Title

Use and effects of follicle-stimulating hormone (FSH) for idiopathic male infertility in Italy: results from a multicentre, observational, cohort, prospective, real world, clinical practice survey

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Running title:

Keywords: follicle-stimulating hormone, male idiopathic infertility, FSH, open registry

Abstract

Background: The management of the male partner of infertile couples remains largely heterogeneous, in particular in case of male idiopathic infertility. Although meta-analyses suggested that this approach is effective in terms of increasing pregnancy rate, the real impact of follicle-stimulating hormone (FSH) in clinical practice was not evaluated so far. In Italy, FSH is approved by the national Medicines Agency for use in idiopathic infertile patient with FSH levels < 8 IU/L. Accordingly, the Italian experience can provide useful data about the pharmacologic use of FSH in male idiopathic infertility.

Aim: Primary end point was to record the therapeutic approach to the male partner of infertile couples in Italian centres for andrology and/or assisted reproduction. Secondary aim was the assessment of FSH effect.

Materials and Methods: A multicentre, prospective, observational, clinical practice survey, based on real world data was carried out, enrolling couples attending ten participating centres across Italy from June 2016 to June 2019. Electronic case report forms were compiled, recording the clinical approach to the male partner of infertile couples. Inclusion criteria: man older than 18 years, with FSH serum levels lower than 8 IU/L, and no specific criteria for sperm parameters, excluding azoospermia. Primary end point was the number of infertile patients treated with FSH. Secondary outcomes collected were: sperm parameters, serum reproductive hormone levels, adverse events and pregnancy rate, when recorded. Investigators decided in autonomy whether to treat or not the male partner of the couple, and to re-evaluate the patient or not according to their clinical practice (the time-interval between visits was not set *a priori*).

Results: 718 infertile couples were enrolled and 241 patients were fully re-evaluated. In 64.9% (466 patients), a specific treatment was prescribed. FSH was prescribed in 397 patients (85.2% of men

who received a medical prescription). Sperm concentration (p=0.002) and normal form percentage (p<0.001) improved significantly during FSH administration. No significant correlation was found between these parameters and duration (p=0.545, p=0.846, p=0.627, respectively) or dosage (p=0.455, p=0.543, p=0.533, respectively) of FSH. Among patients treated with FSH, the incidence of oligozoospermia decreased from 73.0% to 56.0% (p<0.001), teratozoospermia from 43.6% to 27.7% (p <0.001), and asthenozoospermia from 76.6% to 70.4% (p=0.060) after FSH therapy.

Discussion: This first nation-wide survey reveals a FSH prescription rate of 55% in patients qualifying for treatment according to the Italian Drug Agency regulations. Within the limitations of the study design, FSH administration improves sperm concentration and morphology in about half of the treated men without adverse events.

Introduction

Male infertility pathogenesis remains unknown in about one in three infertile patients, representing the male idiopathic infertility category (Kamischke, et al., 1998). In these cases, empirical treatments, both hormonal and non-hormonal, are sometimes used in clinical practice (Kamischke, et al., 1998, Leifke and Nieschlag, 1996). Non-hormonal approaches include many different compounds with antioxidant and prokinetic properties, supported by variable degrees of clinical efficacy (Duca, et al., 2019). On the other hand, hormonal treatments include different empirical approaches, aiming to stimulate sperm production. Among hormonal options, follicle stimulating hormone (FSH) is used in some countries. In general, FSH plays an essential role in the development and maintenance of spermatogenesis (Huhtaniemi, 2015), and it stimulates spermatogenesis and influences sperm quality, and improving quality of acrosome, chromatin, mitochondria and axonema (Bartoov, et al., 1994). The pharmacological use of FSH could hypothetically further stimulate sperm production. However, since the physiologic regulation of spermatogenesis is not completely understood (Huhtaniemi, 2015) and many studies have reported maintained spermatogenesis despite low FSH levels or inactive FSH receptor (Oduwole, et al., 2018), the real effectiveness of FSH in male idiopathic infertility remains largely disputed. Thus, while FSH administration remains, together with hCG, the etiological approach to restore fertility in hypogonadotropic hypogonadal patients (Kliesch, et al., 1995), it represents an empirical treatment in idiopathic infertile patents.

The experimental evidence in idiopathic infertile patients suggests that FSH administration may result in a significant increase of total sperm number (Cannarella, et al., 2019, Colacurci, et al., 2018, Santi, et al., 2015). This improvement is accompanied by a higher pregnancy rate, both spontaneous (Attia, et al., 2013) and after assisted reproductive technique (ART) (Santi, et al., 2015),

although all studies and meta-analyses performed so far are based on very low numbers of patients. Indeed, the largest meta-analysis compared 482 treated with FSH and untreated men (Santi, et al., 2015). Despite the possible clinical efficacy suggested by meta-analyses, the overall evidence is weak, no registration trials were ever performed and FSH is not approved for male idiopathic infertility. In Italy, the note number 74 of the Italian Medicines Agency (AIFA) admits the use of FSH in infertile patients who want to conceive with hypogonadotropic hypogonadism and normal or low gonadotropin levels, with FSH < 8 IU/L (AIFA, 2010), permitting, not recommending, to use FSH in male idiopathic infertility, interpreted in this case as "functional" hypogonadotropic hypogonadism concerning FSH only. This means that Italian infertility clinics may prescribe FSH in-label when the male partner satisfies the indication. Different FSH preparations are commercially available, urinary or recombinant. AIFA does not recommend in favour or against a specific FSH preparation, and the clinician may select any formulation registered for hypogonadotropic hypogonadism. Other empirical treatments for idiopathic infertility (e.g. antioxidants, neutraceuticals, etc) are not reimbursed by the Italian national health system. To what extent FSH is prescribed in Italy for the improvement of sperm parameters to infertile couples attending infertility and andrological clinics is currently not known.

