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Correspondence:
Sandro La Vignera, Department of Clinical and Experimental Medicine, University of Catania, Policlinico 'G. Rodolico', via S. Sofia 78, 95123 Catania, Italy.
E-mail: sandrolavignera@unict.it

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Effects of the insulin-like growth factor system on testicular differentiation and function: a review of the literature

R. Cannarella, R. A. Condorelli, S. La Vignera and A. E. Calogero

Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

SUMMARY

We recently described the occurrence of cryptorchidism, oligoasthenoteratozoospermia, and genital abnormalities in patients with distal 15q chromosome structural abnormalities. This observation brought us to hypothesize that insulin-like growth factor (IGF) receptor (*IGF1R*), mapping on the 15q 26.3 chromosomal band, may be involved in testicular function. To further evaluate this topic, we reviewed in vitro and in vivo studies exploring the role of the IGF system [IGF1, IGF2, IGF1R, insulin receptor substrates (IRS)] at the testicular level both in animals and in humans. In animals, IGF1/IGF1R has been found to be involved in testicular development during embryogenesis, in Sertoli cell (SC) proliferation, and in germ cell (GS) proliferation and differentiation. Interestingly, IGF1R seems to mediate follicle-stimulating hormone (FSH) effects through the PI3K/AKT pathway. In humans, IGF1 directly increases testicular volume. The molecular pathways responsible for testicular differentiation and IGF1/IGF1R signaling are highly conserved among species; therefore, the IGF system may be involved in FSH signaling also in humans. We suggest a possible molecular pathway occurring in human SCs, which involves both IGF1 and FSH through the PI3K/AKT pathway. The acknowledgment of an IGF1 mediation of the FSH-induced effects may open new ways for a targeted therapy in idiopathic non-FSH-responder oligoasthenoteratozoospermia.

INTRODUCTION

Infertility affects 15% of couples in Western countries and, among these, about 50% of cases are due to a male factor (Asero *et al.*, 2014). It has been estimated that a genetic abnormality is responsible for infertility in 15% of infertile patients (Lee *et al.*, 2011). Although several specific genetic causes of male infertility have been acknowledged (e.g., quantitative and qualitative chromosome abnormalities, AZF microdeletions in the Y chromosome, congenital bilateral agenesia of vas deferens [CBAVD]) (Asero *et al.*, 2014), the etiology of many forms of male infertility remains unknown (Dohle *et al.*, 2002).

Thus, in recent years, next-generation sequencing (NGS) analysis has been performed in testicular tissues and blood from infertile patients (D'Aurora *et al.*, 2015; Dong *et al.*, 2015; Nakamura *et al.*, 2017), providing a huge amount of data relative to the differential expression of genes that might be involved in the etiology of idiopathic oligozoospermia and/or azoospermia. However, very often such genes are involved in many different pathways resulting in an increasing complexity of the molecular model that might explain some idiopathic cases of male infertility.

We recently analyzed the differential insulin-like growth factor 1 receptor gene (*IGF1R)* expression in three patients carrying 15q chromosome structural abnormalities. After reviewing the literature, we found that cryptorchidism and other gonadal abnormalities often occur both in patients with 15q chromosome duplications and in those with ring chromosome 15 syndrome. Hence, we hypothesized that *IGF1R*, which maps on the 26.3 15q chromosomal band, may play a role in the onset of such features (Cannarella *et al.*, 2017). To further evaluate this topic, we focused our attention on the effects that the insulin-like growth factor (IGF) system (mainly IGF1, IGF2, IGF1 receptor [IGF1R], insulin receptor substrates [IRS]) plays on testicular development and function. To accomplish this, we reviewed all in vitro and in vivo studies carried out on different animal species and in humans. The understanding of the specific role that

this system may display in human infertility, if any, might open new strategies for future targeted therapy.

EVIDENCE IN ANIMAL MODELS: IN VITRO AND IN VIVO STUDIES

Data from the animal model attribute to the IGF system a role in testicular differentiation during embryogenesis, in reaching a normal testicular volume, in the proliferation and differentiation of Sertoli cells (SCs) and germ cells (GCs). Interestingly, IGF1R seems to mediate follicle-stimulating hormone (FSH) actions. These effects are summarized in Table 1.

