Endocr (2009) 36:1–5
DOI 10.1007/s12020-009-9199-6

INVITED REVIEW

FSHβ knockout mouse model: a decade ago and into the future

T. Rajendra Kumar

Received: 18 February 2009 / Accepted: 9 April 2009 / Published online: 23 April 2009

Abstract In 1997, more than 10 years ago now, we first reported the phenotypes of follicle stimulating hormone (FSH) β null mice. Since then, these mice have been useful for various physiological and genetic studies in reproductive biology. More recently, extra-gonadal functions of FSH have been discovered in bone. These studies opened up exciting avenues of new research on osteoporosis in postmenopausal women. Several genomics and proteomics tools and novel strategies to spatio-temporally restricting gene expression in vivo are available now. It is hoped that with the aid of these and other emerging technologies, an integrated network of FSH signaling pathways in various tissues would emerge in the near future. Undoubtedly, the coming 10 years should be more exciting to explore this "fertile" area of reproductive physiology research.

Keywords FSH · Pituitary · Gonads · Transgene · Gene targeting

Introduction

Prior to the advent of mouse gene/genome manipulation techniques, several physiological studies have elucidated the functions of the pituitary gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH). Some of these studies used the state-of-the-art approaches of those times that include (a) surgical hypophysectomy, (b) passive or active immunoneutralization of circulating hormones, (c) pharmacological antagonists that suppress release of FSH and LH, and (d) hormone replacement strategies involving implants that release either protein hormones or appropriate steroids in naturally occurring *hypogonadal* rodent strains. These studies have established and laid the foundations to understanding the physiological roles of gonadotropins in testis and ovarian development and function including gametogenesis and steroidogenesis.

Very soon several drawbacks became apparent with the above approaches. For example, paracrine interactions within the pituitary were also removed by hypophysectomy, lack of sufficient purity of the hormone preparations used for generating the antibodies was a critical issue with immunoneutralization studies, cumbersome, and repeated injection protocols were necessary with the antagonists that suppressed in many cases both the gonadotropins, and similarly both LH and FSH were suppressed in *hypogonadal* strains. Furthermore, while FSH actions in the female in ovarian granulosa cell proliferation and differentiation were somewhat understood, FSH actions in the male remained controversial. Depending upon the experimental paradigm and species studied, the need for FSH in spermatogenesis and male fertility was found to be variable [1–4].

Three other issues remained unresolved. First, how FSH stimulated or suppressed signaling pathways in gonadal cells and what gene networks are involved during cell proliferation and differentiation were unknown. Second, elevated FSH levels were observed in women with ovarian cancer [5]; whether this increase in circulating FSH is merely a correlation or cause was not clear. Third, mice lacking inhibin α-subunit gene develop gonadal tumors with 100% penetrance; these mice also demonstrate high levels of FSH in serum [6, 7]. Whether absence of inhibin or gonadal cell hyperstimulation by

T. R. Kumar (✉)
Departments of Molecular & Integrative Physiology and Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160, USA
e-mail: tkumar@kumc.edu

FSH causes tumor development has been debated. Thus, alleviation of the above technical problems and defining the consequences of isolated deficiency of only FSH in normal reproductive physiology and pathophysiology required the development of a more precise in vivo gene manipulation approach. These have led to the development of the *Fshb* null mouse model by embryonic stem (ES) cell technology [8].

Generation and characterization of FSHβ knockout mice

A partial cDNA of mouse *Fshb* was originally cloned from pituitary total RNA. This was achieved using a set of degenerative PCR primers designed after aligning the known *Fshb* gene sequences [9]. At that time, both human and rat FSHβ encoding gene sequences have already been fully characterized by the Jameson and Chin laboratories [10, 11]. The PCR cloned partial mouse *Fshb* cDNA was subsequently used to screen a mouse genomic library and several overlapping phage clones containing mouse *Fshb* gene sequences were identified. Large fragments up to 18 kb of DNA sequences encompassing the entire coding region of mouse *Fshb* gene along with flanking arms were cloned into plasmid based vectors for restriction enzyme mapping [12], engineering gene targeting constructs, and subsequent ES cell transfection experiments.

