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OPINION

FSH TREATMENT OF MALE IDIOPATHIC INFERTILITY: TIME FOR A PARADIGM CHANGE

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Abstract

Follicle-stimulating hormone (FSH) has been used in inconclusive clinical trials for male idiopathic infertility in the past. FSH is sometimes prescribed empirically for male idiopathic infertility, showing some improvement in sperm parameters in about half of the patients. In this opinion article we briefly analyze the pathophysiological evidences in favor of a more aggressive approach in planning future studies on pharmacological FSH use in male infertility, in analogy with the FSH use for multiple follicular growth in women undergoing ovarian stimulation for assisted reproduction. There is sufficient evidence that spermatogenesis does not run at its top in the primate and that some extra FSH can stimulate spermatogenesis over its baseline. Existing data suggest that the pharmacological regimens applied so far were insufficient, both in dosage and in duration, to elicit this response in about half of the patients. A paradigm change is needed now: we should move away from the classical, endocrinological approach, which simply applied the substitutive, therapeutic regimen used in hypogonadotropic hypogonadism, towards testing a "testicular hyperstimulation" scheme for a time sufficient to cover more than only one spermatogenic cycle, a concept to be verified in an appropriately controlled, prospective, randomized clinical trial.

Introduction

The problem of male idiopathic infertility is surely well-known to the readership of this journal and does not need to be extensively introduced. Here, we would like to remind only two crucial aspects relevant for this article: i) some sort of male factor is present in about 50% of cases of couple infertility (Colpi, et al., 2018) and ii) in most of the cases an etiological therapy does not exists and a pregnancy requires assisted reproduction technology (ART). The latter means that it is the woman who has to undergo ovarian stimulation, with its implications and consequences, even if she is perfectly fertile. In this sense, the discovery of intracytoplasmic sperm injection (ICSI), while

crucial to allow paternity, not only was a disaster for research on male infertility but also introduced another burden to women. ART for male infertility is probably the most dramatic example of gender inequity in medicine, along with hormonal contraception.

Couple infertility is a very peculiar field of medicine for several reasons. The causes remain unknown in about one third of the cases (De Jonge and Barratt, 2019); it is not perceived as medical problem worth of thorough research, which means that it remains under-supported by the funding agencies (while animal reproduction, instead, is much more subsidized); treatments are mostly empirical and not evidence-based; the burden of the treatment is carried by the women in most of the cases; the field is dominated by gynecologists, while males are rarely referred to the proper doctor, i.e. the andrologist; treatments are often conducted in private clinics, at the costs of the patients, in most countries of the world.

In this hostile scenario, is there anything that can be done now to improve medical practice in cases of male idiopathic infertility? In this opinion article we would like to analyze the case of follicle-stimulating hormone (FSH) therapy, an option which has been snubbed enough and now needs to be re-evaluated in the light of the most recent evidences. The pharmacological use of FSH for the treatment of male idiopathic infertility needs to undergo a paradigm change.

Targets of FSH action in spermatogenesis

In humans, FSH stimulates spermatogenesis both independently from testosterone and with a mechanism partially overlapping with testosterone. Spermatogenesis occurs in proliferation and maturation steps, requiring autocrine, paracrine and endocrine stimuli that are guaranteed by both FSH and testosterone (Jan, et al., 2012, Oduwole, et al., 2018). FSH mainly acts in the first stages ("FSH dependent phase" – Figure 1), whereas testosterone works in the last steps (Figure 1). The leading FSH roles on spermatogenesis are: i) determination of Sertoli cells number; ii)

spermatogonial proliferation as well as metabolic and structural support; iii) stimulation of meiotic progression until spermatids stage; iv) metabolism and transport of nutritive substances to germ cells.

FSH acts on Sertoli cells, interacting with the FSH receptor (FSHR) (Kangasniemi, et al., 1990). On the other hand, testosterone comes from the luteinizing hormone (LH) action on Leydig cells, reaching a 50-100-fold higher concentration within testes rather than in blood circulation (Roth, et al., 2010).

FSH determines the final Sertoli cells number during neonatal and peri-pubertal life (Sharpe, et al., 2003), increasing the transcription of Sertoli cell genes involved in DNA replication and cell cycle regulation (Zimmermann, et al., 2015). Although the *FSHR* is expressed since the second half of gestation, it is the neonatal pituitary FSH onset and its peri-pubertal raise that activate the FSHR (Huhtaniemi, et al., 1987) and stimulate Sertoli cell proliferation (Sharpe, et al., 2003), respectively. In adult life, FSH stimulates Sertoli cells to produce regulatory molecules and nutrients required for germ cells maturation (Cheng and Mruk, 2002), and its circulating levels correlate directly with Sertoli cells number and testicular volume (Allan, et al., 2004).

