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Mouse models of altered gonadotrophin action: insight into male reproductive disorders

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Abstract

The advent of technologies to genetically manipulate the mouse genome has revolutionised research approaches, providing a unique platform to study the causality of reproductive disorders in vivo. With the relative ease of generating genetically modified mouse models, the last two decades have yielded multiple loss-of-function and gain-of-function mutation mouse models to explore the role of gonadotrophins and their receptors in reproductive pathologies. This work has provided key insights into the molecular mechanisms underlying reproductive disorders with altered gonadotrophin action, revealing the fundamental roles of these pituitary hormones and their receptors in the hypothalamic-pituitary-gonadal axis. This review will describe genetically modified mouse models of gonadotrophins and their receptors with enhanced or diminished actions, specifically focussing on the male. We will discuss the mechanistic insights gained from these models into male reproductive disorders, and discuss the relationship and understanding provided into male human reproductive disorders originating from altered gonadotrophin action.

1. Introduction

The precise control of the hypothalamic-pituitary-gonadal axis is essential for coordinating and maintaining reproductive functions. In response to the pulsatile release of hypothalamic gonadotrophin-releasing hormone (GnRH), the synthesis and secretion of the pituitary gonadotrophic hormones, luteinising hormone (LH) and follicle-stimulating hormone (FSH), modulates testicular function through the binding and activation of the gonadotrophin receptors, luteinising hormone/chorionic gonadotrophin receptor (LHCGR or LHR)[1] and FSH receptor (FSHR) respectively. The downstream activity of the gonadotrophin receptors is critical for initiation and maintenance of gonadal steroidogenesis and for support, production and maturation of viable germ cells. Our understanding of gonadotrophic hormone/gonadotrophin receptor biology has been greatly enhanced by the generation and study of genetically modified (GM) mouse models. The advent of GM mouse

[1] The luteinising hormone/chorionic gonadotrophin receptor, abbreviated as LHCGR or LHR, is the official gene, derived from the two endogenous ligands of LHR, LH and chorionic gonadotrophin (CG), in CG secreting species e.g., humans and horses.

models, with their relative ease in generation, coupled with short gestation time and life-cycle relative to larger mammalian species, have provided a powerful tool to study reproductive disorders. Moreover, the study of GM mouse models has provided key molecular insight into the causality and contributions of gonadotrophic hormones and their cognate receptors to human reproductive pathologies. A number of GM approaches have been taken to understand the molecular mechanisms governing reproductive pathologies; gain-of-function approaches have utilised the over-expression of gonadotrophins or the generation of constitutively activating mutations (CAM) of gonadotrophin receptors, while loss-of-function approaches have relied upon knock-out technology to remove/silence gonadotrophin receptor or gonadotrophin gene expression. This review will describe GM mouse models with direct genetic modifications in gonadotrophin subunits or gonadotrophin receptors. We will discuss the implications of these findings on male reproductive function, and the important insights these models provide into human health and disease.

2. GM models of altered gonadotrophin action

The functional role of the testis is two-fold; the production of male gametes and androgen support, primarily through testosterone secretion for local androgenic action, for stimulation and maintenance of spermatogenesis and extra-gonadal sexual and anabolic functions (McLachlan, et al. 1995, Sharpe, et al. 1994). In the postnatal mouse, the coordinated and temporal release of the gonadotrophins, LH and FSH, are required for the differentiation and maturation of the testis and extragonadal sex organs; LH is necessary for the production and secretion of testosterone via the Leydig cells, although minimal tonic testosterone production is observed in the absence of LH/LHR function, while FSH is responsible for the maintenance of spermatogenesis by stimulation and maintenance of a multitude of Sertoli cell functions.