Effects on testicular development

In recent years, increasing knowledge suggests that the insulin receptor tyrosine kinase family (mainly *Igf1r,* insulin receptor [*Insr*], and insulin-related receptor [*Insrr*]) is important for testicular development and sex determination (Nef *et al.*, 2003). In mice, during embryogenesis, at the embryonic day (E) 10.5, primordial germ cells and steroidogenic factor 1 (*Nr5a1*)-positive somatic cells are usually present in the bipotential gonads. In XY mice, *Sry* upregulation and *Sf1* upregulation induce *Sox*9 upregulation and, consequently, cell precursors' differentiation into SCs (Malki *et al.*, 2005; Wilhelm *et al.*, 2005; Sekido & Lovell-Badge, 2008, 2009). This latter orchestrate the differentiation of all testis-specific cell types. In XX gonads, the absence of *Sry* expression induces the activation of a female-specific differentiation program, leading to ovarian determination (Clark *et al.*, 2000; Yao & Capel, 2002; Yao *et al.*, 2002; Brennan *et al.*, 2003; Callier *et al.*, 2014; Rebourcet *et al.*, 2014).

The *Igf1r*, *Insr,* and *Insrr* triple knockout (*Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$) in XY and XX mice resulted in a failure of testicular differentiation of XY triple mutant gonads, while the XX ones appeared undistinguishable from XX wild-type gonads. At E17.5, *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$ XY gonads were histologically similar to XX triple mutant ones. In situ hybridization analysis showed the occurrence of female-specific markers (*Wnt4* and *Figla*) in both XX and XY *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$ gonads, and the absence of male-specific ones (*Cyp11a1*, *Insl3*, *Dhh*, *Amh,* and *Sox9*). Because of the failure in testicular determination, genital ducts in XY mice resembled the XX structure and molecular profile, consistent with the absence of SCs and Leydig cells. Furthermore, *Sry* was downregulated in *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$ XY mice, suggesting that the insulin family signaling functions upstream to *Sry*. Interestingly, sex-reversed phenotype was observed only when both *Igf1r* alleles were mutated (it was observed in *Igf1r*$^{+/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$ and in *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{+/+}$ XY mice), suggesting that the overall contribution of *Igf1r* is greater than that of *Insr* or *Insrr* (Nef *et al.*, 2003). In addition, *Igf1r* and *Insr* double knockout mice resembled the *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ *Insrr*$^{-/-}$ mice phenotype, indicating that *Insrr* is dispensable for testicular differentiation (Pitetti *et al.*, 2013a). Moreover, independently from the genetic sex, gonads from *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ mice remained in an undifferentiated state for a longer time compared to wild-type gonads (in GCs, the upregulation of the meiotic protein synaptonemal complex 3, SCP3, occurs at E13.5 in wild-type and at E16.5 in *Igf1r*$^{-/-}$ *Insr*$^{-/-}$ gonads), showing a delay in carrying out the molecular differentiation program (Pitetti *et al.*, 2013a).

Molecular pathways responsible for testicular differentiation have an evolutionary conserved role among species (Capel, 2000). The insulin signaling pathways are present in *Caenorhabditis elegans*, *Drosophila*, amphibians, and higher vertebrates. Therefore, it might play role in testicular differentiation in many species (Nef *et al.*, 2003).

Effects on Sertoli and germ cell proliferation and differentiation

Several in vitro and in vivo studies performed in different animal species (such as mice, zebra fish, newt, *Caenorhabditis elegans*, *Drosophila melanogaster*) evaluated the role of IGF system on SC and GC proliferation and differentiation. Following, the evidence coming from non-mammalian and mammalian species is discussed.

Non-mammalian species

The IGF system seems to be effective in SCs and GCs from zebra fish. Indeed, IGF3 protein (a fish-specific member of the IGF family) has been detected in SCs and type A and type B spermatogonia. FSH exposure (which promotes type A and type B spermatogonia proliferation) showed to increase IGF3 transcript

Table 1 Effects of the insulin-like growth factor system on testicular development, Sertoli cell and germ cell proliferation and differentiation. In vitro and in vivo studies on animal models

