Protective effect of pregnancy for development of uterine leiomyoma

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Many factors that can modulate the risk of developing uterine leiomyoma have been identified, including parity. Epidemiological data on decreased risk of developing this disease has been subject to different interpretations regarding whether pregnancy per se is protective or, as leiomyomas are a major cause of infertility, women that develop these tumors are less fertile and thus have lower pregnancy rates. We have utilized an animal model genetically predisposed to uterine leiomyoma to investigate the potential protective effect of pregnancy on the risk of developing this disease.

Female Eker rats that carry a mutation in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene develop uterine leiomyoma with a frequency of 65% when nulliparous. These animals were bred with intact or vasectomized males and tumor incidence determined after a single pregnancy (to confirm fertility) or multiple pregnancies over the lifetime of the animals. Females with multiple litters displayed a dramatic shift in tumor incidence and presentation. Tumor incidence decreased from 71% in single litter females to 10% in females that had multiple litters (average: five litters/animal). Interestingly, females bred with vasectomized males also exhibited a reduced tumor incidence of 41%, suggesting that the hormonal changes associated with early stages of pregnancy that occur in pseudopregnant females may have contributed to the protective effect of pregnancy.

Uterine leiomyoma, commonly referred to as fibroids, arise from the uterine myometrium and are the most common tumor of the female reproductive tract. Risk increases with age during the premenopausal years, but tumors typically regress and/or become asymptomatic with menopause (1–3). GnRH agonists, which induce a hormonal milieu similar to menopause, effectively shrink tumor volume (4). The hormonal responsiveness of these tumors has been further demonstrated by in vitro experimental studies. Leiomyomas express estrogen and progesterone receptors and laboratory studies with leiomyoma-derived cell lines indicate that these cells are responsive to steroid hormones. Leiomyoma cells proliferate in response to estrogen in culture, and this response can be inhibited by estrogen antagonists such as ICI 18572, tamoxifen and raloxifene (5–7).

Several risk factors for uterine leiomyoma have been identified in epidemiologic studies including pregnancy, which is associated with a decreased risk of developing this disease (3,8–10). Each additional pregnancy appears to provide added protection and risk increases with time since last birth (3,8,11). Whether the protective association seen with parity is accounted for by infertility or the fact that pregnancy and the associated change in hormonal milieu is in of itself protective is controversial (11). Studies that report changes in existing leiomyomas during pregnancy are not consistent. Some tumors grow, others shrink, but many show little change (12,13). Thus, as the mechanism(s) by which increasing parity might reduce the risk of fibroids are not understood, dissection of the factors responsible is of importance, as the biological basis of this protective effect could yield valuable information with potential implications for therapy.

The Eker rat is an animal model for uterine leiomyoma. Susceptibility to tumor development in this model is the result of a germline mutation in the tuberous sclerosis 2 (Tsc-2) tumor suppressor gene. Female Eker rats have a high frequency of spontaneous uterine leiomyoma that are phenotypically similar to their human counterpart (14). Tumors develop in ~65% of female rats carrying the Eker mutation by 16 months of age. Like their human counterpart, tumors are hormonally dependent and tumor development can be virtually ablated by ovariectomy (15). Although previous studies have been conducted with virgin females, the fact that tumors develop in intact and reproductively competent females makes it possible to address questions regarding the impact of reproductive status on tumor development using this well characterized animal model.

The steroid hormone profile of female Eker rats during pregnancy, and the similarity of this profile to wild-type Long–Evans rats (the background strain on which the mutation is carried) was first defined. Groups of 10 animals were killed on days 12, 18, 20, 21, and 23 (postpartum) and circulating levels of estradiol and progesterone determined. Eker females exhibited typical high sustained levels of progesterone during late stages of pregnancy which as expected declined prior to parturition (day 20). Progesterone levels during this period (days 12–21) dropped from 83.5 (± 7.6) ng/ml to 55.6 (± 11.9) ng/ml, and continued to decrease to prepregnancy levels of 23.1 (± 4.8) ng/ml postpartum. As anticipated, estrogen levels were relatively low and stable until just prior to parturition but exhibited a peak at day 21, when a sharp increase to 379.4 (± 49.7) pg/ml occurred. Estrogen levels then returned to prepregnancy levels of <10 pg/ml (8.8 ±1.5). The profiles for both estrogen and progesterone in female Eker rats therefore appeared similar to that reported in wild-type rats (16), confirming that regulation of these pregnancy-associated hormones was normal in female Eker rats.

To examine the effects of pregnancy and parturition on tumor development, 129 age matched female Eker rats were bred with either intact or vasectomized males and maintained until 16 months of age. Group 1, comprised of 17 rats, was allowed to have a single litter of pups to confirm their fertility and held without further breeding until the termination of the
study (Fertile group). Group 2 had 58 fertile rats that became pregnant and delivered multiple litters during the course of the study (Pregnancy group). The average number of litters in this group was five per animal (range of 3–7). Group 3, which had 39 females, was bred continuously with vasectomized males (Pseudopregnant group). Animals with compromised fertility \( n = 14 \), identified during the course of the study by their failure to become pregnant following several rounds of breeding with at least two different males were also examined as a group to determine if their infertility correlated with the presence of uterine tumors (Infertile group 4). At the termination of the study, all animals were examined for gross and microscopic uterine tumors.