In order to evaluate FSH efficacy in male infertility treatment, randomized clinical trials (RCTs) should be performed, comparing FSH to placebo. In the absence of interest and support by the major FSH producing companies, due to high costs and difficulties in identifying the target patients, the collection of real world data could be useful to better understand how to focus future studies on the topic (Eichler, et al., 2019, Sherman, et al., 2016). We reasoned that the regulatory situation in Italy could allow the collection of data to evaluate the use and, possibly, the usefulness of FSH for male infertility. However, no national registry exists regarding male infertility and its treatment. Thus, we started a prospective, observational study to record the approach to infertile

couples, depicting the infertility treatments actually prescribed. The primary aim of this study is to understand to what extent FSH is used in Italian andrology and ART centres, in those couples whose male partner fulfils the AIFA note number 74 criteria.

Materials and Methods

A multicentre, longitudinal, prospective, observational clinical practice survey was carried out enrolling couples attending ten participating andrology or ART centres across Italy (Fig.1). Since infertile couple management is performed by either andrology or ART centres, we involved both these medical settings in the study. The present study is an example of a small-scale real world trial, managing data regarding the usage, the potential benefits and the risks of a specific drug, using sources other than traditional clinical trials (Sherman, et al., 2016).

The study protocol consisted in the compilation of an electronic case report form (eCRF) provided by Fullcro s.r.l. (Roma, Italy), recording the clinical approach to the male partner of infertile couples. All couples attending the participating Centres from June 2016 to June 2019 were evaluated for inclusion and exclusion criteria. The only inclusion criterion was: couples in which the male partner was potentially in indication for FSH treatment, according to AIFA note number 74. Thus, the male inclusion criteria were: (i) age older than 18 years, (ii) FSH serum levels lower than 8 IU/L, (iii) no specific criteria for semen parameters according to the world health organization (WHO) criteria (WHO, 2010). On the other hand, no specific inclusion/exclusion criteria were provided for the female partner. Specific exclusion criteria for the male partner were: (i) all known aetiologies of male infertility (endocrine disorders, genetic disorders, chromosome abnormalities, congenital bilateral absence of the vas deferens, microdeletions of the AZF region of the Y chromosome,

infections, immunological infertility, and obstructive infertility), (ii) azoospermia, and (iii) FSH serum levels > 8 IU/L.

Study endpoints

All clinical information routinely collected about attending couples were recorded. The primary end point of the study was the number of infertile couples in which the male partner was treated with FSH. Secondary outcomes were: sperm concentration, total sperm number, progressive motility, normal forms, serum reproductive hormone levels (when measured), and serious adverse events (SAE). Finally, pregnancy rate, occurring either spontaneously or after ART, was defined as hearth beat at ultrasound investigation of the female partner. This information, however, could not be considered for all cases, because it was not requested to the participating centres to follow up the patients in this respect.

Study design

This was an independent, investigator-started, observational, cohort study in which participating clinicians were asked to record how they managed the male partner of infertile couples. No specific drugs were provided and men could be treated according to the national/regional regulations and to the clinical practice of each participating centre. The investigators did not receive any remuneration.

If the male partner of the enrolled couples fulfilled inclusion criteria, an informed consent was obtained. History, physical examination, conventional semen analysis and hormonal

parameters were collected at baseline (V0) for each patient. Hormonal data collected were testosterone, luteinizing hormone (LH), FSH, estradiol and prolactin.

The investigators at each Centre decided whether to offer a medical treatment to the male partner of the couple, after andrological workup and according to their clinical practice. Any FSH preparations registered for hypogonadotropic hypogonadism could be used during the study. Similarly, individual clinicians decided autonomously whether to re-evaluate the patient (totally or in part) or not, and the time-interval between visits was not set *a priori*. The study design did not foresee specific visits. When the patient was re-evaluated, a second visit was performed, recording the time-interval since the V0 and all the secondary endpoints (V1). Finally, when the couple underwent ART, the patient could be re-evaluated thereafter, to register pregnancies and secondary endpoints (V2 - post-ART). As for common practice, not all men enrolled underwent V1 and V2.

All parameters were registered in an eCRF, including, in addition, the type of gonadotropin preparation, dosage and regimen chosen for each man, treatment duration and cumulative dosages. For the entire duration of the study, patients continued to have unprotected intercourses and the occurrence of spontaneous pregnancies was registered.

Sample size determination

Considering the observational study design, sample size determination was not needed and was not calculated *a priori*. The duration of the survey was fixed at three years.

Ethical

The local Ethics Committee of the University Hospital and province of Modena, approved the study protocol (prot. number 274/15). This was followed by approval by all ethics committees of the participating centers.

Statistical analysis

Since the primary objective of the study was the evaluation of the clinical practice in Andrology/ART Centers, descriptive statistics were performed, in order to evaluate how many men were treated according to AIFA note 74.

Then, secondary endpoints were considered. The parameter distribution was evaluated by Kolmogorov-Smirnov test. Treated patients were evaluated before and after treatment (V0 *versus* V1), performing ANOVA univariate analysis (for normally distributed values) and Mann-Whitney test (for non-normally distributed values). Simple and multivariate regression and correlation analyses were performed, in order to evaluate possible predictive variables of FSH response. Correlation analyses were performed comparing semen parameters to FSH duration and doses. Abnormal semen analyses were classified in (WHO, 2010): (i) oligozoospermia (sperm concentration < 15 millions/mL and total sperm number < 39 millions), (ii) asthenozoospermia (progressive motility < 32%), (iii) Teratozoospermia (normal forms < 4%). Moreover, the co-existence of the three abnormalities reported above, identified the oligo-astheno-teratozoospermia (OAT) condition (Colpi, et al., 2018).