Species	Results	References
Male newts (*Cynops pyrrhogaster*)	• IGF1 is expressed in SCs and in secondary spermatogonia • IGF1 directly stimulates GC proliferation and differentiation and SC proliferation • FSH promotes secondary spermatogonia differentiation through SC-derived IGF1	Nakayama *et al.* (1999) Yamamoto *et al.* (2001)
Zebra fish	• FSH promotes secondary spermatogonia proliferation and differentiation through the IGF1R signaling (via the cAMP/PKA route)	Nóbrega *et al.* (2015)
Caenorhabditis elegans	• Insulin- and IGF-like receptors are required for GC proliferation and act through a highly conserved PI3K/AKT-dependent pathway	Michaelson *et al.* (2010)
Drosophila melanogaster	• The insulin/IGF signaling pathway stimulates GC proliferation	McLeod *et al.* (2010)
Angus male cattle	• IGF1 is involved in the events that precede and initiate puberty	Lirón *et al.* (2012)
Mice	• INSR and IGF1R are required for testis determination and thus for male sexual differentiation • Insulin/IGF signaling regulates the final number of SCs, testicular volume, and sperm concentration • Insulin/IGF signaling is required for FSH-mediated SC proliferation through the PI3K/AKT pathway	Nef *et al.* (2003) Pitetti *et al.* (2013a,b) Griffeth *et al.* (2013) Escott *et al.* (2013) Castilla-Cortázar *et al.* (2015)

IGF1, insulin growth factor 1; SCs, Sertoli cells; GCs, germ cells; FSH, follicle-stimulating hormone; IGF1R, insulin growth factor receptor 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; cAMP, cyclic AMP; PKA, protein kinases A; INSR, insulin receptor.

testicular levels, but this effect was reduced in the presence of PKA and MAPK inhibitors. Moreover, the FSH-induced proliferation of type A spermatogonia was hindered by the incubation with an IGF3R inhibitor. Therefore, the FSH-induced production of IGF3 by SCs promotes spermatogonia proliferation and differentiation through the cAMP/PKA route in zebra fish (Nóbrega *et al.*, 2015).

In newt testis, IGF1 directly promotes SC and GC proliferation, spermatogonia differentiation into spermatocytes (Nakayama *et al.*, 1999). FSH has been found to increase IGF1R transcript expression in SCs and GCs, consistent with the hypothesis that FSH-induced GC differentiation is mediated by SC-derived IGF1 (Yamamoto *et al.*, 2001).

The IGF system is relevant for GC proliferation also in *Caenorhabditis elegans* (Michaelson *et al.*, 2010; Hubbard, 2011) whose genome encodes for a single insulin-/IGF-like receptor, called *daf-2*. Accordingly, *daf2* mutants showed a significantly reduced number of adult GC progenitors. The insulin-/IGF-like receptor cascade, which acts through a highly conserved PI3K/AKT-dependent pathway, was necessary within GCs to promote progression through the G2 phase in *Caenorhabditis elegans* (Hubbard, 2011).

In *Drosophila melanogaster,* insulin-like receptor is expressed in somatic testicular cells, GCs, and spermatogonia, indicating that all of these cell types are competent to directly respond to insulin (McLeod *et al.*, 2010). In this species, an in vitro study found that the activation of insulin/IGF pathway prevented GC loss during starvation (McLeod *et al.*, 2010).

Mammalian species

Constitutive *Igf1r* and *Insr* invalidation systematically leads to perinatal lethality. Some evidence come from mouse models with a selective GC and SC *Igf1r* and *Insr* knockout (respectively, GC-*Insr*;*Igf1r* and SC-*Insr*;*Igf1r* mice). In GC-*Insr*;*Igf1r* mice, adult testicular volume, testicular histology, and sperm production were not affected, suggesting that *Igf1r* and *Insr* are replaceable for GCs and spermatogenesis. In contrast, a 75% reduction in testicular size was observed in SC- *Insr*;*Igf1r* mice and histologic signs of immaturity were found in SCs. Furthermore, epididymal sperm concentration decreased by 38.9% and 58.7%, respectively, in SC-*Igf1r* and SC-*Insr*; *Igf1r* mice, and the SC number decreased by 74.3% in the latter. In addition, testicular sperm concentration decreased by 79% in SC-*Insr*; *Igf1r* mice, although the frequencies of parturition were not affected (Pitetti *et al.*, 2013b). To study the effects of FSH in this model, the authors further evaluated testicular volume and sperm concentration in hemicastrated SC-*Insr*; *Igf1r* and control mice. As expected, control animals showed an increased volume of the remaining testis and a compensatory increased sperm production due to the rise in FSH levels. Interestingly, in SC-*Insr*; *Igf1r* mice, weight, epididymal sperm count, and seminiferous tubule length of the remaining testis were not influenced by hemicastration (Pitetti *et al.*, 2013b). In addition, human recombinant FSH therapy, administered at concentrations known to promote SC proliferation and testicular growth in rats (Nurmio *et al.*, 2012), did not increase testicular size and sperm output in SC-*Insr*; *Igf1r* mice, differently from what was found in control animals. These data suggest that insulin/IGF1 signaling mediates FSH proliferative action on immature SCs (Pitetti *et al.*, 2013b).

