1. Introduction

The male gonad, the testis, is the unique site where male germ cells (sperm) are produced and grown. The vast majority of testicular space is dedicated to this mission, with seminiferous tubules enriched in spermatogenic cells, at different stages of maturation, and somatic cells (Sertoli cells) that support germinal cell growth and differentiation. When Enrico Sertoli originally described the Sertoli cells, he used the term ‘mother cells’, suggesting a unique role for these cells in germ cell maturation and development [1]. Sertoli cells are also involved in hormonal activity, producing several peptides, with both paracrine and endocrine functions, as is the case for the inhibin-related peptides [2-4]. In a minority of specialized mesenchymal cells, located in the interstitium between seminiferous tubules, there is the production of other hormones, sex steroids, which, locally, favor spermatogenesis and, systemically, target several tissues, essentially turning phenotype and behavior from male into men. These sex-steroid secreting specialized endocrine cells number ~ 500 million and are termed Leydig cells, after the German anatomist Franz Leydig, who discovered them in 1850 (see for review [2-4]).

In adult men, Leydig cells actively synthesize and release testosterone (T), the major circulating androgen, with an estimated production rate of 5 – 7 mg/day. In target tissues, T itself, or its potent metabolite dihydrotestosterone (DHT, produced after 5α-reduction), binds with high affinity (subnanomolar range) to the androgen receptor (AR). In addition, T and its precursor, Δ4 androstenedione, can be actively...
**Article highlights.**

- Lifestyle modifications and weight loss should be the first step in obese or overweight men with hypogonadism (HG).
- Restoring sexual activity might improve testosterone levels in mild form of HG.
- Gonadotropin therapy is the treatment of choice in men with secondary HG who require fertility.
- Antiestrogens might represent a successful treatment for some forms of secondary HG.
- Other potential options include: Stimulation of hypothalamic activity: kisspeptin and neurokinin-B agonists; Stimulation of Leydig cell steroid production, independently from gonadotropin stimulation: phosphodiesterase type 5 inhibitors.

transformed, through P450 aromatase, to other bioactive metabolites, such as estrone and 17β-estradiol (E2) (daily production about 45 µg), which activate estrogen receptors (ERα and ERβ) [2-4]. A small amount of E2 can also be synthesized within the testis and released in the peripheral circulation [2-4]. In the adult male, sex steroids have numerous biological functions (Figure 1), essentially devoted to stimulating/maintaining male characteristics and behaviors. They are essentially derived from the selective activation of the AR, by T or DHT, or by the activation of ERα or ERβ by E2, or a combination thereof, as in the case of the central nervous system (CNS), including hypothalamus and pituitary gland, or bone matter [2-4].

Spermatogenesis is a highly complex process, involving subtle and continuous interactions between paracrine and autocrine regulators – among which T is the most important – and the pituitary-derived gonadotropins (Gn): follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which mainly control seminiferous and Leydig cell activity, respectively (Figure 1) [3,4].

LH and FSH secretion from the anterior pituitary are finely regulated by inhibitory influences coming from the testis itself (sex steroids and inhibin B) and stimulated by the secretion in the portal vessels of the pituitary stalk of the decapetide, gonadotropin-releasing hormone (GnRH), synthesized in the arcuate nucleus of the hypothalamus (Figure 1). The GnRH pulsatile secretion is negatively controlled by the activity of other hypothalamic neurons, including corticotrophin-releasing hormone and β-endorphin neurons. Conversely, Kiss-1 neurons, through the activation of the specific Kiss-1 receptor, G protein-coupled receptor-54 (GPR54; [3,5]), are the main components involved in the stimulation of GnRH secretion. Infusion of Kiss-1 in humans stimulates GnRH secretion from the hypothalamus, leading to an increase in LH and FSH secretion and a corresponding rise in T (Figure 1). Another peptide, neurokinin-B or TAC, is involved in GnRH-Gn secretion acting through its receptor TACR3. Inactivating mutation of its gene or that encoding TACR3 cause delayed puberty or secondary hypogonadism (sHG) in adults [6]. Neurokinin-B is co-expressed with Kiss-1 and dynorphin-A by kisspeptin neurons in the hypothalamus, that, for this reason, are named KNDY neurons [7]. TACR3 is not expressed by GnRH neurons [7] but it is by KNDY cells, so that neurokinin-B is hypothesized to act in an autocrine or paracrine manner to enhance secretion of Kiss-1, thus indirectly affecting GnRH-Gn secretion (Figure 1) [7]. Leptin, the major adipokine produced by fat stores, also facilitates puberty onset, by activating Kiss-1 signaling [5]. Leptin maintains its permissive role on overall reproductive activity throughout adulthood, however, a detrimental effect of leptin excess has also been described [8].

## 2. Testicular testosterone formation

The binding of LH, or of its structurally related molecule, human chorionic gonadotropin (hCG), to their common receptor (LHCGR) – a seven transmembrane, G-protein-associated protein – stimulates testicular steroidogenesis [9]. Upon hormone binding, LHCGR receptor shifts conformation and thus mechanically activates stimulatory G protein, which detaches from the receptor and activates adenyl cyclases (ACs), converting ATP into the second messenger, cAMP. Thereafter, cAMP activates cAMP-dependent protein kinase (PKA), which, in turn, promotes T synthesis, through phosphorylation and activation of key enzymes involved in cholesterol handling and transport, such as the steroidogenic acute regulatory (StAR) protein, a rapidly synthesized labile phosphoprotein, and the hormone-sensitive lipase (HSL) (also known as cholesterol ester hydrolase). The transport of cholesterol from intracellular stores and from the outer to the inner mitochondrial membrane forms the first and rate-limiting step in steroid hormone biosynthesis, allowing CYP11A1 (cytochrome P450 side chain cleavage) to convert it into steroid intermediates. In humans, StAR mutations cause lipid congenital adrenal hyperplasia that ranges from an almost complete inability to synthesize steroids [10], to less severe forms that retain partial StAR protein activity [11].

The 18-kDa translocator protein (TSPO) [12], previously known as the peripheral-type benzodiazepine (BZD) receptor (PBR) – a pharmacologically distinct and unrelated G-aminobutyric acid (GABA)-associated BZD binding site [13] – is also expressed predominantly in mitochondria from steroid synthesizing cells, where it is localized to the outer mitochondrial membrane. TSPO binds to cholesterol with high affinity and is supposed to cooperate with StAR in transporting it from the outer to the inner mitochondrial membrane, where cholesterol is then converted into the first steroid, pregnenolone, by CYP11A1, an enzyme located on the matrix side of the mitochondria. In addition, PKA activation also has a longer lasting influence on steroidogenesis. In the chronic phase of steroid production, mRNA transcripts of several of the key steroidogenic genes increase due to cAMP/PKA-mediated activation of transcription factors, including shape factor-1 (steroidogenic factor 1) and DAX-1 (dosage-sensitive sex
reversal-adrenal hypoplasia congenita critical region on the X chromosome gene 1) [14]. All of these regulatory processes are controlled by cAMP, although possibly by different pools or functional compartments. Beside cAMP, also cGMP might have a role in controlling T synthesis. Stimulation of cGMP-dependent protein kinase G (PKG) production phosphorylates...
StAR protein in vitro and initiates steroidogenesis [15,16]. The Leydig cells express several cGMP hydrolyzing phosphodiesterases (PDEs) including PDE type 5 (PDE5) [17], whose activity might participate in regulating T synthesis.