Recent findings obtained by genetically modified mouse models increased our knowledge about FSH role in spermatogenesis. In adult mouse testis, FSH stimulates Sertoli cells to produce anti-apoptotic survival factors and adhesion molecules, facilitating germ cells maturation until the round spermatids stage (Figure 1) (Ruwanpura, et al., 2010). However, the lack of FSH or FSHR reduces the Sertoli cells number but does not cause sterility (Abel, et al., 2000, Kumar, et al., 1997), suggesting that FSH is not absolutely necessary for spermatogenesis in the mouse. In humans, only one inactivating *FSHR* mutation was described in five males (Tapanainen, et al., 1997). Despite the receptor defect, these men were subfertile, with normal testosterone levels, reduced spermatogenesis, but not necessarily azoospermia. This result reflects the clinical picture of *Fshr*-

KO mice models and was interpreted as demonstrating that spermatogenesis may be possible without FSH action, although an alternative explanation was offered recently by a more complete exploration of FSHR signaling (Tranchant, et al., 2011). On the contrary, the only five men described so far with *FSHB* mutations were all azoospermic (Zheng, et al., 2017), suggesting that spermatogenesis cannot take place without FSH action in man. However, the paucity of human FSHB/FSHR mutations does not allow to fully describe the role and molecular mechanism of FSH in human spermatogenesis.

FSH works in synergy with testosterone, which is imperative for blood-testis barrier development and function, whereas FSH is permissive in the organization of tight junctions and ectoplasmic specialization (Sluka, et al., 2006, Stanton, 2016, Zimmermann, et al., 2015). In some circumstances, intratesticular testosterone can compensate for the insufficiency of FSH action (Abel, et al., 2008) . FSH and testosterone stimulate different aspects of spermatogenesis, showing both addictive and synergic pathways (Abel, et al., 2008). Without any doubt, however, qualitatively and quantitatively normal spermatogenesis does not occur in the absence of FSH action.

Is FSH efficacious for treating male idiopathic infertility?

In male idiopathic infertility, FSH may be empirically used to stimulate residual spermatogenesis and to improve sperm production when FSH serum levels are within the normal range (Barbonetti, et al., 2018, Colpi, et al., 2018). However, the clinical efficacy of FSH remains a matter of debate. Seventeen controlled clinical trials (too few) and four meta-analyses (too many) evaluated so far the efficacy of FSH administration to the male partner of infertile couples. Although meta-analyses are generally useful to achieve an overall view on FSH efficacy, they consider pregnancy rate as primary endpoint, introducing potential biases related to the female partner. Indeed, while an overall pregnancy rate increase was found in couples in which the man was treated

with FSH (Attia, et al., 2013, Attia, et al., 2007, Santi, et al., 2015), the number needed to treat (NNT) was high. From 10 to 18 men should be treated to achieve one pregnancy (Santi, et al., 2015), a number too large to recommend extensive use of FSH.

Anyhow, some effect is there and the elevated NNT could be due to inadequacy of the current FSH treatment regimen. Indeed, the FSH type, scheme and dosage used in clinical trials were highly heterogeneous (Table 1) and no conclusive results have ever been produced about the most effective FSH regimen. Recently, a dose-dependent sperm concentration and total sperm count improvement was described in a meta-analysis, which was, however, based on only three studies and a very limited number of patients (Cannarella, et al., 2019). With these limitations, the higher the FSH dose used, the greater was the increase in sperm production (Cannarella, et al., 2019). Thus, limited evidence suggests that high FSH doses may be useful to stimulate Sertoli cells, providing a stronger support to germ cells proliferation and maturation. Accordingly, when FSH was used at high dosages (300 IU daily) the NNT decreased to 12 and 6 for spontaneous and ART pregnancies, respectively (Ding, et al., 2015). Recently, we performed a nation-wide, clinical practice survey in Italy, showing that FSH administration leads to a significant increase in sperm concentration and morphology, when used at 150 IU on alternate days (Santi, et al., Submitted).

Similarly, to the FSH dose, also the treatment duration is not supported by strong scientific evidence. Spermatogenesis is a long process, requiring 72-90 days for completion. Thus, a prolonged stimulation would be required to increase sperm production. Whether the three months of treatment generally applied (i.e. the time needed to cover at least one spermatogenic cycle) are sufficient to obtain a clinical effect is not clear so far. We recently demonstrated that the sperm quality improvement after FSH administration in male idiopathic infertility is evident only after three months of therapy and continues after further three months of treatment withdrawal (Simoni, et al., 2016). This “hangover” effect is in line with the knowledge that FSH acts at the early

spermatogenic stages and suggests that the stimulation required to obtain a sustained improvement of the end product, i.e. the sperm, should cover more than only one spermatogenic cycle.

Finally, the end point to evaluate FSH efficacy remains challenging. While pregnancy and live birth rates are the strongest endpoints to assess efficacy of any infertility therapy, their strength is weakened by a number of hidden biases. Indeed, pregnancy rate is the final result of sperm and oocyte quality, combining the endogenous and exogenous variables interfering with gamete production in both sexes. In the absence of a direct, pharmacodynamic marker of FSH action, the evaluation of sperm production and quality is the only and most appropriate endpoint we have.