Consistent with these results, *Irs2*$^{-/-}$ mice showed a 45% decreased testicular weight, fewer SCs, spermatogonia, spermatocytes, spermatids, and spermatozoa. Hence, IGF1 signaling and IRS2 play a critical role in testicular development (Griffeth *et al.*, 2013).

IGF1 seems also to impact on Leydig cell function. Indeed, *Igf1* deletion impairs Leydig cell development and proliferation (Hu *et al.*, 2010). Furthermore, these cells are able to secrete IGF1 in mice (Huang *et al.*, 2014) and the Leydig cell-derived IGF1 is involved in the maintenance of spermatogonial stem cells pluripotency (Huang *et al.*, 2009) and in their proliferation (Wang *et al.*, 2015).

The molecular pathway of IGF1R and INSR in SCs has been studied in Wistar rats. Through IGF1R, both insulin and IGF1 are able to promote membrane depolarization, calcium, glucose, and amino acid cellular uptake via the PI3K/AKT route (Escott *et al.*, 2013, 2014). The PI3K/AKT pathway is known to be activated by both IGFs and FSH (Khan *et al.*, 2002; Zhou *et al.*, 2013). In SC-*Insr*;*Igf1r* mice, the AKT signal was not observed in immature SCs, whereas it was found at high levels at post-natal day 5 in SC cytoplasm of control mice. Therefore, consistent with previous data (Gonzalez-Robayna *et al.*, 2000; Khan *et al.*, 2002; Meroni *et al.*, 2002), the PI3K/AKT signaling may mediate both the IGF1 and the FSH signal (Pitetti *et al.*, 2013b).

This signaling may occur also in GCs. In mice, IGF1 plays a role in GC proliferation via the IGF1R-PI3K/AKT-mTOR-HIF2α pathway under hypoxia (Huang *et al.*, 2014). In addition, in ovarian granulosa cells, the pathway through which FSH promotes GC proliferation has been found to involve IRS1, PI3K, and AKT (Hunzicker-Dunn *et al.*, 2012). As IRS1 is involved in the IGF1R cascade, IGF1 may enhance GC proliferation through the PI3K/AKT signaling.

Altogether, these evidences suggest that the IGF1/IGF1R system induces SC proliferation and GC proliferation and differentiation in different animal models. Importantly, IGF1, through the PI3K/AKT signaling, seems to be required for FSH action. The IGF1/IGF1R system appears to have an evolutionary conserved role in regulating GC proliferation (Nóbrega *et al.*, 2015), and therefore, a role in human SC and GC differentiation may be hypothesized.

EVIDENCE IN HUMANS

The IGF system seems to influence testicular development and function in humans. Indeed, during puberty, IGF1 serum levels have been found to increase concomitantly with increasing testicular volume (Juul *et al.*, 1994). Furthermore, growth hormone (GH) administration in boys with non-GH deficient (GHD) short stature and constitutional delay of puberty is effective in the normalization of testicular volume and sperm parameters (Radicioni *et al.*, 2007). Similar results were also observed in congenital isolated GHD (cIGHD) patients after GH therapy. In these cases, the final testicular volume resulted positively correlated with the duration of GH administration and negatively correlated with the patient's age at the beginning of GH treatment (Smuel *et al.*, 2015).

According to a previous study (Juul *et al.*, 1994), the effects of GH treatment on testicular volume may be ascribed to the rise in IGF1 levels. Consistent with this hypothesis, IGF1 treatment in males with primary GH resistance (Laron syndrome) increased

gonadotropin and testosterone serum levels, testicular volume, and penis size (Laron & Klinger, 1998).

Interestingly, cryptorchidism and genital abnormalities have often been observed in patients with 15q structural abnormalities involving the *IGF1R* locus (Cannarella *et al.*, 2017). Such findings indicate a possible role of the IGF1/IGF1R signaling in testicular development, descent, and volume, and in genital differentiation. Studies investigating the role of IGF system on human testicular development and function are summarized in Table 2.