Within the testis, T concentrations are two log units higher than those in peripheral circulation, reaching micromolar levels. In a study, the mean concentration of T in testis aspirates obtained from the 21 patients was 609 ± 50 µg/l (almost 2 µM), that is, at least 200-fold greater than the concentration of T found in normal human serum [18]. It has been previously reported that in spermatogenic plasma the efferent level of T was 259 ± 133 µg/l, suggesting that the majority of Leydig cell-produced T is exported towards the periphery [19]. Accordingly, intratesticular T concentrations required for maintaining spermatogenesis is very low (<5%) of the usual intratesticular T concentrations [20-24]). Local actions of T, besides spermatogenesis, are still a matter of debate [2-4].

3. Hypogonadism: a testicular failure in testosterone production

The inadequate gonadal production of T is known as HG, a common condition due to an intrinsic testicular failure (primary HG) or to a suboptimal stimulation by pituitary Gn (secondary or central HG). The term male HG, however, in clinical practice, is rarely used to identify isolated abnormalities in sperm production and is more often applied to describe any T (and spermatogenetic) deficiency. Both primary HG and sHG, if not treated, are characterized by symptoms and signs of dramatically different severity, spanning from a complete pseudo-female phenotype to no or very mild phenotype changes, dictated more by the age of onset of the testicular failure than by the cause of this failure [2-4].

4. Prevalence and main manifestations of HG

When HG develops during early fetal life, symptoms can be very severe, ranging from an almost complete feminine body shape (complete androgen insensitivity or enzymatic defects blocking androgen synthesis) to various defects in virilization such as in the case of impaired secretion or activity of GnRH or Gns [2]. In the case of a peripubertal appearance of HG, due to central (e.g., pituitary tumors, as germinoma) or peripheral defects (such as Klinefelter’s syndrome), there might be a slowing down of or delay in puberty progression, with a eunuchoidal phenotype. Hypogonadism onset in the prepubertal period is rather uncommon although not so rare, spanning from 1:100,000 for complete androgen insensitivity syndrome to 1:500 for Klinefelter’s syndrome [2].

Hypogonadism with a clinical exordium in young adulthood or later on due to any reason is the most common cause of male HG. It includes relatively mild, insidious and difficult to recognize, but often bothersome and frustrating, symptoms such as weakness and fatigue, reduced libido and erectile dysfunction (ED), mood symptoms, low bone mineral density and mild anaemia, all of which can contribute to decreasing the overall quality of life [2]. However, it should be recognized that many of these symptoms are rather non-specific, as they are also characteristic of the aging process per se, which, in turn, may act as a confounder in the interpretation and identification of the syndrome. Hence, according to major international guidelines, the diagnosis of adulthood HG (also known as late-onset HG [LOH]) should be made with two separate biochemical determinations of serum T levels along with the presence of consistent symptoms and signs [2]. Different T thresholds have been proposed for the biochemical definition of low T [2,25]. The most widely shared consensus is that T substitution has to be offered to symptomatic individuals when circulating total T is below 8 nmol/l (231 ng/dl). In addition, there is also general agreement that a total T level above 12 nmol/l (346 ng/dl) does not require substitution. When total T is repetitively >8 and <12 nmol/l in the presence of typical hypogonadal symptoms (as listed before), a T treatment trial may be considered [2,25].

Guidelines proposed by the American Association of Clinical Endocrinologists [26] and by the Endocrine Society [27] differ in the T-threshold suggested, considering a cutoff of 7 nmol/l (200 ng/dl) and 10.4 nmol/l (300 ng/dl), respectively. Recently, by evaluating data from the European Male Ageing Study (EMAS), obtained from more than 3400 community-dwelling middle-aged and older men (40–80 years) across 8 European centres, Wu et al. [28] introduced a new definition of LOH. In particular, they demonstrated a syndromic association between decreased T levels (total T <11 nmol/l) and a triad of sexual symptoms: low libido, and reduced spontaneous and sex-related erections [28]. Therefore, according to EMAS criteria, LOH should be diagnosed in the presence of at least three sexual symptoms and a morning total T level of less than 11 nmol/l (230–319 ng/dl) and free testosterone levels of less than 222.2 pmol/l (<64 pg/ml).

By applying only biochemical criteria, the prevalence of LOH has been reported to be up to 15% in the general population [2]. However, by using the EMAS criteria, the prevalence of LOH is much lower, that is, 2.1% [28]. It is quite evident, however, that subjects complaining of sexual dysfunction represent a population overloaded with LOH with prevalence five to six times higher than that reported in the general population [29].

5. Treatment of HG

T deficiency can be successfully treated by supplying the deficient hormone, T, which is available in oral, transdermal and injectable formulations (reviewed in [29,30]). T replacement therapy (TRT) is considered the most common and simple way of treating HG. However, it has several limitations, because turning off Gn production will suppress any residual testicular activity, including spermatogenesis. Hence, the paradoxical effect of treating male HG with exogenous T is that it
induces further testis atrophy and infertility, by inhibiting Gn secretion.

The aim of this review is to critically scrutinize alternative options to T as a medical intervention in treating HG. According to the physiology of hypothalamus—pituitary—testis axis, the goal can be reached by increasing: i) hypothalamic (i.e., kisspeptin and neurokinin-B agonists) and/or ii) pituitary activity (i.e., GnRH analogs, antiestrogens), or by mimicking pituitary activity, (i.e., Gn), or, finally, iii) by increasing Leydig cell steroid production, independently from Gn stimulation (i.e., PDE5 inhibitors, TSPO agonists). Obviously, removing the main cause of HG is the first step in a successful management of T deficiency. Considering that a consistent fraction of adult-onset HG is associated with obesity and metabolic syndrome (MetS), the effect of medical treatment of these conditions will be also considered.

5.1 Stimulation of GnRH secretion: kisspeptin and neurokinin-B agonists

The most physiological approach to sHG therapy, but still not available for clinical use, is based on the use of kisspeptin or neurokinin-B agonists that, theoretically, activate the entire GnRH-Gn-testis axis (Figure 1 and Table 1; see section 1&2 and [31] for review).

Both native and synthetic kisspeptin agonists have been studied. Native forms are peptides of 54 or 10 amino acids (kisspeptin-54 and -10, respectively the native 54 amino acid kisspeptin and the C-terminal decapeptide) both with full biological activity, but low molecular stability. Synthetic compounds are modifications of kisspeptin-10 made for providing resistance to degradation and improving pharmacokinetics. Examples of synthetic kisspeptin agonists are TAK-448 or TAK-683, currently investigational synthetic peptides (nine amino acids), modified from kisspeptin-10, which bind to GPR5 with an affinity similar to that of native peptides [32,33], thus stimulating GnRH release. The native kisspeptin-54 and -10, acutely administered in healthy men, have been proven to elicit a strong stimulation of Gn secretion [34,35], and a continuous infusion of kisspeptin-10 for 22.5 h produced an increased amplitude and pulse frequency of Gn secretion [35]. Either acute or continuous administration of kisspeptin-10 has also been tested in men with