Notably, the current available guidelines recommend against the treatment of male idiopathic infertility without a specific diagnostic workup (Barbonetti, et al., 2018, Colpi, et al., 2018) and underline the lack of evidence in favour of a generalized beneficial FSH effect on male.

Responders and non-responders

Overall, meta-analyses suggest FSH efficacy but heterogeneity of patient populations results in weak statistics, which will never improve unless we are able to recognize *a priori* "responders" and "non-responders". This concept was introduced in this field over twenty years ago (Foresta, et al., 1998) and has been repeatedly applied *a posteriori* to analyze the results of FSH treatment (Foresta, et al., 2005, Foresta, et al., 2002, Foresta, et al., 2000, Foresta, et al., 2009). It is now clear, also from our recent survey of clinical practice in Italy involving the largest number of men treated with FSH so far, that 30-50% of the patients respond to the current, empirical FSH regimens with an improvement of sperm count (Santi, et al., Submitted). *De facto*, this is a rather crude parameter of FSH action, for which we still do not have any reliable pharmacodynamic marker.

Foresta et al. (Foresta, et al., 2005, Foresta, et al., 2002, Foresta, et al., 2000, Foresta, et al., 2009) proposed that patients with a spermatogenetic arrest are expected not to respond to FSH, the action of which is exerted mainly at the spermatogonial level. This concept did not receive any independent confirmation so far.

Assuming a dose-dependency of the response to FSH, another explanation could lie in the individual genetic background. It has been shown that common polymorphisms in the *FSHB* gene promoter, influencing gene transcription and, thereby, FSH production, and in the *FSHR* gene, influencing expression levels and signal transduction, can determine serum levels of FSH and, more in general, the amount of FSH activity to which the testis is exposed (Simoni and Casarini, 2014, Tuttelmann, et al., 2012). A pharmacogenetic approach to FSH treatment of male idiopathic infertility has been tried with contrasting results (Ferlin, et al., 2011, Selice, et al., 2011, Simoni, et al., 2016) and the correct approach is not established yet (Busch, et al., 2015, Schubert, et al., 2019). It is reasonable to think that the genetic background, perhaps involving a wider spectrum of genes than that explored so far, can influence response to FSH but the way is still long before the entire picture is revealed. An *a posteriori*, comprehensive genetic analysis of responders and non-responders could be useful to this purpose.

Whatever the explanation is (FSH regimen, spermatogenetic blockade, genetic background or other), the impossibility to recognize *a priori* responders and non-responders derives essentially from the intrinsic limitation of the instrument that we have to evaluate the infertile man, i.e. semen analysis. This is a very “primitive” examination, full of drawbacks and not indicative at all of the testicular phenotype, not even in case of azoospermia (Cito, et al., 2018). We need new instruments, possibly non-invasive, to characterize better the testicular phenotype of infertile men and to dissect the mess of “idiopathic infertility” into discrete subgroups characterized by common features at the testicular level. Radically new approaches, e.g. determination of nanoscale components of biological

samples (Ouyang and Han, 2019), comprehensive genetic analysis and markers (Cariati, et al., 2019, Hotaling and Carrell, 2014), big data analysis by machine learning (Santi, et al., 2018), ultrastructural in vivo imaging (Blazquez-Llorca, et al., 2015), are necessary to obtain some progress. Meanwhile, we must do with what we have, and a paradigm change in pharmacologic FSH treatment of male infertility should take place.

A paradigm change is necessary

Several lines of evidence support the concept that spermatogenesis can be boosted beyond the physiological level using larger FSH doses than those applied so far and for longer periods.

First of all, it seems that spermatogenesis does not run at its top. In the prepubertal human testis, FSH stimulates Sertoli cell proliferation, proliferation of A spermatogonia, cytoscheletal rearrangements (spermatogonia migration, tight junctions, etc..), increase in inhibin B and testis volume (Dwyer, et al., 2013). Once the number of Sertoli cells is fixed, FSH effect can only be exerted on spermatogonial proliferation. This is suggested by several animal models of hemicastration, which is usually followed by a compensatory increase in volume of the remaining testis. This increase is supported by a rise in endogenous FSH, driven by the decrease in inhibin B, a marker of spermatogonial-Sertoli interaction, following the removal of one testis (Bercovici, et al., 1985, Brown, et al., 1990, Cunningham, et al., 1978, Johnson and Neaves, 1983, Ramaswamy, et al., 2000). The effect of hemicastration is evident also in humans, although the picture is tarnished by the usual presence of a tumor (Leydig or germ cell tumor) in the removed testis (Bercovici, et al., 1985). In the primate, the compensatory FSH effect on the remaining, adult testis is stimulation of proliferation of B spermatogonia (Ramaswamy, et al., 2000).

