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RECIPROCAL OVARY TRANSPLANTATION AS A MEANS TO STUDY REPRODUCTIVE FUNCTION IN FEMALE LETHAL YELLOW MICE ($\underline{A}^{\underline{y}}/\underline{a}$: C57BL/6J)

BY

GERALD ALAN DICKENS

A thesis submitted in partial fulfillment
of the requirements for the degree
Master of Science
Major in Biology
South Dakota State University
1987

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RECIPROCAL OVARY TRANSPLANTATION AS A MEANS TO STUDY REPRODUCTIVE FUNCTION IN FEMALE LETHAL YELLOW MICE ($\underline{A}^{\underline{y}}/\underline{a}$: C57BL/6J)

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Nels H. Granholm, Ph.D. Date
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INTRODUCTION

The lethal yellow gene ($\underline{A}^{\underline{y}}$) located at the agouti locus (chromosome 2) in the house mouse (*Mus musculus*) causes a number of phenotypic abnormalities. Homozygosity of the $\underline{A}^{\underline{y}}$ allele results in death of the embryo *in utero* around the time of implantation (Eaton, 1968). For this reason $\underline{A}^{\underline{y}}$ is called the lethal yellow gene. However, the heterozygote ($\underline{A}^{\underline{y}}/\underline{a}$) is viable but shows a number of phenotypic abnormalities. Among these abnormalities is reproductive failure in females. This reproductive senescence of $\underline{A}^{\underline{y}}/\underline{a}$ females is the primary focus of this thesis.

The mammalian reproductive system departs furthest from that of the primitive vertebrates. Primitive, ancestral vertebrates were presumably egg-layers, and this style of reproduction is fairly typical of all classes of vertebrates except the mammals. All mammalian species with the exception of prototherians possess a uterus, nourish their young with milk, and care for their young. Relatively few young are produced, but the likelihood for survival is fairly high. The following paragraph briefly describes the highly successful reproductive system of the female mammal.

Reproduction is characterized by a series of cyclic events that are under nervous and hormonal control. The regulation of the reproductive cycle is maintained through reciprocal controls between endocrine organs and their

secretions. Female reproductive organs consist of ovaries, fallopian tubes, uterus, and vagina. The reproduction cycle, termed the menstrual cycle, can be thought of as beginning with the development of follicles in the ovaries. This development depends directly upon the estrogen produced by the follicle, and upon two hormones secreted by the anterior pituitary, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Secretion of FSH and LH is stimulated by the secretion of gonadotrophin releasing hormone (GnRH) by the hypothalamus. In turn the hypothalamus is controlled by a negative feedback system via estrogen and progesterone from the ovaries. As with any complex biological system, there are numerous opportunities for things to go wrong. Somewhere in this complex neural and endocrine system, the lethal yellow gene causes one or more malfunctions resulting in reproductive senescence.

There are a number of tissue candidates for the specific $\underline{A}^{\underline{y}}$ - directed malfunction. A hypothalamic and/or pituitary lesion seems probable, since they are the target organs for other obese rodent syndromes (e. g., ob/ob, db/db, and fa/fa). However, unlike other rodent syndromes, the yellow mouse seems to have a progressive rather than a sharply defined infertility. The progressive infertility in $\underline{A}^{\underline{y}}/\underline{a}$ mice seems to be related to fat deposition. Therefore, a fundamental question is whether there exists a primary $\underline{A}^{\underline{y}}$ hypothalamic lesion causing the reproductive failures, or

whether fat deposition or fat metabolism is the more primary lesion which secondarily affects hypothalamo-pituitary function.

In addition to causing infertility in females, A^{y} induces a number of other phenotypic phenomena that may be of considerable interest to biomedical science. Some of these phenomena include:

1. Implantation failure. Implantation is a particularly sensitive time in the reproduction of all mammals. Johnson and Everitt (1984) report that 40 - 50% of all spontaneous abortions are due to cervical incompetence or implantation in eccentric uterine positions. A^{y}/a and a/a dams together with their lethal yellow (A^{y}/a) and control progeny (a/a), provide a well-controlled and productive system to investigate prerequisites for mammalian implantation.
2. Unique nutritional pathways that channel incoming food calories into fat rather than protein. The national health craze with obesity is well known. It would be productive to determine how A^{y} changes cellular metabolism; once understood, it might be possible to develop effective treatments to prevent or retard human obesity.
3. Induction of a diabetic-like condition. A^{y} stimulates a diabetic-like condition in $A^{y}/-$ mice (Hellerstrom and Hellman, 1963; Hummell et al., 1972). An understanding

of A^{y}'s precise role in stimulating diabetes could lead to novel treatments.

4. Cancer. A^{y} causes increased susceptibility to spontaneous and induced lung, liver, skin, and mammary tumors (Heston and Vlahakis, 1961; Morgan, 1950; Vlahakis and Heston, 1963). Since A^{y} has also been reported to have a growth promoting effect (Wolff et al., 1986), it may be that growth promoting and cancer producing characteristics may be related. The A^{y} gene may provide clues and insights regarding cancer and growth regulation.

LITERATURE REVIEW

Relationship of A^y to Reproduction

The lethal yellow gene (A^y) causes a number of phenotypic alterations. Cuenot (1905) described the genetic mutation and attributed three aberrant phenotypic alterations to the lethal yellow gene. These included yellow coat color, obesity, and embryonic lethality in A^y homozygous embryos (A^y/A^y). Since that time, a number of other phenotypic alterations have also been attributed to A^y. Cancer, diabetes, aberrant body temperature regulation, and increased body and tail lengths have also been observed in mice heterozygous for A^y (Danforth, 1927; Cizadlo, 1976; Bray and York, 1979).

Reproductive failure in mature obese yellow mice (A^y/a: Strain C57BL/6J), especially females, occurs progressively (Jeppesen, 1985). Granholm and Brock (1980) reported that females put into production at puberty (A^y/a ♀ x a/a ♂ and A^y/a ♀ x A^y/a ♂ matings) rarely produce third litters, and have never been observed to produce fourth litters. Obese yellow females are poor breeders.

In several mammalian species, chemical factors called pheromones have been shown to exert an important regulating influence on reproduction, aggressiveness, and other behavioral patterns (Bronson, 1970). Therefore, the inability of obese A^y/a females to breed could be due to

pheromonal aberrations. With respect to the Bruce and Whitten effects, the neurosecretory competence of $\underline{A}^{y}/\underline{a}$ mice of both sexes to produce, release, receive and respond to pheromones revealed no consistent differences between $\underline{A}^{y}/\underline{a}$ and nonyellow controls (Bartke and Wolff, 1966; Kakihana et al., 1974; Whitten, 1973). Granholm and Brock (1980) performed a mating selection study using 6-12 week old C57BL/6J $\underline{A}^{y}/\underline{a}$ and $\underline{a}/\underline{a}$ littermates; they concluded that the $\underline{A}^{y}$ gene did not influence mate selection or copulatory success in young preobese $\underline{A}^{y}/\underline{a}$ females. Thus there are no convincing data that support the notion of aberrant pheromones in $\underline{A}^{y}/\underline{a}$ females or males.

Previous investigators (Robertson, 1942; Kasten, 1952; Eaton, 1968) have suggested that $\underline{A}^{y}/\underline{a}$ females are deficient in steroid hormones. Since the interplay of these hormones is crucial in regulating estrus and in maintaining a healthy uterine environment $\underline{A}^{y}$ may alter the synthesis and/or deployment of ovarian steroids.

Cizadlo et al. (1975) showed that the average mean litter size of 4.2 $\pm$ 0.7 (mean $\pm$ standard error of the mean) from strain C57BL/6J $\underline{A}^{y}/\underline{a}$ x $\underline{a}/\underline{a}$ matings was lower ($P<0.05$) than the mean of 6.1 $\pm$ 0.1 from the reciprocal cross in which embryos develop in nonyellow uteri. Wolff and Bartke (1966) also showed a reduced litter size in yellow females by black male matings (5.4 $\pm$ 0.1) as opposed to (6.5 $\pm$ 0.1) in reciprocal crosses. Wolff and Bartke (1966) also

reported a deficiency of A^y/a progeny born to A^y/a females suggesting a specific deleterious uterine effect of genetically yellow uteri (A^y/a) on heterozygous yellow (A^y/a) embryos. Granholm et al. (1986) found that obese A^y/a females had significantly reduced uterine weights (49.2 $\pm$ 4.1 versus 84.3 $\pm$ 7.1 mg) for A^y/a and a/a females, respectively. Granholm et al. (1986) also reported significantly reduced copulatory success and conception rate in A^y/a females. Obese A^y/a females may experience sex steroid imbalances, perhaps as a secondary effect of reduced gonadotrophin activity.

Wolff et al. (1986) postulate that unknown local environmental factors in the maternal reproductive tract influence the differentiation of A^{vy}/a zygotes. The developmental retardation and death of lethal yellow (A^y/A^y) embryos provides evidence for early activation of the agouti locus (Granholm and Johnson, 1978). Wolff et al. (1986) also cite the 1942 Robertson experiment in which A^y/A^y embryos supposedly survived a day longer when they developed in nonyellow uteri as support for his "uterine factor" theory. Apparently these uterine factors influence the expression of the agouti locus genes during early cleavage stages, since developmental retardation occurs in lethal A^y/A^y embryos (Pederson and Spindle, 1976; Granholm and Johnson, 1978). It appears that the agouti locus may be involved in some aspect of cleavage stage metabolism. Thus

in 2- and 4- cell stages the agouti locus could be synthesizing specific gene products that could be influenced or regulated by factors within the reproductive tract (Jeppesen, 1985).

Granholm et al. (1986) also found that estrous cycles differed in obese A^{y}/a mice when compared to age-matched a/a controls. Of 70 A^{y}/a females tested, only 18.6% (13/70) had typical estrous smears while 44.3% (31/70) a/a females displayed typical estrous smears. Kasten (1952) also had difficulty in obtaining typical estrous stage smears from obese A^{y}/a females during a two week trial period. Following semiquantitative histological analyses of ovaries from mildly obese and obese $A^{y}/-$ females , Kasten (1952) concluded that ovaries from obese females showed reduced corpora lutea and other signs of infertility. Silberberg and Silberberg (1957) also reported marked luteinization of ovaries of both $A^{y}/-$ and gray controls of strain YBR/WI females.

To date, Kasten (1952) and Granholm et al. (1986) have analyzed the effects of A^{y} on female reproduction. An analysis of ovarian steroids in both cycling and pregnant $A^{y}/-$ mice might establish the molecular and cellular bases of A^{y} action. Data on plasma progesterone, and estrogen levels in cycling and pregnant mice have been reported (Murr et al., 1973; Nelson et al., 1981). However, neither of these studies dealt with $A^{y}/-$ mice.

The precise target of $\underline{A}^{y}$ gene action within the reproductive system is not known. Therefore, a brief discussion of the mammalian reproduction system is warranted to facilitate further discussion of the reproductive basis of the $\underline{A}^{y}$ lesion. A large part of the following discussion is adapted from Johnson and Everitt (1984).

Since the ovary is a predominant structure of the female reproductive system we can start with its function. Each day after puberty a few follicles recommence growth. It is useful to trace one of these follicles through its full development.

The earliest phase of follicular growth is called the preantral phase and is characterized by an increase in follicular diameter. The major part of this growth occurs in the primary oocyte. The granulosa cells divide and will become several layers thick. During this dividing stage granulosa cells secrete a glycoprotein material that forms an acellular layer (zona pellucida) between themselves and oocytes. However, contact between granulosa cells and oocytes is maintained via cytoplasmic processes that penetrate the zona and form gap junctions at the oocyte surface. Another layer of cells, thecal cells, also forms during this phase. Unlike granulosa cells, thecal cells are highly vascularized. Thus, the first phase of development is mainly characterized by an increase in both size and complexity of the follicle. This phase is also independent

of any direct external control. At the end of the preantral phase, granulosa cells develop receptors for estrogen and follicle stimulating hormone (FSH), while thecal cells develop luteinizing hormone (LH) receptors. Entry into the second phase is critically dependent upon pituitary gonadotrophin stimulation.

During the second or antral phase of follicle maturation, many of the follicles undergo atresia. During atresia the granulosa cells show reduced synthetic activity, accumulate lipid droplets, and develop pycnotic nuclei. Death of oocytes follows. However, atresia can be prevented if adequate tonic levels of FSH and LH in the circulation coincide with the development of FSH and LH receptors on granulosa and thecal cells, respectively. The effect of the gonadotrophins is to convert the preantral follicles to antral follicles (also called Graafian follicles). During this conversion process granulosa and thecal cells proliferate, resulting in a further increase in follicular size. Thecal cells divide into two distinct layers, a highly vascular theca interna, surrounded by a fibrous capsule, the theca externa. A fluid filled cavity (antrum) will also form between the dividing granulosa cells. A cumulus oophorus, a dense mass of granulosa cells suspended in the antrum, may also form. During this phase follicles also begin to synthesize steroids; gonadotrophins control follicular steroidogenesis. As the follicles increase in

After the termination of meiosis II, a number of changes occur in the cytoplasm of the oocyte. These include: (1) intimate contact between the oocyte and the granulosa cells is broken by withdrawal of the cytoplasmic processes, (2) the Golgi apparatus begins to synthesize lysosomal-like granules, and (3) although protein synthesis continues at the same rate, new and distinctive proteins are synthesized. These activities will prepare the oocyte for fertilization.

In addition to the physical changes that occur in the oocyte, major changes also take place in steroid secretion. Shortly after the beginning of the LH surge, the follicle increases its output of estrogens and androgens for a short time which then decline to low levels. This elevated steroid output coincides with distinctive changes in the thecal cells. Within a few hours after the LH peak, granulosa cells also show some marked changes. First, granulosa cells can no longer convert androgens to estrogen, as they were able to do in antral follicles. Instead they synthesize progesterone. Second, LH stimulates the synthesis of progesterone via the newly acquired LH receptors. Third, granulosa cells lose their capacity to bind estrogen and FSH. This preovulatory phase of follicular growth is usually the shortest, but culminates in the remarkable process of ovulation.

By the end of the preovulatory phase, the rapid

expansion of the antrum has resulted in a thin peripheral rim of granulosa and thecal cells to which the oocyte is attached only by a tenuous and thinning stalk of cells. Only a thin layer of epithelial cells exist between the follicular wall and the peritoneal cavity. The follicle then ruptures when this thin layer of epithelial cells becomes very thin and avascular. The fluid within the follicle then flows out over the surface of the ovary carrying with it the oocyte and its surrounding mass of cumulus cells. In some species including the mouse, a peritoneal capsule or bursa encloses the ovary and acts to retain the egg masses close to the ovary. The egg masses are then collected by the concerted beating of oviductal cilia and drawn into the oviductal ostium.

The collapsed follicle is now transformed into a corpus luteum. This process is known as luteinization and is associated with a steady increase in the secretion of progestagens. The maintenance of the corpus luteum requires hormonal support. However, this support varies from species to species. LH provides the main support in humans. In mice prolactin predominates. The life of the corpus luteum in non-pregnant females is also species specific. In mice it lasts for about 2 days and then luteolysis occurs.

The length of follicular development varies with species. Johnson and Everitt (1984) reported that in mice the duration of preantral phase as 14 days, antral phase as

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4 days, preovulatory phase as 11 hours, and the luteal phase as 2 days. Whatever the actual length of follicular development, the preovulatory phase is always the shortest.

The ovarian cycle of the nonpregnant mouse shows much the same pattern as large farm animals. Estrus occurs at 4-6 day intervals throughout the reproductive life span (Whittingham & Wood, 1983). The estrous cycle is divided into four phases, proestrus, estrus, metestrus, and diestrus. Proestrus and estrus culminate with ovulation and represent the follicular phase of the ovarian cycle, while metestrus and diestrus constitute the luteal phase. The phases can be distinguished by vaginal smears, and numerous investigators have characterized these estrous phases (Allen, 1922; Bingel, 1974; Bingel and Schwartz, 1969).

The mouse also has one more curious feature that allows its ovarian cycle to vary in length, depending on whether females become pregnant (Johnson and Everitt, 1984). If a female has an infertile mating at the time of ovulation, her luteal phase is 11-12 days in duration. However, if she fails to mate at ovulation her luteal phase is only 2-3 days long. The explanation for this is that mechanical stimuli to the cervix (occurring at copulation) relay messages to the central nervous system (CNS) eliciting the release of prolactin. The luteal period is extended. Prolactin is essential for maintenance of the corpus luteum, and without it, luteal life is abbreviated to only 2 or 3 days (Johnson

and Everitt, 1984). This abbreviating device allows mice to be fertile every 4 or 5 days instead of every 13 or 14 days. The increased reproductive efficiency of this evolutionary modification should be apparent.

Little is known about the precise secretory timing of ovarian steroids in the mouse (Whittingham and Wood, 1983). However, a brief review of the regulation of gonadal function in mammals in general may be useful to facilitate our discussion of possible $\underline{A}^{y}$- induced reproductive lesions.

An important concept previously discussed was the ability of the mouse to reduce it's ovarian cycle to 4-5 days instead of the expected 12-13 day cycle. This concept was the control of the ovarian cycle by the CNS. Two areas of the brain, the anterior pituitary and the hypothalamus, exercise a delicate control over the ovarian cycle.

The hypothalamus, a relatively small area that lies at the base of the brain releases one or more neurohormones that regulate the synthesis and release of both FSH and LH from the pituitary. This hormone is called gonadotrophin-releasing hormone (GnRH) and is secreted into portal vessels which travel to the pituitary gland. GnRH is released as a series of pulses, travels to the anterior pituitary by way of the portal vessels, and induces or triggers gonadotrophin secretion in a similar, pulsatile manner. By increasing or decreasing the amplitude or frequency of these GnRH pulses, the output of LH and FSH may be regulated.

The hypothalamus also exercises control over the secretion of prolactin. It does this through another neurohormone known as dopamine. However, dopamine acts on the anterior pituitary in a way quite different from that of GnRH. Dopamine acts as an inhibitor on the anterior pituitary, and thereby depresses prolactin secretion. For this reason dopamine has become known as prolactin inhibitory factor (PIF). Both GnRH and dopamine also act as neurotransmitters at other sites within the central nervous system.