The IGF system, particularly IGF2, may also influence sperm parameters, as emerging from epigenetic studies. *H19-IGF2* is one of the best-characterized imprinted genes: *H19* is maternally imprinted; *IGF2* is paternally imprinted, meaning that *H19* is expressed by the maternal allele, *IGF2* by the paternal one. Therefore, maternally unmethylated differentially methylated region (DMR) prevents *IGF2* from accessing the common enhancers, silencing *IGF2* expression. In contrast, paternal DMR allows *IGF2* expression and *H19* silencing (Tremblay *et al.*, 1997; Bell & Felsenfeld, 2000; Takai *et al.*, 2001). Methylation of imprinted genes begins in prospermatogonia, at the E14.5, and is completed at the stage of type A spermatogonium (Davis *et al.*, 2000; Ueda *et al.*, 2000). Curiously, paternally *H19* hypomethylation, probably leading to *H19* biallelic expression and *IGF2* downregulation, occurs in male germ line in association with oligozoospermia (Marques *et al.*, 2004, 2008; Kobayashi *et al.*, 2007), thus indicating that IGF2 may influence human spermatogenesis and final sperm concentration.

DISCUSSION

This review collects the evidence supporting the role of the IGF system on testicular differentiation, SC proliferation, and GC proliferation and differentiation. In addition, IGF1R seems to mediate the effects of FSH. Accordingly, studies in humans reveal the presence of a positive correlation between IGF1 serum levels and testicular volume.

Evidence from the ovarian tissue further supports the relevance of the IGF system on gonadal function and development. *Igf1r* expression is important for fertility, steroidogenesis, GC survival, and FSH actions in female mice (Baumgarten *et al.*, 2017). Indeed, selective *Igf1r* knockout in granulosa cells ($Igf1r^{gc,ko}$) resulted in the absence of oocytes, lower 17β-estradiol levels, GC apoptosis in $Igf1r^{gc,ko}$ mice compared to control animals (Baumgarten *et al.*, 2017). In addition, IGF1R inhibition prevented FSH-induced AKT phosphorylation and GC proliferation (Law & Hunzicker-Dunn, 2016; Baumgarten *et al.*, 2017), thus confirming that IGF1R mediates the effects of FSH through the PI3K/AKT pathway also in the female gonad.

Despite the great amount of data in experimental models, evidence in humans is scanty. This may relate to the lethality of *IGF1R* knockout and the ensuing difficulty in finding a human model of IGF1 deficiency, apart from the rare cases of Laron syndrome. As the molecular mechanisms responsible for testicular differentiation are highly conserved among species (Capel, 2000), a role of the IGF system in *SRY* upregulation and consequently normal testicular appearance cannot be excluded.

