Retrospective analysis of treatments with recombinant FSH and recombinant LH versus human menopausal gonadotropin in women with reduced ovarian reserve

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Abstract

Purposes The aim of this study is to determine whether a clinical advantage is gained with use of LH in combination with FSH or as a component of human menopausal gonadotropin (hMG) to achieve optimal ovarian stimulation.

Methods In this study, we compared retrospectively two regimens, r-FSH/r-LH and hMG, for the treatment of women with reduced ovarian reserve, identified as subjects with antral follicle count (AFC) < 11 and AMH ≤ 1.1 ng/ml.

Results Overall, the clinical pregnancy per started cycle was higher in the r-FSH/r-LH group (12.5 vs. 8.1%, $P < 0.02$), while implantation (11.1 vs. 9.5%) and miscarriage rates (29.9 vs. 35.9%) were comparable. Data were further analysed performing separate comparisons in subpopulations with different ranges of AFC, i.e. < 4, 4–6 and 7–10. Major differences between the two regimens were observed in women with AFC < 4. In this subpopulation, not only was the clinical pregnancies per started cycle higher in the r-FSH/r-LH group (10.2 vs. 1.5%, $P < 0.01$), but also implantation was significantly higher (13.0 vs. 2.8%, $P < 0.02$).

Conclusions A r-FSH/r-LH regimen appears to be beneficial for the treatment of women with extremely poor ovarian reserve. It should be considered however that, being retrospective, this study is affected by obvious limitations, such as post-treatment patient selection criteria and absence of randomisation.

Keywords IVF · LH · FSH · hMG · Ovarian reserve · Poor responders · Ovarian stimulation · Pregnancy

Background

Follicle growth can be obtained with different preparations or combinations of gonadotropins that however must include FSH, purified or recombinant, but not necessarily LH or LH-like molecules [1–3]. The dispensable use of LH in ovarian stimulation schemes is surprising in many ways. In fact, during the menstrual cycle, serum levels of LH follow a precise pattern that is suggestive of a specific role during the final stages of folliculogenesis, irrespective of the importance of mid-cycle surge to trigger ovulation [4]. Levels, although low, of LH observed during most of the luteal phase and the beginning of the follicular phase are believed to be instrumental for the production of androgen by theca cells, a hormone that is afterwards converted into estradiol by granulosa cells [5]. Progressively higher levels of LH during the second half of the follicular phase appear instead to subserve other needs. While approaching the size of approximately 10 mm, the leading follicle becomes in fact increasingly sensitive to LH, which at this stage is able to elicit many of the effects of FSH, while the support of FSH to follicular growth decreases. Together, these changes allow the selection and dominance of a single follicle and emphasise the crucial role of LH as an element complementary to FSH and essential in final analysis for oestrogen production [6].

Together with FSH in the formulation of human menopausal gonadotropin (hMG), LH has been used extensively to achieve ovarian stimulation for decades. However, hMG is rather inadequate to establish the specific merit, if any, of LH in qualitative and quantitative aspects of follicle

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development in ART treatments. In fact, hMG is nominally characterised by a fixed FSH:LH dose ratio (75:75 IU). However, purification processes required for the preparation of commercial formulations cause preferential loss of LH activity, which is compensated with the supplementation of pharmacologically significant quantities of hCG [7], a hormone having an action similar, but not identical, to LH. Therefore, in the impossibility to discriminate between effects caused by LH or hCG, the use of hMG has had a misleading influence on the study of LH in ovarian stimulation.

The more recent release of recombinant gonadotropins has created much more favourable opportunities to understand and exploit different roles of FSH and LH in multifollicular development. In one of the first experiences with r-LH, Durnerin and colleagues [8] observed that in ART cycles, administration of r-LH during the first 7 days of cycle and prior to stimulation with FSH produced an increase in the number of antral follicles and yield of normally fertilised oocytes, although no changes in hormone profiles were reported. These observations, no matter how preliminary, generated the expectation that the use of r-LH could indeed mark a progress in ovarian stimulation. However, many other studies undertaken in the following years until now have not confirmed unambiguously a clinical advantage of the use of LH [9–12].

