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Targeting Gonadotropin Receptor Genes

Reproductive Biology, Aging, and Related Health Implications

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This review highlights observations gleaned from recent reports on the deletion of FSH and LH receptors in mice. Gonadal differentiation does not depend on the presence of gonadotropin receptors but development is affected to varying degrees in both sexes. In both knockouts the null females are infertile with severely underdeveloped gonads and accessory structures. Sexual maturity and/or pubertal delay occur depending on the sex and knockout. Male null FSH-R mice have reduced fertility but null LH-R males are sterile due to cryptorchid testes and deficient spermatogenesis. In null FSH-R females hormonal imbalances are due to deficient estrogen and hyperandrogenemia. LH-R deficient females have low estrogen and testosterone. Females in both knockouts display phenotypes such as obesity, bone deficiency, and changes in brain structure and function in addition to manifestation of different types of reproductive tract tumors. Both types of mice represent good models for testing hormone replacement therapy in different combinations. The FSH-R heterozygous females could also be useful for studying age-dependent phenotypes.

Key Words: FSH receptor; LH receptor; ovary; testis; gene knockout; infertility; ovulation; spermatogenesis; menopause; andropause.

Introduction

Following the cloning of gonadotropin receptors in the late 1980s to early 1990s from many species including humans, information on the molecular biology and physiology of these gate keepers of gonadal signaling process have expanded by leaps and bounds. For the understanding of deficiencies in fertility and other gonadal disorders in humans, analysis of several naturally occurring mutations in the genes have provided important clues to their structure and function. As these aspects are discussed in more detail elsewhere in this issue by Huhtaniemi and Themmen *(21)*, we will focus our attention on recent mouse gene targeting studies described for both the follitropin receptor (FSH-R) and the lutropin receptor (LH-R). In addition to several more recent options of temporal blockade by targeting the mRNA of receptors in cells or tissues in vivo, options that have not yet been exploited successfully for gonadotropin receptors, the now classical gene knockouts achieved by homologous recombination in the mouse have been highly informative with significant impact on various issues relevant health of both women and men. In addition, the recognition of important phenotypes that become apparent during haploinsufficiency and aging are interesting for experimental investigations and instructive for considering mechanisms and potential treatment strategies.

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Targeted Disruption of the Follitropin Receptor Gene (FSH-R) (FORKO Mice)

Among the three classical pituitary glycoprotein hormone receptors, the knockout of the mouse FSH-R was first reported in 1998 *(15)*. To clarify what would happen if the gonads could not respond to the hormone, we engineered mice lacking the FSH-R gene repertoire. The strategy used for homologous recombination ensured that none of the alternatively spliced receptors would be generated in mutants. Another group also subsequently developed a mouse mutant and confirmed or extended salient features of FSH-R loss *(1)*. One of the major gender differences in response to the complete removal of FSH-R signaling is the observation that while the resulting female mutants are infertile, FORKO males retain fertility albeit at reduced levels *(15,25)*. Interestingly, similar patterns of aberrations and gender differences occur in the reproductive system of deficient humans. For example, inactivating mutations in the FSH-R gene in women cause absolute infertility and amenorrhea *(3,4)*. However, men with the same mutation show deficient testicular function and weakening of fertility to various degrees ranging from slight subfertility to complete infertility *(41)*. The effects of mutations of the FSH-R gene in human females is similar to that described in the FORKO mice.

Wide Variety of Phenotypes in FORKO Females

FORKO female mice are sterile due to anovulation and have atrophic ovaries unable to secrete estrogen (E2) despite higher circulating androgen levels *(8)*. In general the condition of chronic E2 deficiency and hormonal imbalances in null female mutants leads to obesity, alterations in lipid profiles, and skeletal abnormalities *(8,40)*.

Gonadal Morphology and Markers

The most striking difference between wild-type and FORKO ovaries at 2 d of age is the presence of secondary follicles in the FORKO ovary, while wild-type ovaries contain no follicles of this type *(5)*. This is an indication of advanced follicular development present in the FORKO mice. Another notable difference is the significantly smaller size of the 2-d-old FORKO ovary. As fewer naked oocytes are found in the FORKO ovary at this age, it seems that proper formation of germ cells is disrupted in the absence of FSH-R signaling *(5)*. Thus, we have proposed that hormone interaction with its receptor is indeed required for the early transition of follicles from the resting pool into the growing pool.