The gene targeting strategy was to delete exons 1 and 2, most of the exon 3 and both the introns, all of which encompassed only 2.3 kb region at the *Fshb* locus. Sufficient lengths ($\sim$3 kb) of 5′ and 3′ flanking homology were also engineered into the targeting vector, and the appropriate 5′ and 3′ external probe sequences were sub-cloned. Both PGK-HPRT and MC1-TK cassettes were incorporated into the vector for positive and negative selection. The PGK-HPRT also served the purpose of creating a unique *Bam*HI site that would later on prove useful in distinguishing the FSHβ wild-type and mutant alleles. Several gene targeting experiments were performed without success and finally after several rounds of transfections, one targeted clone was obtained [8]. This clone had an abnormal recombination event on the 5′ side but had a correct one on the 3′ side as confirmed by Southern blots with the corresponding external probes. However, as predicted this allele was found to be null [8].

The mutant ES cells were injected into host blastocysts and several good chimeras were obtained. The matings with wild-type female mice were set up exactly on day 42 and the germline transmission of the mutant allele was later confirmed. Several null mice were obtained and later tested for their fertility/fecundity assays. Surprisingly, the null males were fertile but the females were infertile. Consistent with the fertility data, the testes from the null male mouse demonstrated all stages of apparently normal spermatogenesis, but the tubule size was reduced [8]. Additionally, null males demonstrated reduced sperm number and motility. In the null female, clearly there was an ovarian folliculogenesis block at the pre-antral stage [8]. Serum FSH levels were measured by RIA and northern blot analysis of pituitary RNA was also performed. All these studies confirmed that indeed a null mutation at the FSHβ locus was engineered [8].

Ten out of 10 null females did not produce litters over a period of 6 months and in contrast, 5 out of 5 null males sired normal litters. However, immature null female mice when superovulated with exogenous hormones produced similar number of eggs compared to those from control mice [8]. Other data included serum LH and steroid hormone profiles, and analyses of the male accessory glands all of which were comparable to those in control mice [8]. These results suggested that FSHβ null mice phenocopy some of the patients with human FSH-receptor mutations as reported by Huhtaniemi and colleagues [13].

Controversy as to whether FSH is necessary for male fertility

Several missense mutations in exon3 of *HFSHB* gene in men have been identified and correlated to azoospermia and infertility [14]. Hypogonadism and sub- and infertility have also been reported in patients with *FSHR* mutations [14]. Similarly, Matsumoto et al. [15] have shown that blocking endogenous FSH secretion in normal men causes significant reduction in sperm numbers and these could be reversed by exogenous FSH but not by testosterone. These data are similar to those reported in FSH or FSH receptor immunoneutralized non-human primate models where spermatogenesis arrest and various degrees of spermatogenic failure were consistently observed [4]. In contrast, some inactivating mutations have been reported to result in variable fertility. Clearly, more functional studies are required to test the biochemical properties of these mutant ligands as well as their ability to bind and elicit a functional response in target cells.

In several other studies, immunoneutralization of immature but not adult rats showed spermatogenic arrest. Research with hamster, and sheep similarly pointed the critical need for FSH in maintaining spermatogenesis. In GnRH immunized rats, germ cells were suppressed and recombinant human FSH therapy partially restored spermatogenesis defects [4, 16]. However, in *hypogonadal* mice, testosterone alone restored spermatogenesis despite low intratesticular testosterone and undetectable serum FSH levels [17].

One important difference between the studies discussed above and those with *Fshb* null male mice is that FSH immunoneutralization studies have routinely used adult species of interest; whereas FSH is absent from birth in *Fshb* null mice. One way to directly address this variability in FSH action in males of different species could involve developing a conditionally regulated temporal gene inactivation strategy in mice [18–21] in which the *Fshb* gene is deleted selectively during postpubertal period and compare their testicular phenotypes to those in existing *Fshb* null mice. Secondly, one in vitro study documented the presence of low-level constitutively active, FSH ligand-independent FSHR that partly compensates for loss of FSH [22]. However, its existence and functional significance in vivo in mouse Sertoli cells have yet to be confirmed.