Other human models suggesting that FSH overstimulation can boost spermatogenesis are pituitary FSH-secreting adenomas, which are usually accompanied by quite large testicular volumes,

decreasing after surgery even in the presence of normal LH and testosterone levels (Dahlqvist, et al., 2010). Activating *FSHR* mutations demonstrate the ability of FSH to fully support spermatogenesis in the absence of androgens (Casas-Gonzalez, et al., 2012, Gromoll, et al., 1996), even in the murine model (Oduwole, et al., 2018). As described above, *FSHB* and *FSHR* inactivating mutations in humans demonstrate that signal transduction pathways different from the canonical cAMP are essential for full FSH action and can support such residual activity (Tranchant, et al., 2011). This is an important concept, because today we know that the molecular action of FSH includes several signal transduction pathways, engaged in specific time- and dose-dependent manner and finely involved in the regulation of cell fate (proliferation vs. apoptosis) and metabolism (Casarini and Crepieux, 2019). Any modern appraisal of FSH-dependent events must consider this complexity. This might be, in principle, one molecular explanation why FSH in therapy is effective only in "responders". These men could be those in whom a relatively low dose of extra FSH is sufficient to stimulate the full spectrum of signal transduction.

We have been too cautious in attempting pharmacological FSH therapy for male idiopathic infertility. The treatment regimens used so far were kind of "substitutive", with the classical endocrinological approach used in hypogonadotropic hypogonadism. But men with idiopathic infertility are not hypogonadal, they have their own FSH but reduced spermatogenesis: treating them with a replacement dosage does not change essentially the situation and might be one reason why the current regimens are ineffective in 50% of the cases. However, remarkably, 50% of men respond in spite of the low dosage applied (Santi, et al., Submitted). This does not prove that FSH is ineffective, although statistically it may be so. This rather suggests that half of the men did not receive a pharmacological dose sufficiently high to challenge spermatogenesis over the baseline. Men with idiopathic infertility should not be considered "functionally hypogonadal" any longer: they

have a problem in spermatogenesis, which we do not understand (they are idiopathic), and need an extra stimulus with a different approach, rather than a gonadotropin replacement.

Together with the results of the few clinical studies conducted so far, these are the reasons why a paradigm change is necessary. We should adopt a more aggressive attitude and start trials based on higher FSH dosages for longer times. Boosting spermatogenesis over the baseline should be regarded as something alike controlled ovarian hyperstimulation, which, similarly, aims at obtaining more oocytes than normal. It is time to develop pharmacological testicular hyperstimuation protocols to be applied to men with idiopathic infertility and normal FSH levels.

Is pharmacological testicular hyperstimulation expected to be risky?

The clinical consequences of FSH hyperstimulation are quite different in males and in females. In women an excess of FSH may result in ovarian hyperstimulation syndrome (OHSS), as demonstrated by FSH-secreting tumors (Caretto, et al., 2017, Galway, et al., 1990, Macchia, et al., 2012, Snyder, 1987, Valimaki, et al., 1999) and activating *FSHR* mutations (Lussiana, et al., 2008), in addition to the OHSS which can result from ART. In men, this does not occur: the expected consequence of pharmacological doses of FSH is, if any, stimulation of spermatogenesis and testis volume over the baseline, which is exactly what we want to achieve. No serious adverse events were ever reported as a consequence of FSH replacement treatment in males and FSH producing tumors have no other effect than increasing testis size. Extragonadal effects of FSH are a very much debated issue (Chrusciel, et al., 2019, Lizneva, et al., 2019) and, for the time being, should not be a concern. The experimental evidence in humans demonstrates that FSH has no direct role in bone turnover or other metabolic functions (Crawford, et al., 2017, Drake, et al., 2010, Uihlein, et al., 2014).

Would a pharmacological FSH stimulation result in *FSHR* downregulation and, thereby, loss of FSH effect? There is no evidence *in vivo* that this would occur. FSH hyperstimulation in women can

result in OHSS, which would not happen if the receptor was downregulated. Actually, all endocrine syndromes resulting from an excess of pituitary hormones, e.g. due to a pituitary tumor, demonstrate that receptor down-regulation does not occur, or, if it occurs, is not sufficient to reduce the effects of the pituitary hormone excess. The only G protein-coupled receptor down regulation clinically known and exploited for therapeutic purposes is the GnRHR: superagonist analogs of GnRH are able to downregulate the receptor (after a flareup phase) and this is useful to shut down gonadotropin secretion. But the GnRHR is an exception. Rather than turning off signal transduction, a supraphysiologic FSHR stimulation is expected to activate all signal transduction pathways involved, including those entering in action after receptor internalization (Sayers and Hanyaloglu, 2018), permitting the full spectrum of FSH action, which might be defective in some cases of idiopathic infertility.