Secretion and synthesis of FSH and LH by the anterior pituitary are dependent upon the pulsatile stimulation of GnRH from the hypothalamus. However, the regulation of FSH and LH secretion can also be achieved directly at the level of the anterior pituitary. The regulation of GnRH, FSH, and LH are exercised mainly by secretory products of the ovary through both negative and positive feedback effects.

After ovariectomy or menopause, plasma levels of FSH and LH increase markedly. The reason for this is that the ovary produces a group of hormones known collectively as estrogens which act both upon the hypothalamus to regulate the release of GnRH and upon the anterior pituitary to regulate the release of FSH and LH. Since estrogens in low levels stimulate gonadotrophin release, the process is known as negative feedback, i. e. low estrogen, high FSH/LH

output. In relation to the estrous cycle, the decline of FSH and LH during the early parts of the follicular phase are due to negative feedback effect of the estrogens.

Estrogen also has another effect on gonadotrophin secretion. Interestingly, very high levels of estrogen enhance rather than suppress the release of FSH and LH. Estrogen acts on both the hypothalamus and the anterior pituitary. However, it does seem that the anterior pituitary is probably where the estrogens exert their most potent effect. The ability of estrogen to enhance FSH and LH secretion is termed positive feedback. In relation to the mouse estrous cycle, there is a 200-400% increase in estrogen at the midpoint of follicle maturation. This increase in estrogen produces a surge of LH and a slight surge of FSH at ovulation. As discussed, FSH is necessary to induce LH receptors on granulosa cells, while the LH surge is necessary for ovulation.

A second hormone, progesterone, also plays an important role in the regulation of FSH and LH secretion. Progesterone in high plasma concentrations will enhance the negative feedback effects of estrogen, on both FSH and LH. High levels of progesterone may also block the positive feedback effect of estrogens. There is some evidence that low concentrations of progesterone can actually facilitate the positive feedback effects of estrogen by inducing an LH/FSH surge. However, progesterone appears to act only upon the

hypothalamus, while estrogen acts on both the hypothalamus and the anterior pituitary. In general, high levels of progesterone are associated with negative feedback and low levels with positive feedback.

A third hormone has been implicated in the feedback regulation of FSH and LH. This hormone, known as inhibin, appears to be produced by the granulosa cells of maturing antral follicles. Inhibin is different from estrogen and progesterone, because it depresses FSH secretion but has little or no effect on LH secretion. The secretion of inhibin probably accounts for the observation that FSH decreases during the follicular phase, while LH secretion actually increases.

Feedback effects of the gonadal hormones are adequate to explain the basic features of the reproductive patterns in females. However, the hypothalamic-pituitary-gonadal axis is not a closed system, and external influences can clearly modulate it's activity. For instance in mice, the day-night cycle can exercise profound effects on ovarian activity. In short, the estrous cycle can be extremely labile.

Since the $\underline{A}^{\underline{y}}/\underline{a}$ female displays a progressive infertility that seems to be correlated to adiposity, it is worthwhile to discuss how fat metabolism may affect components of female mammalian reproduction, especially steroid synthesis and regulation. Deslypere et al. (1985)

have reported that fat tissue can act as a steroid reservoir and a site of steroid metabolism. A significant linear correlation existed between plasma estrone and estradiol levels and total body weight and/or fat mass (Deslypere et al., 1985). Previous researchers (Robertson, 1942; Kasten, 1952; Eaton, 1968; Granholm et al., 1986) have also suggested A^y- caused ovarian steroid deficiencies and the potential role of fat in causing A^y- induced sterility.

Cuenot (1905), in his description of the yellow mouse, discussed A^y as a genetically transmitted obese syndrome. However, in comparison to other rodents with inherited forms of obesity, studies on A^y/- mice have been limited. Since other genetically obese rodents also have reproduction problems, it would be productive to briefly review how obesity may cause their sterilities. Three obese rodent syndromes seem to have some likenesses to the yellow mouse. These include obese (ob/ob), diabetes (db/db), and the fatty rat (fa/fa).

The obese mouse (ob/ob) inherits its obesity as an autosomal recessive mutation on chromosome 6 (Coleman, 1978). These animals are also known to have multiple abnormalities of the endocrine systems, including impaired growth, impaired temperature regulation, and impaired reproductive function (Swerdloff et al., 1978). Therefore, ob/ob mice may have a hypothalamic defect. Early studies on the hypothalamo-pituitary axis in males revealed smaller

than normal testes and reduced ventral prostate weights (Swerdloff et al., 1976). The obese animals also had lower serum testosterone and FSH levels, and they did not demonstrate an LH rise at 39-45 days of age (Swerdloff et al., 1978). These findings indicate that the hypogonadism is secondary to altered hypothalamic-pituitary function. However, a second study attempted to separate hypothalamic from pituitary dysfunction. An acute LHRH response test was administered to obese (ob/ob) and lean (+/ob) littermates. Both groups showed an increase in serum LH concentration, but the increase was two-fold greater in lean animals (Swerdloff et al., 1978). A further study was conducted to determine if the defect in obese animals was due to problems in the pituitary gland or in the hypothalamus. In this study chronic amounts of LHRH were administered for 20 days after which an acute LHRH test was repeated. Surprisingly, in both lean and obese groups, the LHRH response was smaller from the chronic LHRH treatment as compared to the acute LHRH treatment (Swerdloff et al., 1978). Therefore, obese (ob/ob) mice have an impaired response to LHRH, and this would be consistent with a defect in pituitary function (Swerdloff et al., 1978).

The diabetes mouse (db/db) inherits its obesity as an autosomal recessive mutation on chromosome 4 (Coleman, 1978). These mice also exhibit other abnormalities that include hyperglycemia, hyperinsulinemia, thermoregulatory

disturbances, and sterility in both sexes (Hummel et al., 1966). Other studies on food intake (Coleman and Hummel, 1969) suggest that the brain (hypothalamo-pituitary axis) may be the site of action of the _db_ genetic locus. Johnson and Sidman (1979) gathered endocrine and reproductive data that suggest an abnormal hypothalamic function; they reported that reproductive problems of homozygous _db_ mutant females were associated with inadequate gonadotrophic stimulation and not with an unresponsive reproductive tract. Equivalent increases in serum LH for mutant and control mice after GnRH treatment supported the notion that the problem in _db/db_ mice relates to inadequate GnRH release from the brain; Johnson and Sidman (1979) concluded that the reproductive neuroendocrine defect is a hypothalamic disorder, but other CNS sites could play more fundamental roles.

The _fatty_ (_fa/fa_) rat is inherited as an autosomal recessive mutation (Zucker and Zucker, 1961). These rats exhibit obesity, hyperinsulinemia, hyperlipemia, and reduced fertility. Female fatty (_fa/fa_) rats had delayed vaginal opening, prolonged estrous cycles, decreased uterine weight, and absence of deciduomata formation during reserpine-induced pseudopregnancy (Saiduddin et al., 1973). These effects would suggest a decreased estrogen secretion or impaired estrogen effect (Saiduddin et al., 1973). However, since serum LH and FSH were found to rise normally after

ovarian hypertrophy in response to unilateral ovariectomy, these data suggest a decreased threshold in the hypothalamus for feedback inhibition of gonadotrophin secretion (Saiduddin et al., 1973). There may be two defects in the reproductive system of fatty (fa/fa) rats - estrogen and hypothalamus defects. Further evidence favors impaired hypothalamic function including diminished feedback control of FSH secretion (York et al., 1972), altered thirst response to plasma osmolarity (York and Bray, 1971), and defective regulation of food consumption in response to cold stress (Bray and York, 1972).

In summary, A^y does not appear to affect pheromonal communications, at least in young A^y/a mice. A^y/- females undergo a progressive infertility apparently correlated with age and obesity. Data suggest an A^y - caused hostile uterine environment, although this hasn't been documented. Many reproductive parameters (copulatory success, number of ova ovulated, fertilization success, uterine weight, receptivity, and others) decline in aging obese A^y/a females even though their age-matched a/a littermates perform well (Granholm et al., 1986). The actual effects of A^y on the reproductive system of A^y/- mice are not known. However, adiposity may play a key role.

Objectives

The first study was conducted to determine whether the previously observed reproductive failures in aging obese lethal yellow (A^y/a) females (Granholm et al., 1986) are due primarily to: (1) intrinsic defects within A^y/a ovaries or (2) systemic defects extrinsic to A^y/a ovaries such as sites within the hypothalamus or pituitary gland.

A second experiment was conducted to assess uterine capacity in A^y/a females. In order to accurately measure uterine capacity in A^y/a females, it was necessary to separate uterine effects from embryo (A^y/a) effects. Thus by conducting the appropriate ovary transplantations, one could test uterine capacity in A^y/a females in the absence of A^y/a embryos.

EFFECTS OF RECIPROCAL OVARY TRANSPLANTATION ON REPRODUCTIVE PERFORMANCE OF LETHAL YELLOW MICE (A^{y}/a): C57BL/6J)

SUMMARY

This study was conducted to determine whether reproductive failures in aging, obese lethal yellow (A^{y}/a) females are due primarily to defects within A^{y}/a ovaries or to systemic defects which may operate outside the ovaries. Reciprocal ovary transplantation between control (a/a) and lethal yellow (A^{y}/a) females provided an experimental system to test the reproductive potential not only of A^{y}/a ovaries in control (a/a) females but also of control (a/a) ovaries in mutant (A^{y}/a) females. Results on reproductive performance of all four combinations of grafts between A^{y}/a and a/a mice proved that A^{y}- induced reproductive failures are not due to intrinsic ovarian lesions but rather to defects operating extrinsically to the ovary. The hypothalamo-pituitary axis is a likely site for this reproductive lesion.

INTRODUCTION

The lethal yellow gene ($\underline{A}^{y}$) causes a number of metabolic aberrations including yellow hair, embryo death in $\underline{A}^{y}$ homozygotes, obesity, cancer, and female sterility (Bray and York, 1979). Granholm et al. (1986) have reported decreased copulation success, reduced uterine weight, and depressed conception rate in $\underline{A}^{y}/\underline{a}$ females older than 120 days of age. These findings are consistent with deficiencies in ovarian steroids. Since exogenous gonadotrophins at superovulatory levels (5.0 I.U. PMG/5.0 I.U. HCG) can partly restore ovulation rate, embryo viability to 2-cell stages, and conception rate of reproductively senescent $\underline{A}^{y}/\underline{a}$ females (Granholm et al., 1986), ovarian tissues can respond to FSH and LH. This exogenous gonadotrophin-induced restoration of folliculogenesis, although productive in delineating potential bases of $\underline{A}^{y}$- induced reproductive senescence, does not clarify whether the $\underline{A}^{y}$ lesion results in a weak gonadotrophin signal to the ovary, an impairment of ovarian thecal or granulosa cells to respond to a normal gonadotrophin signal, or both. Reciprocal ovary grafting provides an experimental procedure to determine the overall capability and therefore functional integrity of the $\underline{A}^{y}/\underline{a}$ ovary. Severely affected reproductively nonfunctional ovaries of ob/ob, db db and hpg/hpg mice have been shown to

ovulate and produce expected frequencies of mutant and nonmutant progeny upon grafting to nonmutant recipients (*ob/ob*: Hummel, 1957; Batt and Harrison, 1963; *db/db*: Hummel et al., 1966; Johnson and Sidman, 1979; *hpg/hpg*: Bamber et al., 1980).

MATERIALS AND METHODS

Breeding stocks of C57BL/6J $\underline{A}^{\underline{y}}/\underline{a}$ and $\underline{a}/\underline{a}$ mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Mice were produced by means of trio matings, 2 $\underline{a}/\underline{a}$ x $\underline{A}^{\underline{y}}/\underline{a}$. Mouse facility environmental conditions were kept constant at 16h light: 8h darkness and 21 degrees C. Wayne Breeder blox and water were available ad libitum. The bedding of white pine shavings was changed weekly.

Virgin $\underline{A}^{\underline{y}}/\underline{a}$ and $\underline{a}/\underline{a}$ females, 50-70 days of age, were used as donors and recipients. Anaesthesia was induced by tribromoethanol, and ovaries were transplanted using surgical procedures outlined by Jones and Krohn (1960). Donor ovaries were surgically removed, placed in petri dishes of Brinster's BMOC III medium (Gibco, NY) on ice, bisected, and half ovaries were grafted to empty ovarian bursae. Stevens (1957) documented that grafts of half ovaries resulted in greater reproductive efficiency (i.e. average litter size and average number of litters per operation) than did whole or quarter ovaries.

TABLE 1. RECIPROCAL OVARY TRANSPLANTATIONS.

Grafting Type	Genotype of grafted Ovary	Genotype of host	Grafting Designation	Expected progeny from hosts x black (a/a) males
Surgical controls				
I	a/a	a/a	B-B[1]	All a/a or black
II	A^Y/a	A^Y/a	Y-Y	1A^Y/a:1a/a
Experimental grafts				
III	a/a	A^Y/a	B-Y	All a/a or black
IV	A^Y/a	a/a	Y-B	1A^Y/a:1a/a

1 Ovary genotype-host genotype; B=a/a, Y=A^Y/a; in the case of B-B, B or a/a ovaries were grafted to B or a/a hosts.

Table 1 displays the four qualitatively different types of ovarian grafts that were conducted. Groups I and II were necessary controls to account for non-A^Y related surgical losses. Groups III and IV were the experimental reciprocal ovarian transplantations combining a/a ovaries within A^Y/a hosts (B-Y) and A^Y/a ovaries within a/a hosts (Y-B), respectively. Since C57BL/6J a/a and A^Y/a mice possess uniformly black (B) and yellow (Y) coats respectively, grafting designations B-B, Y-Y, B-Y and Y-B were used to indicated the relationship between ovary and host genotypes. For example, grafting designation B-Y indicates a/a (B)

ovaries grafted into $\underline{A}^{\underline{y}}/\underline{a}$ (Y) hosts.

After surgery, females recovered for one-week. Then host females were individually paired with proven a/a males to assess their reproductive performance. The paired matings were maintained until hosts failed to have a litter within a 10-week period after their last litter. Each host was scored for total litters, mean progeny per litter, genotypes of progeny, and other measures (Tables 2 and 3). Numerical differences in these values were tested for statistical significance using X^2 analyses.

Ten weeks following their last litters, females were injected with superovulatory levels of gonadotrophin-like hormones - PMS and HCG. PMS (Gestyl, Organon) was prepared immediately prior to the start of the experiment. Each PMS vial contained 400 I.U. (International Units of biological activity in a lyophilized form. One ml. of sterile saline was added to the powder to rehydrate the PMS and then removed and placed in a sterile 25 ml. vial with 7 ml. of a balanced salt solution (Brinster's medium) as a diluent. The final concentration of the hormone was 50.0 I.U. per ml. or 5.0 I.U. per 0.1 ml. Each mouse received a 0.1 ml. injection containing 5.0 I.U. of PMS.

The HCG (Pregnyl, Organon), which was lyophilized, was reconstituted and diluted with Brinster's medium to a final concentration of 50.0 I.U./ml. Again, each mouse received a 0.1 ml. injection containing 5.0 I.U. of PMS.

Analyses of reproductive parameters included the following. Thirty-eight hours following HCG injection, each female was weighed to the nearest 0.1 g. and sacrificed. Ovaries and uterine tracts were removed and each pair of organs was weighed to the nearest 0.1 mg.

Ovaries were visually observed under a Nikon dissecting microscope at 50x and then "pricked" with 25 gauge hypodermic needles. We used a procedure similar to that described in Rafferty (1970), who used this technique to liberate ova from Graafian follicles for in vitro fertilization experiments. This ovary "punching" technique was judged to be a good method to standardize treatment between A^y/a and a/a ovaries and obtain a quantitative measure of the ability of an ovary to liberate ova within ovarian follicles. Each ovary was "punched" or "pricked" 75 times. Ovarian fragments and ova were suspended by adding approximately 0.5 ml. of physiological saline. Using the dissecting microscope, the number of liberated ova was counted.

Each uterine horn and oviduct was observed under the dissecting microscope. If no ova could be detected in the proximal ampullary regions of the oviduct (i.e. the end of the oviduct which receives ova), the oviduct was flushed with 0.1-0.2 ml. of physiological saline following insertion of a truncated 30-gauge needle. Recovered ova and/or embryos were classified as normal ova (N) or

vesiculated/abnormal ova (V).

Numerical differences in reproductive parameters between experimental and control groups were statistically analyzed using X^2 and analysis of variance.

RESULTS

Grafting success was tested by analyzing genotypes of the offspring. To complete grafts in Group III successfully, A^y/a ovaries of hosts must be completely removed and replaced by half ovaries of a/a donors. These B-Y females when mated to a/a males should produce only black (a/a) progeny. The presence of any A^y/a neonates would indicate incomplete removal of host A^y/a ovarian tissue. Of 66 offspring derived from B-Y x B matings, all 38 surviving neonates were genetically a/a. Similarly, following successful transplantation in Group IV, matings of Y-B females to a/a males should result in a 1:1 ratio of A^y/a to a/a progeny. Of the 151 surviving progeny from this mating (151/251) the ratio of A^y/a to a/a mice was 59:92. Also, each Y-B female of Group IV produced at least one A^y/a mouse, thereby genetically verifying successful grafting of A^y/a ovarian tissue to a/a females.

The overall design of this study allowed us to test the performance of both mutant ovaries in control hosts (Y-B) and control ovaries in mutant hosts (B-Y). If A^y- induced defects are intrinsic to A^y/a ovaries, reproductive performance in Groups II and IV should be equally low in comparison to Groups I and III, since intrinsically defective A^y/a ovaries in a/a hosts should not perform appreciably better than A^y/a ovaries in A^y/a hosts (Table 1). In contrast, if A^y- induced reproductive senescence

results from causes extrinsic to the ovary, females in Groups I and IV, which have control hosts (B-B and Y-B), should perform comparably better than mice in Groups II and III which posses mutant host (Y-Y and B-Y).