The adult mutant ovary has only primordial to preantral follicles with hyperplasia of interstitial tissue in the middle and no functional corpora lutea *(8)*. The lack of any recovery of ovarian activity despite the 10X rise in serum FSH *(9,15)* indicates that in absence of the FSH-R signaling, follicles fail to progress beyond the secondary stage and that no other mechanism including the presence (or the up-regulation) of the counterpart LH-receptor albeit in certain ovarian compartments is able to compensate for the loss.

Lack of ovarian stimulation in FORKO mutants causes a major reduction in the content of inhibin α (and thus total dimeric inhibin) in the aging 12-mo-old FORKO ovaries *(9)*. The dramatic decrease in the number of healthy early antral follicles that usually secrete inhibin correspond to the low levels of this protein in 7- and 12-mo-old FSH-R +/– ovaries *(11)*. Recent studies using 8-wk-old *hpg*, FSH β KO, and FSH-R KO mutants have provided further evidence for FSH-dependent ovarian expression of all three inhibin subunit genes above basal levels *(20)*. The lack of difference in the expression of inhibin α by immunohistochemistry between all three mutants (*hpg*, FSH β KO, and FSH-R KO) and their normal/heterozygous littermates, in contrast to our observations in FORKOs, might be explained by the early age of mutants (8 wk) indicating that at earlier stages expression of inhibin α is less dependent on FSH.

Uterine Morphology and Markers

Uterine and vaginal weights in 3-mo-old null mutants are reduced by 70% and 40%, respectively. The uterine histology of FORKO mice reveal distinguishing features of estrogen deprivation with severely reduced epithelium, stromal, and myometrial layers. The glandular elements of the endometrium are less complex in mutants compared with that in +/+ and +/– mice *(8)*. Heterozygous mice at this age show uterine histology similar to +/+ littermates. Loss of estrogen impacts typical uterine target genes such as lactoferrin, an iron-binding glycoprotein and progesterone receptor. A low level of constitutive lactoferrin expression is evident in FORKO uterus compared to abundant expression wild-type uterus females. The loss of ovarian estrogen reduced A and B forms of the progesterone receptor by 60% causing their imbalance *(8)*.

Reproductive Hormones

LH levels in the plasma of FORKO females are significantly higher than wild-type females at all ages studied *(5, 8,9)* indicating a disrupted negative feedback mechanism(s) at the level of the hypothalamic–pituitary beginning from the perinatal period. In FORKO females, the circulating estrogen is undetectable, while its precursor testosterone remains significantly higher at all ages studied *(5,8,9)*. The high testosterone level in the circulation of KO females, a clear indication of hyperandrogenemia, is explained by the early rise in LH and persistent stimulation of thecal/stromal cells as well as the failure of conversion to estradiol due to an inactive aromatase. A normal pattern of the aromatase gene and protein expression at 3 mo indicates that, while the expression of the aromatase gene and its product in the granulosa cells of the ovary is not dependent on FSH-R signaling, activation of the preexisting enzyme may be involved to allow conversion of androgen into estrogen, once the hormone receptor is activated *(8)*. However, with advancing age ovarian aromatase gene expression is also compromised in the mutants (unpublished data). At 3 mo of age, progesterone in mutants and +/– mice is also reduced by 70% and 30%, respectively *(8)*, indicating a significant perturbation of ovarian steroidogenesis in different compartments.

Nonreproductive Tissues (Body Weight, Bones)

The lack of estrogen in FORKO females produces obesity and skeletal abnormalities, all of which become apparent and visible externally within a few months. It is notable that metabolic alterations in the adult null female induce obesity by 3–5 mo *(8)*. It is also interesting that estrogen replacement eliminated the excess adipose tissue mass in FORKO mice and partially corrected alterations in lipid profiles suggesting metabolic effects of estradiol *(40)*. Whether all different fat depots are differentially affected remains to be ascertained, as this has an important bearing for hormone-deprived states such as menopause *(16)* and hyperandrogenemia in women with polycystic ovarian syndrome *(17)*. That this normalizing effect occurred in a background of high testosterone in FORKO mice indicates that hyperandrogenemia was of no consequence to derive the benefits of estrogen replacement.