Which gonadotropin for pharmacological testicular hyperstimulation?

Historically, gonadotropins have been used already in the eighties in the attempt to stimulate spermatogenesis in men with idiopathic infertility with negative results. The studies conducted according to the golden design (prospective, double-blind, randomized, placebo-controlled) failed to demonstrate a clinical effect in the overall population investigated (Table 1). The lack of significant difference between *placebo* and *verum* group set every time a stop in further research on this issue for long years. There are several reasons why these studies failed: treatment protocol designed on analogy of the substitution treatment of hypogonadotropic hypogonadism (albeit for a much shorter time); insufficient dosage and duration of therapy, type of gonadotropin used, and, perhaps more importantly, impossibility of *a priori* patient stratification, i.e. personalization of therapy, a concept now widely applied in other fields of medicine. In any case, such studies clearly demonstrated that that sort of gonadotropin stimulation was overall ineffective,

at the regimen used, to improve sperm parameters. Now that we know more about FSH action, pharmacogenetics, dose-dependency of the effect, efficacy in some groups of patients, and possible effect on pregnancy rate, both spontaneous and after ART, we need to try another approach.

For the time being, the best option to test, in an appropriate prospective trial, the paradigm change proposed here is FSH alone. It might be recombinant or urinary, since both have been used with substantially equal results (Cannarella, et al., 2019, Santi, et al., 2015). The only difference between the existing FSH preparations is the route of administration (sc *vs.* im) which, given the required long duration of therapy, might pose a compliance problem (Cannarella, et al., 2019).

Not all existing FSH preparations are registered for use in the male. Highly purified urinary FSH, follitropin alfa, follitropin beta and biosimilars are registered for male hypogonadotropic hypogonadism and could be used already now and are indeed used in clinical practice in Italy (Santi, et al., Submitted). A meaningful increase in dosage and duration would require 300 IU three times/week or 150 IU daily for four-six months to cover at least two spermatogenetic cycles, which is a demanding schedule. This should be tested in a controlled clinical trial, which should, in addition, collect all information and be powered to stratify the patients *a posteriori*, e.g. sDF or genetic background. This will be the only way to personalize pharmacological FSH treatment of male idiopathic infertility. The feasibility of a placebo arm in such a long-lasting trial should be considered carefully. At least a no-treatment control arm must be included. Before such a trial is performed to ultimately demonstrate if pharmacological FSH doses really improve male fertility potential and in which patients, no meaningful pharmacoeconomic projections can be made.

The newest molecules, follitropin delta, follitropin gamma and chorifollitropin have a more favorable pharmacokinetics profile and would permit less injections. The ideal preparation should allow one injection/month. While a very-long-acting FSH is not suited for use in ART, it would be

ideal for treatment of male infertility/hypogonadism. New strategies are being attempted in this respect (Zhang, et al., 2016) and should be further pursued.

A crucial point in performing a trial of high-dose FSH treatment of male infertility is the collaboration of drug companies, which, unfortunately, are overtly uninterested. Those producing follitropin alfa see no advantage in embarking in a costly trial because their patent expired and the results could be exploited by any other company producing FSH. Follitropin delta and chorifollitropin could represent ideal drugs, having a longer half-life, but the respective companies do not see the business, partly because they do not believe that men would accept treatment, but there are other reasons. Gonadotropin companies are *de facto* driving the clinical use of their products, limiting their clientele to receptive doctors especially in ART centers, and do not intend to expand the spectrum of possible users. It is not a matter of scientific evidence, since gonadotropin use in ART is the least evidence-based treatment in medicine, it is a matter of marketing at large. It goes without need of complex calculations that the amount of FSH required for one cycle of ovarian stimulation (2,000-5,000 IU) is much less than the amount which would be used in men (approximately 10,000-30,000 IU). If FSH is really effective in improving semen quality and pregnancy rate, gonadotropins sales would increase (at least two-six times) but the ART business might decrease if spontaneous pregnancies occur, reducing the revenues of ART centers (Gleicher, et al., 2019). However, ART centers could become interested in different business models, involving male treatment. Here a mentality change is required, based also on accurate pharmacoeconomic calculations and ad hoc business cases, which so far, were not considered attractive enough by gonadotropin producing companies. In such a situation, the battle for trials to find the best treatment regimen of male infertility is lost from the outset.

Is there any role for other gonadotropins, e.g. hCG, hMG or LH? Currently, one popular hMG preparation is a 1:1 mixture of urinary FSH and “LH activity”, the latter consisting of both LH and

hCG. Other preparations consist of a 1:1 mixture of urinary FSH and hCG (no LH), while a further, recombinant hMG is in development by mixing recombinant FSH and recombinant hCG. Using hMG instead of FSH would only jeopardize the results of future clinical trials, not permitting to dissect the role of FSH alone and introducing another variable. A crucial problem is that, using hMG, the dosage of FSH cannot be increased without increasing LH activity as well, which means introducing the possibility of a Leydig cell overstimulation and excessive testosterone production and androgenization and suppression of endogenous gonadotropins. Therefore, future treatment protocols/clinical trials cannot be based on hMG.