2.1 Enhanced LH-LHR activity

To examine the effects of promiscuous LHR activation, our laboratory generated two transgenic mouse models with enhanced LH/human chorionic gonadotrophin (hCG) action. The first model generated expressed the hCGβ subunit under the human ubiquitin C promoter, allowing ubiquitous,

persistent, and low-level expression of hCGβ from late gestation onwards (Rulli, et al. 2003). The rationale behind this was that when the transgene was co-expressed in pituitary gonadotroph and thyrotroph cells with the glycoprotein hormone common α-subunit (αGSU), bioactive heterodimers α/β hCG would be produced. We termed this model hCGβ, and in males, it attained moderately 3-4-fold elevated levels of bioactive hCG compared to endogenous LH (Rulli, et al. 2003). hCGβ+ males were fertile with full spermatogenesis and normal sperm quality despite reduced testis size and serum FSH (Rulli, et al. 2003), echoing the phenotype observed in activating LHR mutations in humans. However, the onset of puberty was normal, with no evidence of precocious puberty, which is the hallmark of human males with enhanced LHR activation (Themmen and Huhtaniemi 2000). As modest elevation in LH/hCG action had no effect on fertility or the timing of puberty, we went on to test the effect of grossly elevated LH/hCG on these factors. To achieve this, we generated another mouse model expressing the αGSU, also under the human ubiquitin C promoter when crossed with the hCGβ+ mice, creating a double transgenic line (hCGαβ+), with a 1000-fold higher circulating concentration of bioactive LH/hCG observed than in wild-type (WT) mice (Rulli, et al. 2003). hCGαβ+ males were infertile, despite exhibiting comparable spermatogenesis as evidenced by histological analysis of testis and caudal epididymal sperm motility and morphology to hCGβ+ and WT littermates. Infertility appeared to be mechanical and/or behavioural in origin, with hCGαβ+ males displaying extremely aggressive behaviour, often resulting in severe injury or death of WT females housed with the males, and mating ability impaired as evidenced by the lack of vaginal plugs during breeding studies. Testes size was smaller with enlarged seminal vesicles and prostate, dilated vasa deferentia and bladder, as well as kidney defects in adulthood (Rulli, et al. 2003). Testicular steroidogenesis was also enhanced, despite a near total down-regulation of cell surface LHR expression, echoing studies showing that less than 0.1% occupation of LHR is required for full testicular steroidogenesis (Mendelson, et al. 1975). As with hCGβ+ males, precocious puberty was not detected in hCGαβ+ males, despite highly elevated serum testosterone with the timing of the balano-preputial separation and onset of spermatogenesis indistinguishable from WT males (Ahtiainen, et al. 2005). Interestingly, juvenile hCGαβ+ males developed Leydig cell adenomas, reaching their maximum size at 10 days postpartum but disappearing by puberty, coinciding with the normal regression pattern of fetal Leydig cells. The gene expression of fetal and adult Leydig cell markers suggested that the adenomas originated from

the fetal Leydig cell population, providing evidence that the adult Leydig cells may be resistant to developing gonadotrophin-induced adenomas (Ahtiainen, et al. 2005).

Recent studies of the hCGαβ+ animals, have revealed that the hypothalamic function of prepubertal males was altered, displaying accelerated GnRH pulse frequency and increased GnRH content of GnRH neurons, coupled to decreased pituitary expression of GnRH receptor (Gonzalez, et al. 2011). A profound and persistent malfunction of the neuroendocrine feedback control of the gonadotrophin axis was evidenced, with FSH levels persistently low throughout life and unresponsive to castration or the anti-androgen flutamide both pre and postpubertally, but with re-establishment by blockade of perinatal androgen action (Gonzalez, et al. 2011). These findings suggest that androgen excess, during a critical window between gestational day 18 and postnatal day 14, is able to disrupt the developmental programming of the male hypothalamic-pituitary-gonadal axis. A direct testosterone-dependent regulation of hypothalamic aromatase expression was also demonstrated, indicating that locally produced oestrogens might play a key role in the hypothalamic–pituitary phenotype of hCGαβ+ mice.