A total of 67 host females (16 within each of Groups I and II, 17 in Group III, and 18 in Group IV) were scored for reproductive performance (Table 2). The mean percentage pregnancy in all groups was 59.7% (40/67), with controls (B-B and Y-Y) having a pregnancy rate of 62.5% (20/32) versus the experimental (B-Y and Y-B) pregnancy rate of 57.1% (20/35). The 35.3% pregnancy in the B-Y females (Group III) was significantly reduced ($P<0.01$) from that in the other three groups.

Host females from all 4 groups produced a total of 599 progeny from 129 total litters (4.6 progeny per litter). Group (B-B) and Group IV (Y-B) produced 73.1% (438/599) of the progeny, while Group II (Y-Y) and Group III produced only 26.9% (161/599) of the progeny. Groups I and IV also produced 94 of the 129 litters (72.9%). Groups I and IV did have one more mouse in their groups, but one additional mouse would not be expected to yield that great a difference in progeny (i.e., 72.9% versus 27.1%). Groups I and IV produced nearly identical mean litters per female, 4.0 and 3.9, while Groups II and III also show a similar but decreased number of litters per female, 2.0 and 2.5. In fact, except for one B-Y female that produced six litters,

mean litters per pregnant female in Group III were identical with those of Group II. The mean progeny per litter in each of the four groups are nearly the same.

A considerable number of neonatal mice died before Day 4 or 5, and their coat colors could not be determined, i.e. 46.4% (278/599) of the population. These neonatal losses were uniform in Groups I, III and IV at 43.9%, 42.4% and 40.0%, respectively. However, mice in Groups II (Y-Y) sustained a 71.6% neonatal loss which, when compared to neonatal deaths of Groups IV (Y-B), was significantly higher ($P<0.01$). These mortality data, although possibly spurious, may be a reflection of poorer mothering ability, less tolerance to stress, or other factors inherent in $\underline{A}^{\underline{y}}/\underline{a}$ versus $\underline{a}/\underline{a}$ hosts. If so, comparable neonatal losses should also have occurred in the B-Y females (Group III) but did not. One cannot rule out the possible potentiation between grafted $\underline{A}^{\underline{y}}/\underline{a}$ ovaries and $\underline{A}^{\underline{y}}/\underline{a}$ hosts that could lead to the observed 71.6% neonatal death rate.

Data on parity and litter size in each of the four groups are presented in Table 3. Mice in Groups I and IV had 13 and 22 litters within the 4th to 9th parities, while only three 4th to 9th parity litters were produced by mice in Groups II and III ($P<0.01$). All 3 of the 4th to 6th parity litters within Group III were derived from one highly unusual $\underline{A}^{\underline{y}}/\underline{a}$ female that never became obese. The most productive of the four grafting types was the Y-B

combination. Five of the 18 females in that group produced 6th litters, and 4 of these went on to have 7th litters in spite of the fact that they possessed $\underline{A}^{\underline{y}}/\underline{a}$ ovaries. In contrast, only 1 of 17 mice in the B-Y group, an unusually thin $\underline{A}^{\underline{y}}/\underline{a}$ host, had six litters.

Data on female weights, uterine weights, ovarian weights, and other parameters are presented in Table 4. After the females failed to produce a litter during a 10-week period, they were then injected with superovulatory levels of gonadotrophin-like hormones (PMS and HCG). Thirty-eight hours after HCG injection, the females were weighed and sacrificed. The reproductive tracts were then analyzed.

Mice in Groups I and IV had mean body weights of 31.0 g. and 30.8 g., while Groups II and III had mean weights of 47.5 and 47.2. Female weights were significantly different ($P<0.01$).

Ovarian weights and uterine weights were not significantly different in the four groups. However, uteri of black females (B-B and Y-B) showed a trend; they weighed mathematically more than uteri of yellow females (Y-Y and B-Y). The uteri of black females (B-B and Y-B) weighed 52.6 and 55.6 mg., respectively, while the uteri of yellow females (Y-Y and B-Y) weighed 41.2 and 43.7 mg., respectively. Also, ovaries of yellow hosts weighed mathematically more (7.8 and 8.8 mg.) than those of black

hosts (6.6 and 5.5 mg.).

The mean number of oocytes released after "punching" ovaries also shows a trend with Groups I and IV releasing the highest amounts of ova, 6.8 and 7.5, and Groups II and III releasing the least, 4.2 and 4.0. However, statistical significance was not observed.

Following ova recovery via flushing of the oviducts, Groups I and IV had the highest mean number of ova present, 4.6 and 3.4, respectively. Groups II and III had the least amount of ova, 3.2 and 2.9, respectively.

Of the 43 total mice in which the oviducts were flushed, 67.4% (29/43) yielded at least one ovum from either the left or right oviduct. This indicates that the ovary and oviduct were functionally coupled in 67.4% of the grafted hosts after ovarian transplantation. This compares favorably with the overall 59.7% (40/67) pregnancy rate and indicates highly successful grafting of ovarian tissue.

TABLE 2. REPRODUCTIVE PERFORMANCE OF FEMALES AFTER RECIPROCAL OVARIAN TRANSPLANTATION[1]

Parameter Measured	Genotypic Combinations: Controls Gp. I (B-B)	Controls Gp. II (Y-Y)	Experimentals Gp. III (B-Y)	Experimentals Gp. IV (Y-B)
No. of grafted females	16	16	17[2]	18
Pregnant females (%)	10(62.5)	10(62.5)	6(35.3)	14(77.8)
Total Litters	40	20	15	54
Mean litters per pregnant female	4.0±0.5[3]	2.0±0.2	2.5±0.7	3.9±0.7
Total progeny	187	95	66	251
Mean progeny per litter	4.7±0.3	4.8±0.3	4.4±0.5	4.6±0.2
Genotypes of progeny				
No. A^y/a progeny	0	14	0	59
No. a/a progeny	105	13	38	92
Neonatal losses (%)	82(43.9)	68(71.6)	28(42.4)	100(39.8)

1 The end point for evaluation of fertility in females was 10 weeks after either the last litter or pairing females with males if no pregnancies occurred.

2 One female died during surgery in this group.

3 Means ± SEMs

TABLE 3. SUMMARY OF PARITY AND LITTER SIZE CONTRIBUTION TO OVARY TRANSPLANTATION DATA

	Genotypic Combinations			
	Controls		Experimentals	
Parity	Gp. I (B-B)	Gp. II (Y-Y)	Gp. III (B-Y)	Gp. IV (Y-B)
1	4.3(10)[a]	4.4(10)	4.2(6)	4.0(14)
2	5.1(9)	5.1(7)	5.0(4)	4.3(11)
3	5.1(8)	5.1(7)	5.0(4)	4.9(7)
4	5.0(6)		5.0(1)	5.6(5)
5	3.8(5)		4.0(1)	6.4(5)
6	4.0(2)		2.0(1)	6.0(5)
7				3.5(4)
8				3.5(2)
9				3.0(1)

a Mean progeny per litter (number of litters).

TABLE 4. REPRODUCTIVE PERFORMANCE OF GONADOTROPHIN-TREATED[1] FEMALES AFTER RECIPROCAL OVARIAN TRANSPLANTATION

	Genotypic Combinations			
	Controls		Experimentals	
Parameter measured	Gp. I (B-B)	Gp. II (Y-Y)	Gp. III (B-Y)	Gp. IV (Y-B)
No. of females	10	9	13	11
Mean weight per female (g)	31.0±1.0[5]	47.5±2.3	47.2±2.2	30.8±1.2
Mean ovarian weight (mg)[2]	6.6±1.3	7.8±0.6	8.8±0.8	5.5±1.2
Mean uterine weight (mg)	52.6±8.9	41.2±5.9	43.7±2.8	55.6±6.7
Mean oocytes mechanically liberated from both ovaries[3]	6.8±1.0	4.2±1.7	4.0±1.0	7.5±1.0
Mean ova flushed from oviducts[4]	4.6±0.7	3.2±1.0	2.9±0.7	3.4±1.3

1 Injected with 0.1 cc, 5.0 I.U. of PMS, followed by 0.1 cc, 5.0 I.U. of HCG 48 hours later.

2 Combined weights of ovaries (two half ovaries).

3 Number of oocytes released following mechanical "punching" of ovary. Only ovaries that released oocytes were included.

4 Only oviducts that contained ova were included.

5 $\bar{x} \pm$ SEM

DISCUSSION

Of the 67 host females, 59.7% became pregnant and produced viable offspring. This ovary transplantation efficiency compares favorably to the 80.0% pregnancy rate reported by Stevens (1957) and that of 46.5% by Jones and Krohn (1960). Litter size in host females also compared favorably to litter size following normal matings in C57BL/6J - $\underline{A}^{\underline{y}}/\underline{a}$ and $\underline{a}/\underline{a}$ females; Granholm and Brock (1981) reported litter sizes in BxB and YxB matings of 6.7 $\pm$ 0.2 (855/127) and 5.8 $\pm$ 0.2 (823/141), respectively. In the present study litter sizes in Groups I and III which represent BxB matings were approximately 70% of litter sizes obtained by natural matings. Also, litter sizes in Groups II and IV which represent YxB matings were approximately 80% of that produced by natural matings. Present litter sizes (4.6) also compared favorably to litter sizes of 3.2 and 4.0 reported by Jones and Krohn (1960) and Stevens (1957), respectively.

Host females produced a mean of 4.6 offspring per litter. Those females that gave birth to litters of two or less offspring represented only 8.5% (11/129) of all litters, and these small litters were evenly distributed throughout all grafting groups. Thus, the $\underline{A}^{\underline{y}}$ mutation did not result in an infertility characterized by a gradual diminution in litter size. Excluding the single thin $\underline{A}^{\underline{y}}/\underline{a}$ host in Group III that had 4th, 5th and 6th litters (Table

3), females in Groups II and III produced only 3 litters averaging 4.8 offspring per litter and then abruptly ceased production. These results suggest that A^y- induced infertility, as reflected in this study, is not a graded but rather an all-or-none phenomenon.

After natural matings between A^y/a and a/a mice (BxB, BxY, YxB, and YxY), Granholm and Brock (1981) reported that A^y/a females put into production at puberty rarely had third litters (only 7.0% of all litters) and were never observed to produce 4th litters. In the present study, 5 females of Group IV (Y-B) had 4th, 5th and 6th litters and 2 of the 5 continued production for 8 litters. Since Y-B females possessed genetically yellow ovaries (A^y/a), these data document that A^y/a ovaries when grafted to a favorable reproductive environment such as nonyellow (a/a), can continue to function well beyond the time they would ordinarily become senescent in A^y/a hosts. Clearly, A^y-induced reproductive senescence is not due to intrinsically defective ovaries.

Host females had mean body weights of 47.4g. and 30.9g. for A^y/a and a/a females, respectively. These weights are comparable to those of Granholm et al. (1986) who reported mean weights of 46.1 and 25.8g. for yellow and black females, respectively. Granholm et al. (1986) reported uterine weights of 67.6 and 56.2 mg. for a/a and A^y/a respectively, which contrast from our findings of 54.1 and

42.5 mg. for a/a and A^y/a, respectively. Granholm et al. (1986) also reported greater number of oocytes, 15.8, for both groups after "punching" the ovaries, and greater number of ova, 13.4 and 11.1 for A^y/a and a/a, respectively after flushing the oviducts. In the present study we had means of 7.2 (a/a) and 4.1 (A^y/a) for numbers of oocytes released after "punching" the ovaries, and 4.0 and 3.1 ova for a/a and A^y/a, respectively after flushing the oviducts. One reason for this decline in uterine weight and ovarian activity when compared to data of Granholm et al. (1986) may be the size of grafted ovaries; grafts were half ovaries and therefore could be expected to be less productive than whole intact ovaries. Another factor could be the age of the host females. The experimental mice of Granholm et al. (1986) were about 120 days of age and older. Mice in the present study were all over 225 days at sacrifice; many of these mice had probably entered menopause. The lowered ovarian activity at menopause could also account for decreased uterine weights, decreased number of ova flushed from oviducts, and lower ova recovery following ovary "punching". In fact, 44% of the females released no ova after ovary "punching", and 33% released no ova after flushing the oviducts.

Certain aspects of the lethal yellow syndrome such as hyperphagia, obesity, induction of eumelanogenesis by alpha-MSH in A^y/- mice (Geschwind et al., 1972; Granholm and Japs,

1984), and suspected thermoregulatory defects (Turner, 1948; Cizaldo et al., 1977) are consistent with a general hypothalamic lesion. Other genetically obese rodents such as *db/db*, *ob/ob* and *fa/fa* have severe reproductive problems which have been traced to specific hypothalamo-pituitary defects. Since A^{y}- induced infertility results from nonovarian defects, the hypothalamo-pituitary axis is a likely site for the reproductive lesion.

SEPARATION OF MATERNAL AND EMBRYO CONTRIBUTIONS TO REPRODUCTIVE FAILURE IN AGING YELLOW MICE ($\underline{A}^{\underline{y}}/\underline{a}$: C57BL/6J)

SUMMARY

This study was conducted to determine if the reduced reproductive performance of aging yellow females ($\underline{A}^{\underline{y}}/\underline{a}$) is due primarily to the maternal uterine environment or to the presence of heterozygous yellow embryos ($\underline{A}^{\underline{y}}/\underline{a}$) within genetically yellow uteri. Genetically black ($\underline{a}/\underline{a}$) ovaries were grafted into empty ovarian bursae of 79 experimental ($\underline{A}^{\underline{y}}/\underline{a}$) and 54 control ($\underline{a}/\underline{a}$) hosts. At 120 days of age or older, hosts were superovulated (5.0 I.U. PMS/5.0 I.U. HCG), mated to proven black males, and scored for reproductive performance. Since $\underline{a}/\underline{a}$ ovaries were grafted to $\underline{A}^{\underline{y}}/\underline{a}$ females which were then mated to $\underline{a}/\underline{a}$ males, all pregnant $\underline{A}^{\underline{y}}/\underline{a}$ as well as $\underline{a}/\underline{a}$ hosts contained embryos of only one genotype, $\underline{a}/\underline{a}$. No $\underline{A}^{\underline{y}}/\underline{a}$ embryos were present in either experimental ($\underline{A}^{\underline{y}}/\underline{a}$) or control ($\underline{a}/\underline{a}$) hosts.

Although uterine weights for $\underline{A}^{\underline{y}}/\underline{a}$ (228.7 $\pm$ 33.1 mg, n=12) and $\underline{a}/\underline{a}$ females (365.8 $\pm$ 82.4 mg, n=13) were not significantly different, they showed a trend. The mean uterine weight per decidua was significantly less ($P<0.05$) in $\underline{A}^{\underline{y}}/\underline{a}$ versus $\underline{a}/\underline{a}$ hosts (45.5 $\pm$ 6.6 mg v. 76.1 $\pm$ 11.9 mg, respectively). Mean somites per embryo and mean normal embryos were also significantly less ($P<0.01$) in $\underline{A}^{\underline{y}}/\underline{a}$ versus $\underline{a}/\underline{a}$ hosts. Because of the way in which the grafts were conducted, both $\underline{A}^{\underline{y}}/\underline{a}$ and $\underline{a}/\underline{a}$ hosts when pregnant contained

only genetically black (a/a) embryos. Therefore differences in reproductive performance between A^y/a and a/a hosts must be due to the environment of the host's reproductive tract and not to the presence of heterozygous A^y/a embryos. Results from this study document that A^y causes a significant decline in reproductive performance via its action in the female reproductive tract independent of its action in heterozygous embryos (A^y/a).

INTRODUCTION

The lethal yellow gene (A^y) causes a number of phenotypic alterations including yellow hair, embryo death in A^y homozygotes, obesity, cancer and female sterility (Bray and York, 1979). Previous researchers (Danforth, 1927; Granholm and Brock, 1981) have noted that yellow females (A^y/a) had few litters and stopped reproducing at an early age. Reduced reproductive efficiency in mature obese A^y/a females mice occurs progressively (Granholm et al., 1986). Obese yellow females greater than 120 days of age simply do not breed well.

It has been suggested that the reproductive tracts of A^y/a females may be poorer (more hostile) environments for developing embryos than oviducts and uteri in nonyellow females. Cizadlo et al.(1975) showed that the average mean litter size of 4.2 ± 0.7 from strain C57BL/6J A^y/a x a/a matings was significantly lower ($P<0.05$) than the mean of 6.1 ± 0.1 from the reciprocal cross in which embryos develop in uteri of nonyellow females. Wolff and Bartke (1966) also found that litter size from yellow female by black male matings (5.4 ± 0.1) was significantly reduced ($P<0.05$) compared to the reciprocal cross (6.5 ± 0.1); in addition, there was a deficiency of A^y/a progeny born to A^y/a females suggesting a specific deleterious uterine effect of A^y/a uteri on A^y/a embryos. Granholm et al. (1986) also reported reduced uterine weight, decreased copulation success, and

depressed conception rates in A^{y}/a females older than 120 days of age.

The above mentioned studies characterized reproductive efficiency in A^{y}/a females which contained one-to-one ratios of A^{y}/a to a/a embryos. Therefore one cannot accurately determine if the progressive sterility is due to the hostile A^{y}/a uterine environment, to heterozygous A^{y}/a embryos developing within A^{y}/a uteri, or to both.

The aim of this study was to determine if the progressive infertility observed in A^{y}/a females was due to the presence of mutant A^{y}/a embryos within the tract, to systemic defects within the reproductive system of A^{y}/a females, or due a combination of both. By grafting genetically a/a ovaries to A^{y}/a females and mating to a/a males, one can experimentally produce a system in which only a/a embryos will be present in A^{y}/a mice. Such a system allows one to determine the extent of reproductive loss due to the A^{y}/a reproductive system independent of heterozygous A^{y}/a embryo contributions.

MATERIALS AND METHODS

Experimental animals were derived from C57BL/6J -$\underline{A}^{\underline{y}}$/$\underline{a}$ and $\underline{a}$/$\underline{a}$ mice obtained originally from The Jackson Laboratory, Bar Harbor, Maine. Males and females were derived from trio matings, 2 $\underline{a}$/$\underline{a}$ females x $\underline{A}^{\underline{y}}$/$\underline{a}$ male. Mouse facility environmental conditions were kept constant at 16h light: 8h darkness and 21 degrees C. Bedding of white pine shavings was changed weekly. Wayne Breeder blox and water were available ad libitum.