Overall, these experiences have generated the prevailing impression that the use of r-LH is rather neutral for the treatment of normo and high responders [13], women in whom antral follicles amenable to stimulation are abundant or numerous, and normal compensatory mechanisms acting during final follicle development can make LH action redundant or not essential. Instead, there seems to be relatively more consensus on the hypothesis that patients with defective or inefficient folliculogenesis due to ageing or other intrinsic causes may benefit from exposure to LH during ovarian stimulation [14]. However, older-aged women can have rather different ovarian reserve and, on another hand, women of different age can be equally affected by poor ovarian reserve [15]. This implies that studies on possible beneficial effect of LH should be conducted on well-defined and appropriate patient populations. Here, we report a retrospective analysis suggesting that, in patients with poor ovarian reserve, an ovarian stimulation regimen based on r-FSH and r-LH appears producing a better clinical outcome compared with a more conventional stimulation scheme involving hMG.

The novelty of our study resides in the comparison of LH with hCG present in the hMG formulation. Importantly, these hormones have different half lives and potentially different cellular effects. Also, the study identifies a well-defined study group that can indeed benefit from LH supplementation. Although retrospective, our study has the merit of being among the largest of its kind conducted so far. It also offers a precise hypothesis to test in future ‘ad hoc’ randomised control trials.

Materials and methods

Study design, settings and participants

Data reported in the present analysis represent a retrospective cohort study including assisted reproduction treatments carried out between January 2013 and April 2016. The study was approved by the competent ethical committee (San Gerardo Hospital, Monza, Italy). Written informed consent was obtained from all couples before starting treatment. In particular, patients accepted that data generated from their treatment would be used in an anonymized form for the retrospective study described in the present manuscript. The aim of the analysis was to assess comparatively the efficacy at the first cycle of treatment of two ovarian stimulation regimens, r-FSH/r-LH vs. highly purified human menopausal gonadotropin (hp-hMG), applied to achieve multifollicular growth in women characterised by poor ovarian reserve defined as antral follicle count (AFC) < 11 and AMH ≤ 1.1 ng/ml [16]. Both regimens had been previously approved by the clinic’s medical direction as appropriate for the treatment of poor ovarian reserve and used without preference by individual doctors.

Exclusion criteria were as follows: female age > 42 years, basal FSH > 13 mIU/ml (parameter usually associated with scarce sensitivity to exogenous gonadotropins), BMI > 30, cryopreserved or severely affected semen, stage III and IV endometriosis and women with AFC > 10 at the first ultrasound scan during treatment. Clinical pregnancy per started cycle was the primary outcome, while rate of cycle cancellation and implantation rates were secondary outcomes. Patients included in the study were initially assessed as an overall population, but also divided in subgroups according to intervals of AFC values of equivalent or comparable extension (< 4, 4–6 and 7–10). In fact, the definition of poor ovarian reserve is rather broad, including women with significantly different prognosis.

Ovarian stimulation and oocyte recovery

Pituitary downregulation was achieved by gonadotropin-releasing hormone (GnRH) agonist (triptorelin acetate 0.10 mg/die, fertipeptil, Ferring, Milan, Italy) from cycle day 1. Basal antral follicle count (AFC) was carried out on day 3. Subsequently, follicle monitoring was performed together with assessment of blood levels of progesterone, and 17-β-estradiol stimulation of follicle growth was carried out from day 3 with r-FSH (300 IU/die, Gonal-F, Merck, Rome, Italy or Puregon, MSD, Rome, Italy) and r-LH (150 IU/die, Luveris, Merck, Rome, Italy) or hp-hMG (300 IU/die, Meropur, Ferring, Milan, Italy).

r-hCG (Ovitrelle, 0.25 mg, Merck, Rome, Italy) was administered 36 h prior to oocyte collection to achieve final oocyte maturation when at least one follicle larger than

18 mm in diameter was observed after a maximum of 18 days of stimulation. After retrieval, oocytes were cultured in IVF Medium (Origio, Måløv, Denmark). Within 3 h from collection, cumulus cells were removed by brief exposure to culture medium containing Cumulase (80 U/ml; Origio, Måløv, Denmark), followed by mechanical action achieved by passage through a fine bore pipette. Mature oocytes were placed in fresh drops of Cleavage Medium (Origio, Måløv, Denmark), until further use.

Semen preparation, oocyte insemination and embryo culture

Semen samples were prepared by discontinuous gradients (47.5 and 90%) of Sil-Select (Ferti-Pro, Beemen, Belgium), and spermatozoa were washed and re-suspended in IVF Medium (Origio, Måløv, Denmark) and stored in an incubator at 37 °C in a 6% CO₂ humidified atmosphere until use. Mature oocytes were fertilised by ICSI, carried out by the same five embryologists throughout the study period. Fertilisation was assessed 16–18 h after microinjection and confirmed by the presence of two pronuclei and two polar bodies. Zygotes and embryos were cultured in microdrops of Cleavage Medium under oil for 2–3 days (Origio, Måløv, Denmark).