Additional GM models to test the effects of elevated hCG or LH have also been utilised by others. A transgenic model over-expressing hCGβ expressed under the metallothionein (MT-1) promoter did not show elevated circulating dimeric hCG nor obvious changes in testicular phenotype, yet MT-1-hCGβ males were infertile, speculated to be due to free circulating hCGβ subunit binding to LHR and competing with endogenous LH for receptor occupancy (Matzuk, et al. 2003). Co-expression of MT-1-hCGα and hCGβ subunits was conducted, to form the active hCG heterodimer. Male mice with low expression of MT-1-hCGαβ were initially fertile and indistinguishable from WT littermates. However, by 6-7 months, these mice were progressively infertile but no histological abnormalities were observed or obvious phenotypic explanation to indicate the cause of infertility. Male mice with high expression of the MT-1-hCGαβ transgenes, as with ubiquitin C-expressed hCGαβ+ male mice, were infertile, the origin of which appearing to be through disrupted mating behaviour as evidenced by lack of vaginal plugs when housed with either super-ovulated or naturally cycling female mice. Male mice

were also noted to be aggressive when caged with other male or female mice, and displayed altered sexual behaviour. Serum testosterone was highly elevated, and circulating gonadotrophins decreased. Testis size was reduced, and histological analyses showed Leydig cell hyperplasia and in some tubules sertoli-cell only like syndrome, with germ cell loss, echoing observation of LHR over-activity in humans. A transgenic model for elevated LH consisting of a fusion protein of the bovine LHβ subunit and the hCGβ C-terminal peptide (bLHβ-CTP) under the common α- subunit promoter has also been studied. However this model failed to produce sufficiently elevated LH/hCG bioactivity in male animals, as they presented with no apparent phenotype (Risma, et al. 1995).

To constitutively activate LHR, a novel transgenic approach using covalently linked hCGβ and αGSU to reconstitute heterodimeric hCG, fused to rat LHR expressed under inhibin-α subunit promoter, termed 'yoked' LHR (YHR), was utilised (Meehan, et al. 2005, Meehan and Narayan 2007). In pre-pubertal males, enhanced LH/LHR action was observed, with increased circulating testosterone and seminal vesicle weights, probably due to the early expression of the transgene driven by the inhibin-α promoter. However, despite this elevation in testosterone, as with the hCGβ+ and hCGαβ+ animals, the timing of puberty was normal. Post-puberty, there was a trend for enhanced LHR action, with decreased seminal vesicle weights and reduced testis size due to a decrease in seminiferous tubule volume. However, normal spermatogenesis was noted. As with hCGβ+ animals, serum FSH was supressed in both pre- and postpubertal animals, however LH was only suppressed in pre-pubertal animals. This defect, may be the consequence of a dysregulation in hypothalamic-pituitary communication, and may reflect differences in the regulation of LH and FSH secretion.

To date, a single constitutively activating mutation (CAM) LHR mouse model has been described, the result of a knock-in D582G LHR mutation, the most commonly observed CAM mutation in human boys with familial male-limited precocious puberty (McGee and Narayan 2013). As with the human mutation, D582G LHR resulted in precocious puberty, with decreased testis weight and increased seminal vesicle weight at 3 weeks post-partum. Serum and intra-testicular testosterone were increased from day 7 post-partum; however serum FSH and LH remained below the limit of detection

throughout the tested life-span of the animals, due to steroid hormone feedback. Sertoli cell development was unaltered, however Leydig cell hyperplasia was observed, with enhanced expression of steroidogenic genes in most age groups tested. Although precious puberty was observed, spermatogenesis was not altered in these male mutants. Although initially fertile, progressive infertility was detected, but normal levels of epididymal sperm were noted, indicating a potential abnormality in seminal vesicle and prostate function and/or lower urinary tract, however detailed analysis of accessory gland function was not carried out.