Donor and recipient females were 50-70 days of age. Anesthesia was produced by tribromoethanol. Ovaries were transplanted using the surgical procedures outlined by Jones and Krohn (1960). Donor ovaries were surgically removed, placed in petri dishes of Brinster's BMOC medium (Gibco, NY) on ice, bisected, and half ovaries were grafted to empty ovarian bursae. Stevens (1957) documented that grafts of half ovaries resulted in greater reproductive efficiency (i.e. average litter size and average number of litters per operations) than did whole or quarter ovaries.

Two different types of ovarian grafts were conducted. For the control group, $\underline{a}$/$\underline{a}$ ovaries were grafted to $\underline{a}$/$\underline{a}$ hosts. The experimental group involved the grafting of $\underline{a}$/$\underline{a}$ ovaries to $\underline{A}^{\underline{y}}$/$\underline{a}$ hosts.

After surgery, females recovered for 1-week. Then, each host female was individually paired with one proved $\underline{a}$/$\underline{a}$ male to assess ovarian transplantation success. Females

were checked daily for vaginal plugs indicating copulation. After two weeks all females were separated from males and placed in individual cages. Females were checked daily for the presence of offspring and for genotypes of the offspring assessed by coat colors.

At 120 days of age or older each female was superovulated with 5.0 I. U. of PMS and 5.0 I. U. of HCG, and placed with a "proven" a/a male. Those females that copulated were analyzed for a number of reproductive parameters 10 days later. Those that did not copulate were separated for a 2-3 week period, and then superovulated again. The females that copulated were again analyzed 10 days later while those which did not copulate were analyzed 36 hours later. During the third replication, females were superovulated 3 times.

Analyses of reproductive parameters for those mice that copulated included the following. Ten days following the detection of a vaginal plug, each female was weighed to the nearest 0.1g and sacrificed. Ovaries and uterine tracts were removed and each pair of organs was weighed to the nearest 0.1 mg. Each ovary was visually scored for the presence of corpora lutea and follicles. The uterus was observed for total deciduae. Then deciduae were dissected out of the uterus and weighed to the nearest 0.1mg. Each decidua was dissected. If an embryo was present, it was analyzed for its developmental progress. Criteria used for

evaluating 10-day embryos included:

1. Functioning (beating) heart which occurs at 9 days according to Rugh (1968) and Theiler (1983),
2. Number of somites - 13-25 for Day 9 and 26-36 for Day 10 according to Rugh (1968) and 13-29 on Day 9 and 30-39 on Day 10 (Theiler, 1983),
3. Forelimb buds - visible on Day 9 and growing rapidly on Day 10 (Rugh, 1968),
4. Hindlimb buds - appear on 10-1/2 day embryos (Rugh, 1968, Theiler, 1983),
5. Tail bud - appears at 10-1/2 days (Theiler, 1983),
6. Embryo turning - embryo turning should be completed by the -14 to -15 somite stage (Theiler, 1983). On day 9, the embryo is still twisted especially at posterior end (Rugh, 1968),
7. Brain differentiation - on Day 9 the most prominent brain vesicles are the two vesicles of the prosencephalan plus mesencephalon, metencephalon, and myelencephalon. On Day 10, the paired telencephalic vesicles continue to expand but the mesencephalon is the most prominent (Rugh, 1968) - greater C-shaped

curvature of brain and greater definition of brain vesicles.

Criteria used to determine if an embryo was N(normal), R(retarded in development but normal), or A(abnormal) were the following. If the embryo had either 20 somites and/or three of the five yes/no criteria (beating heart, forelimb bud, hindlimb bud, embryo turning, and tail bud), it was defined as N. Differentiation of brain vesicles was also used to define development; embryos were scored as 10 Day, 9 Day, or less than 9 Days based on differentiation and anatomical definition of brain vesicles. Those embryos apparently developing normally which did not display those developmental criteria assigned as N were defined as R or developmentally retarded. Some of these embryos defined as R were very early Day 7 and Day 8 stages. In those instances where no embryo could be found within deciduae, they were defined as A(abnormal) embryos. Also, embryos grossly abnormal in their development were defined as A(abnormal).

Reproductive parameters for those mice that did not copulate after superovulation included the following. At approximately 36 hours after HCG injection, females were sacrificed and weighed to the nearest 0.1g. Ovaries and uteri were removed and weighed to the nearest 0.1 mg. Ovaries were scored visually with a dissecting microscope

for the presence and staging of follicles. Oviducts were flushed with a saline solution (about 0.1ml) and the number and condition of ova recovered were recorded.

Statistical analyses were performed using analysis of variance for all continuous data, while the CATMOD procedure was used to compare discontinuous data. CATMOD is a method to measure frequency distributions and utilizes the Chi square statistic.

RESULTS

By grafting genetically black (a/a or B) ovaries into yellow (A^y/a or Y) and black (a/a or B) hosts and mating these hosts to proven B males, one can test the effects of the aging Y uterus on reproduction. Since genetically B ovaries were grafted into both Y and B hosts, all progeny sired by B males must be B. If fragments of Y ovaries remained in Y hosts as a result of incomplete host ovary removal, matings would result in Y(A^y/a) rather than B(a/a) progeny. Such Y progeny were produced in only 3.8% (1/26) of all test litters. This single host female was eliminated from the study.

Table 1 provides information on the overall design of the experiment. Ovary grafts were performed on a total of 133 females. Of that total, 111 or 83.4% survived the surgery and were used in the study. A large percentage of this 16.6% loss was due to contaminated anesthesia in one of the replications. Of the 111 total, 67 or 60.3% represented B ovaries grafted to Y females; i.e., the experimental group. The control group of B ovaries grafted to B females numbered 44. After surgical recovery, both Y and B hosts were bred to "proven" B males. In the experimental group 49.3% (33/67) copulated after pairing with the proven B males. Ultimately 22 or 32.8% B-Y females produced litters. Four females in the experimental group produced litters undetected by copulatory plugs. Thus, of the 67 surviving

experimental hosts, 26 or 38.8% produced litters.

In the control group 45.5% (20/44) copulated after pairing with proven B males, and 8 or 18.2% ultimately produced litters. One female in this group produced a litter even though a copulatory plug was not detected. Thus of the 44 surviving control hosts, 9 or 20.5% produced litters. Therefore of the 111 total hosts, 35 or 31.5% produced litters. As mentioned above, 25 of 26 litters (96.2%) from the experimental cross bred true. Therefore in the test population, the complete removal of $\underline{A}^{\underline{y}}/\underline{a}$ ovaries and grafting of $\underline{a}/\underline{a}$ ovaries was 96.2% successful.

All of the host females (67 B-Y and 44 B-B) were superovulated and mated to proven B males. Of the 111 hosts, 49 or 44.1% mated as judged by copulatory plugs. Percentages of matings for experimental and control groups were 37.3% (25/67) and 54.5% (24/44), respectively. Of the 25 experimental hosts that copulated, 12 or 48.0% were pregnant on Day 10 of gestation. In the control group, 54.2% (13/24) were pregnant on Day 10. Thus, of total superovulated females in the study, 22.5% (25/111) were found to be pregnant after a gestation of 10 days. Percentages of 10-day pregnant hosts for experimental and control hosts are 17.9% (12/67) and 29.5% (13/44), respectively. Data on reproductive parameters of these 10-day pregnant mice are presented in Table 3.

Table 2 provides data on mice that failed to copulate

after superovulation. Of the 111 mice that were superovulated, 50.4% (56/111) failed to copulate. Percentages of experimental and control hosts that failed to copulate were 58.2% (39/67) and 38.6% (17/44), respectively. The mean age of host females at autopsy was not statistically different.

Mean weight of females was greater ($P<0.01$) in Y as compared to B hosts. Yellow ($\underline{A}^{y}/\underline{a}$) females weighed 29.6 $\pm$ 0.7g, an increase in weight of 18.9% over that of black ($\underline{a}/\underline{a}$) females (24.9 $\pm$ 0.5g). The mean weight of right ovaries was identical for both groups (5.0 $\pm$ 0.4 v. 5.0 $\pm$ 0.6), while the left ovarian weights showed only slight mathematical differences (5.1 $\pm$ 0.5 v. 5.0 $\pm$ 1.0). A visual score of the ovaries yielded the total ovarian follicles per female. These numbers were also slightly different mathematically for Y and B hosts (24.6 $\pm$ 1.3 v. 28.7 $\pm$ 2.6). The mean uterine weights were greater ($P<0.05$) in B ($\underline{a}/\underline{a}$) females as compared to Y ($\underline{A}^{y}/\underline{a}$) females. The B ($\underline{a}/\underline{a}$) uterus weighed 61.2 $\pm$ 7.7mg as compared to 47.2 $\pm$ 1.6mg for the Y ($\underline{A}^{y}/\underline{a}$). Mean ova recovered after flushing the oviducts were virtually the same for both groups (4.6 $\pm$ 0.6 v. 4.8 $\pm$ 0.9) as was the percentage of females that yielded ova (82.1% v. 76.4%).

Table 3 provides data on mice that copulated after superovulation and were pregnant on day 10 of gestation. Of the 111 mice that were superovulated, 25 or 22.5% were pregnant on day 10. Twelve of these were in the

experimental group while the other 13 were in the control group.

With respect to ovarian weight, there were no major differences between experimental (B-Y) and control (B-B) groups. It is interesting to note that each ovary weighs approximately 5.0mg except the left ovaries in control hosts. Since each grafted ovary is in fact a half ovary, the amount of ovarian hypertrophy or regeneration seems to be equivalent between the two groups. The mean CLs per female were significantly higher in the control host (6.3 ± 1.2) versus the experimental host (5.1 ± 1.0). Also, control hosts possessed 14.0% more ovarian follicles (45.6 ± 5.8) than do experimental hosts (40.0 ± 6.0), although these differences are not statistically significant.

Mean right uterine horn weights were greater ($P<0.05$) in B (a/a) females as compared to Y (A^y/a) females. The right uterine horn of Y females weighed 75.2 ± 17.4mg while the B right horn weighed 237.0 ± 71.4mg. Left uterine horn weights were not different when Y were compared to B (153.5 ± 33.4mg v. 128.8 ± 31.5mg). The mean total uterine weights were not significantly different even though control uteri weighed 59.9% more than experimentals. However, the great variation due to different numbers of deciduae per uterus plus the limited n numbers prevented statistical significance. Mean decidual weights were also not significantly different but the numbers are very different

mathematically (26.0 $\pm$ 4.4mg v. 41.1 $\pm$ 6.5mg). The ratio of total uterine weight to total deciduae was greater ($P<0.05$) in B as compared to Y (76.1 $\pm$ 11.9mg v. 45.5 $\pm$ 6.6mg, respectively).

With respect to embryos, there was a slight mathematical increase in embryo number in control versus experimental hosts (4.2 $\pm$ 0.8 v. 3.5 $\pm$ 0.6 respectively). Mean somites per embryo and mean normal embryos were statistically greater ($P<0.01$) in B females as compared to Y females. The B females had 18.1 $\pm$ 3.0 somites per embryo and 2.6 $\pm$ 1.0 normal embryos per female, while the Y females had 7.8 $\pm$ 2.0 somites per embryo and 1.0 $\pm$ 0.4 normal embryos per female. Although the mean retarded and mean abnormal embryos were not significantly different between groups, the numbers appear to be following an expected trend. The Y females had mathematically more retarded embryos (2.5 $\pm$ 0.7 v. 1.6 $\pm$ 0.6) and abnormal embryos (2.1 $\pm$ 0.6 v. 1.5 $\pm$ 0.6) than did B females. With respect to the numbers of deciduae containing normal and/or retarded embryos, 72.4% (4.2/5.8) of control and 62.5% (3.5/5.6) of experimental hosts possessed either normal and/or retarded embryos.

Table 4 provides information on the mice that copulated but were not pregnant on day 10 of gestation. Of the 111 mice that were superovulated, 21.6% (24/111) copulated and were not pregnant on day 10. Percentages of experimental

and control hosts that copulated and were not pregnant at day 10 are 11.8% (13/111) and 9.9% (11/111) respectively.

The mean weight of females was greater ($P<0.01$) in the experimental group. Yellow ($\underline{A}^{\underline{y}}/\underline{a}$) females weighed 28.8 ± 0.7g while black ($\underline{a}/\underline{a}$) females weighed 25.5 ± 0.6g. The mean weight of the right ovaries was greater ($P<0.05$) in the control group (5.2 ± 0.7 v. 2.8 ± 0.7mg). However, the left ovaries were mathematically greater in the experimental group (5.0 ± 0.4 v. 3.5 ± 0.6mg). A visual score of the ovaries yielded slight mathematical differences in mean follicles per female (27.8 ± 4.5 v. 31.8 ± 2.4). Uterine weights were also mathematically greater in the experimental group (110.8 ± 22.6 v. 76.3 ± 10.8mg). However, some of the uteri in the experimental group contained large amounts of yellowish fluid. Therefore, this mean is probably an inaccurate uterine weight.

TABLE 1. REPRODUCTIVE PARAMETERS OF EXPERIMENTAL (B-Y)[a] and CONTROL (B-B)[b] HOSTS

Parameters	Genotypes B-Y	B-B
Total ovary grafts	79	54
Total surviving hosts (%)	67 (84.8)[j]	44 (81.5)
Host copulation success[c] (%)	33 (49.3)	20 (45.5)
Hosts producing litters[d] (%)	22 (32.8)	8 (18.2)
Hosts producing litters[e] (%)	4 (6.0)	1 (2.3)
Total hosts producing litters (%)	26 (38.8)	9 (20.5)
Hosts breeding true[f]	15	--
Hosts copulating after superovulation[g] (%)[h]	25 (37.3)	24 (54.5)
Hosts pregnant on day 10(%)	12 (17.9)	13 (29.5)
Hosts that copulated and not pregnant on day 10(%)	13 (19.4)	11 (25.0)
Hosts not copulating and ova recovered at 36 h.p.c.	39 (58.2)	17 (38.6)
Hosts judged not to have copulated but pregnant at 36 h.p.c.[i]	2 (3.0)	2 (4.5)

[a] *a/a* ovaries grafted to A^{Y}/*a* hosts.

[b] *a/a* ovaries grafted to *a/a* hosts.

[c] Judged by presence of copulatory plugs.

[d] Only those hosts who plugged and had litters.

[e] Only those hosts that did not plug but had a litter.

[f] 15 females had at least 1 *a/a* with no A^{Y}/*a*.
10 females had litters that died before genotypes could be confirmed.
1 female had a litter with A^{Y}/*a* offspring and was eliminated from the study.

[g] 5.0 I. U. PMS/5.0 I. U. HCG

[h] 3 A^{Y}/*a* and 3 *a/a* females were killed by male after pairing.

[i] Judged by the presence of 2-cell embryos at 36 h.p.c.

[j] Number (percent)

TABLE 2. EFFECTS OF A^y/a REPRODUCTIVE TRACT ON REPRODUCTIVE PERFORMANCE IN B-Y[a] and B-B[b] HOSTS AT 36 HOURS POST COITUM[c]

Parameters	Genotypes B-Y	B-B
Number of females	39	17
Mean age at autopsy (days)	180.1 ± 3.6[d]	175.7 ± 2.7
Mean weight of females (g)	29.6 ± 0.7	24.9 ± 0.5**
Ovaries		
Mean weight of right ovaries (mg)	5.0 ± 0.4	5.0 ± 0.6
Mean weight of left ovaries (mg)	5.1 ± 0.5	5.0 ± 1.0
Mean total ovarian follicles per female	24.6 ± 1.3	28.7 ± 2.6
Uterus		
Mean uterine weight (mg)	47.2 ± 1.6	61.2 ± 7.7*
Ova		
Mean ova recovered	4.6 ± 0.6	4.8 ± 0.9
% of Grafted females yielding ova	82.1% (32)	76.5% (13)

a a/a ovaries grafted to A^y/a hosts.

b a/a ovaries grafted to a/a hosts.

c Superovulated females that failed to copulate with proven a/a males.

d $\bar{x}$ ± SEM

* $P<0.05$ ** $P<0.01$

TABLE 3. EFFECTS OF A^y/a REPRODUCTIVE TRACT ON DEVELOPMENT of a/a EMBRYOS[a] AT 10 DAYS GESTATION

	Genotypes	
	B-Y[b]	B-B[c]
Number of females	12	13
% females pregnant on day 10[d]	42.9	48.1
Mean weight of females (g)	28.5 ± 0.7[i]	27.1 ± 0.6
Ovaries		
Mean wt. right ovaries (mg)	5.0 ± 0.7	5.1 ± 0.7
Mean wt. left ovaries (mg)	5.2 ± 0.6	3.6 ± 0.6*
Mean CLs per female	5.1 ± 1.0	6.3 ± 1.2*
Mean follicles per female	40.0 ± 6.0	45.6 ± 5.8
Uterus		
Mean wt. right horn (mg)	75.2 ± 17.4	237.0 ± 71.4*
Mean wt. left horn (mg)	153.5 ± 33.4	128.8 ± 31.5
Mean total uterine wt. (mg)	228.7 ± 33.1	365.8 ± 82.4
Mean total deciduae	5.6 ± 0.8	5.8 ± 1.2
Mean decidual wt. (mg)	26.0 ± 4.4	41.1 ± 6.5
Mean uterine wt. per decidua[e]	45.5 ± 6.6	76.1 ± 11.9*
Mean uterine weight less total decidual weight (mg)[f]	93.5 ± 10.3	135.1 ± 21.8
Mean Cls per decidua	1.1 ± 0.2	1.5 ± 0.3
Embryos		
Mean embryos[g]	3.5 ± 0.6	4.2 ± 0.8
Mean somites per embryo	7.8 ± 2.0	18.1 ± 3.0**
Mean normal embryos[h]	1.0 ± 0.4	2.6 ± 1.0**
Mean retarded embryos[h]	2.5 ± 0.7	1.6 ± 0.6
Mean abnormal embryos[h]	2.1 ± 0.6	1.5 ± 0.6

a Host females were superovulated, mated to proven a/a males, and sacrificed at 10 days of gestation.

b a/a ovaries grafted to A^y/a hosts.

c a/a ovaries grafted to a/a hosts.

d Of the 55 females that copulated 25 were pregnant on day 10. % A^y/a hosts were 42.9; % B hosts were 48.1.

e Ratio of total uterine weight to total deciduae per female.

f Weight of uterus not involved in the decidualization response.

g Normal plus retarded embryos.

h See Methods for scoring criteria for normal, retarded, and abnormal embryos.

i $\bar{x} \pm$ SEM * $P < 0.05$ ** $P < 0.01$

TABLE 4. REPRODUCTIVE PARAMETERS OF $\underline{A}^{y}/\underline{a}$ AND $\underline{a}/\underline{a}$ MICE THAT COPULATED BUT WERE NOT PREGNANT AT 10 DAYS OF GESTATION

	Genotypes		
	B-Y	B-B	
Number of females	13	11	
Mean weight of females (g)	28.8 ± 0.7	25.5 ± 0.6	**
Ovaries			
Mean weight right ovaries (mg)	2.8 ± 0.7	5.2 ± 0.7	*
Mean weight left ovaries (mg)	5.0 ± 1.4	3.5 ± 0.6	
Mean follicles per female	27.8 ± 4.5	31.8 ± 2.4	
Uterus			
Mean weight right horn (mg)	67.0 ± 16.0	42.6 ± 6.0	
Mean weight left horn (mg)	43.9 ± 6.7	33.7 ± 4.9	
Mean total uterine wt. (mg)	110.8 ± 22.6[a]	76.3 ± 10.8	

a Some of these uteri contained large amounts of a yellowish fluid.