The appearance of kyphosis in mutants (a sharp change in the curvature of the spinal column) around 5 mo of age *(8)* may also be attributed to estrogen loss because similar

effects occur in the upper thoracic vertebrae in postmenopausal women *(43)*. The reduction in absolute weight of femur or per 100 g of body weight, the significant loss of trabecular bone, and higher proportion of B-220–positive cells in the FORKO bone marrow further reveal the signs of osteoporosis *(8)*.

Hormone Replacement Therapy

Our initial findings indicate that lack of estrogen production by the FORKO ovary has no bearing on estrogen receptor gene transcription and translation as both mRNA and protein for the two ERs seem to be unaffected *(8)*. Therefore, it is of interest to know if ERs are also functional elsewhere in the mutants. In previous studies, rapid and immediate response to hormone-replacement therapy has been demonstrated in the uterus, vagina, and adipose tissue *(8,40)*. It is interesting that estrogen replacement eliminated the excess adipose tissue mass in FORKO mice, indicating its metabolic effects *(8,40)*. Taken together, these studies suggest that treatment of the genetically altered and chronically estrogen-deficient female mice with estradiol 17β produces target-specific effects validating its utility in addressing fundamental problems of hormone-replacement therapy. Incidentally, such models might provide the means to resolve some of the emerging controversial issues concerning the benefits and risks of hormone-replacement therapy in its various forms for menopausal women *(42)*.

Consequences in FORKO Males

Reproductive Performance

Overall, the FORKO males show reduced fertility and, in this respect, are somewhat different from the FSH β knockout mice *(28)* that are apparently reported to be fully fertile despite reduction in testicular size and function. The decrease in fertility in FORKO males is reflected both in the time taken to produce the first litter, which doubles compared to the wild-type males, and also in the lower number of pups sired by the FORKO males *(26)*. More recent evidence appears to suggest that such effects might become more prominent on certain other backgrounds (Sairam et al., unpublished data).

Why Are FORKO Males Subfertile?

Loss of FSH-R signaling dramatically affects testicular development beginning from infancy. Different parameters such as the number of Sertoli cells, the tubular diameter, as well as first wave of spermatogenesis and sexual maturity are all affected *(25,26)*. The drastic reduction in testicular weight and shrinkage of seminiferous tubules that occurs as early as 7 d of age persist into the adult stage in the FORKOs, suggesting inhibition of the initial developmental processes *(26)*. There is nearly a complete lack of round spermatids on d 21 as well as of elongated spermatids on d 28 as well as the time required for progression of a round spermatid to an elongating spermatid is delayed in FORKO males *(26)*. This situation could provide opportunities to explore interventions to arrest or prevent germ cell development at selected stages. Additional data revealing impaired expression of multigenic cluster of transition proteins and protamine 2 in FORKO males *(44)* supports the conclusion of destabilization of chromatin compaction process that is normally required for optimal spermatogenesis and fertility *(25)*.

A recent study by Grover et al. *(18)* has addressed the nature of the structural alterations in the Sertoli cells of FORKO mice by revealing a significant reduction in the profile area of seminiferous tubules and large irregularly shaped spaces within the seminiferous epithelium of 3- and 6-mo-old FORKO mice. Upon electron microscopy analyses these spaces correspond to an apparent accumulation of fluid in the Sertoli cell cytoplasm, coincident with an absence of the fine flocculent ground substance seen in wild-type mice. It was first proposed by Jegou et al. *(23)* that FSH is involved in fluid absorption/secretion. Additionally, androgen-binding protein (ABP), a major secretory protein of Sertoli cells, is dramatically reduced in FORKO mice. These results suggest that FSH-R signaling normally maintains water balance in Sertoli cells in addition to regulating ABP production, which may have an indirect effect on epididymal functions and sperm motility, accounting for the reduction in fertility. Interestingly, these studies further suggest the potential involvement of aquaporins in maintaining fluid balance and as mediators of functional alterations, an aspect that needs to be explored in more detail.