Applications of FSHβ KO model

Genetic rescue of *Fshb* null mice and generation of double null mice lacking both inhibin α and *Fshb* followed the initial studies on *Fshb* null mice. After several rounds of breeding and generating double heterozygous mice, the double mutant mice were eventually generated. In addition, FSH gain-of-function mice were also generated and extensively characterized [23]. These studies clearly indicated an important modifier role for FSH in gonadal tumorigenesis in the inhibin α null background. Complementary gain-of-function experiments indicated that high FSH levels are only associated with but do not directly cause ovarian cancer [23].

The genetic rescue experiments used two strains of mice. In one strain of mice, hFSHβ transgene was targeted to and exclusively expressed in gonadotropes [24]. In the second strain human FSH subunits were ectopically expressed in multiple tissues using a mouse metallothionein-I promoter [23]. The gonadotrope-targeted hFSHβ transgene rescued *Fshb* null male and female mice, and ectopically produced hFSH rescued null males but only partially rescued female *Fshb* null mice [25]. These mice have been extensively used later on in collaborative studies with Dr. Irving Boime, to test the bioactivities of various gonadotropin analogs [26, 27].

Over the past 10 years, *Fshb* null mice were used for many studies in our own laboratory. Many other collaborations turned out to be fruitful and provided interesting new results on FSH role in conjunction with activin signaling [28], in testicular Sertoli and germ cells [22, 29], anti-Mullerian hormone (AMH) secretion coupled to non-classical FSH-R mediated signaling in Sertoli cells [30], spermatogonial differentiation [31], IGF-I, and AMH regulation of ovarian folliculogenesis [32, 33] and oocyte-somatic cell communication [34]. Many of these studies have been discussed elsewhere [1–3].

Recently, the most thought-provoking work resulted in collaboration that has started in early 2004 with Dr. Mone Zaidi. Dr. Zaidi and his colleagues used biochemistry, cell biology tools, and genetic models to confirm that FSH exerts extra-gonadal functions, primarily on osteoclasts in bone [35]. Indeed, *Fshb* (and *Fshr* null mice) have higher bone density when compared to that in heterozygous and wild-type controls. Thus, it appears that absence of FSH signaling causes protection from bone loss. This work provided new insights into FSH regulation of bone loss in postmenopausal women, generated some debate with regard to roles of steroids and FSH in bone biology [36]. Dr. Mone and our group continue to collaborate and we are anticipating definitive answers soon.

Final remarks and looking into the future: what and where to next?

The latest ISI citation index reports nearly 520 citations of our Nature Genetics paper (http://portal.isiknowledge.com/portal.cgi?DestApp=WOS&Func=Frame). Excellent research environment, outstanding colleagues, and collaborators contributed to the success of this work. We continue to work with *Fshb* null mice in our laboratory on various aspects related to FSH signaling in the gonads. In parallel to our work with the FSH ligand null model, Drs. Sairam and colleagues [37, 38] and Abel et al. [39] have significantly contributed to the role of FSH signaling in gonads using their FSH-receptor knockout mouse models. Recent work with FSH action in bone has ignited research interests in many labs. In this context, it is worth mentioning that FSH signaling may have a wide-spectrum of implications similar to those proposed for hCG/LH signaling [40, 41]. Perhaps, it will soon be clear that there are many facets to the FSH signaling pathway that previously we never envisioned.

Using a combination of current molecular and genetic tools and those that are new and emerging at a rapid pace, many missing pieces to FSH actions can be precisely addressed in the near future. Few of these are as follows: how is FSH secretion regulated from the pituitary and how are gender-specific FSH actions achieved? Are there really species-specific differences in FSH action in the male? How does FSH coordinate Sertoli cell proliferation and differentiation in such a narrow window of time? What molecular changes occur in granulosa cells when they are first exposed to FSH? What causes the FSH-dependent bone phenotypes to be apparent only during aging? Do FSH variants have any biological roles in normal and aging ovary and bone?