### Table 1. Available preparations to treat male hypogonadism.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trade name</th>
<th>Standard dosage</th>
<th>Advantage</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPR54 agonist</td>
<td>TAK-448</td>
<td>Only Phase I data</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>TAK-683</td>
<td>Only Phase I data</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>TACR3 agonist</td>
<td>Senktide [MePhe7]NKB</td>
<td>Only Phase I data</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Clomifene</td>
<td>Clomiphene</td>
<td>Clomid</td>
<td>25 – 50 mg/day</td>
<td>Useful in functional hypogonadotropichypogonadism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norvadex Istuba</td>
<td>100 – 150 mg/week</td>
<td>Hypogonadism Suggested in idiopathic infertility</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valodex</td>
<td>12.5 – 25 mg/day</td>
<td>Limited risk of venous thromboembolic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Androxa</td>
<td></td>
<td>Required intact hypothalamus–pituitary–testis axis</td>
</tr>
<tr>
<td>Gonadotropin available compounds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hCG</td>
<td>Novarel</td>
<td>1000 – 2000 IU</td>
<td>Low cost</td>
<td>Mixed combination with FSH</td>
</tr>
<tr>
<td></td>
<td>Choror</td>
<td>3 times/week</td>
<td>Efficacy in patients with GnRH receptor mutations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gonatest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gonal F</td>
<td>1000 – 2000 IU</td>
<td>No association with FSH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Puregon</td>
<td>3 times/week</td>
<td>Efficacy in patients with GnRH receptor mutations</td>
<td></td>
</tr>
<tr>
<td>hCG</td>
<td>Recombinant hCG</td>
<td></td>
<td>No combination with FSH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ovidrel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteotropic hormone</td>
<td>Ovitrelle</td>
<td></td>
<td>No association with FSH</td>
<td></td>
</tr>
<tr>
<td>Recombinant hCG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>Menopogen</td>
<td>75 – 150 IU 3 times/ week</td>
<td>Low cost</td>
<td></td>
</tr>
<tr>
<td>Extractive hCG</td>
<td>Meropur</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>Gonal F</td>
<td>75 – 150 IU 3 times/ week</td>
<td>No combination with FSH</td>
<td></td>
</tr>
<tr>
<td>Recombinant hCG</td>
<td>Puregon</td>
<td></td>
<td>Efficacy in patients with GnRH receptor mutations</td>
<td></td>
</tr>
</tbody>
</table>

FSH: Follicle-stimulating hormone; hCG: Human chorionic gonadotropin; NA: Not available.

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Type 2 diabetes mellitus (T2DM) and sHG [36] and it has proven to be able to restore T levels within the normal range. TAK-448 and TAK-683 are characterized by a higher activity and stability than the native peptides [37] and, therefore, they are mainly studied for therapy of prostate cancer. In fact, when continuously infused in healthy men, they suppress T levels into the castrated range [38,39]. Hence, a pulsatile administration of these synthetic analogs could be theoretically offered as a new option for sHG. However, similar to what has been observed for GnRH therapy (see below), the requirement of wearing portable pumps could limit kisspeptin agonist use due to expected poor patient compliance and high cost.

Few neurokinin-B agonists (Senktide and [MePhe7]NKB) have been developed for having a higher stability and affinity for TACR3 than the native molecule [31]. Although they still have not been tested in clinical studies, neurokinin-B agonists could be potentially new therapeutic options for treatment of sHG. However, no clinical studies are at present available on neurokinin-B agonists and stimulation of Gn secretion.

5.2 Stimulation of pituitary activity

5.2.1 GnRH therapy

GnRH therapy can be used, alternatively to T, in starting puberty and testicular growth, but they can also induce spermatogenesis in patients with sHG. GnRH, when administered in a pulsatile manner, physiologically stimulates pituitary to release LH and FSH. This limits its use to subjects with a residual pituitary gonadotropic activity and it cannot be a therapeutic option in sHG due to mutation in GnRH receptor. GnRH therapy for treatment of sHG must be administered in a pulsatile fashion, mimicking the activity of hypothalamic pulse generator [40,41].

At the moment, GnRH therapy represents the most physiological, orthotopic approach for GnRH deficiency, isolated or in combination with other hypothalamic hormone deficiencies. Dosage required for stimulating a pulsatile secretion of Gn is the intravenous or subcutaneous administration of 100 – 400 ng/kg of GnRH every 90 – 120 min and titration must be performed on the basis of T, LH and FSH levels [41]. A meta-analysis of the available longitudinal studies dealing with appearance of sperm in semen of azoospermic sHG subjects upon pulsatile GnRH [40] showed that spermatogenesis is achieved in 75% of cases with a mean sperm count of about 4 million/mL. Studies comparing spermatogenesis obtained with GnRH or Gn therapy substantially report a non-significant difference between these two therapeutic approaches [42-45]. Effectiveness of GnRH in improving sexuality has been mainly studied in prepubertal-onset sHG subjects, as a part of the clinical picture of pubertal development. Few studies also evaluated its effect in improving sexuality in non-HG men, without finding substantial success [46]. However, no study has systematically evaluated GnRH therapy in improving sexual and other HG-related symptoms in adult men with LOH.

The necessity of wearing a pump 24 h a day for a long time is the major inconvenience limiting the use of GnRH as a therapy for sHG [41]. In addition, the lack of sufficient information in improving LOH-related symptoms represents another limitation.

5.2.2 Antiestrogens

The classic, genomic effects of estrogens are mediated through the transcriptional program of estrogen nuclear receptors Erα and Erβ. On these receptors, some estrogen antagonists, having a steroid backbone different from native estrogen, can exert both antagonistic and agonistic effects in different estrogen target tissues. Hence, a class of compounds also known as mixed partial ER agonists (Table 1) has been developed. The first non-steroidal partial ER agonist, ethamoxytriphetol or MER-25, was synthesized more than 50 years ago [47], but never developed clinically because of high toxicity and low potency. The triphenylethylene derivative compound, MRL-41, now known as clomiphene citrate, was developed in 1961 and is made of enclomiphene (trans-isomer, 62%) and zuclophen (cis-isomer [43]). The former shows only antagonistic activity, while the latter has mixed properties and longer half-life: 30 days versus 10.5 h [48]. There are currently two main chemical classes of mixed partial ER agonists approved for clinical use in females: the first-generation triphenylethylene derivatives (clomiphene, tamoxifen and toremifene), which are used for female infertility and in the treatment and secondary prevention of breast cancer, and raloxifene, a second-generation benzo-thiophene derivative indicated for the treatment and prevention of osteoporosis. Bazedoxifene acetate is a new, third-generation, mixed partial ER agonist. Other classes include benzopyrans and naphthalenes (e.g., lasofoxifene).

Tamoxifen shows an agonistic effect on another class of ER: the orphan receptor G protein-coupled receptor 30 or G protein-coupled ER (GPR30/GPER) [49]. GPR30/GPER is localized in the cell membrane, as well as in intracellular membranes, and is widely expressed in the genitourinary tract, including testis, corpora cavernosa, prostate and bladder [50]. We recently demonstrated that, in corpora cavernosa [51] and prostate, tamoxifen behaves as an Erα antagonist, whereas in the prostate it acts as a GPR30/GPER agonist [50].

In the male, all mixed partial ER agonists show a rather similar biological profile, although with some differences related to the different characteristics of the compound (Table 1). Essentially, all show an antagonistic activity versus ERs in the hypothalamus–pituitary centres, regulating Gn release. In fact, all mixed partial ER agonists increase LH and FSH secretion in the bloodstream and therefore stimulate testis activity both in terms of spermatogenesis and steroidogenesis. However, due to an agonist activity in the liver they also increase sex hormone binding globulin (SHBG), therefore, the resulting fraction of unbound (free) T is often unchanged [49,52-55]. Another, unwanted, agonist effect of mixed partial ER agonists is venous thromboembolic disease,
Alternative therapy for male HG

Although the absolute risk is relatively small [49,56,57]. Concerning other mixed partial ER agonists’ activity in the male, tamoxifen has been shown to maintain some estrogenic action in genital tissues such as the epididymis [58] and bone. In fact, following administration of other mixed partial ER agonists, such as raloxifene and toremifene, an increased bone mineral density has been demonstrated in men treated with GnRH agonist, and therefore hypogonadal [59]. In this patient population, toremifene (80 mg/day) demonstrated, in a large, placebo-controlled Phase III study, not only to prevent bone loss, but also to decrease the risk of fracture [56,57]. In addition, in prostate cancer subjects undergoing androgen-deprivation therapy (ADT) with bicalutamide, tamoxifen administration (20 mg) was able to significantly ameliorate gynecomastia and mastalgia [54,60-62]. Hence, the possible use of mixed partial ER agonists in prostate carcinoma patients undergoing ADT is emerging for treating gynecomastia and preventing bone loss. Similarly, this class of drugs can be combined with T for the treatment of TRT-induced gynecomastia [4].