The fall in circulating androgen in FORKO mutants after the 10th week *(26)* supports the argument that hormone action via FSH-R but not testosterone is the principal endocrine stimulus that maintains testicular size in the prepubertal animal *(39)*. Adult FORKO male mice (>3 mo) exhibit increased levels of FSH and decreased serum testosterone but normal LH *(15,25–27)* suggesting that the perturbations leading to reduced androgen might be at the target site, the Leydig cells *(27)*. Although the Leydig cells can remain functional in the complete absence of FSH-R signaling, their capacity to produce testosterone appears to be reduced *(27)*. In the absence of FSH-R signaling, Leydig cells appear to undergo changes manifesting themselves in higher expression of the enzyme 3β-hydroxysteroid dehydrogenase (3β HSD) as well as LH-R density *(27)*. Therefore, in the adult FORKO testis, although full expression of LH-R in the Leydig cells is independent of FSH-R signaling, the overall response of the androgen-producing Leydig cells are reduced in the adult owing to improper intercellular communication. Therefore, Sertoli cell factors under the influence of FSH-R signaling must be responsible for these modulating effects. This conclusion is also reinforced by another study that compared Leydig cell development in FSH-R KO and FSH β KO and concluded that deficiencies are evident only in receptor deficient mutants and implicate a role for constitutive FSH-R activity *(6)*. The regulation of LH-R in the ovary stands in contrast to males because our obser-

vations in FSH-R +/– females demonstrate that full expression of FSH-R is required for the normal appearance of LH-R in granulosa cells, but not obligatory for the constitutive expression of LH receptor in thecal cells and interstitial tissue *(11)*.

The adult FORKO male has low sperm counts, decreased sperm motility, and aberrant sperm morphology *(15,25)*. In addition to showing decreased motility, >80% of cauda epididymal sperm from FORKO males also exhibits morphologic abnormalities such as retention of the cytoplasmic droplet *(25)*. The retention of the cytoplasmic droplet by ejaculated spermatozoa has been correlated with reduced fertility in animals *(32)*. The fluorescence intensity properties of sperm collected from the cauda region of FORKO males indicate that they remain in an immature state suggesting that inadequate compaction of chromatin may have contributed to increased head size of sperm in FORKO males *(25)*.

Epididymal Phenotype

Loss of FSH-R gene also caused some reduction in the epididymis weight. Although the epididymis of the FORKO males appear normal, not all the lumina are filled with spermatozoa, with tubules of the caput epididymides being devoid of sperm *(25)*. The reduction in the size of epididymis in FORKO males may be due to the decreased levels of testosterone because in FSH β deficient mice with unaltered levels of testosterone there is no change in epididymis *(28)*. Recently, Grover et al. found changes in epithelial cells of the epididymis in adult FORKO males that could impact on sperm morphology and sperm maturation *(19)*. In contrast to LH-R mRNA detected in the epithelial cells of the monkey epididymis, FSH-R mRNA was not present in the monkey epididymal cells *(46)* suggesting that effects on epididymal function may be indirect.

Pubertal Development in Both Sexes During Receptor Deficiency

Vaginal opening, an indication of puberty, occurs at an earlier age (by 2–3 d) in FORKO mice (unpublished observations) but, interestingly, sexual maturation as evidenced by vaginal introitus is significantly delayed (by 5–7 d) in LuRKO mice when compared to wild-type littermates *(47)*. This could be attributed to high levels of plasma LH in FORKO females because elevated serum LH and hyperandrogenemia results in accelerated vaginal opening (precocious puberty) in LH transgenic females *(37)*. It is also possible that the fat-derived hormone leptin, the first peripheral molecule demonstrated to accelerate the maturation of the reproductive axis in normal rodents *(2)* could be elevated in FORKO females (unpublished data). In FORKO males the loss of FSH-R signaling causes delay in puberty *(26)*.

Targeted Disruption of LH Receptor Gene in Mice

Two groups independently reported the targeted disruption of the LH-R gene in mice *(29,47)*. The designation LuRKO suggested by Zhang et al. *(47)* will be used in the following discussion of the various phenotypes observed in both sexes.

Consequences of the Loss of LH-R in Females

Delayed Puberty, Gonadal Morphology and Ovarian Markers

In LuRKO females, the age of vaginal opening was delayed to 35–38 d, compared to 30–32 d in +/+ mice *(47)*. Comparison of estrous cycles between +/+ and –/– females at 12 wk of age revealed acyclicity in LuRKO females.