Embryo transfer, luteal support and pregnancy assessment

Maximum three cleavage stage embryos were transferred on day 2 or 3 of development. Luteal support was assured by administration of progesterone (Prometrium, 600 mg/die, Rottapharm, Monza, Italy) starting from the day of oocyte recovery. Serum levels of b-hCG were measured 12–13 days following embryo transfer. Occurrence of a clinical pregnancy was assessed by ultrasonography observation of a gestational sac, with or without foetal heartbeat, 25–30 days following embryos transfer. In case of clinical pregnancy, progesterone supplementation was continued until the 12th week of gestation.

Statistics

Absolute and percentage frequencies were used to describe categorical items while average values, and standard deviation were assessed for continuous parameters. Student's *T* test and Fisher's exact test were used to compare the differences between results in r-FSH/r-LH and hp-hMG groups for stimulation of follicle growth. Study was also stratified according to AFC ranges (< 4, 4–6 and 7–10) and comparisons between characteristics and outcomes in subpopulations were performed. A *P* value < 0.05 was deemed as statistical significance. Stata software 9.0 (Stata Corporation, College Station, Texas) was used for statistical analysis.

Results

The relative efficacy of gonadotropin regimens based on r-FSH/r-LH or hp-hMG was assessed in 520 and 479 cycles, respectively. Basic population parameters -age, AMH, BMI and basal FSH (Table 1) - were identical or similar between the two groups. Basal AFC measured during treatment was also similar, while serum 17- β -estradiol levels and number of follicles > 14 mm in diameter at hCG triggering were higher in the hp-hMG group (Table 2).

The main study outcome, clinical pregnancy per started cycle, was higher in the r-FSH/r-LH group (12.5 vs. 8.1%, *P* < 0.02) (Table 2). This regimen was also associated with higher rates of clinical pregnancies per oocyte pick up (14.6 vs. 9.3%, *P* < 0.02). On the contrary, the rate of cancelled cycles, where no oocytes were collected or no embryos suitable for transfer were obtained, was lower in the r-FSH/r-LH compared to the hp-hMG group (26.9 vs. 34.4%, *P* < 0.01). Implantation (11.1 vs. 9.5%) and miscarriage rates (29.2 vs. 35.9%) were instead comparable between the r-FSH/r-LH and hp-hMG regimen groups.

Because the exclusion criteria of AFC applied to identify the study population was relatively broad (> 10), the main data set was analysed in more details by performing separate comparisons between subpopulations characterised by different ranges of AFC, i.e. < 4, 4–6 and 7–10 (Table 3).

The large majority of both laboratory clinical and clinical outcome measures were comparable in the subpopulation with AFC of 7–10, irrespective of stimulation regimens. However, percent of metaphase II (MII) oocytes (68.4 vs. 61.1%, *P* < 0.05) and fertilisation rates (72.5 vs. 64.7%, *P* < 0.05) were moderately higher in the r-FSH/r-LH group.

In women whose AFC was within the 4–6 range, percent of MII oocytes and fertilisation rates were also higher in association with the r-FSH/r-LH treatment (64.6 vs. 57.8%, *P* < 0.05; 73.5 vs. 66.6%, *P* < 0.02, respectively). In such subpopulation, stimulation with r-FSH/r-LH also coincided with higher mean number of developing and transferred embryos (2.1 ± 1.5 vs. 1.8 ± 1.6 , *P* < 0.01 and 1.8 ± 0.8 vs. 1.5 ± 0.7 , *P* < 0.05, respectively). Percent of cancelled cycles were instead moderately higher in the hp-hMG treatment group (24.0 vs. 32.3%, *P* < 0.05).

Greater differences between the r-FSH/r-LH and hp-hMG treatments were observed in the subpopulation of women with AFC < 4. Firstly, the main study outcome, clinical pregnancies per started cycle, was higher in the r-FSH/r-LH group (10.2 vs. 1.5%, *P* < 0.01).

