	72.8	75.0	54.6
2	14	7	7	50.0	100.0	50.0
3	11	5	3	45.5	60.0	27.3
4	12	8	7	66.6	87.5	58.4
5	-[g]	1	0	-	0.0	0.0
6 to 15	-	0	0	0.0	-	0.0
Mean[f]	12	5.8	4.6	59.0	64.5	38.1
10.0 mg						
1	-[g]	1	0	-	0.0	0.0
2	-[g]	1	0	-	0.0	0.0
3	12	5	5	41.7	100.0	41.7
4	12	10	10	83.4	100.0	83.4
5	-[g]	4	1	-	25.0	-
6	18	5	3	68.8	60.0	16.7
7	-[g]	1	0	-	0.0	0.0
8 to 15	-	0	0	0.0	-	0.0
Mean[f]	14	3.8	2.7	50.1	40.8	23.7

[a]Total number of corpora lutea on both ovaries 20 days post coitus.
[b]Total number of uterine swellings counted at laparotomy 8 days post coitus.
[c](No. uterine swellings on day 8 p.c. divided by no. C.L.) X 100.
[d](No. born divided by no. uterine swellings on day 8 p.c.) X 100.
[e](No. born divided by no. C.L.) X 100.
[f]Means are for pregnant rats only.
[g]Corpora lutea regressed prior to necropsy.

Table XV. Reproductive Performance of Individual Prepuberal Rats Ovariotomized on Day 8 Post Coitus and Maintained on 3.5 or 5.0 mg Progesterone Until Day 20 Post Coitus

Animal	Total No. Embryos[a]	Progesterone Level Day 8 to 20 p.c.	Total No. Born	Postimpl. Survival[b]
1	11	3.5	9	81.9
2	16	5.0	13	81.2
3	10	3.5	9	90.0
4	11	5.0	7	63.6
5	14	3.5	13	85.7
6	7	5.0	0	0.0
7	6	3.5	2	33.3
8	8	5.0	1	12.5
9	14	3.5	14	100.0
10	11	5.0	removed from trial	
11	10	5.0	9	90.0
12	11	5.0	7	63.5
13	9	3.5	0	0.0
14	6	5.0	4	66.6
15	9	3.5	1	11.1
16	0	-	-	-
17	2	5.0	2	100.0
18	3	3.5	0	0.0
19	7	5.0	0	0.0
20	0	-	-	-
21	0	-	-	-
22	0	-	-	-
23	11	0.0	0	0.0
24	10	0.0	0	0.0
25	6	0.0	0	0.0
26	9	0.0	0	0.0
27	12	0.0	0	0.0

[a]Total number of uterine swellings at ovariotomy on day 8 post coitus.
[b](No. born divided by no. uterine swellings at ovariotomy) X 100.

Table XVI. Means and Standard Errors for Body Weight and Reproductive Tract Weight of Animals Used for Histological Examination

Treatment	No. of Rats	Body Weight (grams)	Tract Weight (mg)	Tract weight (mg) per 100 g body weight
Prepuberal				
No treatment	3	110.0±2.8	129.6±21.3	114.6±17.8
PMS+56 hours	3	114.6±1.1	377.3±26.6	328.4±19.5
PMS,HCG+24 hr	3	113.4±3.0	340.3±36.3	298.3±23.8
PMS,HCG+48 hr	3	127.0±4.5	316.6±26.0	248.2±12.4
PMS,HCG+72 hr	3	121.0±3.3	324.0±11.8	267.6± 3.6
3 days p.c.	3	120.0±4.0	255.0± 2.3	213.0±18.2
5 days p.c.	3	143.3±3.6	286.0± 9.9	199.5± 6.5
7 days p.c.	3	156.6±7.2	235.0± 6.8	159.3± 8.8
9 days p.c.	3	146.6±8.9	210.6±18.2	144.1± 4.0
3 days prog.	3	125.3±3.0	266.0± 7.1	212.2± 3.2
5 days prog.	3	126.6±3.0	280.6±11.5	221.6± 2.2
7 days prog.	3	143.3±2.4	271.0±10.5	189.5± 6.8
9 days prog.	3	141.0±8.4	283.3± 9.6	200.9± 4.9
Mature				
Estrus	3	171.3±1.0	446.0± 7.8	261.7± 3.1
Anestrus	3	164.6±8.5	211.3±18.7	127.6± 5.2
3 days p.c.	3	199.6±2.5	290.6±23.6	145.2±10.4
5 days p.c.	3	191.6±7.0	414.3±20.5	215.8± 3.0
7 days p.c.	3	184.3±9.2	516.6±39.5	279.3±12.6
9 days p.c.	3	198.0±4.9	1510.0±35.7	767.0±69.5

VITA

The author, Fredrick Roger Hofsaess, the oldest son of Alfred and Edna Hofsaess of Mountainside, New Jersey, was born on October 15, 1945.