* $P<0.05$

** $P<0.01$

DISCUSSION

This study was conducted to determine if the reduced reproductive performance of aging A^{Y}/a females is due principally to the A^{Y}/a uterus, A^{Y}/a embryos within the uterus, or to both. The experimental design allowed us, via ovary grafting to completely eliminate A^{Y}/a embryos and test solely the uterine capacities of A^{Y}/a (experimental) and a/a (control) females. Initially 133 ovary grafts were completed. Only 111 survived the surgery. One possible reason for the 16% loss was that in one group of grafts, the anesthesia may have been contaminated. Upon replacing the anaesthesia, the next group of mice survived with no losses. Of the 111 hosts which did survive, it was necessary to confirm: (1) complete removal of old or native ovarian tissue and (2) success in grafting the new ovarian tissue (half ovary). Of the 67 A^{Y}/a females that were paired with a/a proven males 26 had litters. Of these 26 litters, 15 litters survived long enough (3-4 days) to determine genotypes based on coat colors. All 15 litters contained only a/a offspring, indicating successful removal of the original A^{Y}/a ovary. One B-Y female did have A^{Y}/a offspring and was eliminated from the study. With these test mating results and those of the previous study (see Experiment I and Granholm and Dickens, 1986) it appears that A^{Y}/a ovaries were completely removed in the experimental (A^{Y}/a) females. Therefore, B-Y hosts which become pregnant via mating with B

males possess only a/a embryos.

The analysis of reproductive performance in mice that did not copulate after superovulation (Table 2) yielded one significant difference between A^y/a and a/a females. Black females had greater (P<0.05) mean uterine weights than did yellow females (61.2 ± 7.7mg v. 47.2 ± 1.6mg, respectively). These weights are comparable to those of Granholm et al. (1986); they reported uterine weights of 83.3 ± 7.1mg for a/a and 49.2 ± 4.1mg for A^y/a females. Granholm et al. (1986) also found significantly greater (P<0.01) ovarian weights in A^y/a females (7.9 ± 0.3mg) as opposed to a/a females (5.7 ± 0.2mg). Greater A^y/a ovarian weight did not occur in the current study in which ovarian weights were found to be virtually identical (Table 2). However, in the current study, donor ovaries were bisected prior to grafting. Such bisected halves would most likely not be able to compensate or hypertrophy to the same size and weight as non-bisected ovaries in the study of Granholm et al. (1986). Interestingly, ovarian weights in both Tables 2 and 3 which represent 81 females and 162 ovaries were extremely close to 5.0 mg. The only deviation (3.6 ± 0.6) from this 5.0 mg weight occurred in the 10-day pregnant control group (B-B, Table 3). Apparently, the amount of regeneration, compensation, or hypertrophy of grafted half ovaries seems to be equivalent between experimental and control groups.

The equivalent degree of ovarian regeneration in both genotypes is a significant observation for at least two reasons. First, since the genotype of all ovary grafts is a/a, the amount of regeneration or any differential patterns of regeneration must be due to host genotypes. Because there were no differences in regeneration of half ovaries, the A^y/a reproductive system appears to be as capable as the a/a system in stimulating regeneration of grafted half ovaries. Since the majority of ovary regeneration occurs soon after grafting at 60-90 days of age (Schoessler, 1987), the A^y/a reproductive system of 60-100 day females must be equivalent to that of 60-100 day a/a females. In short, if hypothalamic-pituitary lesions develop in A^y/a females, such lesions appear not to cause reproductive problems at 60-100 days of age as judged by regeneration (compensation) of half ovaries. Second, since typical ovarian weights for 60-90 day A^y/a and a/a ovaries are 3.0 mg (Schoessler, 1987) the amount or degree of regeneration of half ovaries which weigh approximately 1.5 mg at 60 days of age but which ultimately weigh about 5.0 grams at autopsy (approximately 180 days of age) is striking. Also striking is the fact that the half ovaries seem to reach an optimal or final weight of approximately 5.0 mg in both groups.

Some indication of the success of the surgical technique is also given in Table 2. Of the 56 females, whose uteri were flushed, 80.3% (45/56) contained ova. This

indicates that the ovary grafting procedures were highly successful. Of the 111 females originally superovulated, 22.5% (25/111) were pregnant at day 10. Jones and Krohn (1960) had a pregnancy rate of 46.0%. Our lower rate is probably due to the greater age of our mice, and/or due to the inefficiency of the yellow ($\underline{A}^{\underline{y}}/\underline{a}$) mouse's reproductive system. However, as mentioned previously, continuity between ovary and oviduct existed in 80.3% of the females indicating success of the surgical technique.

Table 3 provides information on the females that copulated after superovulation and were pregnant on day 10. For purposes of this study, information on uteri and embryo development is probably the most important. Although total uterine weights were not significantly different between the two groups, the numbers are mathematically distinctive (228.7 ± 33.1 mg for B-Y v. 365.8 ± 82.4 mg for B-B); in fact control uteri weighed about 60% more than B-Y uteri. Mean total deciduae were not different between groups. Also mean decidual weights were also not significantly different. However, the mean decidual weights (26.0 ± 4.4 mg for B-Y v. 41.4 ± 6.5 mg for B-B) exhibit a trend and would be significantly different with larger n numbers. In fact, if the ANOVA had been conducted using the number of deciduae (n = 67 for B-Y and n = 75 for B-B) instead of the number of females (n = 12 for B-Y and n = 13 for B-B), decidual weights would have been significantly different.

The ratio of mean uterine weight per decidua per female was significantly different between groups. Black (a/a) females had almost twice as much uterine weight per decidua (76.1 ± 11.9 mg) than did the yellow (A^y/a) females (45.5 ± 6.6 mg). This is an important result, because it puts the uterine/decidua relationship on a single decidua basis. It provides an index of the extent of uterine tissue that can be recruited or "sequestered" during the decidualization response.

The embryo analysis also yielded significant differences between groups. The mean somites per embryo were highly significantly different ($P<0.01$). The mean normal embryos were also significantly different ($P<0.01$) with 1.0 ± 0.4 for B-Y and 2.6 ± 1.0 for B-B mice. Mean abnormal embryos and mean retarded embryos were not significantly different, but a trend was evident (Table 3).

Other interesting results occurred in the study. For instance, the left ovary weight was significantly different ($P<0.05$) from the right ovary weight in day 10 females (Table 3). However, this result did not occur in the 36 hour p.c. females (Table 2), which had nearly identical ovary weights. Past research has indicated that the ovary weights of the yellow (A^y/a) females are generally greater than the ovary weights of black (a/a) females (Granholm et al., 1986). However, since both groups had black ovaries in this study, we would not have expected any difference. With

larger n numbers we would probably find little or no difference in the left ovarian weights and can probably conclude that it was simply a sampling error and not a true biological variation.

Day 10 pregnant females had mathematically more mean follicles per female (Table 3) than did 36 hour post coitum mice (Table 2). The reason for this difference is not known, but it may be due to the different ages of the two groups. Mice in the 36 hour post coitum group had ages that averaged about 177 days while those in the day 10 group were about 120-130 days old. It would be reasonable to conclude that the difference was due to the pregnancy, but during pregnancy, plasma LH and FSH are at relatively low levels presumably inhibiting the maturation of any few follicles. Therefore, we would actually expect a lower mean number of follicles in the day 10 mice than in the 2-day post coitum mice.

No statistical differences in mean total deciduae per female were found. Both groups had virtually identical numbers of deciduae (5.6 ± 0.8 for B-Y v. 5.8 ± 1.2 for B-B). The yellow (A^y/a) uterus can sustain about the same number of deciduae as the black (a/a) uterus. Granholm and Brock (1981) reported that black (a/a) females in a/a x a/a matings produce significantly ($P<0.01$) larger litters (6.7 ± 0.2) than do yellow A^y/a females in A^y/a x a/a matings (5.8 ± 0.2). However, based on number of deciduae, there do not

appear to be differences in number of progeny between B-Y and B-B females. However, there were significant differences in normal embryos between groups. Significant differences between groups such as mean uterine weight per deciduae, mean somites per embryo, and mean normal embryos per female suggest a hostile uterine environment in B-Y females. Other mathematical differences though not significant also suggest a hostile uterine environment (i.e. mean total uterine weights, mean decidual weights, mean retarded embryos,and mean abnormal embryos). Since only $\underline{a}/\underline{a}$ and not $\underline{A}^{\underline{Y}}/\underline{a}$ embryos were present within $\underline{A}^{\underline{Y}}/\underline{a}$ uteri of B-Y females, differences in reproductive success between B-Y (experimental) and B-B (control) females must be due to the host reproductive system and not to the presence of $\underline{A}^{\underline{Y}}/\underline{a}$ embryos.

Wolff and Bartke (1966) postulated that a differential intrauterine mortality of yellow embryos in yellow mothers may be a major cause of the lower numbers of yellow weanlings. The present study does not necessarily contradict that study, but it does show that the yellow $\underline{A}^{\underline{Y}}/\underline{a}$ uterus is one of the factors causing decreased reproductive efficiency in $\underline{A}^{\underline{Y}}/\underline{a}$ females. However, we can not rule out the possibility that the $\underline{A}^{\underline{Y}}/\underline{a}$ embryos within $\underline{A}^{\underline{Y}}/\underline{a}$ uteri may have negative potentiating effects. Studies by Granholm and Schoessler (in progress) should clarify the relative contributions of $\underline{A}^{\underline{Y}}/\underline{a}$ embryos and $\underline{A}^{\underline{Y}}/\underline{a}$ uteri in the observed

reduction in reproductive efficiency in yellow (A^y/a) females. By comparing the reproductive efficiency of females in a/a x a/a, a/a x A^y/a, and A^y/a x a/a matings, it should be possible to sort out maternal from embryo contributions during the decline in reproduction in A^y/a females (Granholm and Schoessler, in progress).

Since older A^y/a females have a hostile uterine environment and also exhibit irregular estrous cycles (Kasten, 1952; Granholm et al., 1986), deficiencies in ovarian steroids may occur. Numerous locations within the reproductive system play a role in ovarian steroid synthesis and secretions. These locations and their respective roles in A^y/a induced infertility warrant further discussion.

Since other obese rodent syndromes (e.g., ob/ob, db/db, and fa/fa) have their reproductive problems traced to the hypothalamo-pituitary axis, A^y/a females may also have hypothalamo-pituitary lesions. Let's look at the pituitary gland first.

The pituitary gland releases the gonadotrophins (LH and FSH) which in turn elicit the production of estrogen by ovarian follicular cells. The release of gonadotrophins depends upon gonadotrophin releasing hormones (GnRH) synthesized and released from the hypothalamus. Since ovarian steroids depend upon proper levels of gonadotrophins, A^y might cause a decrease in levels of endogenous gonadotrophins. Potential causes for abnormally

low plasma gonadotrophins are numerous. For instance, the pituitary gland may simply not be able to produce enough gonadotrophins. Or, the pituitary gland may not be able to respond appropriately to GnRH released from the hypothalamus. Or, the hypothalamus may be incapable of synthesizing and releasing normal GnRH. Whatever the reason, the pituitary gland cannot be ruled out as a possible site of $\underline{A}^{\underline{y}}$-induced infertility.

There are methods to test overall functional integrity of $\underline{A}^{\underline{y}}/\underline{a}$ pituitary glands. Injections of exogenous GnRH, followed by radioimmunoassays (RIAs) of plasma gonadotrophins as well as assays of gonadotrophins of pituitary tissues may aid in determining the functional capability of the $\underline{A}^{\underline{y}}/\underline{a}$ pituitary gland.

The hypothalamus also plays a role in ovarian steroids by way of GnRH release which elicits the release of pituitary gonadotrophins. The hypothalamus is probably a more attractive candidate for the $\underline{A}^{\underline{y}}$ lesion than the pituitary gland, since it can be linked to the other effects caused by the lethal yellow gene (i.e. temperature regulatory problems, obesity, and aberrant pigment cell regulation). If the hypothalamus is unable to produce normal amounts of GnRH, or if it is abnormally sensitive to ovarian steroids, gonadotrophin secretion would decrease and ultimately affect ovarian steroid levels. However, since a progressive infertility (Granholm et al., 1986) seems

evident, the hypothalamus is probably capable of producing normal amounts of GnRH, at least in young preobese or mildly obese A^{y}/a females.

The possibility that the A^{y}/a hypothalamus may be overly sensitive to ovarian steroids seems reasonable, since the hypothalamus is known to be able to change its sensitivity to steroids (i.e., becomes less sensitive at puberty, allowing the secretion of GnRH, Vander et al., 1985). This hypothalamus-sensitivity hypothesis might be tested by the use of fertility drugs. Clomiphene, a fertility drug that reduces the hypothalamus' sensitivity to circulating steroids, promotes an increase in GnRH, gonadotrophins, and ultimately ovarian steroids (Johnson and Everitt, 1984). Upon treating A^{y}/a mice with clomiphene, a biological assay conducted on the ovary could yield important results. Restoration of ovarian activity following clomiphene treatment of infertile A^{y}/a females would suggest that A^{y}/a does influence sensitivity of the hypothalamus to circulating ovarian steroids. However, this study may be problematic, because it would be difficult to interpret if, after clomiphene treatments, no differences in ovarian activity occurred. However, perhaps a combination of biological assays (ovarian activity) plus RIAs (GnRH, FSH, LH) could yield definitive data on the primary A^{y} lesion.

Since A^{y}/a infertility seems to be associated with

obesity (Jeppesen, 1985), it would be worthwhile to explore how obesity affects infertility. Deslypere et al. (1985) concluded that fat tissue can act as a steroid reservoir. Possibly ovarian steroids are being produced by fat cells, thereby disturbing their negative feedback effects on the hypothalamus and pituitary gland.

A number of studies could be done to provide information on the effects of obesity on infertility in $\underline{A}^{\underline{y}}/\underline{a}$ mice. First, by restricting the diets of $\underline{A}^{\underline{y}}/\underline{a}$ mice, it might be possible to prevent obesity; if $\underline{A}^{\underline{y}}/\underline{a}$ mice continue to reproduce, one could conclude that obesity is causing the infertility.

Second, one could assay fat tissue either for the presence of ovarian steroids or for the presence of enzymes that catalyze the production of ovarian steroids. Analyzing human fat, Deslypere et al. (1985) report that human fat tissue contains relatively abundant amounts of aromatase and 17B-hydroxysteroid dehydrogenase; they conclude their report, "... The fact that fat tissue (human) contains an active aromatase as well as an even more active 17B-hydroxysteroid dehydrogenase, and taking into account the large fat tissue mass, explain the important role of fat tissue as a site of estrogen formation". Johnson and Everitt (1984) display the following interconversions of steroids:

Aromatase

Androstenedione ---------------> Estrone

17B-hydroxysteroid dehydrogenase

Estrone ------------------------------> Estradiol 17B.

Perhaps $\underline{A}^{\underline{y}}/\underline{a}$ females undergo a progressive decline in reproductive efficiency commensurate with an increase in fat deposition, because large amounts of fat-derived estrogens simply overwhelm the hypothalamus (and pituitary) via positive feedback. The positive feedback of estrogen on the hypothalamus shuts down synthesis and release of GnRH. Pituitary gonadotrophins are neither released nor act on the ovaries to promote folliculogenesis. Thus, $\underline{A}^{\underline{y}}/\underline{a}$-induced fat deposition, which results in extraordinarily high plasma levels of estrogen, causes infertility.

In their review of causes of the yellow mouse syndrome, Wolff et al. (1986) state that an elevated insulin level, due either to islet cell hyperplasia or obesity-induced peripheral insulin resistance, is a common feature of $\underline{A}^{\underline{vy}}$ and $\underline{A}^{\underline{y}}$ expression. Apparently, elevated insulin stimulates lipogenesis, inhibits lipolysis, and leads to obesity and insulin resistance. Perimetrial adipose tissue from yellow mice was more resistant to insulin stimulation of glucose oxidation than that from agouti (nonyellow) mice (Frigeri et al., 1983). Wolff et al. (1986) make the following statement regarding the primary lesion of yellow mice, "... we propose that elevated insulin levels and associated

anabolic bias are fundamental to the yellow mouse syndrome".

Although Wolff et al. (1986) postulate that elevated insulin is fundamental to the yellow syndrome, data of Frigeri et al. (1983) show that plasma insulin levels of yellow mice are not elevated above those of nonyellows until 3-5 weeks of age regardless of sex. Since yellow gene-induced differences in coat color patterns are obvious at one week of age, it seems unlikely that increased plasma insulin causes aberrant pigmentation. Accordingly, clues to the fundamental yellow gene lesion might be obtained by examining the metabolism of hair bulb pigment cells in yellow (A^{vy}/- and A^{y}/-) and nonyellow mice.