Statistical analysis was performed using the 'Statistical Package for the Social Sciences' software for Windows (version 25.0; SPSS Inc., Chicago, IL). Data are expressed as median and interquartile range or mean $\pm$ standard deviation, where appropriate. For all comparisons, p values < 0.05 were considered statistically significant.

Results

Couples' descriptive analysis

The survey was closed after three years.

A total of 718 infertile couples were enrolled, and clinical characteristics are summarized in Table 1. Among these, 241 patients were fully re-evaluated at V1 and 90 partly re-evaluated at V2. For the V2 patients, semen analysis was available in 61 cases.

The mean age of the male partner (37.6 ± 6.5 years) was slightly higher than that of the female counterpart (34.7 ± 5.2 years). Infertility was defined as primary for 577 couples (80.4%) and secondary for 141 patients (19.6%) and the average duration of unprotected intercourses was 29.1 ± 24.0 months, ranging from 12 to 180 months. Ninety-two couples (12.8%) had already undergone at least one cycle of ART procedure at baseline (maximum 9 cycles). Eighty couples (11.1%) had a history of previous spontaneous miscarriages.

We calculated *a posteriori* a statistical power of 85%. The statistical power was calculated considering inequality proportions between two independent groups (Fisher's exact test). This result suggests that this study reflects with good accuracy the current clinical approach to infertile couples in which the men could be treated with FSH in Italy, without giving any evaluation of efficacy.

Baseline male partner characteristics

Considering clinical history, 17 (2.4%) patients had been treated for cryptorchidism. At physical examination, mean weight and BMI of male partners were 80.9 ± 11.6 kg and 25.9 ± 3.6 kg/m^2, respectively. Right and left testicular volumes were within the normal range (16.5 ± 5.3 mL and 16.5 + 5.4 mL, respectively). Varicocele was found in 158 patients (22.0%) on the left side, and in 17 patients (2.4%) on the right side.

During the diagnostic work-up for male infertility, 345 patients (48.1%) underwent genetic analysis, following both clinical indication and national rules for ART. In particular, chromosome Y microdeletions were searched in 206 patients (28.7% of the entire cohort), without detectable alterations. 321 patients (44.7%) had karyotype analyzed, resulting 46,XY in 320 patients and a balanced Robertsonian translocation (14;21) in one subject. This patient was excluded from the analysis. Finally, 10 out of the 130 patients (18.1%) undergoing CFTR gene analysis had heterozygous mutations but not obstructive azoospermia. Genetic tests were more frequently proposed in gynecology *vs.* andrology centers (p=0.004). Remarkably, comparing patients who received to those who did not receive genetic tests, no differences were obtained in any semen parameter (sperm concentration 24.1 $\pm$ 30.7 *vs.* 27.4 $\pm$ 44.5 millions/mL, p=0.079; progressive sperm motility 22.8 $\pm$ 19.3 *vs.* 28.2 $\pm$ 20.0%, p=0.082; normal forms 6.4 $\pm$ 5.4 *vs.* 5.5 $\pm$ 5.0%, p=00.113).

Baseline sperm and biochemical parameters

Semen analysis and biochemical parameters at V0 are shown in Table 1. Oligozoospermia was found in 392 patients (54.6%), asthenozoospermia in 418 patients (58.2%) and teratozoospermia in 234 patients (32.6%). Combining these seminal alterations, 165 patients (25.0%) showed OAT.

Male infertility management

In 64.9% of our cohort (466 patients), a specific treatment was prescribed, whereas in 252 patients (35.1%) there was no medical prescription (Figure 2). Overall, FSH was prescribed in 397 patients (55.3%, i.e. 85.2% of the men who received a medical prescription), with a more frequent use in Andrological (61.3%) compared to Gynecological (30.3%) Centers (p<0.001).

When FSH was prescribed, a recombinant preparation was chosen in 255 patients (64.2%) and a urinary-derived preparation in 142 patients (35.8%). Both FSH dosages and regimen were prescribed in compliance with the AIFA indications: 150 IU every other day (92.9 – 95.3% of relevance). On the other hand, in 27 patients (6.8%) FSH dosage was below the suggested dose, whereas only in one patient (0.3%) a higher dose (225 IU) was chosen. Daily injections were chosen for one patient (0.3%), while a longer interval among injections was chosen in 17 patients (4.3%). Sub-cutaneous injections were used in 342 patients (88.6%), the rest of the patients received i.m. administration.

FSH was prescribed alone in 341 patients (85.9% of the patients who received FSH prescription), whereas it was combined with other endocrine treatments in 15 patients (3.8%), with nutraceuticals in 40 patients (10.1%), and with both in one patient (0.2%) (Figure 2). Endocrine treatments different from FSH were prescribed alone in 2 patients (0.4% of men who received any prescription), whereas the use of nutraceuticals alone was prescribed to 67 patients (9.3%) (Figure 2). Non-FSH, endocrine therapy consisted of the use of hCG in 15 patients (3.2% of the patients who received any prescription) or clomiphene citrate (CC) in 2 patients (0.4%). hCG was prescribed together with FSH, whereas CC was used alone. When hCG was used together with FSH, serum testosterone levels were within the normal ranges (330.0 $\pm$ 114.2 ng/mL). Patients treated with FSH plus other endocrine treatments did not differ compared to those treated with FSH alone, considering sperm concentration (p=0.230), progressive motility (p=0.462), and normal forms (p=0.310). Considering nutraceuticals, more than 20 antioxidant mixtures were used. Supplementary table 1 shows the composition of the formulations prescribed.