2.2 Enhanced FSH-FSHR activity

GM mouse models with elevated FSH have been generated to explore enhanced ligand-dependent activation of FSHR. As with MT-hCGβ, and MT-hCGαβ, Kumar et al took the approach of overexpressing human αGSU, and the human FSHβ subunit under the MT-1 promoter. The MT-1-αGSU and MT-1-FSHβ transgenic mice were fertile. Inter-crossing of these transgenic mouse strains generated mice over-expressing dimeric FSH (MT-1-FSHαβ), with high levels of circulating FSH. Male mice were largely infertile, with just 1 in 10 animals producing 1 litter of pups in a 6 month period. Mating studies suggested a lack of mating activity in these animals. Testicular size and morphology was indistinguishable from WT, like wise epididymal weights were comparable. However, serum testosterone was elevated and seminal vesicles enlarged, due to increased androgenic action. Histological analysis of the testes showed little difference from WT, moreover, analysis of epididymal sperm numbers showed MT-1-FSHαβ animals to have increased sperm number, with no difference in motility or viability. These findings suggest that the infertility observed in MT-1-FSHαβ animals appears to result from behaviour changes rather than a direct impact on spermatogenesis. It is possible that the increase in testosterone resulted in altered and/or aberrant seminal vesicle secretions, or functional incompetence of the sperm.

Mouse models of enhanced FSHR activity have primarily utilised the *hpg* mouse model as a background in which to generate the mutations. The *hpg* mouse, resulting from a naturally occurring deletion mutation in *GnRH* (Cattanach, et al. 1977), with a phenotype of hypogonadotrophic hypogonadism, provides the advantage of testing the effects and direct contribution of FSH/FSHR-dependent testicular function, in the absence of circulating LH and activation of LHR. Using the rat androgen binding protein promoter for specific integration into Sertoli cells, Haywood et al created a transgenic line expressing the human Asp567Gly mutation FSHR CAM (TG-FSH+) (Haywood, et al. 2002). Testicular expression was confirmed, and enhanced ligand independent cAMP production detected in cultured TG-FSH+ Sertoli cells. In a WT background, testis weights and fertility were comparable between TG-FSH+ animals and WT littermates. However, in the *hpg* background, testis weights were significantly increased in comparison to *hpg* littermates, moreover, treatment with testosterone at equivalent levels to the maximum observed in *hpg* mouse testis, vastly increased testis size in *hpg* TG-FSH+ animals in comparison to *hpg* littermates. Histological analysis of the testes showed the presents of both round and elongated spermatids, and examination of Sertoli cell structure showed the maturation of this cell type. Although intra-testicular testosterone was increased in *hpg* TG-FSHR+ animals, serum testosterone was no different from *hpg* littermates. A similar phenotype was also observed in a transgenic model over-expressing complete FSH (αGSU and FSHβ subunits) in a WT or *hpg* mouse background (Allan, et al. 2001), showing that without LH-induced testosterone production, FSH/FSHR activity is sufficient for Sertoli cell maturation and can promote spermatogenesis to some extent. However, LH/LHR activity, and consequential testosterone production, is required for the completion of spermiogenesis.

In our laboratory, a knock-in constitutively activating mFshrD580H mouse model has been generated (Oduwole/Peltoketo et al, manuscript in preparation). Interestingly, despite this mutation having deleterious effects on female reproduction (Peltoketo, et al. 2010), male animals did not present with any obviously altered phenotype during embryogenesis, puberty or adulthood. The gross morphology and histology of the reproductive tract and testis appeared no different to WT littermates, showing that enhanced FSHR activity alone in the WT background, as opposed to *hpg* mice, had neither positive nor deleterious effects on male reproductive function.