According to Zhang et al. *(47)*, gonadal histology of female LuRKO mice is indistinguishable from wild-type littermates at birth. At 7 and 12 wk of age ovaries of LuRKO mice have follicles up to the early antral stage, but not preovulatory follicles or corpora lutea. No apparent differences are evident in the thickness of the theca cell layers surrounding the developing follicles *(29,47)*. No differences are found between +/– and +/+ mice in any of the parameters studied. It should be noted that this is in marked contrast to the +/– FSH-R females that manifest age dependent phenotypes *(10)*.

In the LuRKO female, all three inhibin genes are significantly up-regulated compared with normal/heterozygous females *(20)*. This is also reflected in significantly higher inhibin B protein levels in ovaries and serum of LuRKO mice. The inability to respond to LH combined with high levels of FSH that leads to high proportion of antral follicles in LuRKO females could explain the higher levels of inhibin production in these ovaries *(20)*. Low but detectable levels of P450 17-OH mRNA, a marker of theca cell steroidogenesis, are present in the LuRKO ovaries, indicating low but constitutive expression *(47)*. However, LH-R loss caused dramatically decreased steroidogenic acute regulatory protein (StAR) in the ovary *(29)*. Deletion of LH-R had no effect on FSH-R and progesterone receptor mRNA levels in ovaries *(29)*. However, it caused a dramatic decrease in ER α mRNA and increase in ER β mRNA levels in ovaries *(29)*.

Uterine Morphology and Markers

The uterine histology shows thinning of all cell layers and scarce glandular structures *(29,47)*. Uterine ER β, but not ER α, decreased in LH receptor KO animals *(29)*.

Reproductive Hormones

Both groups have reported high serum LH levels (sevenfold increase) and moderately elevated FSH levels (about 30%) in –/– females compared with +/+ and +/– littermates *(29,47)*. The ovarian estradiol concentration is significantly decreased in –/– females compared with +/+ and +/– mice *(29,47)*. Hormone levels are not different in haploinsufficient female mice at 2 mo of age *(29,47)*. Only the group from Kentucky has noted suppressed levels of ovarian progesterone in null female circulation and undetectable serum

testosterone levels in –/– females *(29)*. An alternative mechanism, not involving StAR, has been suggested because the moderate progesterone decrease (by 40%) was accompanied by a dramatic decrease in ovarian steroidogenesis acute regulatory protein (StAR) levels *(36)*.

Treatment of –/– mice with PMSG, an experimental surrogate for FSH, resulted in a greater number of small antral follicles with no other obvious changes in either ovaries or in the reproductive tract *(29)*. Twenty-one day estradiol and progesterone replacement therapy of 30-d-old –/– females normalizes vaginal development with no effect on ovarian morphology *(29)*. Despite uterus thickening, the number of the endometrial glands remains low. Thus, it has been suggested that direct LH actions may be required for complete gland restoration *(29)*. The animals, however, remain infertile. The lack of reversal of ovarian morphology is consistent with a concept that only LH, not estradiol, progesterone, or FSH, can stimulate final stages of follicular growth beyond the antral stage and induce ovulation *(29)*. After hormone treatment, only ER α and StAR decreases were reversed, whereas the ER β increase was not reversed, suggesting that LH signaling is inhibitory and required to maintain normal ovarian ER β levels *(29)*.

Nonreproductive Tissues (Bones, Body Weight)

As in the case of FORKO mice, the LuRKO females are about 50% heavier with a lot of visceral fat at 60 d of age compared with +/+ and +/– littermates *(29)*. Accumulation of body fat pads became more pronounced in 1-yr-old –/– as well as +/– animals compared with +/+ littermates *(36)*. By the age of 1 yr the body weight of +/– females increased about 30% compared with the +/+ siblings *(36)*. All these effects could be attributed to decreased levels of estradiol.