Considering sperm parameters at baseline, a significant difference was found between treated vs. untreated men (Table 2). In particular, patients who were prescribed FSH showed lower semen volume (p<0.001), sperm concentration (p<0.001), progressive motility (p<0.001), and

normal forms (p<0.001). Patients treated with FSH had lower testicular volume at V0 compared to those left untreated (p=0.011). These results suggest that the clinician generally choses to treat with FSH patients with impaired sperm parameters. This is evident from Figure 3, in which the individual patients and treatments are plotted in ascending order, according to sperm concentration detected at baseline.

Patients treated with nutraceuticals alone or combined to FSH did not receive a full evaluation at V1. Thus, an analysis of the effect of nutraceuticals on sperm parameters was not possible.

Effects of FSH administration on sperm parameters

After baseline evaluations, a total of 241 patients treated with FSH alone were evaluated at V1. Mean interval between V0 and V1 was 4.7 ± 2.1 months. Mean duration of FSH treatment was 6.76 ± 4.88 months with a cumulative dose of 14,729.13 ± 10,690.85 IU corresponding to 73 UI/day, in line with the AIFA note 74. No adverse events were recorded during the study. Treatment adherence was not evaluated.

Sperm parameters before and during FSH treatment are reported in Table 3. Sperm concentration (p=0.002), and normal form percentage (p<0.001) improved significantly during FSH administration (Table 3). Considering that not all treated patients were re-evaluated, the analysis was repeated considering only patients evaluated at both V0 and V1, confirming the sperm concentration (p=0.001) and normal form percentage (p=0.001) increase. No significant correlation was found between these two seminal parameters and duration of FSH treatment (p=0.545, p=0.846, p=0.627, respectively) or FSH dosage (p=0.455, p=0.543, p=0.533, respectively).

Among patients treated with FSH, the incidence of oligozoospermia decreased significantly from 73.0% at baseline to 56.0% at V1 (p<0.001). Similarly, the incidence of teratozoospermia

decreased significantly from 43.6% to 27.7% (p <0.001). Accordingly, the percentage of patients with OAT decreased from 35.8% to 21.5% after FSH administration (p=0.002). Asthenozoospermia decreased from 76.6% at baseline to 70.4% after FSH therapy, without any significant difference (p=0.060).

Over half of the FSH-treated patients (51.8%) showed a sperm concentration increase higher than 30% of the baseline value. This subgroup of patients, at baseline, showed lower total sperm count (p<0.001), sperm concentration (p<0.001) and progressive sperm motility (p=0.001) compared to the rest (Supplementary table 2).

ART procedure and pregnancies

Ninety out of 718 patients (12.5%) underwent ART. Intra-cytoplasmic sperm injection (ICSI) was the most frequently performed technique (55 patients, 61.1%), while intra-uterine insemination (IUI) the least frequent (5 patients, 5.5%). During the entire study duration, 23 spontaneous (on 241 that returned to V1) and 7 ART (on 90 that returned to V2) pregnancies were recorded. Among these pregnancies, spontaneous miscarriage occurred in 13 couples. However, since the centers were not requested to follow up the patients' outcome in terms of pregnancies, this data is incomplete.

Discussion

This is the first description of the clinical management of male idiopathic infertility in Italy. Considering the current difficulty to perform a RCT (no interest of the drug companies, no public funds available for male infertility), we reasoned that, a survey based on real world data might provide useful information, possibly helping in refining the criteria to use for future interventional trials. This observational study is the first attempt to describe the clinician's attitude in front of the male partner of infertile couples in a representative sample of infertility centres in a country where gonadotropins can be prescribed in-label for male infertility. In this cohort, 65% of men received a medical prescription after the first visit. This is remarkable, because according to the current guidelines, there is no evidence-based therapy for the male in infertile couples, not even in case of male factor, e.g. oligozoospermia and other alterations of semen parameters (Barbonetti, et al., 2018, Colpi, et al., 2018). To what extent this high rate of prescription is an Italian attitude towards the infertile couple remains to be determined. However, it shows that men are eager to be involved in couple infertility treatment, even with long lasting injection therapy.

All men included in this study were theoretically eligible for treatment with FSH according to AIFA, which does not specify any sperm parameter characteristics. However, clinicians obviously judged that not all males with idiopathic infertility potentially compliant with the AIFA rule are candidates for a therapy, since this opportunity was used in 55% of them, largely representing the cases with more altered seminal parameters. Therefore, the clinician decides whether to treat or not, primarily based on conventional semen analysis results, with FSH being the preferred choice in 85.2% of treated men. Our study also shows a clear difference in male management depending on the clinician background. In fact, in andrological centres, FSH was prescribed in 61.3% of the patients but only in 30.3% in ART centres. In the latter, couple management is generally handled by the

gynaecologist alone, confirming the secondary role attributed to the male by gynaecologists without an andrological background. Moreover, in this setting, other parameters leading to this discrepancy could be considered, such as the female partner characteristics.