215

216 *2.3 Diminished LH-LHR activity*

217 The first GM approach exploring the effects of loss of function of gonadotrophins utilised deletion of
218 αGSU. Deletion of the αGSU gene in male mice showed normal pre-natal and pre-pubertal sexual
219 differentiation and gonadal development, confirming that pre-pubertal gonadal development in mice is
220 independent of gonadotrophin action (Kendall, et al. 1995). However, male animals, being also
221 hypothyroid, failed to undergo puberty, and exhibited a lack of sex steroid production. Post pubertal
222 animals lacked gonadal development and function, with diminished testis size and smaller
223 seminiferous tubules, and spermatogenesis blocked at the first meiotic division. The presence of vas
224 deferens and epididymis showed that the αGSU KO mice were able to produce sufficient testosterone
225 *in utero*. As the αGSU gene is an integral part of both heterodimeric thyroid stimulating hormone
226 (TSH), and FSH, it should be noted that phenotypic effects observed from deletion of αGSU are not
227 just the result of lacking LH action, but also TSH and FSH action. The mouse model demonstrated
228 that mice devoid of glycoprotein hormone production are viable, which is perhaps not unsurprising
229 given that mice do not express or secrete placental CG, and rather rely upon placental lactogens and
230 alternative hormonal support for maintenance of pregnancy, in contrast to humans in whom hCG is
231 vital.

232

233 To decipher the effects of deleting LHR, our laboratory took the approach of generating an LHR
234 knockout (LuRKO) mouse. As with the αGSU knockout mice, LHR deletion resulted in alterations of
235 the reproductive tract from the pubertal period onwards, exhibiting normal pre-pubertal development
236 (Zhang, et al. 2004). Elevated FSH and LH were observed, with a decrease in sex steroid
237 concentrations, due to lack of steroid feedback to the hypothalamic-pituitary axis (Pakarainen, et al.
238 2007). Adult LuRKO males were infertile with underdeveloped testes and hypoplastic accessory sex
239 organs. Testes were cryptorchid and significantly reduced in size, with narrow seminiferous tubules,
240 decreased number and size of Leydig cells and arrested spermatogenesis at the round spermatid
241 stage. The expression of Leydig cell specific genes, whilst similar at birth, became gradually low or
242 undetectable in adulthood. Accessory sex organs, including the prostate and seminal vesicles, were

undetectable (Lei, et al. 2001, Zhang, et al. 2001). A similar phenotype to the LuRKO mice was also observed with the deletion of LHβ, mimicking the reproductive phenotypes displayed in αGSU null male mice (Ma, et al. 2004), however knock-out LHβ males exhibited unaltered serum FSH, contrasting from the hypogonadotrophic and hypergonadotrophic phenotypes of αGSU and LuRKO male mice respectively.

An interesting difference that exists between human and mouse inactivating LHR mutations, is that normal pre-pubertal development is observed in male mice, however, in human counterparts, complete inactivation of LHR results in pseudohermaphroditism (Themmen and Huhtaniemi 2000). This indicates that LH action *in utero* is not a prerequisite for fetal Leydig cell androgen and insulin-like growth factor 3 (INSL3) production required for intrauterine testicular development and descent, and masculinization in male mice, highlighting the presence of additional safety mechanisms present for maintaining fetal Leydig cell function by a network of paracrine factors (El-Gehani, et al. 1998, Peltoketo, et al. 2011, Themmen and Huhtaniemi 2000).

Testosterone replacement therapy in LuRKO animals leads to partial reversal of the hypogonadal phenotype, with achievement of full spermatogenesis; however, male mice remained sub-fertile due to poor accessory gland development and poor sexual behaviour (Pakarainen, et al. 2005). Abnormalities such as vigorous inflammation of the epididymis and the prostate were conspicuous in a proportion of the testosterone-treated mice. The incidence of low ejaculatory frequency and low sperm count in cauda epididymis were also observed. Whether testosterone replacement, or lack of sufficient androgen priming prepubertally prior to testosterone replacement, is responsible for these abnormalities, is not however clear. A striking physiological finding in the LuRKO mice is a late onset recovery of qualitatively full spermatogenesis around 12 months of age, when the passage of round spermatids to elongated spermatids can be found. This suggests that spermatogenesis can proceed qualitatively to completion with support of the basal LH-independent low intra-testicular testosterone present in the LuRKO testis (Zhang, et al. 2003), though a much higher threshold of testosterone may be required to induce qualitatively and quantitatively full spermatogenesis (Huhtaniemi, et al. 2006).