As shown in Table I, fertile rats that comprised group 1 had a total gross + microscopic tumor incidence of 71%, similar to the previously reported spontaneous tumor incidence of 65% in virgin females in this model (15). A dramatic decrease in tumor incidence was observed in group 2 animals that had multiple pregnancies. These animals, which had an average of five litters each, exhibited a tumor incidence of \(-10\%\), a >6-fold reduction compared with group 1. Pseudopregnant females in group 3 also displayed a less dramatic, but significant reduction in tumor incidence, with tumors developing in 41% of these animals.

Because uterine leiomyoma in women is associated with infertility, we looked separately at the Eker rats that arose at the Eker rats that arose in the cervix and uterine body, making the uterine horns the predominant site (86%) of tumor development. Pseudopregnant animals from group 3 displayed an intermediate pattern with 61% of the lesions arising in the cervix/uterine body and 39% arising in the uterine horns. Although tumor incidence was reduced in infertile females of group 4, all tumors observed in these animals occurred in the cervix/uterine body. Tumor histology reflected this shift in localization. Tumors arising in the cervix/uterine body were predominantly of the epithelioid variant in all groups, except animals in group 2 which had multiple litters and a reduced incidence of epithelioid and mixed tumors (Table I). The shift in tumor development in the parous and females of group 2 toward the uterine horns was accompanied by a decrease in the number and percentage of epithelioid variants, resulting in a preponderance of tumors of typical and mixed histology (86%).

<table>
<thead>
<tr>
<th>Location</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
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<td>6**</td>
<td>16*</td>
<td>5*</td>
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<td>Typical</td>
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**Significant \( P < 0.001 \) (\( \chi^2 \) analysis).
*Significant \( P < 0.05 \) (\( \chi^2 \) analysis).

Table I. Tumor outcomes in pregnant rats

![Pedunculated leiomyoma from parous Eker rat. Leiomyomas from parous animals frequently presented as pedunculated lesions, which were observed only rarely in virgin animals.](image-url)
that those myometrial cells in which spontaneous loss of function of the wild-type allele occurred remained susceptible to the protective effects of pregnancy, either failing to progress to tumors or being eliminated from the myometrium over the reproductive life span (16 months) of these animals. There is the possibility that genetic factors may exist in human populations that predispose women to the development of uterine leiomyoma. At least two examples of families in which there appears to be a hereditary predisposition to this disease have been identified (18,19). Ethnicity is also a risk factor for uterine leiomyoma, with African–American women having higher rates of this disease than Caucasian women (2.20). Thus, extrapolating our data to the human situation suggests it may be possible to intervene to reduce disease occurrence even in those populations which may be at risk due to a genetic predisposition.

While epidemiologic evidence indicates a protective effect of pregnancy for human leiomyoma, the mechanism(s) by which pregnancy exerts its protective effects is unclear. Pregnancy is a modulating factor in other hormone dependent tumors, although the mechanisms by which pregnancy exerts its protective effects appear to operate at different levels in different cell types. In breast cancer, early pregnancy is the most protective, suggesting an effect on the normal target cell population from which these tumors arise (21–24). Experimental animal studies have confirmed this hypothesis and suggest that induction of differentiation of ductal epithelial cells in the mammary gland mediated by the hormones of pregnancy may contribute to the protective effect of pregnancy (25–28). In contrast, in the endometrium, time of last pregnancy appears to be a more important determinant. In women with only a single pregnancy, the most protection is afforded to women having a late rather than early pregnancy, suggesting that the protective effect is acting against the nascent neoplastic/preneoplastic cell population (29–34). The fact that no protection against the development of uterine leiomyoma was afforded female rats with a single early pregnancy would suggest that the protective mechanisms of pregnancy in the myometrium are more similar to the endometrium than the breast.

As pregnancy and pseudopregnancy significantly decreased tumor incidence, comparing physiological mechanisms similar and unique to these two conditions may highlight factors that effect tumor development. In both states, progesterone production by ovarian corpora lutea is stimulated and maintained by neurogenic surges of prolactin and luteinizing hormone while ovarian corpora lutea regression is stimulated by a combination of an increase in estrogen, progesterolins, and oxytocin, and a loss of progesterone, prolactin and luteinizing hormone stimulation (35). Both progesterone and estrogen stimulate growth of leiomyomas, and these have been reported to grow in women during pregnancy. Thus, the abrupt drop in these hormones at the time of corpora lutea regression may cause tumor regression or delay the development of tumors. Alternatively, the rise in hormones involved in luteal regression, and particularly oxytocin and progastalins (36), may stimulate regression of tumors. Pregnancy caused a decrease in the number of uterine tumors greater than that observed for pseudopregnancy. One major difference between pregnancy and pseudopregnancy is that implantation and uterine decidualization occur during pregnancy and not during pseudopregnancy. A key hormone produced during decidualization and throughout pregnancy is placental prolactin. Prostaglandins, metalloproteinases, and other tissue degrading proteases are also induced in uterine cells by the implanting blastocyst. A second event that occurs during pregnancy but not pseudopregnancy is parturition and the ensuing induction of cervical relaxation, tissue degradation and uterine remodeling (37). During this process myometrial cells express pregnancy associated contractile proteins that include prostaglandins and there is a marked influx of eosinophils and other inflammatory cells that produce collagenases and other tissue degrading enzymes. Such local and systemic products during the decidualization process and during parturition may serve to either induce apoptosis in preneoplastic or neoplastic cells in the myometrium, or inhibit the growth of neoplastic cells. Additional studies to identify which of these hormonal changes are principally responsible for the protection against uterine leiomyoma afforded by pregnancy could contribute valuable information regarding signaling pathways that can inhibit leiomyoma growth and/or development.

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References


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