When prescribed, FSH is used according to AIFA recommendations in 95% of cases. In this regard, clinicians follow the national, regulatory and scientific societies' guidelines. Despite the lack of clear-cut scientific evidences supporting FSH use in male infertility, the Italian Society of Andrology and Sexual Medicine (SIAMS) and the European Academy of Andrology (EAA) suggest that FSH may be empirically used in *selected cases* of idiopathic male infertility (Barbonetti, et al., 2018, Colpi, et al., 2018), although criteria for patient selection are not unequivocal. Here, FSH is used at the AIFA/SIAMS suggested regimen, concerning dosage and duration of treatment, while patients are empirically selected according to the semen parameters.

In spite of limits proper to purely observational studies, this is the largest collection of idiopathic infertile patients ever treated by FSH and the analysis of secondary end points suggests that FSH may be effective in improving some sperm parameters. In particular, despite only 69% of treated patients were re-evaluated, an improvement was demonstrated, using the suggested doses of 150 IU three times weekly, in terms of sperm concentration and morphology. Since we do not have any information about the female partner, we cannot conclude anything about couple fertility improvement after treatment. Moreover, treatment adherence might have affected the results. However, it is interesting to note that FSH administration overall reduces the oligozoospermia and teratozoospermia incidence after treatment, even if the data at this time point are not complete. This suggests that FSH treatment might be much more effective than what could be shown here, reinforcing the need of a RCT powered to resolve this very controversial issue.

It is presently unclear if idiopathic infertile patients might benefit from higher FSH dosages than those used here. Although several FSH preparations, administration schemes (from daily to weekly) and dosages have been tested, the current FSH treatment regimen remains empirical. It is possible that higher doses/longer durations might be more effective (Ding, et al., 2015). This is suggested by a recent meta-analysis, considering five randomized clinical trials, although based on a low number of patients (372 treated with FSH and 294 non-treated) (Cannarella, et al., 2019).

In our cohort, recombinant FSH preparations are preferred over urinary-derived FSH. This does not appear to be related to the different reimbursement rules of the Italian Regional Health Systems, since no clear regional trends were detectable. The choice between recombinant and urinary-derived FSH preparations might depend more on the formulation, selecting the most comfortable treatment for the patient, in order to increase compliance. Finally, FSH is prescribed together with hCG or nutraceuticals in 15.1% of men treated. Antioxidants seem useful to reduce sperm DNA fragmentation index (sDF), potentially improving the FSH beneficial action on spermatogenesis (Colacurci, et al., 2018, Muratori and Baldi, 2018, Muratori, et al., 2019, Muratori, et al., 2019). Hypothetically, the combined treatment could act both on the first phases of spermatogenesis with FSH (i.e. on spermatogonia), and on the last developmental steps of spermatocytes, with the antioxidant action, but this requires experimental demonstration in clinical trials and this survey did not provide data useful here for.

In conclusion, we have conducted a nation-wide survey, recording the real world management of the male partner of infertile couples in Italy. This revealed a FSH prescription rate of 55% in men qualifying for treatment according to the Italian Drug Agency regulations and, as secondary outcome, showed some efficacy of FSH treatment on sperm parameters in about half of the treated men without adverse events. Since FSH treatment of male idiopathic infertility is a

much-debated issue, our study reinforces the urgent need of a prospective randomized controlled trial assessing FSH efficacy on large numbers of patients, evaluating dose- and time-dependency of the therapy.

Acknowledgements

The study was partly supported by the “Department of Excellence” Programme of the Italian Ministry of University and Research (art. 1, 324 e 331 law 11 December 2016, n. 232) and own, unrestricted research funds of the principal investigator.

MS is a LE STUDIUM RESEARCH FELLOW, Loire Valley Institute for Advanced Studies, Orléans & Tours, France—INRA—Center Val de Loire, 37380 Nouzilly, France, receiving funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 665790.

References

AIFA. Note 74: Italian Medicines Agency, 2010.

Attia AM, Abou-Setta AM, Al-Inany HG. Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst Rev. 2013:CD005071.

Barbonetti A, Calogero AE, Balercia G, Garolla A, Krausz C, La Vignera S, Lombardo F, Jannini EA, Maggi M, Lenzi A, Foresta C, Ferlin A. The use of follicle stimulating hormone (FSH) for the treatment of the infertile man: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). J Endocrinol Invest. 2018;41:1107-1122.

Bartoov B, Eltes F, Lunenfeld E, Har-Even D, Lederman H, Lunenfeld B. Sperm quality of subfertile males before and after treatment with human follicle-stimulating hormone. Fertil Steril. 1994;61:727-34.

Cannarella R, La Vignera S, Condorelli RA, Mongioi LM, Calogero AE. FSH dosage effect on conventional sperm parameters: a meta-analysis of randomized controlled studies. Asian J Androl. 2019.

Colacurci N, De Leo V, Ruvolo G, Piomboni P, Caprio F, Pivonello R, Papaleo E, La Verde E, Depalo R, Lispi M, Longobardi S, Paoli D, Pallotti F, Lombardo F. Recombinant FSH Improves Sperm DNA Damage in Male Infertility: A Phase II Clinical Trial. Front Endocrinol (Lausanne). 2018;9:383.

Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, Krausz C, Giwercman A. European Academy of Andrology guideline Management of oligo-astheno-teratozoospermia. Andrology. 2018;6:513-524.

Ding YM, Zhang XJ, Li JP, Chen SS, Zhang RT, Tan WL, Shi XJ. Treatment of idiopathic oligozoospermia with recombinant human follicle-stimulating hormone: a prospective, randomized, double-blind, placebo-controlled clinical study in Chinese population. Clin Endocrinol (Oxf). 2015;83:866-71.

Duca Y, Calogero AE, Cannarella R, Condorelli RA, La Vignera S. Current and emerging medical therapeutic agents for idiopathic male infertility. Expert Opin Pharmacother. 2019;20:55-67.