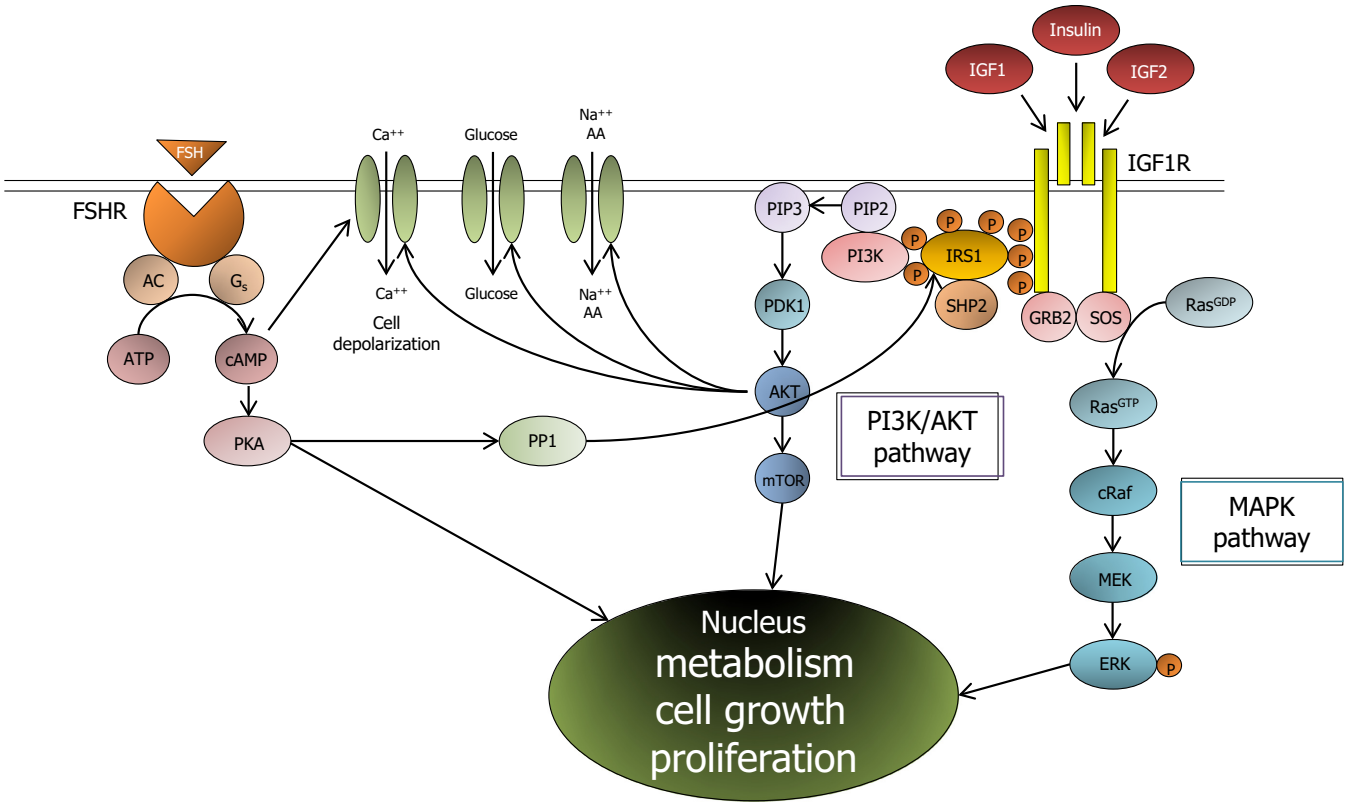
IGF1 replacement therapy has been already suggested. In great detail, clinical trials have been proposed to evaluate risks and benefit of IGF1 treatment in preterm infants, to avoid the consequences on brain, heart, and metabolism due to the preterm delivery (Hellström *et al.*, 2016). In murine models, IGF1 replacement therapy showed to restore the damage of blood–testis barrier and of testicular structure induced by IGF1

Table 2 Effects of the insulin-like growth factor system on human testicular development and function

Authors and year of publication	Study design	Results
Juul *et al.* (1994)	In 1030 healthy children, adolescent and adults (both males and females) the influence of IGF1 on age, stage of puberty, testicular volume, and BMI was studied.	IGF1 serum levels increased with the age in very early Tanner stage and decreased in late stages. IGF1 was positively correlated with testicular volume. It did not influence the BMI.
Laron & Klinger (1998)	The effects of IGF1 administration in seven men with primary GH resistance (Laron syndrome) (4 aged <5, two pre-pubertal 10- and 14-year-old boys, one 28-year-old fully sexually developed adult) were studied.	IGF1 administration (150 mcg/kg/die to the boys, 120 mcg/kg/die to the adult patient) increased hormone serum levels, testicular volume and penile size; they returned to pre-treatment values after stopping IGF1 administration. These effects were not observed at very young age.
Giagulli (1999)	Four azoospermic HH patients were treated with a 6-month-long Gn treatment, followed by a 6-month-long combined Gn-GH therapy.	Gn treatment alone induced a rise in T levels, a very little increase in testicular volume and azoospermia persisted. Combined Gn-GH therapy induced a further increase in testicular volume, but the patients remained azoospermic.
Radicioni *et al.* (2007)	Eight boys affected by non-GHD short stature and constitutional delay of puberty were treated with rhGH.	All patients showed normal testicular volume once the final pubertal stage was reached. Conventional sperm parameters normalized except for one patient.
Andreassen *et al.* (2014)	In 838 healthy young men, the possible influence of GHrd3 SNP (resulting in exon 3 deletion in the *GH receptor* gene) in semen quality and reproductive hormone levels was investigated.	GHrd3/d3 men had an upward trend for semen volume and higher serum inhibin B concentrations.
Smuel *et al.* (2015)	The effects of rhGH administration on growth, development, and puberty were evaluated in 41 patients with congenital isolated GH deficiency (cIGHD) (data were collected in 37/41 patients, 21 men and 16 females).	Puberty was delayed in boys, less in girls. The age at start of rhGH therapy and that of onset of puberty were positively correlated. Final testicular volume was positively correlated with the duration of rhGH therapy and a negatively correlated with between the age at the beginning of hGH treatment. All patients reached full sexual development, but penile size and testicular volume were below the normal values.