Fshb null mice will prove valuable and serve as a platform to answering the above questions. For example, new FSH analogs could be engineered and directed to gonadotropes on an *Fshb* null genetic background. Genomics and proteomics tools coupled with in vivo RNA interference approaches may yield new information on signaling networks regulated by FSH in Sertoli, granulosa and osteoclast cells. Cells of interest isolated from control and *Fshb* null mice should provide the necessary resources/ reagents for this type of functional genomics experiments. Biochemical and cell biological studies involving FSH receptor characterization on osteoclasts, an analysis of FSH normal glycosylation patterns and age-dependent changes will shed new light on roles of sugars in FSH secretion, organ/cell specific functions, and intracellular signal transduction cascades. Certainly, the forthcoming 10 years should be worth pursuing research on FSH actions, and in anticipation of answers to the above questions that may have several fundamental and clinical implications.

Acknowledgments I am fortunate to have worked under the tutelage of Professor K. Muralidhar, University of Delhi, India, who introduced me to gonadotropin research. I thank Professor Malcolm Low, Vollum Institute, Portland, for providing me an opportunity to work on FSHβ transgenic mice, clone mouse FSHβ gene in his laboratory and allowing me to continue the FSH project. I am grateful to Professor Marty Matzuk, Baylor College of Medicine, Houston, who lent his support and encouragement throughout the course of generating and characterizing FSHβ null and various other transgenic lines of mice. I also thank all my USA and foreign collaborators, particularly, Mone Zaidi, Mount Sinai School of Medicine, for convincing me that FSH could have extra-gonadal actions and for many thought-provoking telephone discussions on estrogen and FSH actions on bone biology. My thanks are also due to Dr. Huizhen Wang, Mr. Phillip Stevenson my colleagues at the University of Kansas Medical Center, for their dedication to the studies in my laboratory with FSHβ null mice that received support from the NIH (HD043945; RR024214), and The Hall Family Foundation, Kansas City, MO.