Only a limited number of studies are available on mixed partial ER agonists in treating male HG (Table 2). Shabsigh et al. [63], reported positive results in terms of T rise with clomiphene citrate (25 mg/day) in a study on 36 men with T deficiency (<10.4 nmol/l), thus confirming the previous experiences of Guay et al. [64,65], in subjects with ED. Katz et al. [66], confirmed these positive results in a prospective series of 86 men with low-normal LH (<6 mU/l) and low T (<10.4 nmol/l) treated with 25–50 mg/day of clomiphene for a mean duration of almost 20 months. They observed a more than twofold rise in circulating T and LH, along with improvements in several HG-related symptoms, including lack of libido. Another recent study indicates that clomiphene is able to restore normal T and LH levels and to improve lack of libido. Another recent study indicates that clomiphene citrate (25 mg/day) was able to significantly ameliorate gynecomastia and mastalgia [54,60-62]. Hence, the possible use of mixed partial ER agonists in prostate carcinoma patients undergoing ADT is emerging for treating gynecomastia and preventing bone loss. Similarly, this class of drugs can be combined with T for the treatment of TRT-induced gynecomastia [4].

Two meta-analyses performed more than 10 years ago failed to support a significant effect of different antiestrogens, including clomiphene, on pregnancy rate [77,78]. In contrast, a more recent meta-analysis of randomized controlled trials (RCTs) on antiestrogen as an empiric medical therapy for male infertility indicates that they can improve spontaneous pregnancy rate, sperm concentration and motility [79]. Use of clomiphene for increasing sperm retrieval from ejaculate or testicular biopsies in otherwise azoospermic subjects was suggested [80]. Tamoxifen citrate was introduced three decades ago as an empiric treatment for idiopathic oligospermia [81]. Tamoxifen, in subjects with infertility, was also able to increase T and Gn [82,83] and similar results were also reported in a small sample of healthy subjects [84,85]. Accordingly, the aforementioned Chou meta-analysis showed a significant positive effect of antiestrogens on FSH and T levels [80].

Enclomiphene (Androxal), the trans-isomer of clomiphene citrate (see before), is being developed by Repros Therapeutics Inc., The Woodlands, TX, USA, for promoting Gn-dependent T secretion in sHG. Enclomiphene was first tested in a Phase IIb, randomized, open-label, fixed-dose (25 mg) clinical trial in 12 men with obesity (mean weight = 105 kg) and sHG (total T <10.4 nmol/l), previously treated with T gel. The primary end point was change in hormonal and seminal profile from baseline and comparison with results obtained during T transdermal application. Results indicated a similar rise in total T for gel and enclomiphene, the latter being able to increase LH and FSH concentration and sperm count [86]. No significant change was noticed in SHBG and calculated free T [86]. In another single-blind, 6-week, randomized Phase II study, the effect of increasing concentrations of enclomiphene (6.25, 12.5, 25 mg) was tested and compared with T gel (50 mg/day) in 44 subjects with obesity (mean body mass index [BMI] = 34.7 kg/m2) and sHG (total T <12 nmol/l and LH <12 intrauterine [IU]/l) with primary end point variation in total T and LH [87]. A pharmacokinetic study of different doses of enclomiphene was also performed. With enclomiphene citrate, there was a significant increase in total T level, which, at the maximal dose, was comparable with T gel. However, in the enclomiphene arms, LH was dose-dependently stimulated, whereas it was suppressed in the T transdermal arm. The positive effect of enclomiphene on LH and T persisted 1 week after discontinuation of the drug. In this, as in the previous study, an enclomiphene-associated IGF-1 decline was noted. The authors recognized that the highest doses (12.5 and 25 mg) warrant further clinical development [87]. Recently, results from another Phase II trial, this time placebo-controlled, were reported [88]. This study was conducted in obese men with sHG, but with a more severe form than in the previous trials (total T <8.67 nmol/l), and without having undergone T substitution. Two doses of enclomiphene were tested for 3 months (12.5 and 25 mg) and compared with T gel. Even in this placebo-controlled trial, both doses of enclomiphene increase, to the same extent of T gel, total T levels, however, with opposite effects on Gn, which were reduced by transdermal T and stimulated by the antiestrogen. Sperm parameters, not always available, were maintained...
### Table 2. Characteristic and outcome summary of studies performed with mixed partial estrogen receptor agonists.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Sample size</th>
<th>Population studied</th>
<th>Age (yr)</th>
<th>Drug</th>
<th>Duration of treatment (days)</th>
<th>Serum TT before therapy (nmol/l)</th>
<th>Serum TT after therapy (nmol/l)</th>
<th>Serum LH before therapy (U/l)</th>
<th>Serum LH after therapy (U/l)</th>
<th>Serum FSH before therapy (U/l)</th>
<th>Serum FSH after therapy (U/l)</th>
<th>Other outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lim et al. (1976) [70]</td>
<td>5</td>
<td>Chronic renal failure</td>
<td>48.6</td>
<td>CC</td>
<td>100 mg/d</td>
<td>150 – 360</td>
<td>7.7 ± 5.7</td>
<td>30.5 ± 5.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bjork et al. (1977) [72]</td>
<td>1</td>
<td>Case report of Laennec’s cirrhosis</td>
<td>37</td>
<td>CC</td>
<td>50 – 200 mg/d</td>
<td>370</td>
<td>4.3</td>
<td>6.9</td>
<td>22.0</td>
<td>71.1</td>
<td>8.8</td>
<td>17.5</td>
</tr>
<tr>
<td>Landefeld et al. (1983) [71]</td>
<td>2</td>
<td>Case reports of sickle cell anaemia</td>
<td></td>
<td>CC</td>
<td>50 – 100 mg/d</td>
<td>90</td>
<td>2.7 ± 1.2</td>
<td>9.1 ± 5.5</td>
<td>14.5 ± 6.4</td>
<td>82.8</td>
<td>4.3 ± 2.5</td>
<td>9.5 ± 2.1</td>
</tr>
<tr>
<td>Ronnberg et al. (1985) [74]</td>
<td>8</td>
<td>Healthy men</td>
<td>29 – 49</td>
<td>CC</td>
<td>100 mg/d</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>19.0 ± 1.4</td>
<td>16 ± 0.3</td>
<td>2.4 ± 0.6</td>
<td>2.3 ± 2.8</td>
</tr>
<tr>
<td>Ronnberg et al. (1985) [74]</td>
<td>8</td>
<td>Healthy men with sulpiride-induced hyperprolactinemia</td>
<td>29 – 49</td>
<td>CC</td>
<td>100 mg/d</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>19.0 ± 1.4</td>
<td>15 ± 0.3</td>
<td>2.4 ± 0.6</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>Guay et al. (1995) [64]</td>
<td>17</td>
<td>Secondary HG men with ED</td>
<td>60.5</td>
<td>CC</td>
<td>50 mg 3/wk</td>
<td>56</td>
<td>8.3 ± 1.3</td>
<td>18.3 ± 5.2</td>
<td>6.4 ± 1.5</td>
<td>10.2 ± 3.1</td>
<td>3.4 ± 1.4</td>
<td>5.6 ± 3.4</td>
</tr>
<tr>
<td>Tan et al. (2003) [73]</td>
<td>1</td>
<td>Case report of steroid abuse ED</td>
<td>30</td>
<td>CC</td>
<td>100 mg/d</td>
<td>60</td>
<td>2.5</td>
<td>24.5</td>
<td>1.7</td>
<td>26.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Guay et al. (2003) [65]</td>
<td>173</td>
<td>ED</td>
<td>54.3</td>
<td>CC</td>
<td>50 mg 3/wk</td>
<td>120</td>
<td>-</td>
<td>-</td>
<td>3.9</td>
<td>7.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shabsigh et al. (2005) [63]</td>
<td>36</td>
<td>Infertile men</td>
<td>39.0</td>
<td>CC</td>
<td>25 mg/d</td>
<td>90</td>
<td>8.6 ± 1.4</td>
<td>21.2 ± 6.2</td>
<td>2.3 ± 2.3</td>
<td>-</td>
<td>7.5 ± 6.8</td>
<td>-</td>
</tr>
<tr>
<td>Hussein et al. (2005) [80]</td>
<td>42</td>
<td>Infertile men</td>
<td>29.6</td>
<td>CC</td>
<td>25 – 75/d</td>
<td>155</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.2 ± 7.9</td>
<td>7.9 ± 7.5</td>
</tr>
<tr>
<td>Whitten et al. (2006) [69]</td>
<td>4</td>
<td>nIHH panhypopituitarism</td>
<td>30.5</td>
<td>CC</td>
<td>50 mg 3/wk</td>
<td>NR</td>
<td>15.6 ± 23.8</td>
<td>21.4 ± 16.8 &lt; 1.0</td>
<td>6.4</td>
<td>6.0 ± 0.01</td>
<td>3.5 ± 2.4</td>
<td>-</td>
</tr>
</tbody>
</table>