There are some data suggesting that Leydig cell function may be reduced in men with idiopathic infertility, as indicated by the generally low (albeit still within the normal range) serum testosterone levels in these men (Andersson, et al., 2004, Begum, et al., 2016). Would these patients benefit from LH supplementation? The old studies using hCG (Knuth, et al., 1987) do not indicate this. However, hCG is not the natural Leydig cell-stimulating hormone in the adult and shows pro-inflammatory activity in the testis (Bergh, et al., 1996, Bergh, et al., 1993). Finally, we now know that hCG and LH have different molecular actions and are biased ligands for the same receptor exerting different functions (Casarini, et al., 2018). Therefore, should LH activity supplementation be required in some men, recombinant LH should be tested. The short half-life of this hormone might be an obstacle, although this should be investigated in a proper dose-finding, pharmacodynamic study in men. If LH will become a useful asset in treatment of male infertility in the future, radically new biomolecules, displaying the full spectrum of LH molecular activities, will need to be designed to allow more distant administrations, as mentioned above for FSH. Several studies in animal models showed a partial overlap between FSH and testosterone action on Sertoli cells (Oduwole, et al., 2018). The pharmacological action of FSH alone could be insufficient to compensate a concomitant missing

androgen action. The possible combined pharmacologic effect of FSH and LH on spermatogenesis should be evaluated more in details by properly designed clinical trials.

Conclusions: call for international action

Couple infertility treatment remains the most gender-unfair therapy known. Infertile couples are not aware of this problem and the ART professionals have no interest in sensitizing public opinion on this topic. Infertility treatment is confined to the private sphere and insufficient, serious, public research is conducted in this field. Infertility in general is not perceived as a medical problem in an overpopulated world, and there are no dedicated programs by granting agencies. The care for the infertile male falls into the more general problem of men's health, a topic that starts now to gain some attention by the scientific community (De Jonge and Barratt, 2019, Ravitsky and Kimmins, 2019, WHO, 2018).

ART works well but it is an industry-driven, rapidly and steadily evolving technology, so that we cannot estimate its long-term consequences. Every year more than 1 million ART cycles are performed in the world and about 300,000 ART children are born (Kushnir, et al., 2017). There is already some indication that boys conceived by ICSI for male infertility have reduced semen quality compared to naturally conceived controls (Belva, et al., 2017). Apart from this, epigenetic effects are more common in ART children and we do not have the full spectrum yet (Santi, et al., 2017). Meanwhile, thanks to the introduction of novel inventions (e.g. single embryo transfer, blastocyst culture, preimplantation genetic screening, time lapse, etc) and the enormous commercial interests behind them, ART performance is decreasing and the main goal of the big, private owners of ART clinics seems to be the revenue rather than clinical outcome. New technologies led to a rapid evolution of reproduction toward a very profitable, sex-free practice (Gleicher, et al., 2019). Despite ART is co-funded by public health services in several countries worldwide, in the vast majority of

ART centers around the world it remains in private hands. Thus, in these countries, the treatment seems to be possible only for wealthy people: this is unjust and overtly against the WHO SDG target 5.6 – gender equality/sexual and reproductive health (WHO, 2018).

The Scientific Societies in Andrology, Endocrinology and Reproductive Medicine are urgently called to action in several directions. We should change our attitude towards gonadotropin treatment of the idiopathic infertile male by performing a really powerful, multicenter, phase 2 trial, radically changing the cautious/skeptical approach used so far, with the aim of defining novel, evidence-based, personalized therapeutic regimens. If drug companies are deaf to this call, we should make aware of the problem the Medicines Agencies (EMA, FDA, National Agencies): they cannot agree with the current, empirical way of approaching couple infertility and should stimulate companies to support innovative trials. If industry does not want to get involved in fully sponsored, registration studies, they should support investigator-started trials.

Meanwhile basic researchers should invent new, really long-acting gonadotropins for the treatment of male infertility. We should sensitize patients' and women' organization to the problem of male infertility: women should not accept anymore to be treated instead of their partner if a medical treatment of the male is available. Finally, interdisciplinary collaborations should promote industry-independent, pharmacoeconomic studies, to show the real impact of male infertility care on the socio-economic and health system.