This regimen was also associated with higher rates of MII oocytes (68.8 vs. 56.4%, *P* < 0.01) and fertilisation (68.9 vs. 63.5%, *P* < 0.05). Clinical pregnancy rates per oocyte pick up and embryo transfer, as well as the rate of ongoing pregnancies, were also higher in the r-FSH/r-LH group. The rate of cancelled cycles was considerably higher instead in women

Table 1 Population parameters of patients characterised by poor ovarian reserve defined as AMH ≤ 1.1 ng/ml and where ovarian stimulation was achieved with r-FSH/r-LH or hp-hMG

	r-FSH/r-LH	hp-hMG
Number of cycles	520	479
Age	39.5 $\pm$ 3.2	39.5 $\pm$ 3.2
BMI	22.7 $\pm$ 3.7	22.8 $\pm$ 3.7
AMH (ng/ml)	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3
Basal FSH (mIU/ml)	9.8 $\pm$ 3.9	9.6 $\pm$ 3.6

Exclusion criteria were female age > 42 years, basal FSH > 13 mIU/ml, BMI > 30, cryopreserved or severely affected semen and women with AFC > 10 at the first ultrasound scan during treatment

treated with hp-hMG (37.9 vs. 55.6%, $P < 0.01$). Finally, implantation rate was much higher in the r-FSH/r-LH group (13.0 vs. 2.8%, $P < 0.02$).

Discussion

Among women requiring IVF treatment, those with poor ovarian reserve represent a category characterised by a particularly poor prognosis. In these subjects, as an effect of advanced age or other intrinsic or extrinsic factors, the number of small antral follicles able to respond to exogenous gonadotropin is small, with the implication that the few oocytes that are retrieved at each cycle are not sufficient or do not have

sufficient quality to ensure substantial chances to achieve a pregnancy. Over the years, a plethora of attempts have been made in these patients in order to improve oocyte yield, and therefore clinical outcome [17], especially by changing dose of FSH and modality of pituitary suppression, or by using gonadotropin mixtures with LH-like action. Such attempts generated conflicting or inconclusive results, leaving many questions open, especially concerning the possible beneficial effect of LH. Here, we report a large retrospective study, to our knowledge probably the largest of its kind, in which regimens based on r-FSH/r-LH or hp-hMG were compared. Besides, our experience is among the very few that compared a r-FSH/r-LH stimulation scheme with hp-hMG, thereby representing an element of novelty. In fact, many previous studies assessed the outcome of cycles in which r-FSH was used alone or in combination with r-LH. This is surprising, considering that the choice of hp-hMG for the treatment of women with poor ovarian reserve is rather popular. Overall, our analysis suggests that the use of r-LH was associated to higher rates of pregnancies (per started cycles and pick up) and lower rates of cancellation in the overall study population. A subanalysis performed on groups of patients characterised by different AFC intervals (< 4, 4–6 or 7–10) was perhaps even more revealing of the beneficial effect of LH. In fact, in the group with severely affected ovarian reserve (AFC < 4), the superiority of the r-FSH/r-LH regimen was more significantly associated with higher clinical pregnancy and implantation rates. We believe that these findings are very relevant for at least two reasons: firstly, AFC values were

Table 2 Clinical outcome of poor responders described in Table 1 and treated with r-FSH/r-LH or hp-hMG

	r-FSH/r-LH (520)	hp-hMG (479)
Basal AFC on treatment cycle	4.9 $\pm$ 2.1	5.9 $\pm$ 2.4
Days of stimulation	8.8 $\pm$ 2.7	8.5 $\pm$ 2.5
Number of follicles ≥ 14 mm in diameter at r-hCG triggering	2.6 $\pm$ 1.9*	3.0 $\pm$ 2.0*
17- β -estradiol (pg/ml)	964.0 $\pm$ 581.9*	1256.7 $\pm$ 780.1*
Progesterone on the day of trigger (ng/ml)	0.7 $\pm$ 0.5	0.7 $\pm$ 0.9
Endometrial thickness (mm)	8.3 $\pm$ 1.6	8.4 $\pm$ 1.8
Collected oocytes (mean $\pm$ SD)	3.8 $\pm$ 2.7	4.2 $\pm$ 3.1
MII oocytes (%)	1144/1716 (66.6)*	1044/1764 (59.2)*
Fertilised oocytes (%)	832/1151 (72.3)*	694/1063 (65.3)*
Embryos (mean $\pm$ SD)	2.10 $\pm$ 1.59	1.97 $\pm$ 1.75
Transferred embryos (mean $\pm$ SD)	1.71 $\pm$ 0.80	1.64 $\pm$ 0.76
Clinical pregnancy rate per started cycles (%)	65/520 (12.5)**	39/479 (8.1)**
Clinical pregnancies per oocyte pick up (%)	65/446 (14.6)**	39/418 (9.3)**
Clinical pregnancies per embryo transfer (%)	65/380 (17.1)	39/314 (12.4)
Live birth rate (%)	46/380 (12.11)***	25/314 (7.96)***
Cancelled cycles (%)	140/520 (26.9)*	165/479 (34.4)*
Implantations (%)	69/620 (11.1)	47/492 (9.5)
Miscarriages (%)	19/65 (29.2)	14/39 (35.9)