*Age was reported as mean when available or range when mean value was not reported.*

BMI: Body mass index; BMD: Bone mineral density; CC: Clomiphene citrate; d: Day; DA: Dopamine agonists; DHT: Dihydrotestosterone; ECC: Enclomiphene citrate; ED: Erectile dysfunction; FSH: Follicle-stimulating hormone; HDL: High-density lipoprotein; HG: Hypogonadism; HOMA: Homeostatic model assessment; LDL: Low-density lipoprotein; LH: Luteinizing hormone; nIHH: Normo-osmic idiopathic hypogonadotropic hypogonadism; OGTT: Oral glucose tolerance test; PRL: Prolactin; Raloxifene; SHBG: Sex hormone binding globulin; TC: Toremifene citrate; TRH: Thyrotropin-releasing hormone; To: Toremifene; TT: Total testosterone; yr: Years; wk: Week.
Table 2. Characteristic and outcome summary of studies performed with mixed partial estrogen receptor agonists (continued).

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Sample size</th>
<th>Population studied</th>
<th>Age (yr)</th>
<th>Drug</th>
<th>Duration of treatment (days)</th>
<th>Serum TT before therapy (nmol/l)</th>
<th>Serum TT after therapy (nmol/l)</th>
<th>Serum LH before therapy (U/l)</th>
<th>Serum LH after therapy (U/l)</th>
<th>Serum FSH before therapy (U/l)</th>
<th>Serum FSH after therapy (U/l)</th>
<th>Other outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribeiro and Abucham (2009) [67]</td>
<td>14</td>
<td>Hyperprolactinemia treated with DA without correction of T values</td>
<td>42.7</td>
<td>CC 50 mg/d</td>
<td>84</td>
<td>5.8 ± 3.1</td>
<td>11.4 ± 2.6</td>
<td>1.4 ± 1.5</td>
<td>4.1 ± 3.7</td>
<td>2.9 ± 3.4</td>
<td>5.6 ± 6.4</td>
<td>↑ estradiol ↑ SHBG ↓ calculated free T ↓ erectile function ↑ sperm motility ↑ BMD ↓ fat mass ↓ triglycerides ↓ glycemia after OGTT</td>
</tr>
<tr>
<td>Kadioglu (2009) [82]</td>
<td>120</td>
<td>Infertile men</td>
<td>26.74</td>
<td>TC 20 mg/d</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.7 ± 3.3</td>
<td>7.1 ± 5.2</td>
<td>↑ sperm concentration ↑ sperm concentration ↑ sperm concentration ↓ sperm concentration</td>
</tr>
<tr>
<td>Tsourdi et al. (2009) [83]</td>
<td>94</td>
<td>Infertile men</td>
<td>-</td>
<td>TC 20 mg/d</td>
<td>90</td>
<td>17.2 ± 6.1</td>
<td>26.5 ± 7.6</td>
<td>4.5 ± 1.8</td>
<td>7.8 ± 4.6</td>
<td>5.7 ± 2.7</td>
<td>8.4 ± 4.9</td>
<td>↑ sperm concentration ↑ sperm concentration</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>Infertile men</td>
<td>-</td>
<td>To 60 mg/d</td>
<td>90</td>
<td>17.3 ± 5.4</td>
<td>25.8 ± 8.3</td>
<td>4.1 ± 1.9</td>
<td>6.5 ± 2.7</td>
<td>5.6 ± 3.4</td>
<td>9.5 ± 4.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>Infertile men</td>
<td>-</td>
<td>R 60 mg/d</td>
<td>90</td>
<td>20.3 ± 5.6</td>
<td>20.9 ± 6.2</td>
<td>4.2 ± 1.7</td>
<td>4.8 ± 2.1</td>
<td>6.4 ± 3.4</td>
<td>6.9 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Birzniece et al. (2010) [84] and</td>
<td>10</td>
<td>Healthy men</td>
<td>51 – 77</td>
<td>R 60 mg/d</td>
<td>14</td>
<td>14.8 ± 3.2</td>
<td>18.6 ± 4.7</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.9</td>
<td>4.3 ± 2.2</td>
<td>5.7 ± 2.8</td>
<td>↓ SHBG</td>
</tr>
<tr>
<td>(2012) [85]</td>
<td>10</td>
<td>Healthy men</td>
<td>51 – 77</td>
<td>R 120 mg/d</td>
<td>14</td>
<td>14.8 ± 3.2</td>
<td>18 ± 4.7</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.6</td>
<td>4.3 ± 2.2</td>
<td>5.3 ± 2.8</td>
<td>↓ SHBG</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Healthy men</td>
<td>51 – 77</td>
<td>TC 10 mg/d</td>
<td>14</td>
<td>14.8 ± 3.2</td>
<td>18.6 ± 5.4</td>
<td>1.3 ± 0.3</td>
<td>1.7 ± 0.6</td>
<td>4.3 ± 2.2</td>
<td>5.5 ± 3.2</td>
<td>↓ SHBG</td>
</tr>
<tr>
<td>Ribeiro and Abucham (2011) [68]</td>
<td>9</td>
<td>Non-functioning pituitary adenomas</td>
<td>47.1</td>
<td>CC 50 mg/d</td>
<td>84</td>
<td>5.4 ± 2.8</td>
<td>2.9 ± 2.3</td>
<td>0.8 ± 0.6</td>
<td>1.1 ± 1.2</td>
<td>1.9 ± 3.0</td>
<td>2.8 ± 4.5</td>
<td>↑ Estradiol ↑ BMI ↓ fat mass ↓ lean mass ↓ glycemia ↓ HOMA ↓ total cholesterol</td>
</tr>
</tbody>
</table>
Table 2. Characteristic and outcome summary of studies performed with mixed partial estrogen receptor agonists (continued).