References

1. T.R. Kumar, What have we learned about gonadotropin function from gonadotropin subunit and receptor knockout mice? Reproduction **130**, 293–302 (2005)
2. T.R. Kumar, Gonadotropin gene targeting and biological implications. Endocrine **26**, 227–233 (2005)
3. T.R. Kumar, Mouse models for gonadotropins: a 15-year Saga. Mol. Cell. Endocrinol. **260–262**, 249–254 (2007)
4. N.R. Moudgal, M.R. Sairam, Is there a true requirement for follicle stimulating hormone in promoting spermatogenesis and fertility in primates? Hum. Reprod. **13**, 916–919 (1998)
5. B.E. Greer, J.S. Berek, *Gynecologic oncology: treatment rationale and technique* (Elsevier Publishing, New York, 1991)
6. T.R. Kumar, L.A. Donehower, A. Bradley, M.M. Matzuk, Transgenic mouse models for tumour-suppressor genes. J. Intern. Med. **238**, 233–238 (1995)
7. M.M. Matzuk, M.J. Finegold, J.G. Su, A.J. Hsueh, A. Bradley, Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. Nature **360**, 313–319 (1992)
8. T.R. Kumar, Y. Wang, N. Lu, M.M. Matzuk, Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat. Genet. **15**, 201–204 (1997)
9. T.R. Kumar, M.J. Low, Gonadal steroid hormone regulation of human and mouse follicle stimulating hormone beta-subunit gene expression in vivo. Mol. Endocrinol. **7**, 898–906 (1993)
10. S.D. Gharib, A. Roy, M.E. Wierman, W.W. Chin, Isolation and characterization of the gene encoding the beta-subunit of rat follicle-stimulating hormone. DNA **8**, 339–349 (1989)
11. J.L. Jameson, C.B. Becker, C.M. Lindell, J.F. Habener, Human follicle-stimulating hormone beta-subunit gene encodes multiple messenger ribonucleic acids. Mol. Endocrinol. **2**, 806–815 (1988)
12. T.R. Kumar, M. Kelly, M. Mortrud, M.J. Low, M.M. Matzuk, Cloning of the mouse gonadotropin beta-subunit-encoding genes, I. Structure of the follicle-stimulating hormone beta-subunit-encoding gene. Gene **166**, 333–334 (1995)
13. J.S. Tapanainen, K. Aittomaki, J. Min, T. Vaskivuo, I.T. Huhtaniemi, Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat. Genet. **15**, 205–206 (1997)
14. G. Meduri, A. Bachelot, M.P. Cocca, C. Vasseur, P. Rodien, F. Kuttenn, P. Touraine, M. Misrahi, Molecular pathology of the FSH receptor: new insights into FSH physiology. Mol. Cell. Endocrinol. **282**, 130–142 (2008)
15. A.M. Matsumoto, A.E. Karpas, W.J. Bremner, Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. J. Clin. Endocrinol. Metab. **62**, 1184–1192 (1986)
16. N.R. Moudgal, M.R. Sairam, H.N. Krishnamurthy, S. Sridhar, H. Krishnamurthy, H. Khan, Immunization of male bonnet monkeys (*M. radiata*) with a recombinant FSH receptor preparation affects testicular function and fertility. Endocrinology **38**, 3065–3068 (1997)
17. J. Singh, D.J. Handelsman, Neonatal administration of FSH increases Sertoli cell numbers and spermatogenesis in gonadotropin-deficient (hpg) mice. J. Endocrinol. **151**, 37–48 (1996)
18. M. Gossen, H. Bujard, Studying gene function in eukaryotes by conditional gene inactivation. Annu. Rev. Genet. **36**, 153–173 (2002)
19. S. Lamartina, G. Roscilli, C.D. Rinaudo, E. Sporeno, L. Silvi, W. Hillen, H. Bujard, R. Cortese, G. Ciliberto, C. Toniatti, Stringent control of gene expression in vivo by using novel doxycycline-dependent trans-activators. Hum. Gene Ther. **13**, 199–210 (2002)
20. K. Schonig, F. Schwenk, K. Rajewsky, H. Bujard, Stringent doxycycline dependent control of CRE recombinase in vivo. Nucleic Acids Res. **30**, e134 (2002)
21. E. Vigna, S. Cavalieri, L. Ailles, M. Geuna, R. Loew, H. Bujard, L. Naldini, Robust and efficient regulation of transgene expression in vivo by improved tetracycline-dependent lentiviral vectors. Mol. Ther. **5**, 252–261 (2002)
22. P.J. Baker, P. Pakarinen, I.T. Huhtaniemi, M.H. Abel, H.M. Charlton, T.R. Kumar, P.J. O'Shaughnessy, Failure of normal Leydig cell development in follicle-stimulating hormone (FSH) receptor-deficient mice, but not FSHbeta-deficient mice: role for constitutive FSH receptor activity. Endocrinology **144**, 138–145 (2003)
23. T.R. Kumar, G. Palapattu, P. Wang, T.K. Woodruff, I. Boime, M.C. Byrne, M.M. Matzuk, Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. Mol. Endocrinol. **13**, 851–865 (1999)
24. T.R. Kumar, V. Fairchild-Huntress, M.J. Low, Gonadotrope-specific expression of the human follicle-stimulating hormone beta-subunit gene in pituitaries of transgenic mice. Mol. Endocrinol. **6**, 81–90 (1992)