This finding was confirmed and extended in our recent study (Oduwole, et al. 2014), observing that a narrow margin separated the testosterone doses that activated peripheral male sexual androgen action and spermatogenesis. When extrapolated to humans, this may jeopardize the current approach to hormonal male contraception, as it will be practically impossible to define a single dose of testosterone that can suppress gonadotrophins and attain azoospermia. It is only a total abolition of intra-testicular testosterone action therefore, that can bring about total and complete suppression of spermatogenesis.

2.4 Diminished FSH-FSHR activity

Targeted ablation of bioactive FSH was achieved through deletion of exons 1, 2 and partial deletion of exon 3 of FSHβ (Kumar, et al. 1997). Phenotypic examination of FSHβ KO males showed reduced testis size, with decreased seminiferous tubule diameter and volume. However, Leydig cell populations were unaffected, and speculated qualitatively to be enhanced in number, however due to the reduced testis size, net Leydig cell number probably did not differ from WT littermates. Accessory sex glands were of comparable size to age-matched litter mates, consistent with comparable circulating serum testosterone and adequate Leydig cell number and function. Epididymal sperm were decreased by 75% in comparison to heterozygous and WT littermates, with motility decreased by 40%, however no difference in viability was observed. Despite this, FSHβ KO animals were fertile, with normal serum LH, probably reflecting negative feedback from circulating testosterone. The maintenance of spermatogenesis and Sertoli cell function in the absence of FSH-activated FSHR is suggestive of potential testicular or extra-testicular paracrine factors that can compensate for FSH function in the testis, or that basal constitutive, ligand-independent FSHR activity is sufficient to maintain tonic testis function and spermatogenesis in male mice.

The generation of FSHR knockout mice (FORKO) provided additional insight into the dependence of spermatogenesis on FSH (Abel, et al. 2000, Dierich, et al. 1998). As with FSHβ null males, FORKO males were fertile, with reduced testis size and decreased spermatogenesis. To examine key

differences in these models, a study was conducted to directly compare the phenotypes observed of FORKO and FSHβ GM mice (Baker, et al. 2003). Comparison of serum and intra-testicular testosterone showed a reduced level of circulating testosterone in FORKO animals that was not observed in FSHβ mouse model; yet both models exhibited diminished intra-testicular testosterone, indicating that local production of testosterone was impaired in both FORKO and FSHβ mice. Serum LH was elevated in FORKO animals, but not FSHβ animals. Interestingly, Leydig cell specific steroidogenic genes such as P450scc were diminished in the FORKO model, with decreased Leydig cell number to approximately 60% of control, suggesting a potential failure of Leydig cell proliferation and/or differentiation at puberty in FORKO animals that was not observed in FSHβKO animals. This effect is likely to be reflective of the decreased Leydig cell number observed in these animals and represents a key difference between these animal models. As both models were fertile, these studies revealed that FSH action is not critical for maintenance of fetal Leydig cells, as shortly after birth, when the maintenance of these cells is critically dependent on gonadotrophin action. As FSHR is expressed solely in Sertoli cells, the action of FSHR on Leydig cell development must be via Sertoli cell secreted paracrine factors. Previously studies have implicated factors such as desert hedgehog and PDGF; however, to date nothing has conclusively been described to be the key factor(s) mediating these paracrine effects. It is likely that FSHR action mediates and ensures sufficient Sertoli cell activity for output of such trophic factors, and why spermatogenesis is impaired when either FSHβ or FSHR action is abrogated. Whereas FSHβ and FSHR KO male mice are fertile, there is some discrepancy in humans on the phenotype of men with inactivated FSH function. The three men described with inactivating FSHβ mutations are all azoospermic (Layman, et al. 2002, Lindstedt, et al. 1998, Phillip, et al. 1998), whereas the 5 men with inactivating FSHR mutations have oligozoospermia of variable severity (Tapanainen, et al. 1997). This discrepancy can be resolved only through detection of new cases of these extremely rare mutations.