IGF1, insulin growth factor 1; BMI, body mass index; GH, growth hormone; HH, hypogonadotropic hypogonadism; Gn, gonadotropins; T, testosterone; GHD, growth hormone deficiency; rhGH, recombinant human GH; SNP, single nucleotide polymorphism; cIGHD, congenital isolated GHD.

Figure 1 Signaling pathway activated by the insulin-like growth factor (IGF) system and FSH in Sertoli cells. FSH binding to the FSH receptor induces adenylate cyclase (AC) activation and an intracellular raise in cAMP levels. cAMP opens Ca^{++} channels, leading to cellular depolarization. cAMP activates protein kinase A (PKA), which phosphorylates and activates downstream effectors, such CREB, a regulator of gene transcription, and protein phosphatase 1 (PP1). This latter dephosphorylates inhibitory Ser/Thr residues on insulin receptor substrate 1 (IRS1), sensitizing IRS1 to enhanced phosphorylation by IGF1 receptor (IGFR). IGF1R, in turn, activates MAPK and PI3K/AKT pathways, leading to calcium, glucose and amino acids uptake, cellular growth and proliferation.



deficiency (Castilla-Cortázar *et al.*, 2015). Further studies are needed to evaluate the role, if any, of the IGF system on testicular function in humans and whether it mediates the effects of FSH. This knowledge may help in understanding why FSH treatment is not effective in increasing sperm count in some circumstances. In such cases, infertile patients unresponsive to FSH may benefit by a contemporary rise of IGF1 (Radicioni *et al.*, 2007).

Studies on human testicular tissue are also useful to shed light on FSH and IGF1 signaling pathways. It is well known that FSH acts through cAMP as its receptor belongs to the G protein-coupled receptor family. However, FSH seems to activate also AKT signaling (Walker & Cheng, 2005). As PI3K/AKT pathway is involved in IGF1R signaling, according to evidence coming from the animal model, it might be hypothesized that, in humans, FSH-FSHR-cAMP signaling may induce SC IGF1 synthesis, which, in turn, through the AKT signal, may enhance FSH effects (Fig. 1). Studies on rat granulosa cells attribute to IRS1 the role of 'hub linking' between FSH signaling and PI3K. More in depth, in the presence of both FSH and IGF1, PKA activates protein phosphatase 1 (PP1) which dephosphorylates Ser/Thr residues on IRS1, thus inducing IRS1 phosphorylation by IGF1R. The phosphorylated IRS1 enhances PI3K/AKT cascade, which is responsible for synergic gene response to IGF1 and FSH (Law & Hunzicker-Dunn, 2016). Consistent with these results, in granulosa cells, the concomitant exposure to exogenous FSH and IGF1 was shown to induce IRS1 phosphorylation, leading to the transcription of common FSH- and IGF1-induced genes and to granulosa cell differentiation (Law *et al.*, 2017). Therefore, in vitro studies on human SCs and GCs are needed to show whether this pathway occurs also in men.

In conclusion, a great amount of data provide evidence for an essential role of IGF1/IGF1R in testicular development, SC and GC proliferation and differentiation in the animal model. This has been observed also in ovarian granulosa cells. Furthermore, FSH and IGF1 pathways are intimately connected, and IGF1R appears essential for FSH action in animals. In addition, IGF1 seems to influence testicular volume in humans. Therefore, the implications of IGF1/IGF1R in human fertility should be evaluated and studies investigating the possible relationship between IGF1 and sperm parameters need to be performed. Finally, a role for recombinant human IGF1 in infertile non-(or poor) FSH-responder patients might be proposed.

CONFLICT OF INTEREST

The authors declare no conflict of interests in this study.

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AUTHORS' CONTRIBUTIONS

Rossella Cannarella involved in conception and design of the study, data acquisition, analysis and interpretation of data,

drafting and writing of the article, and final approval of the manuscript; Rosita A. Condorelli involved in data acquisition, analysis and interpretation of data, and final approval of the manuscript; Sandro La Vignera involved in data acquisition, analysis and interpretation of data, and final approval of the manuscript; Aldo E. Calogero involved in conception and design of the study, analysis and interpretation of data, critical final revision of the manuscript, and final approval of the manuscript.

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