Interestingly, hair bulb pigment cells of agouti (A/A) and other nonyellow mice (such as a/a or black) display three electrophoretic variants (isozymes) of tyrosinase, while yellow mice display only one (Holstein, et al., 1973). Reflecting on this observation, Pawelek and Korner (1982) made the following provocative comment, "... Since the multiple forms of tyrosinase result from glycosylation reactions, it seems possible that there is a disturbance in protein glycosylations in mice of this (yellow) genotype. As an example, both obesity and some forms of diabetes in humans have been traced to defects in insulin receptors--insulin receptors are glycoproteins". Also, Tamate and Takeuchi (1984) suggest that an aberrant alpha-melanocyte stimulating hormone (alpha-MSH) receptor on the surface of

hair bulb pigment cells may be the result of a mutation (recessive yellow mutation) at the extension locus. Perhaps $\underline{A}^{\underline{y}}$ induces aberrant receptors on adipocytes which secondarily alter the regulation of adipocyte metabolism enhancing lipogenesis and fat deposition. However, following reciprocal adipocyte grafts in yellow and nonyellow mice, Meade et al. (1979) showed that genetically yellow adipocytes responded to circulating factors in nonyellow hosts by shrinking in volume to that of nonyellow host adipocytes. But, since perimetrial adipose tissue of yellow mice was more resistant to insulin stimulation of glucose oxidation than adipose tissue of nonyellow mice (Frigeri et al., 1983), perhaps insulin receptors are in fact defective on adipocyte cell surfaces of yellow mice. An aberrant insulin receptor hypothesis should be readily testable.

To summarize, infertility in $\underline{A}^{\underline{y}}/\underline{a}$ females may be caused by positive feedback of estrogen to the hypothalamo-pituitary axis essentially shutting down the synthesis and release of pituitary gonadotropins. Increased estrogen levels in turn may be caused by greatly increased stores of fat as $\underline{A}^{\underline{y}}/\underline{a}$ females mature. And, the fat may be the result of $\underline{A}^{\underline{y}}$-induced elevation in circulating insulin levels and/or an $\underline{A}^{\underline{y}}$-induced glycosylation defect in adipocyte cell surface receptors which abrogates the normal equilibrium between fat deposition (lipogenesis) and fat breakdown (lipolysis).

RIAs of estradiol 17B (E_2) and progesterone in mice of different ages, weights, and reproductive status should provide adequate data to test the "high estrogen-positive feedback" hypothesis of $\underline{A}^{y}$-induced infertility. Presumably, total fat, estrogen concentration, and infertility ought to be strongly correlated.

Alternatively, treatment of obese $\underline{A}^{y}/\underline{a}$ females with clomiphene might be instructive. Since clomiphene has antiestrogenic properties and makes the hypothalamus less sensitive to estrogens, an improvement in the reproductive status of obese $\underline{A}^{y}/\underline{a}$ females would be consistent with the "high estrogen-positive feedback" hypothesis.

Third, it might be possible to surgically remove the fat tissue from the obese mice (i.e. ovarian fat depots) and see if they could return to an active reproductive state. One could conduct unilateral and bilateral ovarian "fat padectomies" of obese $\underline{A}^{y}/\underline{a}$ females and monitor the effects on reproductive activity. One could also put obese reproductively-depressed mice on restricted diets and monitor reproductive activity.

Fourth, since $\underline{A}^{y}/\underline{a}$ mice become obese, the balance between deposits and withdrawals of fat from adipocytes is shifted drastically to the deposit side of the equilibrium. Perhaps one or more of the enzymes responsible for the hydrolysis of intra-adipocyte triglycerides is (are) defective in $\underline{A}^{y}/\underline{a}$ females. Alternatively, there may exist

$\underline{A}^{\underline{y}}$-induced membrane and/or structural abnormalities of the smooth endoplasmic reticulum within those SER compartments responsible for triglyceride breakdown to fatty acids and glycerol; Wolff et al. (1978) suggested that structural defects in the endoplasmic reticulum of melanocytes could account for aberrant pigmentation in yellow mice.

Fifth, receptors on $\underline{A}^{\underline{y}}/\underline{a}$ adipocytes may be defective. Fawcett (1986) discusses the mode of hormone interaction with adipocytes; plasma membranes of adipocytes posses specific receptors for a number of hormones including ACTH, TSH, LH, and epinephrine. Hormones activate cytoplasmic lipases within adipocytes via receptors, adenylate cyclase, cyclic AMP, and ultimately organ-specific protein kinases (the series of inductions termed "second message"). The amount of intra-adipocyte triglyceride broken down into fatty acids and glycerol depends upon the overall efficiency and functional capability of the entire "second message" inductive system from outer cell surface receptor to the protein kinase activation of specific lipases. Perhaps $\underline{A}^{\underline{y}}$ causes defects in one or more of these steps; $\underline{A}^{\underline{y}}$ may block the receipt of specific hormones such as alpha-MSH in pigment cells (see thesis of Japs, 1987).

CONCLUSIONS

Results presented in Experiment 1 document that $\underline{A}^{y}/\underline{a}$ ovaries are indeed functional when placed in a proper environment such as $\underline{a}/\underline{a}$ females. Interestingly, one $\underline{A}^{y}/\underline{a}$ host produced six litters, which has never occurred in our colony. However, this $\underline{A}^{y}/\underline{a}$ female never became obese, suggesting that obesity may play a role in the $\underline{A}^{y}/\underline{a}$ infertility. There is nothing intrinsically defective with $\underline{A}^{y}/\underline{a}$ ovaries.

Results presented in Experiment 2 document that $\underline{A}^{y}/\underline{a}$ reproductive systems cause a decline in reproduction independent of the possible potentiating effects of $\underline{A}^{y}/\underline{a}$ embryos.

The reproductive process is a complex scheme of events relying on the intricate interplay between developing embryos and the endocrine milieu of the reproductive tract. It seems to be a recurrent theme that an endocrine imbalance exists within the $\underline{A}^{y}/\underline{a}$ mouse. The results of these studies tend to support that theme. However, the role that obesity may play in this hormonal imbalance should not be overlooked and probably should be the focus of future studies in $\underline{A}^{y}$-induced reproductive senescence.

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APPENDIX I. RAW DATA FROM EXPERIMENT I. NUMBER OF DAYS ELAPSED BETWEEN LITTERS

Mouse Identification Number	Genotype Designation	Number of days elapsed between litters									Age of Dam at Ovary Grafting
		Pairing and 1st Litter	1st to 2nd	2nd to 3rd	3rd to 4th	4th to 5th	5th to 6th	6th to 7th	7th to 8th	8th to 9th	
1-1-16	B-B[a]	-[b]									63 ± 7 days
1-1-17	B-B	-									63 ± 7 days
1-1-18	B-B	-									63 ± 7 days
1-1-19	B-B	47	25	22	55	22	23				63 ± 7 days
1-1-20	B-B	22	20	132	47	27					63 ± 7 days
1-2-1	B-B	30	25	70	25	31					55 ± 5 days
1-2-2	B-B	26	21	24							55 ± 5 days
1-2-3	B-B	40	20	25							55 ± 5 days
1-3-10	B-B	21	21								61 ± 2 days
1-3-11	B-B	-									61 ± 2 days
1-3-12	B-B	21	21	24	19						61 ± 2 days
1-4-1	B-B	54	33	21	19	24					66 ± 2 days
1-4-2	B-B	-									66 ± 2 days
1-4-3	B-B	40									66 ± 2 days
1-5-1	B-B	29	21	21	30	22	21				61 ± 3 days
1-5-2	B-B	-									61 ± 3 days
1-1-1	Y-Y[c]	22	27	20							63 ± 7 days
1-1-2	Y-Y	23									63 ± 7 days
1-1-3	Y-Y	20	24	21							63 ± 7 days
1-1-4	Y-Y	35									63 ± 7 days
1-1-5	Y-Y	50	25								63 ± 7 days
1-2-10	Y-Y	34	20								55 ± 5 days
1-2-11	Y-Y	23	20	22							55 ± 5 days
1-2-12	Y-Y	26	22								55 ± 5 days
1-3-1	Y-Y	-									61 ± 2 days
1-3-2	Y-Y	21									61 ± 2 days
1-3-3	Y-Y	-									61 ± 2 days

APPENDIX I CONTINUED

Mouse Identification Number	Genotype Designation	Pairing and 1st Litter	1st to 2nd	2nd to 3rd	3rd to 4th	4th to 5th	5th to 6th	6th to 7th	7th to 8th	8th to 9th	Age of Dam at Ovary Grafting
			Number of days elapsed between litters								
1-4-10	Y-Y	-									66 ± 2 days
1-4-11	Y-Y	-									66 ± 2 days
1-4-12	Y-Y	-									66 ± 2 days
1-5-11	Y-Y	-									61 ± 2 days
1-5-12	Y-Y	51	23								61 ± 2 days
1-1-6	Y-B[d]	46	21	20							63 ± 7 days
1-1-7	Y-B	129	27								63 ± 7 days
1-1-8	Y-B	-									63 ± 7 days
1-1-9	Y-B	22	25	35							63 ± 7 days
1-1-10	Y-B	23	21								63 ± 7 days
1-2-7	Y-B	42	62	48	52	31	24				55 ± 5 days
1-2-8	Y-B	23	31	22	36	39	24	27			55 ± 5 days
1-2-9	Y-B	23									55 ± 5 days
1-3-4	Y-B	21	24	40	50	24	42	25	55		61 ± 2 days
1-3-5	Y-B	-									61 ± 2 days
1-3-6	Y-B	-									61 ± 2 days
1-4-7	Y-B	-									66 ± 2 days
1-4-8	Y-B	21	22	20	24	22	21	23	44	22	66 ± 2 days
1-4-9	Y-B	127									66 ± 2 days
1-5-7	Y-B	29	21	29	20	24	77	23			61 ± 3 days
1-5-8	Y-B	27	39								61 ± 3 days
1-5-9	Y-B	23									61 ± 3 days
1-5-10	Y-B	29	23								61 ± 3 days
1-1-11	B-Y[e]	-									63 ± 7 days
1-1-12	B-Y	46									63 ± 7 days
1-1-13	B-Y	43	30								63 ± 7 days
1-1-14	B-Y	23	20	25							63 ± 7 days

APPENDIX I CONTINUED

Mouse Identification Number	Genotype Designation	Pairing and 1st Litter	Number of days elapsed between litters: 1st to 2nd	2nd to 3rd	3rd to 4th	4th to 5th	5th to 6th	6th to 7th	7th to 8th	8th to 9th	Age of Dam at Ovary Grafting
1-1-15	B-Y	36	21								63 ± 7 days
1-2-4	B-Y	-									55 ± 5 days
1-2-5	B-Y	-									55 ± 5 days
1-2-6	B-Y	23	22	24	49	25	24				55 ± 5 days
1-3-7	B-Y	-									61 ± 2 days
1-3-8	B-Y	-									61 ± 2 days
1-3-9	B-Y	-									61 ± 2 days
1-4-4	B-Y	-									66 ± 2 days
1-4-5	B-Y	-									66 ± 2 days
1-4-6	B-Y	-									66 ± 2 days
1-5-3	B-Y	-									61 ± 3 days
1-5-4[f]	B-Y	-									
1-5-5	B-Y	34									61 ± 3 days
1-5-6	B-Y	-									61 ± 3 days

[a] a/a ovaries grafted to a/a hosts

[b] - never had a litter

[c] A'/a ovaries grafted to A'/a hosts

[d] A'/a ovaries grafted to a/a hosts

[e] a/a ovaries grafted to A'/a hosts

[f] female died during surgery

APPENDIX II.RAW DATA FROM EXPERIMENT I. LITTER SIZE AND GENOTYPE DATA

Mouse Identification Number	LITTERS 1st				2nd				3rd				4th				5th				6th				7th				8th				9th			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1-1-16		-																																		
1-1-17		-																																		
1-1-18		-																																		
1-1-19	5	5			4	0	0	4	6	5	0	1	4	0	0	4	4	2	0	2	2	0	0	2												
1-1-20	3	2	0	1	5	0	0	5	5	0	0	5	4	4			3	0	0	3																
1-2-1	7	1	0	6	3	3			4	0	0	4	5	1	0	4	2	2																		
1-2-2	7	1	0	6	6	0	0	6	3	3																										
1-2-3	6	6			6	0	0	6	4	4																										
1-3-10	2	2			5	5																														
1-3-11		-																																		
1-3-12	3	3			4	0	0	4	4	1	0	3	3	3																						
1-4-1	3	0	0	3	10	4	0	6	11	11			9	0	0	9	3	3																		
1-4-2	--																																			
1-4-3	3	0	0	3																																
1-5-1	4	0	0	4	3	3			4	1	0	3	5	2	0	3	7	3	0	4	6	2	0	4												
1-5-2	--																																			
1-1-1	6	0	4	2	7	7			7	3	3	1																								
1-1-2	4	4																																		
1-1-3	4	4			4	1	1	2	2	2																										
1-1-4	6	4	1	1																																
1-1-5	6	6			7	7																														
1-2-10	5	5			5	5																														
1-2-11	2	2			5	3	1	1	6	3	1	2																								
1-2-12	4	0	2	2	4	4																														

APPENDIX II CONTINUED

Mouse Identification Number	LITTERS 1st				2nd				3rd				4th				5th				6th				7th				8th				9th			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1-3-1		-																																		
1-3-2	3	3																																		
1-3-3		-																																		
1-4-10		-																																		
1-4-11		-																																		
1-4-12		-																																		
1-5-11		-																																		
1-5-12	4	4			4	1	1	2																												
1-1-6	4	1	2	1	3	3			4	1	1	2																								
1-1-7	2	2			3	3																														
1-1-8		-																																		
1-1-9	4	1	2	1	7	3	2	2	6	0	6	0																								
1-1-10	4	1	2	1	5	5																														
1-2-7	4	0	2	2	3	0	1	2	6	1	0	5	4	1	0	3	3	2	1	0	3	0	0	3												
1-2-8	2	2			5	2	0	3	3	3			5	0	1	4	8	1	1	6	6	6			5	0	3	2								
1-2-9	5	3	0	2																																
1-3-4	7	2	5	0	3	3			6	0	3	3	9	0	4	5	9	2	3	4	8	1	2	5	3	3			3	0	0	3				
1-3-5		-																																		
1-3-6		-																																		
1-4-7		-																																		
1-4-8	2	2			5	1	1	3	3	3			3	1	1	1	6	2	1	3	7	3	3	1	3	3			4	1	2	1	3	3		
1-4-9	2	2																																		
1-5-7	4	0	1	3	3	3			6	0	1	5	7	1	3	3	6	6			6	1	1	4	3	3										

APPENDIX II CONTINUED

LITTERS

Mouse Identification Number	1st				2nd				3rd				4th				5th				6th				7th				8th				9th			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1-5-8	4	4			6	6																														
1-5-9	5	0	3	2																																
1-5-10	7	2	1	4	4	4																														
1-1-11		-																																		
1-1-12	4	4																																		
1-1-13	8	5	0	3	7	0	0	7																												
1-1-14	4	4			7	1	0	6	6	6																										
1-1-15	4	4			3	1	0	2																												
1-2-4		-																																		
1-2-5		-																																		
1-2-6	3	0	0	3	3	1	0	2	4	2	0	2	5	3	0	2	4	2	0	2	2	0	0	2												
1-3-7		-																																		
1-3-8		-																																		
1-3-9		-																																		
1-4-4		-																																		
1-4-5		-																																		
1-4-6		-																																		
1-5-3		-																																		
1-5-4[f]	-	-																																		
1-5-5	2	2																																		
1-5-6		-																																		

a. Litter size

b. Number of neonates that died before confirmation of the genotype

c Number of A^y/a neonates

d Number of a/a neonates

e - never had a litter

f female died during surgery

APPENDIX III. RAW DATA FROM EXPERIMENT I. EXOGENOUS GONADOTROPHIN TREATMENT DATA[a]

Mouse ID Number	Grafting Type	Age at Exogenous Treatment	Female wt. (g)	Copulatory plug (+ or -)	Right ovary wt.(mg)	Left ovary wt.(mg)	Uterus wt.(mg)
1-1-17	B-B	330 ± 7 days	29.2	-	1.2	3.2	100.0
1-1-18	B-B	330 ± 7 days	31.7	-	2.0	7.5	58.1
1-1-19	B-B	443 ± 7 days	32.9	-	4.7	1.3	44.0
1-1-20	B-B	443 ± 7 days	29.1	-	5.9	3.6	34.2
1-2-2	B-B	324 ± 5 days	26.1	-	6.3	4.5	33.5
1-3-11	B-B	296 ± 1 day	31.6	-	6.0	-	34.0
1-3-12	B-B	296 ± 1 day	29.3	-	3.7	1.5	93.5
1-4-1	B-B	364 ± 2 days	36.6	-	1.2	1.4	32.1
1-4-2	B-B	278 ± 2 days	26.1	-	0.7	1.5	52.3
1-5-1	B-B	300 ± 2 days	35.0	-	5.6	4.4	43.9
1-1-1	Y-Y	330 ± 7 days	57.0	-	5.8	3.4	47.5
1-1-2	Y-Y	330 ± 7 days	44.1	-	2.7	2.9	31.0
1-2-10	Y-Y	270 ± 1 day	46.9	-	1.9	6.7	29.0
1-2-12	Y-Y	270 ± 1 day	46.9	-	4.7	4.2	65.1
1-3-2	Y-Y	247 ± 1 day	52.9	-	4.3	3.2	35.9
1-3-3	Y-Y	247 ± 1 day	39.5	-	2.0	5.0	27.9
1-4-11	Y-Y	278 ± 2 days	46.2	-	4.7	5.6	33.5
1-5-11	Y-Y	229 ± 2 days	55.9	-	2.1	2.5	78.1
1-5-12	Y-Y	300 ± 2 days	34.5	-	4.0	4.4	23.1
1-1-13	B-Y	330 ± 7 days	39.2	-	2.3	7.7	33.5
1-1-15	B-Y	330 ± 7 days	56.9	-	1.0	6.7	37.0
1-2-4	B-Y	270 ± 5 days	56.6	-	6.2	2.3	49.4