3. Conclusions and perspectives

The precise and coordinated control of gonadotrophin actions is crucial for the maintenance of male reproductive functions. Modifications in these functions can result in impaired fertility, with chronic dysregulation of gonadotrophin action often resulting in sub- or infertility. Our understanding of the

328 molecular mechanisms underlying human reproductive pathologies resulting from dysregulation of
329 gonadotrophin action has been greatly enhanced by the generation and study of GM mouse models.
330 The use of loss-of-function and gain-of-function models enables us to probe both modest and chronic
331 changes in gonadotrophin secretion and gonadotrophin receptor activity, providing key detail in the
332 developmental programming of males. These models identify how fundamental temporal control of
333 the hypothalamic-pituitary-gonadal axis co-ordinates the development and function of the Sertoli and
334 Leydig cells, necessary for the production and maintenance of full spermatogenesis.

335

336 Comparative analysis of human and mouse reproductive pathologies shows us that Sertoli and Leydig
337 cell function is highly sensitive to changes in gonadotrophin action, particularly LH/LHR. Clinical
338 pathologies of enhanced LH action result in precocious puberty and Leydig cell hyperplasia, however
339 normal fertility is usually maintained in humans, (Themmen and Huhtaniemi 2000), as observed with
340 the CAM LHR mouse model (McGee and Narayan 2013). Many activating mutations of the LHR
341 resulting in male reproductive pathologies have been identified, with the hotspots for activating
342 mutations primarily localised to the G-protein coupling region of the receptor (Simoni, et al. 1998). It is
343 interesting to note the disparity between GM models with constitutively active LHR and increased
344 circulating LH/hCG in the timing of puberty. Precocious puberty was not observed in male mice with
345 increased circulating LH/hCG despite the pre-pubertal increase in testosterone observed in many of
346 the GM models discussed. This may reflect differences in the regulatory and membrane trafficking
347 mechanisms controlling the expression and activity of WT and constitutively active LHR. Indeed, in
348 hCG$\alpha\beta$ mice, the WT LHR was subject to chronic down-regulation, whilst the constitutively active LHR
349 may not be subject to such control. Unsurprisingly, only few activating mutations of FSHR have been
350 identified in humans, probably due to the relatively benign phenotype observed (Casas-Gonzalez, et
351 al. 2012, Gromoll, et al. 1996). Human males are fertile, mimicking the CAM FSHR mouse models
352 described.

353

354 Although there are many similarities between human reproductive pathologies originating from the
355 dysfunction of gonadotrophin/gonadotrophin receptor, and mouse models of the same origin, it should
356 be noted exceptions do exist and exact phenocopies of observed dysfunctions are not always

observed between these species. Of notable difference are the mechanisms of prenatal and prepubertal development and the relative importance and contributions of gonadotrophin/gonadotrophin receptor function, particularly LH/LHR, to testicular development in these processes. That said, GM mouse models have been excellent tools for dissecting the molecular mechanisms underlying reproductive pathologies, underpinning many research efforts to understand the physiology of the function of gonadotrophins and their receptors.

With the ever growing sophistication in GM approaches, allowing similar point mutations with human genetic diseases, and more targeted spatial and temporal integration, replacement or deletion. With the coming of age of BAC transgenics, the use of mouse models provides new exciting opportunities to understand the mechanisms underlying reproductive pathologies. Whether mouse models can be used to test small molecule activators, inhibitors, or pharmacochaperones of gonadotrophin receptor function is yet to be investigated. However, in vivo proof of concept studies with pharmacochaperone of the LHR (Newton, et al. 2011) and of the GnRHR (Janovick, et al. 2013) presents exciting opportunities and future directions in drug design, with the use of in vivo models providing important hypothesis testing tools for researchers for many years to come.

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Declaration of Interest

All authors have nothing to declare.

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