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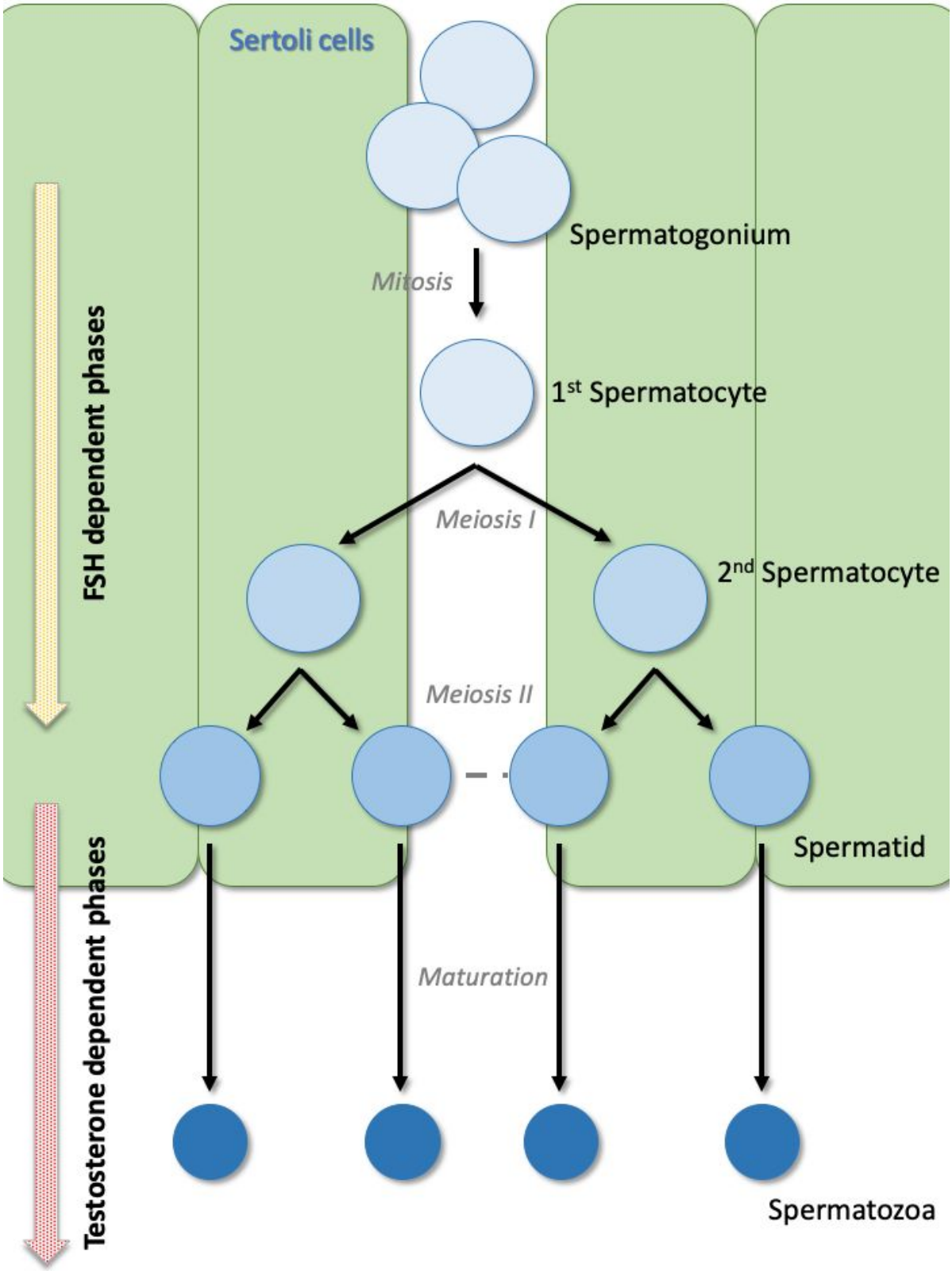
Table 1. FSH regimen used in clinical trials available in the literature.