Mouse ID Number	Left Ovary Follicles[b]	Right Ovary Follicles[b]	Left Ovary Ova[c]	Right Ovary Ova[c]	Left Oviduct[d]			Right Oviduct[d]		
					Normal	Abnormal[e]	Total	Normal	Abnormal[e]	Total
1-1-17	13+	6-12	0	0	0	0	0	0	0	0
1-1-18	13+	6-12	4	3	0	0	0	2	2	4
1-1-19	13+	13+	0	0	0	0	0	0	0	0
1-1-20	6-12	13+	0	0	0	0	0	0	0	0
1-2-2	6-12	6-12	3	2	1	0	1	2	0	2
1-3-11	-	13+	-	11	-	-	-	5	0	5
1-3-12	0-5	13+	1	4	0	0	0	8	0	8
1-4-1	6-12	6-12	0	0	1	0	1	2	0	2
1-4-2	6-12	6-12	5	1	2	0	2	1	0	1
1-5-1	13+	13+	0	0	0	0	0	7	0	7
1-1-1	6-12	13+	1	2	5	0	5	1	0	1
1-1-2	0-5	6-12	0	0	1	0	1	0	0	0
1-2-10	13+	6-12	0	1	0	0	0	0	0	0
1-2-12	13+	13+	1	0	0	0	0	0	0	0
1-3-2	6-12	13+	0	0	1	0	1	0	0	0
1-3-3	13+	6-12	1	0	0	0	0	0	0	0
1-4-11	6-12	6-12	8	4	0	0	0	0	0	0
1-5-11	13+	6-12	4	3	0	0	0	6	0	6
1-5-12	13+	13+	0	0	0	0	0	2	0	2
1-1-13	13+	6-12	0	0	4	2	6	0	1	1
1-1-15	6-12	0-5	1	0	0	0	0	0	0	0
1-2-4	0-5	0-5	0	0	0	0	0	0	1	1

APPENDIX III. CONTINUED

Mouse ID Number	Grafting Type	Age at Exogenous Treatment	Female wt.(g)	Copulatory plug (+ or -)	Right Ovary wt.(mg)	Left Ovary wt.(mg)	Uterus wt.(mg)
1-2-5	B-Y	270 ± 5 days	31.4	-	1.5	1.3	20.9
1-3-7	B-Y	247 ± 1 day	47.4	-	2.6	3.4	50.4
1-3-8	B-Y	247 ± 1 day	51.9	-	3.8	3.3	52.4
1-3-9	B-Y	247 ± 1 day	55.6	-	1.7	6.8	55.6
1-4-4	B-Y	278 ± 2 days	55.1	-	9.5	5.5	56.7
1-4-5	B-Y	278 ± 2 days	49.5	-	8.0	2.3	43.1
1-4-6	B-Y	278 ± 2 days	44.7	-	5.7	4.9	44.7
1-5-3	B-Y	229 ± 2 days	40.5	-	3.1	3.9	47.7
1-5-5	B-Y	229 ± 2 days	45.8	-	1.6	7.9	46.2
1-5-6	B-Y	229 ± 2 days	38.4	-	6.1	3.0	31.3
1-1-6	Y-B	330 ± 7 days	30.8	+	3.8	-	34.9
1-1-7	Y-B	443 ± 7 days	29.9	-	3.2	2.0	46.7
1-2-7	Y-B	406 ± 5 days	30.1	-	2.3	2.6	56.5
1-2-8	Y-B	406 ± 5 days	34.7	-	0.6	1.2	32.3
1-3-4	Y-B	384 ± 1 day	37.5	+	1.6	2.0	82.6
1-3-5	Y-B	247 ± 1 day	21.8	-	3.1	4.5	56.2
1-3-6	Y-B	296 ± 2 days	29.5	-	0.5	1.6	56.4
1-4-7	Y-B	278 ± 2 days	26.4	-	1.2	2.4	72.9
1-5-7	Y-B	300 ± 2 days	34.3	-	4.3	12.9	104.6
1-5-8	Y-B	300 ± 2 days	32.4	-	5.4	1.2	36.7
1-5-10	Y-B	229 ± 2 days	31.1	-	0.8	2.6	32.2

Mouse ID Number	Left Ovary Follicles[b]	Right Ovary Follicles[b]	Left Ovary Ova[c]	Right Ovary Ova[c]	Left Oviduct[d]			Right Oviduct[d]		
					Normal	Abnormal[e]	Total	Normal	Abnormal[e]	Total
1-2-5	6-12	0-5	0	1	0	0	0	0	0	0
1-3-7	6-12	0-5	0	2	0	0	0	0	0	0
1-3-8	13+	6-12	0	1	1	0	1	2	0	2
1-3-9	13+	6-12	4	0	4	0	4	0	0	0
1-4-4	6-12	0-5	1	0	1	0	1	0	0	0
1-4-5	6-12	13+	2	3	1	0	1	0	0	0
1-4-6	13+	13+	3	3	0	0	0	2	0	2
1-5-3	6-12	6-12	8	4	0	0	0	0	0	0
1-5-5	6-12	13+	0	3	0	0	0	6	0	6
1-5-6	13+	6-12	2	6	0	0	0	1	0	1
1-1-6	-	13+	0	0	0	0	0	0	1	1
1-1-7	13+	13+	0	0	0	0	0	0	0	0
1-2-7	13+	13+	0	0	0	0	0	2	0	2
1-2-8	6-12	6-12	0	0	0	0	0	0	0	0
1-3-4	13+	13+	0	0	0	0	0	1	0	1[g]
1-3-5	6-12	0-5	0	0	1	0	1	0	0	0
1-3-6	6-12	13+	6	3	8	0	8	0	0	0
1-4-7	6-12	6-12	4	2	1	0	1	0	0	0
1-5-7	13+	13+	0	0	8	0	8	3	0	3
1-5-8	13+	13+	0	0	2	0	2	0	0	0
1-5-10	0-5	0-5	0	0	0	0	0	0	0	0

a 5.0 I. U. PMS followed 48 hours later by 5.0 I. U. HCG.

b Number of follicles present when ovary scored visually.

c Number of ova released following mechanical "punching" of ovary.

d Number of ova released after flushing oviducts.

e Vesiculated or granular ova were scored as abnormal.

f Ovary was not present.

g One 2-cell embryo was present after flushing oviducts.

APPENDIX IV. RAW DATA FROM EXPERIMENT II. COPULATORY PLUG DATA

Mouse ID Number	Ovary-Host Combination	Plug after Pairing	Had Litter?	Number of young	Number of young that died	Genotypes of young	Plug after 1st S.O.	Pregnant Day 10?	Plug after 2nd S.O.	Pregnant Day 10?
1-1	B-B[a]	+	No				+	Yes		
1-2	B-B[b]	+	Yes	5	1	All Blk[c]	+	Yes		
1-3	B-B	-	-				-		-	
1-4	B-Y	-	-				-		-	
1-5	B-B	-	Yes	4	4		-		-	
1-6	B-Y	+	Yes	3	0	All Blk	-		-	
1-7	B-B	-	-				-		-	
1-8	B-Y	+	Yes	2	0	All Blk	-		-	
1-9	B-B	+	No				-		-	
1-10	B-Y	-	-				-		-	
1-11	B-B	-	-				+	Yes		
1-12	B-Y	+	Yes	6	3	All Blk	+	Yes		
1-13	B-B	+	Yes	3	3		-		-	
1-14	B-Y	+	No				-		-	
1-15	B-B	+	No				+	Yes		
1-16	B-Y	+	No				-		-	
1-17	B-B	+	Yes	5	0		+	Yes		
1-18	B-Y	+	No				+	No		
1-19	B-B	+	No				-		-	
1-20	B-Y	-	-				-		-	
1-21	B-B	+	Yes	4	0	All Blk	+	Yes		
1-22	B-Y	+	Yes	4	4		+	Yes		
1-23	B-B	+	Yes	3	0	All Blk	+	Yes		
1-24	B-Y	-	-				-		-	

APPENDIX IV. CONTINUED

Mouse ID Number	Ovary-Host Combination	Plug after Pairing	Had Litter?	Number of young	Number of young that died	Genotypes of young	Plug after 1st S.O.	Pregnant Day 10?	Plug after 2nd S.O.	Pregnant Day 10?
1-25	B-Y	+	Yes	4	4		-		-	
1-26	B-B	-	-				+	Yes		
1-27	B-Y	-	Yes	3	0	All Blk	-		-	
1-28	B-B	+	Yes	2	2		+	No		
1-29	B-Y	+	Yes	7	0	All Blk	-		-	
1-30	B-B	+	No				-		-	
1-31	B-Y	-	-				-		-	
1-32	B-B	+	No				-		-	
1-34[d]	B-Y	+	Yes	6	3	All Blk	+	No		
1-35	B-B	+	No				+	Yes		
1-36	B-Y	-	Yes	4	0	All Blk	+	Yes		
1-37	B-B	+	No				+	Yes		
1-38	B-Y	+	Yes	4	0	All Blk	-		-	
1-39	B-B	+	No				+	Yes		
2-40	B-Y	+	No				-		-	
2-41	B-B	-	-				-		+	No
2-43	B-B	-	-				-		-	
2-48[e]	B-Y	+	Yes	5	0	3Y.2Blk				
2-49	B-B	-	-				-		-	
2-51	B-B	+	Yes	4	0	All Blk	-		-	
2-53	B-B	+	No				-		-	
2-54	B-Y	+	Yes	3	3		+	Yes		
2-55	B-B	+	No				-		-	
2-58	B-Y	-	-				-		+	No

APPENDIX IV. CONTINUED

Mouse ID Number	Ovary-Host Combination	Plug After Pairing	Had Litter?	Number of young	Number of young that died	Genotypes of young	Plug after 1st S.O.	Pregnant day 10?	Plug after 2nd S.O.	Pregnant Day 10?
2-59	B-B	-	-				-		-	
2-62	B-Y	-	-				-		-	
2-64	B-Y	-	-				-		+	No
2-66	B-Y	-	-				-		-	
2-68	B-Y	-	-				-		-	
2-69	B-B	+	Yes	5	5		+	No		
2-70	B-Y	+	No				-		-	
2-72	B-Y	+	Yes	3	3		+	Yes		
2-73	B-B	-	-				+	No		
2-74	B-Y	+	Yes	4	4		-			
2-75	B-B	-	-				-		-	
2-76	B-Y	+	No				-		-	
2-77	B-B	+	No				-		-	
2-78	B-Y	-	-				-		+	No
2-79	B-B	+	Yes	4	0	All Blk.	-		+	No
2-80	B-Y	+	Yes	3	0	All Blk.	+	Yes		
2-81	B-B	-					-		-	No
2-82	B-Y	-	-				-		+	
2-83	B-B	-	-				+	No		
2-84	B-Y	+	No				-		-	
2-87	B-B	-	-				-		+	Yes
2-88	B-Y	-	-				+	No		
2-89	B-B	-	-				+	No		
2-90	B-Y	-	-				-			
2-91	B-B	-	-				+	No	-	

APPENDIX IV. CONTINUED

Mouse ID Number	Ovary-Host Combination	Plug after Pairing	Had Litter?	Number of young	Number of young that died	Genotypes of young	Plug after 1st S.O.	Pregnant Day 10?	Plug after 2nd S.O.	Pregnant Day 10?
2-92	B-Y	+	Yes	4	4		-		-	
2-93	B-Y	+	Yes	5	0	All Blk.	-		-	
3-1	B-B	-	-				-		+	No
3-2	B-Y	-	-				+	Yes		
3-3	B-B	-	-				+	Yes		
3-4	B-Y	-	-				-		-	
3-5	B-Y	-	-				-		-	
3-6	B-Y	+	No				-		-	
3-9	B-Y	+	No				-		-	
3-10	B-Y	-	Yes	5	5		-		-	
3-11	B-B	-	-				+	No		
3-12	B-Y	-	-				-		-	
3-13	B-B	-	-				-		-	
3-14	B-Y	+	Yes	4	0	All Blk.	-		-	
3-15	B-Y	-	-				+	Yes		
3-16	B-Y	+	Yes	4	4		+	Yes		
3-17	B-Y	-	-				+	No		
3-18	B-Y	+	No				-		-	
3-19	B-Y	-	-				-		-	
3-20	B-Y	-	-				-		-	
3-21	B-B	-	-				+	Yes		
3-22	B-Y	-	-				-		-	
3-23	B-B	-	-				+	No		
3-24	B-Y	+	Yes	3	0	All Blk.	-		-	

APPENDIX IV. CONTINUED

Mouse ID Number	Ovary-Host Combination	Plug after Pairing	Had Litter?	Number of young	Number of young that died	Genotypes of young	Plug after 1st S.O.	Pregnant Day 10?	Plug after 2nd S.O.	Pregnant Day 10?
3-26	B-Y	-	-				-		-	
3-27	B-Y	+	No				-		+	No
3-28	B-Y	-	-				-		-	
3-29	B-Y	+	Yes	5	0	All Blk.	+	No		
3-30	B-Y	+	Yes	5	5		-		-	
3-31	B-B	-	-				-		+	No
3-32	B-Y	-	-				-		-	
3-33	B-B	-	-				-		-	
3-34	B-Y	-	-				-		-	
3-35	B-Y	-	-				-		-	
3-36	B-Y	-	-				-		-	
3-37	B-Y	+	Yes	5	0	All Blk.	+	No		
3-38	B-Y	-	Yes	4	4		-		-	
3-40	B-Y	-	-				+	No		

[a] Black ovaries grafted to a black host.

[b] Black ovaries grafted to a yellow host.

[c] Only neonates that survived were checked for genotypes.

[d] Some mice died during surgery, and their numbers were skipped (i.e. 1-32 - 1-34)

[e] This female had yellow offspring and was not included in study.

[f] Only 3rd replication was superovulated three times. Results are as follows: 3-6 and 3-18 plugged after 3rd S.O. but were not pregnant Day 10, and 3-20 did plug after 3rd S.O. and was pregnant Day 10.

APPENDIX V. RAW DATA FROM EXPERIMENT II. DAY 10 POST COITUM

Mouse Number[a]	Genotype[b]	Mouse wt.(g)	Right Ovary Weight (mg)	Right Ovary Corpora Lutea	Right Ovary Other Follicles	Left Ovary Weight (mg)	Left Ovary Corpora Lutea	Left Ovary Other Follicles	Follicles per Female	Right Uterine Horn Weight (mg)	Right Uterine Horn Number of decidua
1-1	B	27.0	7.7	8	17	0.9	0	1	26	271.2	4
1-11	B	25.1	3.0	2	13	2.8	0	18	33	101.5	1
1-15	B	28.2	7.2	0	0	1.0	0	0	0	943.6	7
1-17	B	24.1	5.4	5	22	3.6	2	28	57	202.3	6
1-21	B	28.4	5.7	1	32	2.3	4	8	45	81.1	1
1-23	B	30.2	1.4	1	11	7.2	4	26	42	325.9	4
1-26	B	28.3	3.7	2	14	4.6	6	20	42	165.5	3
1-35	B	25.7	4.6	0	33	3.4	5	26	64	25.0	0
1-37	B	30.8	7.5	8	7	4.5	7	17	39	308.3	9
1-39	B	29.8	8.8	6	20	5.2	6	7	39	517.6	9
1-2	Y	28.3	8.6	0	25	7.0	4	29	58	20.4	0
1-12	Y	29.9	1.5	0	3	5.9	8	14	25	101.6	1
1-22	Y	32.1	6.0	0	7	5.7	2	30	39	31.5	0
1-36	Y	25.4	4.8	1	13	4.6	2	11	27	89.1	3
2-87	B	24.9	2.5	0	13	1.0	5	18	36	27.0	0
2-54	Y	27.4	3.9	0	0	4.6	0	0	0	63.9	1
2-72	Y	30.4	10.3	0	0	0.1	0	0	0	230.9	8
2-80	Y	31.9	2.6	0	7	6.5	4	26	37	26.0	0
2-82	Y	27.7	3.7	3	24	7.7	1	7	35	76.3	2
3-3	B	24.5	-	-	-	6.2	1	23	24	44.9	0
3-21	B	25.5	4.0	1	18	4.1	2	13	33	67.0	3
3-2	Y	26.5	2.8	5	9	5.0	2	9	25	102.5	3
3-15	Y	25.0	6.0	0	11	3.7	3	12	26	20.9	0
3-16	Y	28.6	6.4	5	15	5.9	8	14	42	113.9	5
3-20	Y	29.3	4.0	0	9	6.3	3	14	26	25.9	0

	Left Uterine Horn									Embryo Scores			
Mouse Number	Decidual designation	Weight (mg)	Number of decidua	Decidual designation	Mean total decidual wt. (mg)	Total decidua	Total uterine wt. (mg)	n number for somites	Mean somites	N	R	A	No. Embryos
1-1	2-5	74.2	1	1	43.5	5	345.4	4	24.0	4	0	1	4
1-11	1	20.2	0	0	56.6	1	121.7	1	29.0	1	0	0	1
1-15	1-7	25.5	0	0	98.0	7	969.1	5	33.8	5	0	2	5
1-17	2-7	53.7	1	1	22.5	7	256.0	5	8.0	0	6	1	6
1-21	3	150.6	2	1-2	37.8	3	231.7	2	18.5	1	1	1	2
1-23	5-8	316.6	4	1-4	56.2	8	642.5	8	28.8	8	0	0	8
1-26	3-5	115.0	2	1-2	32.2	5	280.5	4	16.2	0	4	1	4
1-35	0	61.0	1	1	33.1	1	86.0	1	23.0	1	0	0	1
1-37	3-11	141.1	2	1-2	27.5	11	449.4	3	16.8	2	2	7	4
1-39	8-16	390.6	7	1-7	38.1	16	908.2	11	28.4	11	0	5	11
1-2	0	125.7	3	1-3	23.0	3	146.1	1	18.0	0	2	1	2
1-12	9	365.3	8	1-8	33.1	9	466.9	4	21.2	2	2	5	4
1-22	0	71.0	3	1-3	10.9	3	102.5	0	0	0	0	3	0
1-36	3-5	81.9	2	1-2	18.9	5	171.0	1	18.0	0	1	4	1
2-87	0	184.0	7	1-7	18.3	7	211.0	0	0	0	6	1	6
2-54	8	360.5	7	1-7	32.8	8	424.4	4	23.5	4	0	4	4
2-72	1-8	14.1	0	0	18.2	8	245.0	6	4.2	0	7	1	7
2-80	0	222.4	5	1-5	32.2	5	248.4	5	20.2	4	1	0	5
2-82	5-6	135.1	4	1-4	22.8	6	211.4	6	3.2	0	8	0	6
3-3	0	121.4	1	1	63.0	1	166.3	1	37.0	1	0	0	1
3-21	1-3	21.0	0	0	7.3	3	88.0	3	0	0	2	1	2
3-2	1-3	18.0	0	0	20.4	3	120.5	2	9.7	1	2	0	3
3-15	0	109.4	4	1-4	16.6	4	130.3	4	6.0	0	4	0	4
3-16	7-11	142.2	6	1-6	14.3	11	256.1	4	2.7	0	6	5	6
3-20	0	196.2	2	1-2	69.0	2	222.1	1	44.0	1	0	1	1

[a] First number represents replication number while second number is number of individual mouse.