Bone analysis conducted at 8 and 60 wk reveals a decrease in the density of femur of LH-R –/– and +/– female mice compared to wild-type littermates *(36)*. LuRKO females show a decrease in bone mineral density around 9–10 wk of age *(45)*. Although functional LH-R has been identified in human bone *(48)*, the loss of bone mass in LuRKO females cannot be totally attributed to the deletion of the LH-R gene because skeletal abnormalities resembling osteoporosis have been reported also in FORKO and FSH-R haploinsufficient females that produce high levels of LH and have abundant LH-Rs in addition to elevated testosterone *(9,11, 12)*. Overall, although these investigations support an indirect effect of gonadotropins on the skeleton suggesting that the decrease in bone density is apparently secondary to suppressed gonadal steroid production, additional investigations are warranted to understand the role of receptors in such peripheral sites.

Consequences in LH Receptor KO Males

Sexual Differentiation

It has been known for a long time that LH action is critical for adult testicular function and male fertility. Although fetal testicular testosterone synthesis is crucial for male sexual differentiation, the LuRKO mice provide, for the first time, direct evidence that specific elimination of LH action via the receptor does not hamper this function *(47)*. Because the male –/– mice are indistinguishable from the wild type at birth, the intrauterine process of masculinization, although critically dependent on fetal testicular production of two hormones, testosterone and anti-Müllerian hormone, is apparently normal and not dependent on LH action on the testis *(22)*. Although all male genital organs had apparently differentiated normally in utero, their postnatal growth is totally blocked, including testicular descent.

Why Are LH Receptor Knockout Males Infertile?

Mutant males have a micropenis and abdominal testes. These testes contain underdeveloped seminiferous tubules, with sporadic round spermatids being the most advanced form of spermatogenesis. There is also a marked reduction in the seminiferous tubule diameters with a drastic decrease in the Leydig cell number that are hypotropic fetal-type in –/– animals as compared with +/+ and +/– animals *(29)*. Lack of adult-type Leydig cells suggests that their formation from precursor mesenchymal cells requires LH signaling *(36)*. The Sertoli cell number, on the other hand, seems to be unaffected due to the presence of intact FSH-R signaling *(36)*. It has been suggested that full spermatogenesis was not found in LuRKO mice either due to the cryptorchid position of the KO testes, insufficient intratesticular testosterone concentration, or both *(22)*. The low but detectable level of the P450scc mRNA expression, an indicator of Leydig cell steroidogenic activity, detected in the LuRKO testes suggests that low constitutive expression of this enzyme is possible in the absence of LH-R function in the precursor Leydig cells *(47)*.

Epididymal Phenotype

Epididymis is a site of sperm maturation and storage and also a target for androgen action. As the epididymides are barely recognizable in LuRKO animals, there is a dramatic decrease in weight *(29)*. The morphological phenotype in 7–8-wk-old mutants include a decrease in luminal diameter of the proximal and distal caput and cauda epididymis, the absence of clear and halo cells in the epithelial lining, a decrease in the height of principal cells and the number of cells containing cilia, a decrease in cilia length, and a change from basal to central location of nuclei in the principal cells *(30)*. Decreased levels of AR and ER β and increased levels of ER α in the mutant epididymis signify alterations related to steroid milieu and or the lack of LH-R itself. As previous studies had demonstrated that epididymis contains functional LH receptors, LH might be directly involved in epididymal function *(30,46)*.

Reproductive Hormone Levels

Serum LH levels are dramatically (3.5-fold increase) elevated, while FSH and estradiol levels are moderately ele-

vated but testosterone levels dramatically decreased in –/– males compared with +/+ and +/– littermates *(29)*. The estradiol increase could have come from FSH stimulation of aromatase in Sertoli and germ cells and in the few remaining fetal-type Leydig cells and/or decreased estradiol catabolism *(7)*.

Steroid Hormone Replacement Effects

Interestingly, 21-d testosterone-replacement therapy of 30-d old –/– males reverses cryptorchid status of the testis inducing scrotal descent and growth of the penis *(29)* suggesting that it is androgen dependent. In addition, therapy caused an enlargement of seminiferous tubule diameters with resumption of spermatogenesis but failed to restore Leydig cell number or improve hypotrophy *(29)*. Although the size and weight of epididymides, seminal vesicles, and prostate are restored, yet the animals remain infertile. In a subsequent study, androgen-replacement therapy in 30-d-old KO animals could not reverse luminal diameters of proximal and distal caput and cauda epididymis, the percentage of ciliated principal cells in caput epididymis, and nuclear AR localization *(30)*. These observations suggest LH requirement for certain facets of epididymal morphology and/or function *(30)*.