Eichler HG, Bloechl-Daum B, Broich K, Kyrle PA, Oderkirk J, Rasi G, Santos Ivo R, Schuurman A, Senderovitz T, Slawomirski L, Wenzl M, Paris V. Data Rich, Information Poor: Can We Use Electronic Health Records to Create a Learning Healthcare System for Pharmaceuticals? Clin Pharmacol Ther. 2019;105:912-922.

Huhtaniemi I. A short evolutionary history of FSH-stimulated spermatogenesis. Hormones (Athens). 2015;14:468-78.

Kamischke A, Behre HM, Bergmann M, Simoni M, Schafer T, Nieschlag E. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. Hum Reprod. 1998;13:596-603.

Kliesch S, Behre HM, Nieschlag E. Recombinant human follicle-stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadotropic male. Fertil Steril. 1995;63:1326-8.

Leifke E, Nieschlag E. Male infertility treatment in the light of evidence-based medicine. Andrologia. 1996;28 Suppl 1:23-30.

Muratori M, Baldi E. Effects of FSH on Sperm DNA Fragmentation: Review of Clinical Studies and Possible Mechanisms of Action. Front Endocrinol (Lausanne). 2018;9:734.

Muratori M, Marchiani S, Tamburrino L, Baldi E. Sperm DNA Fragmentation: Mechanisms of Origin. Adv Exp Med Biol. 2019;1166:75-85.

Muratori M, Tarozzi N, Carpentiero F, Danti S, Perrone FM, Cambi M, Casini A, Azzari C, Boni L, Maggi M, Borini A, Baldi E. Sperm selection with density gradient centrifugation and swim up: effect on DNA fragmentation in viable spermatozoa. Sci Rep. 2019;9:7492.

Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of Follicle-Stimulating Hormone in Spermatogenesis. Front Endocrinol (Lausanne). 2018;9:763.

Santi D, Granata AR, Simoni M. Follicle-stimulating hormone treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocr Connect. 2015.

Sherman RE, Anderson SA, Dal Pan GJ, Gray GW, Gross T, Hunter NL, LaVange L, Marinac-Dabic D, Marks PW, Robb MA, Shuren J, Temple R, Woodcock J, Yue LQ, Califf RM. Real-World Evidence - What Is It and What Can It Tell Us? N Engl J Med. 2016;375:2293-2297.

WHO. WHO laboratory manual for the examination and processing of human semen. 5th ed.; 2010.

Table 1. Baseline characteristics of male partners of the entire cohort. Data are expressed as median and interquartile range.

n 718	**Normal range**	**Median (interquartile range)**
Age (years)	-	38.0 (34-42)
Weight (kg)	-	80.0 (73.0-87.0)
BMI (kg/m^2)	19-25	25.2 (23.4-27.5)
Testicular volume – right (mL)		16.0 (13.0-21.0)
Testicular volume – left (mL)		16.0 (12.0-21.0)
Semen parameters		
Volume (mL)	> 1.5	2.6 (2.0-3.5)
pH	> 7.2	7.6 (7.5-8.0)
Sperm concentration (millions/mL)	> 15	10.4 (4.0-30.0)
Total sperm count (millions)	> 39	24.5 (6.0-75.0)
Progressive motility (%)	> 32	20.0 (5.0-40.0)
Normal form (%)	> 4	5.0 (2.0-10.0)
Hormonal variables		
FSH (mIU/mL)	1-12	4.4 (3.0-6.2)
LH (mIU/mL)	1-9	3.5 (2.6-4.7)
Total testosterone (ng/dL)	> 320	459.5 (351.7-589.2)
Estradiol (pg/mL)	< 50	26.0 (20.0-34.0)
Prolactin (ng/mL)	3-13	9.9 (7.4-13.2)

[footnote to Table 1] *BMI: Body Mass Index; FSH: follicle stimulating hormone; LH: luteinizing hormone.*

Table 2. Baseline semen and hormonal characteristics comparing patients who received any therapy to patients who did not. Data are expressed as median and interquartile range. Data were analyzed by Mann-Whitney U-test.

	Patients who received treatment (n=397)	**Patients who did not receive treatment (n=252)**	
Semen parameters	**Median (interquartile range)**	**Median (interquartile range)**	*p-value*
Volume (mL)	2.5 (2.0-3.5)	2.6 (2.0-3.8)	**0.046**
pH	7.6 (7.5-8.0)	7.6 (7.5-8.0)	0.523
Sperm concentration (millions/mL)	7.2 (2.0-13.8)	27.5 (9.0-58.0)	**<0.001**
Total sperm count (millions)	12.6 (4.0-30.0)	75.3 (24.0-158.7)	0.072
Progressive motility (%)	10.0 (0.6-22.2)	40.0 (20.0-50.0)	**<0.001**
Normal form (%)	3.0 (1.0-6.2)	7.0 (4.0-12.0)	**<0.001**
Hormonal variables			
FSH (mIU/mL)	4.5 (3.0-5.9)	4.2 (2.9-6.5)	0.444
LH (mIU/mL)	3.4 (2.5-4.7)	3.5 (2.7-4.7)	0.217
Total testosterone (ng/dL)	460.0 (356.7-601.2)	451.0 (344.7-570.7)	0.357
Estradiol (pg/mL)	25.2 (20.0-34.0)	26.8 (20.0-34.0)	0.631
Prolactin (ng/mL)	9.7 (7.3-13.5)	10.0 (7.5-13.0)	0.893

[footnote to Table 2] *FSH: follicle stimulating hormone; LH: luteinizing hormone.*

Table 3. Differences between seminal parameters of patients treated with FSH alone assessed before and during therapy. Data are expressed as median and interquartile range. Data were analyzed by Mann-Whitney U-test.