[b] B represents B-B grafting, while Y represents B-Y grafting.

APPENDIX VI. RAW DATA FROM EXPERIMENT II. DECIDUAL WEIGHT[a] and EMBRYO SOMITE[b] NUMBER of 10-DAY PREGNANT MICE

Mouse Number	Genotype	n number for somites	Number of deciduae	Decidual Designation 1	2	3	4	5	6	7	8
1-1	B[c]	4	5	37.7(27)[d]	49.3(25)	36.3(-)[e]	46.6(21)	47.6(23)			
1-11	B	1	1	56.6(29)							
1-17	B	5	7	26.0(10)	17.5(5)	18.1(-)	21.8(14)	36.4(6)	17.6(-)	20.0(5)	
1-21	B	2	3	44.3(18)	48.9(19)	20.2(-)					
1-23	B	8	8	46.3(25)	66.9(24)	53.7(36)	59.3(29)	65.9(31)	49.4(26)	71.4(30)	37.0(2(0
1-26	B	4	5	34.2(11)	32.9(19)	33.5(17)	32.4(-)	28.4(18)			
1-35	B	1	1	33.1(23)							
1-37[f]	B	3	11	33.8(-)	56.6(23)	17.6(-)	5.3(-)	45.7(25)	36.7(14)	20.2(-)	18.1(-)[f]
1-39[g]	B	11	16	18.5(-)	37.6(25)	22.3(-)	50.6(26)	28.1(-)	52.0(29)	49.5(27)	65.2(36)[g]
1-15	B	5	7	42.2(-)	45.1(25)	82.3(-)	108.4(30)	107.6(37)	158.7(39)	141.8(38)	
2-87	B	0	7	19.7(-)	16.5(-)	20.0(-)	21.6(-)	18.2(-)	20.3(-)	11.7(-)	
3-3	B	1	1	63.0(37)							
3-21	B	0	3	10.0(-)	6.9(-)	5.0(-)					
1-2	Y[h]	1	3	18.4(-)	26.0(18)	24.5(-)					
1-12[i]	Y	4	9	22.3(-)	29.6(-)	34.1(-)	34.0(9)	9.9(-)	39.6(25)	29.7(-)	51.4(23)[i]
1-22	Y	0	3	7.2(-)	9.0(-)	16.5(-)					
1-36	Y	1	5	16.9(-)	31.5(18)	19.1(-)	9.9(-)	17.1(-)			
2-54	Y	4	8	33.5(21)	32.3(20)	31.8(-)	31.5(-)	45.2(28)	40.3(25)	29.8(-)	17.9(-)
2-72	Y	6	8	16.3(5)	15.6(4)	21.7(3)	24.0(5)	15.7(-)	19.4(6)	21.0(7)	11.9(-)
2-80	Y	5	5	33.3(20)	34.6(21)	33.7(21)	29.9(20)	29.7(19)			
2-82	Y	5	6	21.4(-)	23.4(4)	28.4(5)	24.1(3)	20.1(3)	19.6(4)		
3-2	Y	2	3	29.2(26)	16.7(3)	15.3(-)					
3-15	Y	4	4	15.1(5)	17.9(8)	18.5(4)	15.1(7)				
3-16[j]	Y	4	11	12.0(-)	15.4(3)	12.3(-)	15.8(-)	17.8(4)	14.9(5)	17.4(-)	10.5(-)[j]
3-20	Y	1	2	103.0(44)	34.9(-)						

[a] Deciduae were dissected from uteri and weighed (mg).

[b] Embryos were dissected from deciduae and scored for developmental progress. Somite number was used as a quantitative assessment for developmental normality.

APPENDIX VI. CONTINUED

[c] This genotype represents a/a ovaries grafted to a/a (B) animals.

[d] Each term represents decidual weight (mg) followed by somite number of the embryo within that decidua. For example, 37.7(27) means the decidua weighed 37.7 mg and contained an embryo that possessed 27 somites.

[e] No embryo could be found within these deciduae.

[f] a/a mouse number 1-37 had 11 deciduae; numbers 9-11 are as follows: 16.1(-), 32.0(5) and 20.5(-) respectively.

[g] a/a mouse number 1-39 had 16 deciduae; numbers 9-16 are as follows: 55.4(30), 22.8(28), 54.0(-), 48.6(33), 49.7(27), 42.2(27), 22.6(-), and 40.1(24) respectively.

[h] This genotype represents a/a ovaries grafted to A^y/a (y) animals.

[i] A^y/a mouse number 1-12 had 9 deciduae; decidua number 9 was 47.6(28).

[j] A^y/a mouse number 3-16 had 11 deciduae; numbers 9-11 are as follows: 15.4(4), 16.5(-) and 9.1(-) respectively.

APPENDIX VII. RAW DATA FROM EXPERIMENT II. FEMALES THAT FAILED TO COPULATE AFTER PMS/HCG TREATMENT

Mouse Number	Genotype	Mouse wt.(g)	Right Ovary wt.(mg)	Right ovary total Follicles[a]	Left Ovary wt.(mg)	Left ovary total Follicles[a]	Uterus wt.(mg)	Right Oviduct Flushing: Granular Ova	Vesiculate Ova	Normal Ova	2-cell Stages
1-3	B[d]	27.4	8.3		4.8		59.5	-	-	-	-
1-4	Y[b]	38.5	-[c]		3.1		36.0	-	-	2	-
1-5	B	23.6	4.3		-		53.8	-	-	-	-
1-6	Y	35.1	4.3		9.3		58.3	-	-	-	-
1-7	B	26.4	1.3		14.3		63.5	1	-	-	-
1-8	Y	28.4	5.9		5.1		81.3	2	2	1	5
1-9	B	28.2	6.3		13.2		63.3	-	4	-	-
1-10	Y	37.3	4.3		6.2		42.4	-	-	-	-
1-13	B	25.7	6.4		3.6		62.0	-	-	8	-
1-14	Y	36.7	6.3		5.4		61.7	-	-	-	-
1-16	Y	36.7	11.9		1.1		48.5	1	1	7	-
1-20	Y	34.4	9.4		3.3		52.9	-	-	-	-
1-25	Y	37.1	6.6		3.6		49.8	1	-	-	-
1-27	Y	23.0	-		0.7		26.4	-	-	-	-
1-29	Y	31.8	1.2		0.5		43.6	-	1	-	-
1-30	B	23.7	6.7		5.8		175.1	-	-	-	7
1-31	Y	32.3	5.3		-		43.5	-	-	-	-
1-32	B	22.8	3.3		3.8		36.3	-	-	-	-
2-40	Y	30.2	3.7	7	2.5	19	64.5	-	-	1	-
2-43	B	24.5	4.4	17	2.5	21	49.1	-	-	2	-

Mouse Number	Left Oviduct Flushing: Granular Ova	Vesiculate Ova	Normal Ova	2-cell Stages	Total Ova	Total Entities	Right, left or both Oviducts	Comments
1-3	-	-	-	-	-	-	-	No ova present
1-4	1	-	-	-	1	1	left	
1-5	-	-	-	-	-	-	-	No ova present
1-6	-	-	2	-	4	4	both	
1-7	5	-	-	4	6	10	both	Pregnant (2-cell)
1-8	-	-	-	-	5	10	right	Pregnant (2-cell)
1-9	-	-	-	-	4	4	right	
1-10	-	-	-	-	-	-	-	No ova present
1-13	-	-	4	-	12	12	both	
1-14	2	-	2	-	4	4	left	
1-16	-	1	2	-	12	12	both	
1-20	-	-	-	-	-	-	-	No ova present
1-25	-	-	-	-	1	1	right	
1-27	-	-	-	-	-	-	-	No ova present
1-29	-	-	1	-	2	2	both	
1-30	-	2	-	3	2	12	both	Pregnant (2-cell)
1-31	-	-	-	-	-	-	-	No ova present
1-32	-	-	-	-	-	-	-	No ova present
2-40	2	1	1	-	5	5	both	
2-43	-	-	1	-	3	3	both	

APPENDIX VII CONTINUED

Mouse Number	Genotype	Mouse Wt.(g)	Right Ovary wt.(mg)	Right Ovary total Follicles*	Left Ovary wt.(mg)	Left Ovary total Follicles*	Uterus Wt.(mg)	Right Oviduct Flushing: Granular Ova	Vesiculate Ova	Normal Ova	2-cell Stages
2-48	Y	26.0	10.7	17	5.7	16	40.8	-	2	5	-
2-49	B	24.3	3.0	12	3.3	17	39.0	1	-	-	-
2-53	B	23.4	-	0	5.5	23	48.0	-	-	-	-
2-59	B	21.4	5.0	19	2.1	10	44.2	1	1	2	-
2-62	Y	27.1	4.0	12	2.9	5	52.6	-	-	-	-
2-66	Y	23.0	4.6	8	4.8	17	43.1	-	-	-	-
2-68	Y	24.4	6.6	7	5.1	8	35.9	-	-	-	-
2-70	Y	27.1	1.6	14	9.1	13	51.7	2	1	1	-
2-73	B	25.8	8.8	21	5.6	19	68.0	-	-	4	-
2-74	Y	30.0	1.9	12	10.2	10	49.1	1	2	3	-
2-75	B	26.7	2.0	17	6.2	22	61.0	-	-	-	-
2-76	Y	29.7	3.0	7	6.0	21	39.6	1	-	-	-
2-77	B	27.6	7.4	13	0.2	1	71.8	2	1	1	-
2-81	B	21.4	-	0	1.2	13	48.3	-	-	1	-
2-84	Y	26.4	2.7	21	0.2	3	44.6	-	-	-	-
2-90	Y	28.1	1.3	6	3.8	15	49.4	-	-	2	-
2-92	Y	25.4	2.5	8	5.5	29	50.9	-	-	-	-
2-93	Y	28.1	4.7	8	13.9	16	52.3	-	-	-	-
3-4	Y	31.7	6.3	11	5.5	9	47.6	2	1	6	-
3-9	Y	30.9	7.5	14	5.7	15	46.9	-	-	-	-

	Left Oviduct Flushing							
Mouse Number	Granular Ova	Vesiculate Ova	Normal Ova	2-cell Stages	Total Ova	Total Entities	Right, left or both Oviducts	Comments
2-48	-	-	-	-	7	7	right	
2-49	2	-	2	-	5	5	both	
2-53	2	-	6	-	8	8	both	
2-59	-	-	-	-	4	4	right	
2-62	-	-	-	-	-	-	-	No ova present
2-66	-	-	3	-	3	3	left	
2-68	-	-	-	-	-	-	-	No ova present
2-70	-	1	1	-	6	6	both	
2-73	-	-	-	-	4	4	right	
2-74	-	-	2	-	8	8	both	
2-75	2	-	6	-	8	8	left	
2-76	-	2	3	-	6	6	both	
2-77	-	-	-	-	4	4	right	
2-81	-	-	-	-	1	1	right	
2-84	-	-	1	-	1	1	left	
2-90	-	-	-	-	2	2	right	
2-92	-	-	-	-	-	-	-	No ova present
2-93	-	1	2	-	3	3	left	
3-4	-	-	-	-	9	9	right	
3-9	1	1	5	-	7	7	left	

APPENDIX VII. CONTINUED

Mouse Number	Genotype	Mouse Wt.(g)	Right Ovary wt.(mg)	Right ovary total Follicles[a]	Left Ovary wt.(mg)	Left ovary total Follicles[a]	Uterus Wt.(mg)	Right Oviduct Flushing			
								Granular Ova	Vesticulate Ova	Normal Ova	2-cell Stages
3-10	Y	21.9	5.7	18	3.4	16	45.0	-	1	3	-
3-12	Y	31.8	2.3	6	0.5	3	29.0	-	-	1	-
3-14	Y	27.5	2.3	11	8.2	12	52.0	-	-	2	-
3-19	Y	28.1	7.3	14	2.0	2	47.5	-	-	1	-
3-22	Y	25.4	5.6	9	4.7	16	41.3	-	-	-	-
3-24	Y	23.7	7.5	21	3.0	5	45.9	1	-	3	-
3-26	Y	31.4	10.9	15	8.4	17	54.2	1	-	5	-
3-28	Y	32.0	4.4	11	10.7	12	45.3	2	-	3	-
3-30	Y	30.9	4.3	13	7.0	17	46.0	1	-	3	-
3-31	B	24.8	4.3	12	2.5	18	48.1	-	-	8	-
3-32	Y	32.0	3.0	8	8.8	18	49.5	-	-	-	-
3-33	B	25.3	3.1	15	6.0	13	45.6	4	-	-	-
3-34	Y	24.6	2.5	17	1.4	12	40.0	-	-	3	-
3-35	Y	24.3	4.5	15	4.9	14	55.4	-	-	4	-
3-36	Y	29.5	4.6	18	6.1	13	54.7	-	-	-	-
3-38	Y	32.1	2.1	5	2.4	3	48.8	-	-	1	-

Mouse Number	Left Oviduct Flushing Granular Ova	Vesicluate Ova	Normal Ova	2-cell Stages	Total Ova	Total Entities	Right, left or both Oviducts	Comments
3-10	-	1	1	-	6	6	both	
3-12	-	1	6	-	8	8	both	
3-14	-	-	1	-	3	3	both	
3-19	-	-	-	-	1	1	right	
3-22	4	1	-	-	5	5	left	
3-24	-	-	-	-	4	4	right	
3-26	-	-	-	-	6	6	right	
3-28	-	-	-	-	5	5	right	
3-30	11	-	7	-	21	21	both	
3-31	-	-	8	-	16	16	both	
3-32	8	3	7	-	18	18	left	
3-33	-	-	-	-	4	4	right	
3-34	-	-	-	-	3	3	right	
3-35	1	-	4	-	9	9	both	
3-36	3	-	6	-	9	9	left	
3-38	-	-	-	-	1	1	right	

[a] Visual score of ovaries was not done with replication 1.

[b] Y genotype represents black (a/a) ovaries grafted to yellow (A^y/a) animal.

[c] No ovary was present.

[d] B genotype represents black (a/a) ovaries grafted to black (a/a) animal.

APPENDIX VIII. RAW DATA FROM EXPERIMENT II. COPULATED BUT NOT PREGNANT ON DAY 10

Mouse Identification Number	Grafting Designation	Wt. of female(g)	Right ovary wt.(mg)	Left ovary wt.(mg)	Total Follicles right ovary[a]	Total Follicles left ovary[a]	Uterine horn wt.(mg) Right	Uterine horn wt.(mg) Left	Total uterine wt.(mg)
1-8	B-B	26.4	9.8	1.9	27	12	30.6	21.4	52.0
1-19	B-B	29.5	1.6	6.2	9	31	25.3	25.2	50.5
1-28	B-B	24.7	3.9	4.6	18	10	34.2	23.4	57.6
2-41	B-B	27.4	5.9	4.2	16	16	36.2	36.1	72.3
2-69	B-B	24.8	5.4	4.0	23	10	28.4	24.9	53.3
2-73	B-B	23.9	5.0	1.2	-	-	43.5	30.7	74.2
2-79	B-B	27.0	6.2	2.7	21	20	27.1	22.7	49.8
2-83	B-B	23.3	3.7	2.1	11	10	62.0	39.5	101.5
2-89	B-B	24.7	2.2	6.9	7	18	50.3	39.2	89.5
2-91	B-B	22.5	5.0	3.7	22	15	93.2	78.0	171.2
3-23	B-B	25.8	8.4	1.4	19	3	37.8	29.8	67.6
3-18	B-Y	33.0	4.6	20.9	25	24	83.9	58.4	142.3
3-24	B-Y	31.7	3.2	5.8	22	37	20.2	21.6	41.8
3-34	B-Y	27.7	4.3	3.4	32	23	241.4[b]	67.5	308.9
3-58	B-Y	28.5	8.7	2.5	4	9	28.6	22.7	51.3
3-64	B-Y	31.4	1.1	1.3	12	8	25.2	19.1	44.3
3-78	B-Y	24.4	2.8	1.9	25	6	24.6	18.1	42.7
3-88	B-Y	28.9	0.6	4.4	1	9	56.5	46.7	103.2
3-6	B-Y	30.4	0.8	3.5	8	9	31.7	19.5	51.2
3-17	B-Y	28.3	0.4	2.7	4	11	57.1	59.4	116.5
3-18	B-Y	26.2	1.8	3.9	15	13	35.7	27.2	62.9
3-29	B-Y	27.8	0.8	5.9	2	21	87.1	46.0	133.1
3-37	B-Y	29.4	1.5	5.6	12	11	79.2	82.5	161.7
3-40	B-Y	26.8	6.0	3.3	7	11	99.2	81.6	180.8

[a] Number of follicles present after scoring visually.

[b] This uterine horn was full of a yellowish fluid.