The author was graduated from Westfield Senior High in 1963 and entered Delaware Valley College of Science and Agriculture, Doylestown, Pennsylvania, the following September. In May, 1967 he was graduated from Delaware Valley College with a B.S. in Animal Husbandry.

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In June, 1968, the author married the former Elizabeth Ann Haldimann. The author and his wife are expecting their first child in October, 1970.

EFFECT OF EXOGENOUS HORMONES ON PREGNANCY MAINTENANCE IN THE PREPUBERAL RAT

by

Fredrick R. Hofsaess

ABSTRACT

Seventy five mature and 520 prepuberal Dublin Disease Resistant rats were utilized to study the effects of exogenous hormones upon various reproductive phenomena.

Ovulation was induced in 45 day old females by means of PMS and HCG. Progesterone (1.5, 2.5, 3.5, 5.0, or 10.0 mg) was dissolved in peanut oil and administered subcutaneously daily from day 2 to day 20 post coitus. Estradiol (0.2 mcg) was dissolved in peanut oil and administered subcutaneously on day 3 post coitus. Control rats received oil only. All mated rats were laparotomized, and some ovariotomized, on day 8 post coitus. All rats were necropsied on the 20th or 21st day of gestation.

One hundred seventy of 420 (40.4%) prepuberal rats mated following PMS-HCG treatment. The number of rats conceiving, as compared to the number of rats mated was 2/30, 5/15, 7/15, 12/15, 7/15, and 5/15 for the control, 1.5, 2.5, 3.5, 5.0, and 10.0 mg progesterone groups, respectively. In mature control rats, 13 of 15 conceived on the first mating. There was no significant difference ($P>.05$) in conception rate between the 3.5 mg prepuberal and the mature control groups.

The average litter size per pregnant rat at 20 days post coitus was 8.0, 4.2, 6.0, 7.8, 4.6, and 2.7 for the control, 1.5, 2.5, 3.5, 5.0, and 10.0 mg groups, respectively. The average litter size for the mature control group was 8.9. There was no significant difference ($P>.05$) among

the prepuberal control, 2.5, and 3.5 mg groups in litter size at 20 days.

A single injection of estradiol, alone or in combination with daily treatment with progesterone, proved to be detrimental to reproduction by inhibiting implantation.

The average litter size at 20 days of gestation, in prepuberal rats ovariotomized on the eighth day post coitus, was 6.0, and 4.7 for those animals receiving 3.5, and 5.0 mg, respectively. Although the difference was not statistically significant, the postimplantation survival rates of 63.2% and 55.1% for the 3.5 and 5.0 mg groups, respectively, showed a trend which favored the 3.5 mg level.

Histological examination of the uteri from prepuberal mated rats and prepuberal rats receiving 3.5 mg progesterone indicated that both endometrial cell height and nuclear area were significantly ($P<.05$) increased as a result of progesterone treatment. In addition, endometrial cell height and nuclear area remained relatively constant in rats receiving progesterone while cell height and nuclear area decreased significantly ($P<.05$) in the prepuberal mated rats as the time post mating increased.

Ovariotomy in mature gestating rats resulted in resorption or abortion of all fetuses when the operation was performed on the 8th or 14th day of gestation. However, when ovariotomized on the 17th day of gestation, 68.4% post-operative fetal survival rate was recorded.

The results of this study indicate that the prepuberal DDR rat can be induced to ovulate and mate by treatment with PMS and HCG, but rarely maintains pregnancy due to insufficient progesterone production by the corpora lutea. Administration of 3.5 mg progesterone per day appeared to be the optimum quantity of exogenous progesterone required to induce a uterine environment favorable to embryonic and fetal development.