Seminal paramenters	**Before FSH therapy** *(n 351)*	**During FSH therapy** *(n 241)*	**p-value**
Semen volume (mL)	2.5 (2.0-3.5)	2.7 (2.0-3.0)	0.606
pH	7.6 (7.5-8.0)	7.6 (7.4-8.0)	0.046
Sperm concentration (millions/mL)	7.2 (2.0-13.8)	10.0 (2.7-20.0)	**0.002**
Total sperm count (millions)	12.6 (4.0-30.0)	14.7 (4.5-42.5)	0.287
Progressive motility (%)	10.0 (0.6-22.2)	10.0 (3.0-20.0)	0.659
Normal form (%)	3.0 (1.00-6.2)	5.0 (3.0-8.7)	**<0.001**

[footnote to Table 3] *FSH: follicle stimulating hormone.*

Supplementary table 1. Molecular composition of antioxidant mixtures used in our cohort.

	L-Arginine	L-Carnitine	L-Ornitine	L-Citrulline	L-Taurine	N-acetil-cysteine	Inositol	Co-Enzyme Q10	Zinc	Selenium	Silicium	Magnesium	Glutathione	Licopene	Ac. Lipoic	Ac. Aspartic	Vit C	Vit E
Eufert Q10 (n=2)	x	x				x		x	x	x								
Androlen (n=2)		x				x	x	x	x	x	x	x		x	x			
Chiromannac (n=1)						x	x		x	x								
FertiPlus (n=3)						x			x	x			x		x		x	x
Andrositol Plus (n=6)	x	x				x	x			x								x
CarnitineQ 10 Plus (n=1)		x				x		x										x
PiùFertil Forte (n=1)	x	x						x	x	x			x					x
Gametogen (n=1)							x	x	x	x					x			
Andrositol (n=9)	x	x					x			x								x
Convis (n=1)	x	x	x						x									
FertiMEV Gold (n=1)	x	x						x	x					x		x	x	x
Gyfert (n=1)	x	x						x	x		x	x		x			x	x
Androfix (n=1)	x			x					x									
Lepijet (n=1)	x								x		x	x						
Curmanox (n=2)									x					x				
FertiPlus Sod (n=2)									x				x					
Genadis (n=14)								x	x							x		
Idiprost (n=4)									x									
FertiMEV (n=2)	x	x																
SperginForte (n=8)	x	x	x	x	x			x									x	x
SperginQ10 (n=9)	x	x	x	x				x										x
Carnitene (n=12)		x																
Argifast (n=16)	x						x	x									x	x
Gixoflog (n=1)														x			x	x

Supplementary Table 2. comparison of semen parameters between V0 and V1, dividing patients in responders and non-responders, considering the 30% of baseline of total sperm count as threshold

after treatment. Data are expressed as median and interquartile range. Data were analyzed by Mann-Whitney U-test.

Seminal paramenters	**Responders** (n=177)			**Non-responders** (n=164)		
	Before FSH therapy	**During FSH therapy**	**p-value**	**Before FSH therapy**	**During FSH therapy**	**p-value**
Semen volume (mL)	2.4 (2.0-3.5)	2.7 (2.0-3.0)	0.181	2.7 (2.0-3.5)	2.6 (2.0-3.2)	0.871
pH	7.5 (7.5-8.0)	7.6 (7.5-8.0)	0.657	7.8 (7.5-8.0)	7.6 (7.5-8.0)	0.704
Sperm concentration (millions/mL)	5.7 (2.0 – 20.3)	15 (2.7 – 42.1)	**0.001**	6.2 (2.0 – 20.1)	4.4 (2.6 – 25.2)	0.677
Total sperm count (millions)	9.1 (4.0 – 30.0)	27.6 (4.5 – 56.6)	**<0.001**	11.7 (4.0 – 24.5)	8.5 (4.1 – 30.1)	0.613
Progressive motility (%)	5.0 (0.6 – 21.0)	15.0 (2.2 – 24.2)	**0.004**	10.0 (0.7 – 20.1)	8.0 (0.9 – 19.0)	0.702
Normal form (%)	4.0 (1.1 – 6.0)	6.0 (3.2 – 9.1)	**0.023**	3.0 (0 – 5.8)	4.0 (0 – 6.3.0)	0.179