25. T.R. Kumar, M.J. Low, M.M. Matzuk, Genetic rescue of follicle-stimulating hormone beta-deficient mice. Endocrinology **139**, 3289–3295 (1998)
26. V. Garcia-Campayo, I. Boime, X. Ma, D. Daphna-Iken, T.R. Kumar, A single-chain tetradomain glycoprotein hormone analog elicits multiple hormone activities in vivo. Biol. Reprod. **72**, 301–308 (2005)
27. A. Jablonka-Shariff, T.R. Kumar, J. Eklund, A. Comstock, I. Boime, Single-chain, triple-domain gonadotropin analogs with disulfide bond mutations in the alpha-subunit elicit dual follitropin and lutropin activities in vivo. Mol. Endocrinol. **20**, 1437–1446 (2006)
28. N.G. Wreford, T. Rajendra Kumar, M.M. Matzuk, D.M. de Kretser, Analysis of the testicular phenotype of the follicle-stimulating hormone beta-subunit knockout and the activin type II receptor knockout mice by stereological analysis. Endocrinology **142**, 2916–2920 (2001)
29. H. Johnston, P.J. Baker, M. Abel, H.M. Charlton, G. Jackson, L. Fleming, T.R. Kumar, P.J. O'Shaughnessy, Regulation of Sertoli cell number and activity by follicle-stimulating hormone and androgen during postnatal development in the mouse. Endocrinology **145**, 318–329 (2004)
30. C. Lukas-Croisier, C. Lasala, J. Nicaud, P. Bedecarras, T.R. Kumar, M. Dutertre, M.M. Matzuk, J.Y. Picard, N. Josso, R. Rey, Follicle-stimulating hormone increases testicular anti-Mullerian hormone (AMH) production through sertoli cell proliferation and a nonclassical cyclic adenosine 5′-monophosphate-mediated activation of the AMH Gene. Mol. Endocrinol. **17**, 550–561 (2003)
31. G. Shetty, C.C. Weng, K.L. Porter, Z. Zhang, P. Pakarinen, T.R. Kumar, M.L. Meistrich, Spermatogonial differentiation in juvenile spermatogonial depletion (jsd) mice with androgen receptor or follicle-stimulating hormone mutations. Endocrinology **147**, 3563–3570 (2006)
32. A.L. Durlinger, M.J. Gruijters, P. Kramer, B. Karels, T.R. Kumar, M.M. Matzuk, U.M. Rose, F.H. de Jong, J.T. Uilenbroek, J.A. Grootegoed, A.P. Themmen, Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology **142**, 4891–4899 (2001)
33. J. Zhou, T.R. Kumar, M.M. Matzuk, C. Bondy, Insulin-like growth factor I regulates gonadotropin responsiveness in the murine ovary. Mol. Endocrinol. **11**, 1924–1933 (1997)
34. C.M. Combelles, M.J. Carabatsos, T.R. Kumar, M.M. Matzuk, D.F. Albertini, Hormonal control of somatic cell oocyte interactions during ovarian follicle development. Mol. Reprod. Dev. **69**, 347–355 (2004)
35. L. Sun, Y. Peng, A.C. Sharrow, J. Iqbal, Z. Zhang, D.J. Papachristou, S. Zaidi, L.L. Zhu, B.B. Yaroslavskiy, H. Zhou, A. Zallone, M.R. Sairam, T.R. Kumar, W. Bo, J. Braun, L. Cardoso-Landa, M.B. Schaffler, B.S. Moonga, H.C. Blair, M. Zaidi, FSH directly regulates bone mass. Cell **125**, 247–260 (2006)
36. M.J. Seibel, C.R. Dunstan, H. Zhou, C.M. Allan, D.J. Handelsman, Sex steroids, not FSH, influence bone mass. Cell **127**, 1079 (2006). author reply 1080–1071
37. N. Danilovich, M.R. Sairam, Targeting gonadotropin receptor genes: reproductive biology, aging, and related health implications. Endocrine **26**, 219–226 (2005)
38. A. Dierich, M.R. Sairam, L. Monaco, G.M. Fimia, A. Gansmuller, M. LeMeur, P. Sassone-Corsi, Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc. Natl Acad. Sci. USA **95**, 13612–13617 (1998)
39. M.H. Abel, I. Huhtaniemi, P. Pakarinen, T.R. Kumar, H.M. Charlton, Age-related uterine and ovarian hypertrophy in FSH receptor knockout and FSHbeta subunit knockout mice. Reproduction **125**, 165–173 (2003)
40. C.V. Rao, An overview of the past, present, and future of nongonadal LH/hCG actions in reproductive biology and medicine. Semin. Reprod. Med. **19**, 7–17 (2001)
41. C.V. Rao, Z.M. Lei, The past, present and future of nongonadal LH/hCG actions in reproductive biology and medicine. Mol. Cell. Endocrinol. **269**, 2–8 (2007)