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Sample size</th>
<th>Population studied</th>
<th>Age (yr)</th>
<th>Drug</th>
<th>Duration of treatment (days)</th>
<th>Serum TT before therapy (nmol/l)</th>
<th>Serum TT after therapy (nmol/l)</th>
<th>Serum LH before therapy (U/l)</th>
<th>Serum LH after therapy (U/l)</th>
<th>Serum FSH before therapy (U/l)</th>
<th>Serum FSH after therapy (U/l)</th>
<th>Other outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Katz et al. (2012) [66]</td>
<td>86</td>
<td>Secondary HG men</td>
<td>29</td>
<td>CC 25 - 50 mg/d</td>
<td>180</td>
<td>6.7 ± 3.0</td>
<td>16.8 ± 5.7</td>
<td>2.6 ± 2.2</td>
<td>6.8 ± 2.8</td>
<td>1.9 ± 1.7</td>
<td>7.6 ± 1.9</td>
<td>measured free T ▲ estradiol ▮ SHBG ▮ HG related symptoms</td>
</tr>
<tr>
<td>Kaminetsky et al. (2013) [86]</td>
<td>8</td>
<td>Secondary HG men</td>
<td>46</td>
<td>ECC 25 mg/d</td>
<td>180</td>
<td>6.3 ± 0.3</td>
<td>17.7 ± 10.1</td>
<td>2.2 ± 1.0</td>
<td>6.0 ± 2.5</td>
<td>1.5 ± 0.4</td>
<td>5.2 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Wiehle et al. (2013) [87]</td>
<td>12</td>
<td>Secondary HG men</td>
<td>53.3</td>
<td>ECC 6.25 mg/d</td>
<td>42</td>
<td>9.2 ± 3.2</td>
<td>13.6 ± 7.7</td>
<td>3.7 ± 1.8</td>
<td>6.0 ± 2.7</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Secondary HG men</td>
<td>53.3</td>
<td>ECC 12.5 mg/d</td>
<td>42</td>
<td>10.2 ± 2.8</td>
<td>14.8 ± 4.9</td>
<td>3.9 ± 1.2</td>
<td>6.9 ± 2.4</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Secondary HG men</td>
<td>53.3</td>
<td>ECC 25 mg/d</td>
<td>42</td>
<td>8.8 ± 2.8</td>
<td>20.3 ± 5.0</td>
<td>4.5 ± 3.9</td>
<td>13.1 ± 7.0</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Wiehle et al. (2014) [88]</td>
<td>27</td>
<td>Secondary HG men</td>
<td>49.7</td>
<td>ECC 12.5 mg/d</td>
<td>90</td>
<td>7.5 ± 2.0</td>
<td>16.4 ± 6.4</td>
<td>4.4 ± 1.8</td>
<td>8.9 ± 4.4</td>
<td>6.4 ± 4.2</td>
<td>11.5 ± 8.7</td>
<td>DHT ▲ estradiol ▮ SHBG</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Secondary HG men</td>
<td>49.2</td>
<td>ECC 25 mg/d</td>
<td>90</td>
<td>7.3 ± 1.9</td>
<td>14.1 ± 5.7</td>
<td>5.3 ± 4.0</td>
<td>11.7 ± 8.1</td>
<td>9.4 ± 10.9</td>
<td>14.9 ± 10.4</td>
<td></td>
</tr>
</tbody>
</table>

Age was reported as mean when available or range when mean value was not reported.

BMI: Body mass index; BMD: Bone mineral density; CC: Clomiphene citrate; d: Day; DA: Dopamine agonists; DHT: Dihydrotestosterone; ECC: Enclomiphen e citrate; ED: Erectile dysfunction; FSH: Follicle-stimulating hormone; HDL: High-density lipoprotein; HG: Hypogonadism; HOMA: Homeostatic model assessment; LDL: Low-density lipoprotein; LH: Luteinizing hormone; nIHH: Normo-osmic idiopathic hypogonadotropic hypogonadism; OGTT: Oral glucose tolerance test; PRL: Prolactin; RA: Raloxifene; SHBG: Sex hormone binding globulin; TC: Tamoxifen citrate; TRH: Thyrotropin-releasing hormone; To: Toremifene; TT: Total testosterone; yr: Years; wk: Week.
Gn therapy is the treatment of choice in men with sHG who have total testosterone < 10 nmol/l [90]. All patients started on letrozole 2.5 mg/week, with subsequent dose escalation every month until a serum total testosterone of 20 nmol/l was reached. Similarly to what has been previously reported, a decrease in E2 and an increase in T levels were observed. However, no modifications of psychological symptoms as well as of metabolic parameters were detected [90]. Hence, although aromatase inhibitors could theoretically represent an alternative option for the treatment of obesity-related male HG, the data published so far are too limited to suggest their clinical use. In addition, the possible expected negative impact on bone density represents another major limitation for a long-term treatment [91].

5.2.3 Aromatase inhibitors

Another possibility to increase T levels in subjects with obesity-induced T deficiency is represented by the use of aromatase inhibitors. Loves et al. [89] investigated this possibility in an uncontrolled 6-month pilot study, performed in 12 severely obese men (BMI > 35 kg/m^2). The treatment with 2.5 mg letrozole once a week for 6 months reduced total E2 and increased serum LH and T levels. No information on sexual function was reported. More recently, the same group published the data of the first double-blind, placebo-controlled, RCT in 42 obese men (BMI > 35 kg/m^2) with a serum total testosterone < 10 nmol/l [90]. All patients started on letrozole 2.5 mg/week, with subsequent dose escalation every month until a serum total testosterone of 20 nmol/l was reached. Similarly to what has been previously reported, a decrease in E2 and an increase in T levels were observed. However, no modifications of psychological symptoms as well as of metabolic parameters were detected [90]. Hence, although aromatase inhibitors could theoretically represent an alternative option for the treatment of obesity-related male HG, the data published so far are too limited to suggest their clinical use. In addition, the possible expected negative impact on bone density represents another major limitation for a long-term treatment [91].

5.3 Mimicking pituitary activity: Gn therapy

Gn therapy is the treatment of choice in men with sHG who require fertility (Tables 1 and 3). The most widely used compound is hCG, purified from urine of pregnant women. However, also recombinant hCG (rhCG [92]) and luteinizing hormone (rhLH [93]) are available. Although the efficacy of recombinant compounds in restoring T serum concentration has been proven [94,95], no studies, so far, have evaluated their effects on spermatogenesis. All these compounds stimulate Leydig cells, and increase both intra-testicular and circulating T levels (see before). Considering that a high intra-testicular T concentration is required for successful spermatogenesis, some sHG patients can achieve or restore sperm production and fertility only using hCG alone, with a standard dosage of 1000 – 2000 IU intramuscular or subcutaneous injection two to three times weekly [96]. However, when sperm concentration in ejaculate is lower than 10 millions/ml, after 6 months of hCG alone, co-administration of FSH is required [96]. A recent meta-analysis [40] evaluating the available longitudinal studies on achievement of spermatogenesis with Gn therapy in azoospermic sHG subjects showed an overall successful outcome in 75% of patients, with a mean sperm concentration achieved of almost 6 million/ml. Better results were obtained in patients with a postpubertal onset of sHG and in those with lower endogenous LH and FSH levels before starting therapy [40].