* $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$

Table 3 Comparison between the r-FSH/r-LH or hp-hMG treatment subpopulations of patients characterised by different ranges of AFC, < 4, 4–6 and 7–10

Treatment	AFC					
	<4		4–6		7–10	
	r-FSH/r-LH	hp-hMG	r-FSH/r-LH	hp-hMG	r-FSH/r-LH	hp-hMG
Cycles	166	133	246	204	108	142
AMH (mean ± SD)	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.2	0.6 ± 0.2
Age (mean ± SD)	39.7 ± 3.1	39.0 ± 3.7	39.5 ± 3.3	39.8 ± 3.0	40.0 ± 2.2	39.3 ± 3.3
Collected oocytes (mean ± SD)	2.6 ± 2.1	2.5 ± 2.3	3.9 ± 2.6	3.76 ± 2.62	5.3 ± 2.9	6.0 ± 3.3
MII oocytes (%)	225/327 (68.8)*	132/234 (56.4)*	550/851 (64.6)*	409/707 (57.8)*	368/538 (68.4)*	503/823 (61.1)*
Fertilised oocytes (%)	160/232 (68.9)***	87/137 (63.5)***	403/548 (73.5)**	277/416 (66.6)**	269/371 (72.5)*	330/510 (64.7)*
Embryos (mean ± SD)	1.5 ± 1.3	1.3 ± 1.2	2.1 ± 1.5*	1.8 ± 1.6*	2.8 ± 1.8	2.5 ± 1.9
Transferred embryos (mean ± SD)	1.6 ± 0.7	1.4 ± 0.5	1.8 ± 0.8***	1.5 ± 0.7***	1.8 ± 0.9	1.8 ± 0.8
Clinical pregnancies per started cycle (%)	17/166 (10.2)*	2/133 (1.5)*	32/246 (13.1)	20/204 (9.8)	16/108 (14.8)	17/142 (12.0)
Clinical pregnancies per oocyte pick up (%)	17/124 (13.7)*	2/88 (2.3)*	32/220 (14.5)	20/188 (10.6)	16/102 (15.7)	17/137 (12.4)
Clinical pregnancies per embryo transfer (%)	17/103 (16.5)*	2/59 (3.4)*	32/187 (17.1)	20/138 (14.5)	16/90 (17.8)	17/117 (14.5)
Life birth rate (%)	13/103 (12.6)*	2/59 (3.4)*	22/187 (11.8)	12/138 (8.7)	11/90 (12.2)	11/117 (9.4)
Cancelled cycles (%)	63/166 (37.9)*	74/133 (55.6)*	59/246 (24.0)***	66/204 (32.3)***	18/108 (16.7)	25/142 (17.6)
Implantations (%)	18/138 (13.0)**	2/71 (2.8)**	35/315 (11.1)	24/208 (11.54)	16/167 (9.6)	27/213 (9.9)
Miscarriages (%)	4/17 (23.5)	0/2 (0.0)	10/32 (31.2)	8/20 (40.0)	5/16 (31.2)	6/17 (35.3)

* $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$

measured on day 3 of treatment, not in previous cycles as it often happens in daily routine, therefore representing the actual expression of ovarian reserve at the time of exposure to r-FSH/r-LH or hp-hMG; secondly, women with very poor ovarian reserve, i.e. those for whom the beneficial effect of the r-FSH/r-LH regimen was more tangible, are perhaps the most difficult patients to treat and for whom even relatively moderate increases in success rates can make major differences in their expectations of treatment outcome. Different effects of LH and hCG should not come as a surprise to clinicians. In fact, recent experiments have revealed specific and measurable differences between the actions of the two hormones. In particular, using in vitro system, Casarini and colleagues [18] demonstrated that, although recognised by the same receptor (LHCGR), LH and hCG elicit different biochemical responses in target cells. For example, hCG triggers a more potent cAMP production, while LH is more efficient in the activation of the ERK and AKT signalling pathways. Therefore, LH and hCG are hormonally and biochemically non-equivalent, with predictably important implications for follicular quality and consequently ovarian stimulation regimens.