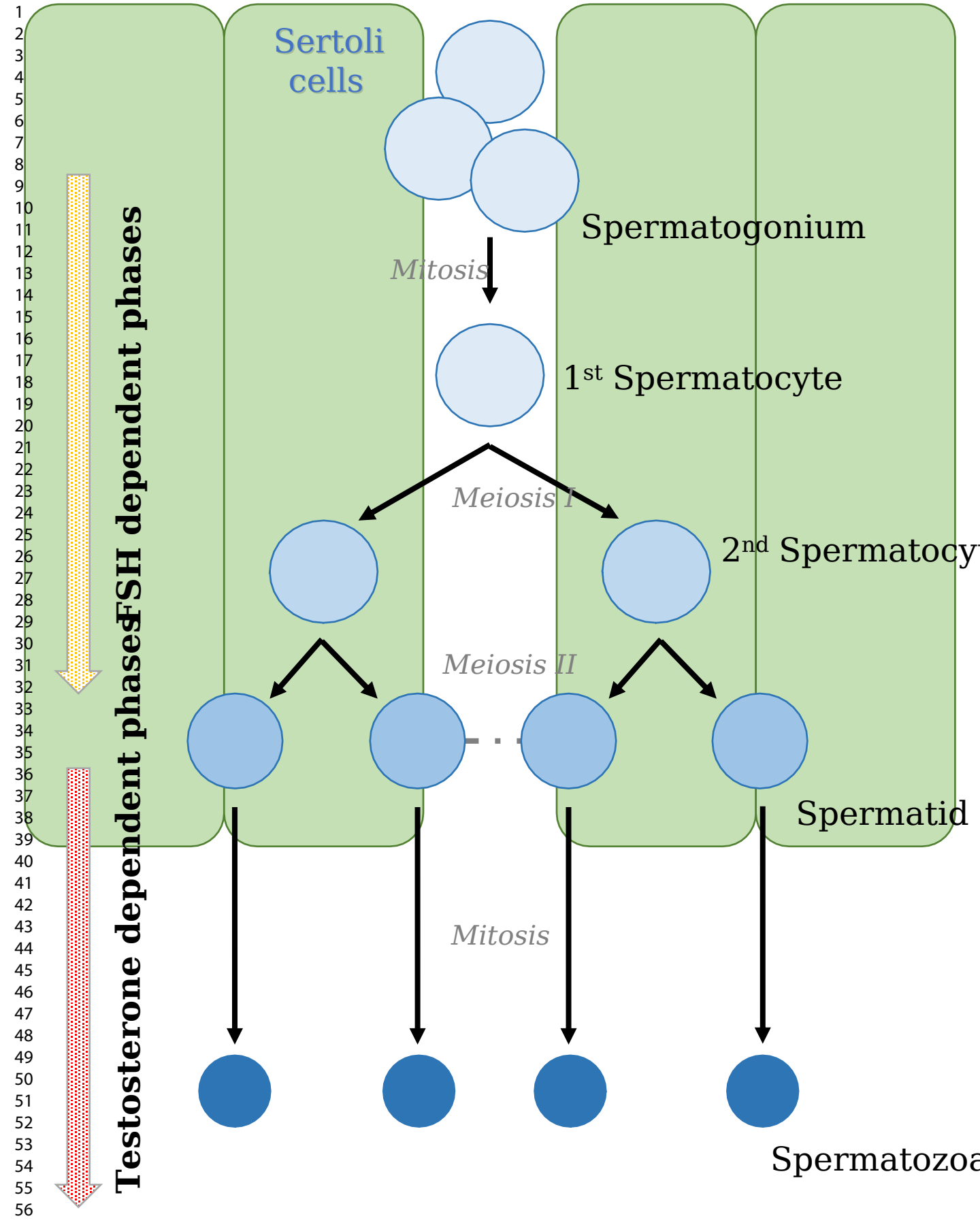
Author	Study design	Number of patients	FSH preparations	FSH scheme
(Ashkenazi, et al., 1999)	Observational	39/39	Urinary-derived FSH	75 IU daily
(Baccetti, et al., 2004)	RCT	24/20	Urinary-derived FSH	150 IU daily
(Bartoov, et al., 1994)	Observational	31/101	Urinary-derived FSH	75 IU daily
(Ben-Rafael, et al., 2000)	RCT	20/20	Urinary-derived FSH	75-150 IU daily
(Caroppo, et al., 2003)	Observational	23/23	Recombinant FSH	150 IU on alternate days
(Colacurci, et al., 2012)	RCT	65/63	Recombinant FSH	100 IU on alternate days
(Ding, et al., 2015)	RCT	272/82	Urinary-derived FSH	50-100-200-300 IU on alternate days
(Foresta, et al., 2000)	Observational	77/20	Urinary-derived FSH	75 IU on alternate days
(Foresta, et al., 2002)	RCT	30/15	Recombinant FSH	50-100 IU on alternate days
(Foresta, et al., 2005)	RCT	62/50	Recombinant FSH	100 IU on alternate days

(Foresta, et al., 2009)	RCT	57/62	Recombinant FSH	150 IU on alternate days
(Kamischke, et al., 1998)	RCT	34/33	Recombinant FSH	150 IU daily
(Knuth, et al., 1987)	RCT	19/20	hMG and hCG	75 IU and 2500 IU daily, respectively
(Matorras, et al., 1997)	RCT	58/78	Urinary-derived FSH	150 IU on alternate days
(Paradisi, et al., 2006)	RCT	15/15	Recombinant FSH	300 IU on alternate days
(Selice, et al., 2011)	RCT	70/35	Recombinant FSH	150 IU on alternate days
(Simoni, et al., 2016)	Non-RCT	38/28	Recombinant FSH	150 IU on alternate days

***[Footnote to Table 1]**: FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; hMG: human menopausal gonadotropin; RCT: randomized clinical trial.*

Figure 1. Spermatogenesis representation, showing the role of FSH and testosterone.




Sertoli cells
Spermatogonium
Mitosis
1st Spermatocyte
Meiosis I
2nd Spermatocyt
Meiosis II
Spermatid
Mitosis
Spermatozoa
FSH dependent phases
Testosterone dependent phases