Treatment with Gn, as an alternative to TRT, is also widely used for inducing androgenization of phenotype in subjects with prepubertal onset of sHG [41]. Administration of hCG induces development of secondary sexual characteristics, leads to testicular growth and, differently from TRT, it allows achievement of spermatogenesis [41]. hCG is usually injected (intramuscularly or subcutaneously) in a dosage of 1250-5000 IU weekly, alone or in combination with 12.5 – 150 IU of FSH weekly (Table 1) [41]. Despite the clinical advantages over TRT reported above, Gn therapy also presents disadvantages, such as modality of administration, which is less convenient, and costs, as it is more expensive than TRT. Hence, the use of Gn therapy in younger subjects for a long time span is cumbersome.

Although the effectiveness of Gn has been extensively studied in prepubertal onset sHG, its role in adulthood for the treatment of LOH has been scarcely investigated. In a RCT involving 40 hypogonadal subjects, aged more than 60 years, Liu et al. [97-99] evaluated for the first time the effect of rhCG 5000 IU or placebo subcutaneously twice/week for 3 months. During the active treatment, the rhCG arm reached

### Table 3. Mean difference in several clinical parameters after weight loss as derived from meta-analysis of the available evidence.

<table>
<thead>
<tr>
<th></th>
<th>Total testosterone (nmol/l)</th>
<th>CFT (pmol/l)</th>
<th>SHBG (nmol/l)</th>
<th>LH (U/l)</th>
<th>FSH (U/l)</th>
<th>E2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low caloric diet</td>
<td>2.05 (0.95; 3.16)^1</td>
<td>42.01</td>
<td>13.05</td>
<td>1.06</td>
<td>1.83</td>
<td>-6.95</td>
</tr>
<tr>
<td>Bariatric surgery</td>
<td>10.15 (6.55;13.76)^2</td>
<td>5.74; 78.28^1</td>
<td>(5.24; 20.85)^1</td>
<td>(0.54; 1.57)^1</td>
<td>(1.13; 2.53)^1</td>
<td>(-12.2; -1.7)^*</td>
</tr>
<tr>
<td>Overall</td>
<td>2.75 (1.69; 3.81)^1</td>
<td>42.01</td>
<td>13.05</td>
<td>1.06</td>
<td>1.83</td>
<td>-6.95</td>
</tr>
</tbody>
</table>

Data taken from [141].

*p < 0.01.

^1p < 0.0001.

CFT: Calculated free testosterone; E2: Estradiol; FSH: Follicular-stimulating hormone; LH: Luteinizing hormone; SHBG: Sex hormone binding globulin.
physiological total and calculated free T levels. As compared with the placebo-treated arm, body weight and lean mass significantly increased in the rhCG-treated patients, whereas fat mass decreased [97,98]. However, no difference in muscle strength was found between active-treated subjects and controls. Besides body composition, lipid profile also improved, with a significant decrease in total and low-density lipoprotein (LDL) cholesterol as well as triglycerides [98]. Conversely, no difference was detected between rhCG-treated subjects and controls in blood pressure, fasting glycemia and insulinaemia as well as insulin sensitivity, as assessed by euglycemic hyperinsulinemic clamp [98]. No improvement of sexual function was observed [98]. Concerning bone metabolism, the short follow-up allowed only for the evaluation of bone biomarkers, and in rhCG-treated patients, in comparison with controls, a higher level of neo-formation markers was found, without differences in bone resorption markers [99]. Finally, no adverse event, including increased polyolacid, urinary symptoms or polyglobulia, was ascribable to Gn treatment [96].

Similar results were reported later on by Tsujimura et al. [100], who evaluated the effects of hCG in 77 shG men aged 50 – 79 years complaining of consistent sexual, physical or psychological symptoms. They were treated for a mean time of 8 months with intramuscular injections of hCG 3000 IU every 2 weeks and a change in several symptoms and signs upon hCG treatment were considered. During follow-up, a significant improvement in sexual, physical and psychological symptoms, as assessed by the Aging Males’ Symptoms (AMS) scale, was observed [100]. However, no improvement of erectile function was detected [100]. In addition, no difference in total or high-density lipoprotein (HDL) cholesterol, as well as triglycerides, was shown after hCG treatment [100]. The treatment was well tolerated and no significant side effects were reported [100]. It must be noted that the dosage used in this study was significantly lower than that commonly used in replacement of shG. Accordingly, total and calculated free T levels, measured after therapy, although significantly higher than baseline levels, were barely above the lower limit of the normal range [100].

Hence, data on hCG treatment of LOH are still scanty and studies comparing TRT and Gn are not available. However, Gn seems to be a reasonable alternative also for treatment of postpubertal forms of shG when fertility is required. They stimulate steroidogenesis overall with possible effects due to steroids other than T, such as E2 and DHT [101]. In addition, it is unlikely to reach excessively high T levels, since Gn’s effect is limited by Leydig cell capacity [102]. Finally, Gn is well tolerated and adverse events are infrequent and include an increased incidence of gynecomastia, development of antibodies limiting pharmacological action and hypersensitivity reactions [41]. However, the required high frequency of injections and the lack of sufficient information in improving LOH-related symptoms represent the major limitations for long-term use.

5.4 Increasing Leydig cell steroid production, independently from Gn stimulation

5.4.1 PDE5 inhibitors

Human testes express gene and protein for PDE5 [15,103], which, in the rat, has been immuno-localized in Leydig and peritubular myoid cells [104]. Although within the human testis the gene expression of PDE5 is 10-fold lower than in penile corpora cavernosa [17], it still might have a physiological significance. Accordingly, in the mouse, PDE5 inhibition, through sildenafil dosing for 30 days, was able to increase androgen levels [105,106]. In the rat, the effect of sildenafil on T secretion was transient and apparent after 60 min, and mediated by a cGMP-dependent, PKG-induced phosphorylation of StAR [107]. However, in a rabbit model of MetS-induced HG we were unable to demonstrate a significant increase in plasmatic T after short- [108] or long-term [109] exposure to tadalafil.

In an open-label, retrospective study on 74 ED subjects it was found that 3-month treatment with either sildenafil or tadalafil increased T levels by 2 – 4 nmol/l [110]. In a recent large RCT, it was found that a 4-week run-in of PDE5 inhibitor (PDE5i), sildenafil, in hypogonadal men significantly raised T levels by a mean of 4 nmol/l [111], supporting a possible role of PDE5i as the sole treatment in selected men presenting with ED and mildly reduced T levels. Although it is possible to hypothesize a direct action of PDE5 inhibition within the testis, these positive results could be explained also by the restored sexual activity, as demonstrated with other treatments (see below). Accordingly, the short-term experimental effects of sildenafil on Leydig cell T production [105-107] could not be compared with the clinical findings reported in a study where subjects were taking on average two doses a week [111]. In addition, the possible role of TRT in improving PDE5i outcomes [112] confirms the limited power of these classes of drugs in improving T levels.

5.4.2 PBR/TSPO agonist

PBR/TSPO agonist ligands, including BDZ with TSPO binding activity [113], stimulate steroidogenesis by facilitating cholesterol delivery to the cytochrome P450 side-chain cleavage enzyme in the inner mitochondrial membrane [114]. It has been demonstrated that the PBR/TSPO agonist PK11195 stimulates steroidogenesis in the Y1 mouse adrenal tumor cell line and that the targeted disruption of PBR/TSPO in the R2C rat Leydig tumor cell line inhibits steroidogenesis (see for review [114]). However, Leydig cell-specific TSPO conditional knockout mice suggested that TSPO was not required for T production in vivo [115,116]. In order to clarify the definitive role of TSPO in steroidogenesis and embryo development, global TSPO null (TSPO-/-) mice were generated. TSPO-/- mice survived with no apparent phenotypic abnormalities and were fertile [110]. Although BDZ have been on the market for several decades, and most of them bind to PBR/TSPO, no stimulating effects on T synthesis of this class of drugs have been reported in humans. Hence,
the role of PBR/TSP0 agonist in the treatment of HG is more than hypothetical.