Recently, Revelli and colleagues [9] also published a study on the relative efficacy of FSH/r-LH and hp-hMG, coming however to different conclusions in comparison to our study. In particular, they observed that the superiority of FSH/r-LH in terms of higher pregnancy rate was evident only in cycles where the number of retrieved oocytes was higher than eight, therefore not in women with poor or very poor ovarian reserve. Although the reasons for different outcomes between the present study and the experience of these colleagues may be several, we tend to think that the stricter inclusion criteria and methods adopted in our analysis create an important discriminating factor. For example, we included in our study only cycles involving GnRH agonist suppression and fixed doses of r-FSH/r-LH and hp-hMG, while the other study included GnRH agonist and antagonist cycles, as well as cases treated with varying doses of gonadotropins. Clearly, these elements can impact the number of antral follicles amenable to stimulation at the beginning of cycle and their response to exogenous gonadotropins. In another relatively recent study, Dahan and colleagues [19] compared the r-FSH/r-LH and hp-hMG regimens, observing a higher pregnancy rate associated to the use of r-LH. However, they did not find such a difference in a subpopulation of poor responders characterised by an AFC < 6 using a study group of poor responders of only 14–17 patients a group size clearly insufficient.

Other studies sought possible differences in the clinical outcome of cycles where either r-FSH/r-LH or hp-hMG was used [11, 12]. A clinically measurable outcome difference between the two regimens did not emerge from these investigations, not surprisingly, though, in light of the small size (33 to 58 cases) of study groups and relative lack of patient selection criteria.

A possible beneficial effect of LH supplementation has been more intensely and systematically investigated by comparing regimens based on r-FSH but not hMG. Starting from the consideration that a fall in LH bioactivity occurring after the age of 35 years may affect ovarian function and fertility of older women, Vuong and colleagues carried out a randomised controlled trial (RCT) to test the hypothesis that the addition of r-LH from cycle day 6 to a standard regimen based on r-FSH could improve the live birth rates in GnRH antagonist cycles involving women older than 35 years [10]. No differences were found between the r-FSH and the r-FSH/r-LH treatments in the main study endpoint or other clinical or laboratory outcomes. Having the design of a RCT, this study involves a high level of evidence, but is not immune from limitations represented mainly by the relative small size of study groups (approximately 100 treatments per group) and the fact that the major patient selection criterion of female age of 35 years or higher was not necessarily appropriate to identify subjects specifically requiring LH supplementation. Also, very recently, another RCT was carried out to explore possible outcome differences between r-FSH/r-LH and r-FSH-only regimens. The study detected only a trend in favour of FSH/r-LH treatment in patients with severely compromised ovarian reserve, although it should be noticed that the primary and secondary endpoints were number of oocytes retrieved and safety parameters [20]. On the contrary, a relatively recent meta-analysis [21] seems to suggest a beneficial effect of r-LH supplementation when added to a standard r-FSH regimen. In particular, Leheret and colleagues analysed 40 RCTs in which the relative performance of r-FSH and r-FSH/r-LH regimens was comparatively assessed. The meta-analysis did not find a difference in the number of retrieved oocytes between the two treatments in the overall population, but did find a higher number of retrieved oocytes in poor responder cycles where the r-FSH/r-LH was used. More importantly, the same analysis revealed that the r-FSH/r-LH scheme was associated with higher pregnancy rates in the overall study population.

Conclusions

In conclusion, in the present study, we observed a possible beneficial effect of an rFSH/r-LH regimen over the hp-hMG scheme for the treatment of women with reduced ovarian reserve, especially in those where this condition is particularly pronounced. This finding seems to confirm predictive models that suggest significant improvements in the prognosis of women with very poor ovarian reserve as an effect of an increase, although small, in the number of retrieved oocytes [22]. Considering its retrospective nature, this study is affected by obvious limitations, such as post-treatment patient selection criteria and absence of randomisation. Nevertheless, our

analysis has strength derived by the large size of the study population. It therefore strongly suggests the necessity to carry out RCTs specifically focused on patients affected by very poor ovarian reserve.

Compliance with ethical standards The study was approved by the competent ethical committee (San Gerardo Hospital, Monza, Italy). Written informed consent was obtained from all couples before starting treatment.

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