[footnote to Table 4] *FSH: follicle stimulating hormone.*

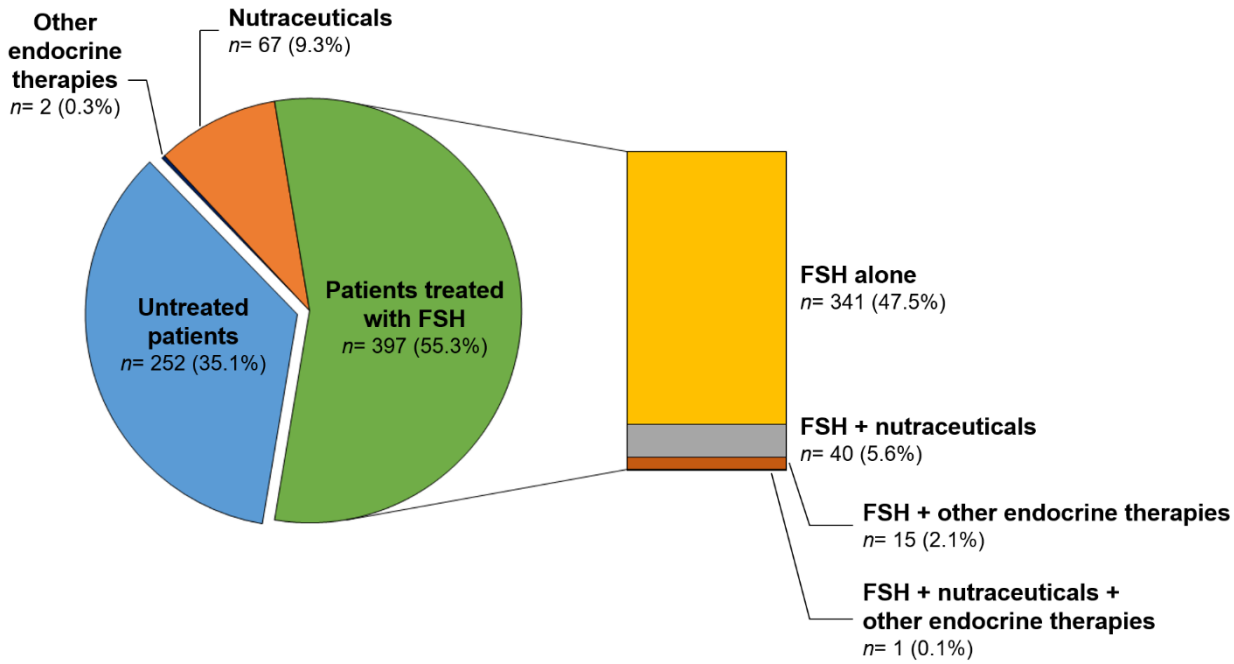
Figure legend

Figure 1. Andrology/ART Centres included in the study across Italy.

City	Institution
	Andrology Centres
Ancona	Endocrinology, Department of Clinical and Molecular Sciences, Polytechnic University of Marche
Catania	Department of Clinical and Experimental Medicine, University of Catania
Modena	Unit of Endocrinology, Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia
Naples	Unit of Endocrinology, Department of Clinical Medicine and Surgery, Federico II University of Naples
Rome	Unit of Reproductive Physiopathology and Andrology, Sandro Pertini Hospital
	Laboratory of Seminology-Sperm Bank Loredana Gandini, Department of Experimental Medicine, Sapienza University of Rome
	ART Centres
Florence	Assisted Reproductive Technology Centre, Careggi Hospital, University of Florence
Naples	S. Maria delle Grazie Hospital, ASL Napoli 2 Nord, Località La Schiana, Pozzuoli
Palermo	Reproductive Medicine Unit, ANDROS Day Surgery Clinic
Rome	S. Anna Center for Women and Children' Health

[Footnote to Figure 1] *ART: Assisted Reproductive Technique.*

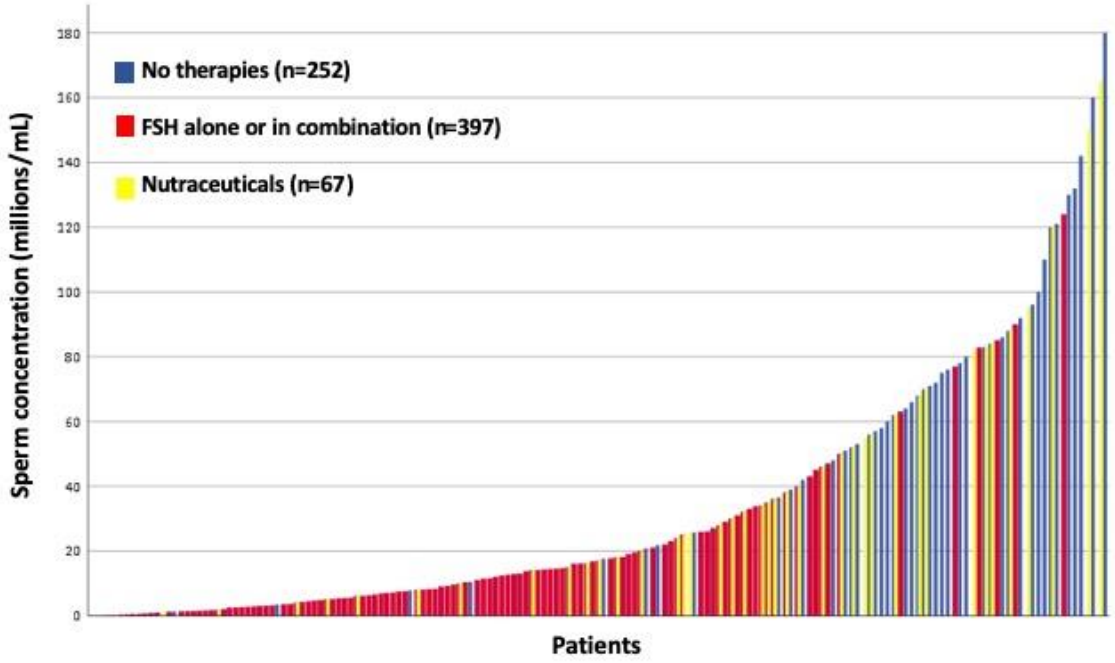
Figure 2. Therapeutic choice within the entire cohort of enrolled couples.



[Footnote to Figure 2] *FSH: follicle stimulating hormone.*

Figure 3. Therapeutic choice for the study population, plotted by increasing sperm concentration.

Each vertical line represents one patient.



[Footnote to Figure 3] *FSH: follicle stimulating hormone.*