5.5 Non-pharmacological options to increase T levels

5.5.1 Restoring sexual activity

The fact that sexual activity *per se* can affect T levels has been hypothesized since the 1970s [117]. During the following years, only scanty reports substantiated this anecdotal report, demonstrating, during sexual intercourse [118,119] or exposure to erotic movies [120], a timely related rise in T level. However, other studies addressing the question of a sexual activity-induced rise in T plasma levels were negative [121-125]. Jannini et al. showed that restoring erection by non-hormonal intervention (behavioural therapy, intracavernous injections) rescued an otherwise borderline low T level by 30-40% of baseline values [126]. The T rise they found was independent from the kind of therapies used, but strictly related to the successful outcome of therapeutic intervention. Hence, they speculated that sexual inactivity resets reproductive axis to a lower activity, somehow inducing a sHG, characterized by a reduced LH bioactivity [126,127]. In agreement with this hypothesis, we observed that sexual inactivity - induced by bilateral cavernous neurotomy - was associated with an overt condition of HG, characterized by reduced T plasma level, reduced ventral prostate weight, reduced testis function (including testis weight and number of Leydig cells), with an inadequate compensatory increase of LH [128]. In addition, in a consecutive series of 2302 male patients with ED, sexual inactivity was associated with overt HG [129]. Hence, it is plausible that increasing sexual activity might ameliorate HG, at least in the milder forms.

5.5.2 Lifestyle modifications

The association between LOH and adverse metabolic conditions, such as obesity, MetS and T2DM, is well known [130,131], with a rather complex, and often multidirectional, pathogenetic background. Several studies have demonstrated that, in individuals at risk, intensive lifestyle intervention, along with nutritional counseling and physical activity is able to reduce weight loss and insulin resistance, preventing the progression to overt diabetes [132-137]. Similarly, weight loss *per se* [138], along with lifestyle modifications, is able to improve sexual function, although only limited data have been published so far [138,139]. Unfortunately, diet and behavioural therapies often ultimately fail [140]. Bariatric surgery is another option proposed to rapidly lose weight in the presence of morbidly obesity and important associated morbidities [140]. In line with the aforementioned evidence, according to the Standard Operating Procedures of the International Society for Sexual Medicine, lifestyle modifications should be strongly encouraged in all hypogonadal subjects with obesity, T2DM and MetS [3]. However, only few randomized clinical studies have specifically evaluated the impact of diet and physical activity on T levels in men [141]. Conversely, data that are more robust suggest an increase of T level, strictly dependent on baseline BMI and weight reduction after bariatric surgery. However, the number of patients evaluated and their follow-up are too limited to draw any conclusions. Interestingly, we recently meta-analyzed available evidence on the effect of weight loss on sex steroid hormone levels and obesity-associated sHG [141]. Both low caloric diet and bariatric surgery were associated with a significant increase in circulating SHBG and total T levels, with bariatric surgery more effective than diet [141]. Similarly, weight loss was associated with an increase of both LH and FSH levels as well as with a reduction of E2 circulating levels (Table 3) [141]. Multiple regression analysis shows that the degree of body weight lost was the best determinant of total T rise (B = 2.50 ± 0.98; p = 0.029), whereas quite unexpectedly, no effect of E2 decrease after weight loss on total T was observed. In line with the latter observation, when a cohort of 55 morbidly obese men observed at 6 and 9 months after different types of bariatric surgery was stratified according to the presence of overt HG (total T < 8.0 nmol/l), the increase in androgen levels (total and free T) was significant only in HG subjects [142]. Moreover, a decrease in E2 levels was observed only in eugonadal subjects, having higher E2 levels at baseline. Hence, the role of E2 in the determination of obesity-related HG is smaller than what has been previously hypothesized [3]. In fact, in eugonadal subjects, the reduction in E2 determined by surgery does not produce an increase in T, whereas recovery of HG occurs without any significant increase in E2 levels. Hence, other fat-associated factors, besides estrogens, can be speculated to mediate the weight reduction-induced improvement in T levels. However, it should be recognized that in all the previous studies serum E2 was evaluated using direct immunoassays which are not considered the gold standard in men [143]. Hence, E2 data would have overlooked the inaccuracy and method-specific bias.

6. Conclusion

Adulthood HG is the most common form of male HG and includes both central and peripheral derangements. TRT is the most commonly used and most simple way to treat male HG. In addition, it represents the advisable therapy for primary HG. However, for the secondary or mixed forms several alternative possibilities can be offered, according to patient-related outcome, including the fertility status. These possibilities range from removing the HG-associated morbidities (MetS, T2DM), encouraging lifestyle modifications (increasing sexual activity, weight loss) or medical therapy. The latter includes medications that decrease the estrogen-negative feedback (antiestrogens) or that activate the hypothalamic-pituitary-testis axis (GnRH agonists and Gn). Antiestrogens are not approved for the treatment of male HG, although for one molecule, enclomiphene, the FDA review is pending. In addition, an intact hypothalamic-pituitary axis is required for the successful outcome of antiestrogens. Conversely, the possibility of increasing Leydig cell steroid production, independently from Gn stimulation such as in the case of
PDE5is or PBR/TSPO agonist ligands, seems unreliable at the moment.

7. Expert opinion

A thorough understanding of the nature of male HG is mandatory before choosing treatment options (Box 1). Considering that sHG is dramatically more prevalent than the primary form and that the former often arises from the presence of obesity or other chronic disturbances, careful screening of such conditions is important not only to improve the HG status, but also for general health. For example, a complete recovery of severe HG (T < 8 nmol/l) was observed in 93% of morbid obese subjects undergoing bariatric surgery [142]. However, when fertility is urgently required by a HG subject, lifestyle modification does not offer satisfactory results. Håkonsen et al. [144], did not observe any modification in sperm concentration in obese subjects after 14 weeks of a weight loss program, even if a T and Gn rise was observed. Hence, in this framework, Gn or GnRH therapy is the correct choice. A recent meta-analysis indicates that three out of four azoospermic subjects with sHG obtain sperm after Gn or GnRH supplementation. An alternative option is antiestrogens. Another recent meta-analysis indicates that this class of compounds is able to increase sperm production and pregnancy rate [80], however, their effect are less dramatic than with Gn. Among antiestrogens, enclomiphene is the most interesting molecule, as it is specifically designed and tested for the treatment of sHG. Enclomiphene was shown to improve hormonal levels, sexual activity and sperm production; however, the number of dedicated studies is still limited. For primary HG no alternative strategy to T is available, but it is important to say that, in this condition, preserving fertility is not a problem, because it is compromised by definition.

This box summarizes key points contained in the article.

Declaration of interest

G Corona has received consultancies from Bayer, Besins, Otsuka, Eli-Lilly and Menarini. M Maggi has received consultancies from Bayer, Prostrakan, GSK, Eli-Lilly and Menarini. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Sertoli E. Dell’ esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano. Morgagni 1865;7:31-40
** Detailed clinical practice guidelines on late onset hypogonadism.
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• An excellent meta-analysis on gonadotropin therapy for male hypogonadism.


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• An excellent meta-analysis on the role of weight loss in improving testosterone levels.


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