Nonreproductive Tissues (Bones, Body Weight)

LuRKO males accumulate visceral fat at about 3 mo (approx 120 d), but their lighter body mass could be due to decreased muscle mass and bone density *(29)*. The decrease in bone mineral density after 5 mo apparently is secondary to suppressed gonadal steroid production *(45)*. Interestingly, although the group from Kentucky has demonstrated indications of obesity and skeletal abnormalities in young and aged LuRKO males and females, no detailed study on extragonadal phenotypes have been reported.

LH Receptor vs FSH Receptor

Because both LH and FSH are indispensable for gonadal regulation, it is of interest to compare the consequences of inactivating their respective receptor genes. The study on both LH-R and FSH-R knockout models shows that gonadotropin stimulation is not required for sexual differentiation of male or female mice. In support of this, the study on *T/ebp/Nkx2.1* knockout mice, devoid of the pituitary gland, also demonstrates that pituitary hormone secretion is not needed for stimulation of sufficient fetal testicular androgen synthesis to induce male sexual differentiation *(33)*. The endogenous testosterone level in these null mutant testes is 5–10% of the control level, which suggests that there is a considerable safety margin in the amount of testosterone that is needed for the male fetal masculinization *(33)*.

The main similarity between the two models is that null females are infertile due to anovulation. Hormone-replacement therapy, in general, has been better investigated in LuRKO mice. However, both estradiol and/or progesterone cannot restore fertility in the LuRKO mice and gonadotropins injections fail to induce ovulation in both models. Thus, signaling via both FSH and LH-Rs are required for oocyte maturation and ovulation.

It is of interest to note that, unlike the FORKO ovaries, which are already different (accelerated folliculogenesis and the reduced number of follicles) by 2 d *(5)*, the ovaries of LuRKO mice are indistinguishable from wild-type littermates at birth *(47)*. These differences might be due to the temporal differences in receptor gene expression at least as discerned in mice. While full-length transcripts of the LH-R are not detectable until postnatal d 5, although shortened transcripts encoding the extracellular domain of the receptor are present from birth *(35)*, FSH-R transcripts encoding all domains of the receptor are detectable at low levels at birth *(34,35)*. Therefore, it could be argued that differences in timing of gonadotropin expression in the ovary might explain changes between the LuRKO and FORKO prenatal ovaries.

Whereas LH-R inactivation has no effect on FSH-R mRNA levels in ovaries or testes *(29)*, disruption of FSH-R gene caused apparent increases of LH-R protein in both ovaries and testes compared with +/+ littermates *(11,27)*. This increase in association with elevated LH levels in FORKO females could account for marked thecal/ stromal hypertrophy and hyperandrogenemia.

Mice with Partial Loss of Gonadotropin Receptor Gene

The critical importance of FSH-R gene dosage for female reproduction is revealed in mice with only one functioning allele (+/–). Heterozygous females also develop significant changes, but these are age-dependent. According to our knowledge, no other gene disruption in the reproductive system, including LH-R disruption, has been shown to develop such partial phenotype in the +/– mice that intensifies in KO mice. Whereas LH-R haploinsufficient females have normal fertility *(29)* despite a modest increase in serum FSH levels and a modest decrease in serum E2 levels *(29)*, the reproductive capacity of 3-mo-old FSH-R +/– females in contrast, is compromised in two ways. In addition to reduction in litter size sired by +/– females *(8)*, another indication of suppressed reproductive potential in +/– females is the increased interval between mating and conception that progressively got longer with each pregnancy. After about six to eight births the FSH-R heterozygous females could no longer conceive. Thus, full FSH-R function is important for ovarian function including generation of healthy eggs, a feature that is critical for the success in assisted reproductive technology for women.

Other Unexpected Phenotypes and Avenues for Exploration

In addition to non-gonadal phenotypes careful observations in aging, FORKO females have produced a rich divi-

dend of reproductive tract and other related pathologies. Three of these merit special mention in this section.

Ovarian Tumors

It has been noted that the incidence of ovarian neoplasms rises around the onset of menopause in women and accompanied by high levels of plasma gonadotropins. Although a causal link between ovarian stimulation in women undergoing in vitro fertilization and the tumor development remains controversial, the precise mechanisms remain unknown. Some investigations in animals have shown a relation between chronic and abnormal gonadotropin exposure and the development of granulosa and/or Sertoli cell tumors *(31,37)*.

Elevated androgen synthesis as a consequence of up-regulation of both LH and LH-R in the FORKO mice contributes to the development of ovarian sex-cord stromal tumors, which are seen in a majority of FORKO mice by 12 mo of age *(9)*. In these tumors, there is loss of granulosa cell proliferation control programs, accompanied by an up-regulation of Sertoli cell markers, Müllerian-inhibiting substance, and GATA-4 transcription factor *(9)*. Interestingly, female bLH β-CTP transgenics have a 10-fold increase in circulating immunoreactive LH and exhibit ovarian cysts and granulosa/theca cell tumors on some genetic backgrounds *(24)*. Taken together, these results suggest that ablation of FSH-R causes development of gonadal tumors in aging female mutants. High levels of ovarian androgens synthesized in response to enhanced LH action in FORKO mice may be among the signals for induction of tumors. We also believe that the loss of receptor expression/signaling that occurs naturally in all women at the time of menopause predisposes the ovary to neoplasia, a condition that might be exacerbated in combination with other confounding (genetic or environmental?) factors.

Uterine Tumors in +/– Mice

Interestingly, by 1 yr of age, all LH receptor +/– mice develop endometrial tumors with cancerous histology *(36)*. By 12 mo of age, many virgin FSH-R +/– females and higher number of retired (failed) +/– female breeders developed visible uterine abnormalities. Uterine abnormality is not seen in +/+ females, either virgin or retired breeders at any age studied. The larger masses that are always unilateral in FSH-R +/– mice are circumscribed and pushing against the uterine walls. Some uterine masses contain trophoblastic-like tissues and decidua. There is also evidence of increased angiogenesis in the uterus of +/– mice. Based on histological and other staining characteristics, we have classified this abnormality as a spectrum of changes that include dilated vessels. These undergo thrombosis to different extents in both the old +/– virgin and retired breeders producing a mass. A strong link between progression of the abnormality and PR imbalance in FSH-R +/– mice is currently favored as a working hypothesis *(12)*.

A third prominent phenotype evident in null FORKO or aging FSH-R +/– females is the alterations in brain markers implicated in memory and behavior *(13,14)*. A significant part of these alterations could in part be attributed to the long-term consequence of estrogen loss and/or other hormonal imbalances. Further work is expected to add to this area of investigation important for women's health.

Conclusions and Perspectives

Although the currently known human mutations of gonadotropin receptor genes are very rare, they constitute a very important group of diagnoses interesting for infertility treatment because of their involvement in aberrant and delayed sexual differentiation and development *(21)*. The observed similarities in phenotypes between naturally occurring mutations and genetically manipulated animal models reinforce their validity as tools for study of human deficiencies. As ethical considerations do not permit frequent sampling of human tissues from patients with mutations, genetically modified animal models might be useful to understand the molecular mechanisms underlying these conditions.

The induction of steroid hormone deficiencies in sterile females in both receptor KOs that mimic menopausal conditions, or andropause in aging men or hormonal imbalances such as hyperandrogenemia in FORKO females including age-dependent appearance of these conditions, afford an excellent opportunity to investigate and clarify issues that currently confound hormone-replacement-therapy regimens *(42)*. Future studies could examine tissue-specific deletion of LH-R that appears to be more widely expressed than the FSH-R. We would also expect that these receptor-deficient mutants would serve experimental medicine and drug developmental therapy in several other ways. First, in the testing of specific (ideally gonad selective) small molecules that can bypass the receptor mechanism and are capable of inducing signaling mechanisms encompassing receptor action in the hope of restoring lost functions. Second, receptor-deficient mutants could serve to test the effects and implication of potential gene therapies involving specific receptor DNAs including interesting receptor variants with altered topography or predicted functions. Finally, microarray or proteomic analysis of gonadal tissues (or even other tissues that are affected) or cells derived there from will provide excellent experimental paradigms to understand changes in gene or protein repertoire and aid in the discovery of potential new genes.

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