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The addition of clomiphene citrate to ovarian stimulation protocols for poor responders

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ABSTRACT

Poor ovarian response (POR) is one of the most challenging problems in assisted reproduction. Several strategies have been used to improve pregnancy rates. The use of Clomiphene Citrate (CC) has been shown to improve ovarian stimulation outcomes and decrease gonadotropin requirements in women of advanced reproductive age. However, the combination of CC and gonadotropins to improve pregnancy rates after in IVF in poor responders is still unexplored due to the small number of trials with few participants.

This is a prospective cohort trial involving 12 patients diagnosed with poor ovarian response who underwent ovarian stimulation during the period between June 2015 and September of 2017. All patients were treated with the maximum dose of gonadotropins (hMG, 300 IU/day, hMG group) according to a short gonadotropin/GnRH antagonist protocol. In a subsequent cycle those patients underwent the same stimulation protocol with the addition of 100 mg of CC from day 3 to day 7 (CC-hMG group).

Supplementation with 100 mg of CC resulted in a statistically significant increase in estradiol levels, number of follicles and number of oocytes retrieved, as well as an increase in the number of total embryos available for transfer. Furthermore, a significant reduction was observed in cancellation rates in the CC-hMG group. Two clinical pregnancies, which resulted in two live births and 3 biochemical pregnancies were achieved in the CC/hMG group.

Furthermore, by employing open-source, biological data we identified a common gene (Estrogen Receptor 1, ESR1) between genetic targets of clomiphene treatment and POR which could explain the benefits of clomiphene in this group of patients.

In conclusion, the addition of CC 100 mg to the stimulation regimen in women diagnosed with POR and previous failed IVF cycles could improve stimulation results, but this study could not demonstrate any benefit in terms of clinical pregnancies and live births. The effectiveness of this treatment requires further investigation.

Introduction

Poor ovarian response (POR) is one of the most discussed problems in assisted reproduction and occurs in 9–24 % of ovarian stimulation cycles [1,2]. Poor response to ovarian stimulation usually indicates a reduction in follicular response, resulting in reduced number of retrieved oocytes or even to cancelation of the stimulation before oocyte pick-up (OPU), leading to emotional distress and significant financial burden [1]. The term "Poor Ovarian Response" is not strictly defined but several investigators have used various criteria such as: age over 38 [3], three or less oocytes retrieved [4,5], history of previous POR [6], Day 3 FSH level higher than 8.5mIU/mL [4,5] and serum E2 levels of less than 600 pg/mL on the day of hCG administration [7]. In a review by Polyzos and Devroey, of 47 randomized trials, 41 different definitions of poor responders were employed [8]. The follow-up publication of the Bologna criteria for the definition of a poor responder [9] was endorsed by the European Society of Human Reproduction and Embryology (ESHRE). According to these criteria, a patient is

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defined as a poor responder if at least two out of the three are met: i) advanced maternal age (≥40 years) [10] or any other risk factor for POR, such as genetic abnormalities (mutations in specific genes or structural abnormalities (Fig.1) [11], pelvic infection [12], ovarian endometriomas [13] or chemotherapy [14], ii) history of a previous poor ovarian response (≤3 oocytes with a conventional stimulation protocol) and iii) an abnormal ovarian reserve test. While these criteria were widely accepted, there is still no unanimity in the definition of poor ovarian response, as minimal differences in the number of retrieved oocytes do not necessarily translate into a significant reduction in pregnancy rates. In a large prospective trial by Nelson et al., which included 144.000 treatment cycles, there was a significant decrease in pregnancy rates after the age of 35, while a similar decrease was observed by the age of 40 according to the Bologna criteria [8,15]. Finally, in 2016, the novel POSEIDON (www.groupposeidon.com) classification system was established to improve counselling and management of low prognosis patients undergoing ART [16,17]. The low prognosis patients are classified into four groups according to the results of ovarian reserve markers (AMH, AFC or both), female age and the number of oocytes retrieved in previous cycles.

Etiology of POR includes multiple genetic [18], autoimmune, idiopathic, and iatrogenic factors [19]. The cause for the prematurely lower number of follicles can be identified at many different levels of follicular and oocyte development. Multiple genes were identified involved in folliculogenesis, however, the impact of mutations or polymorphic variants of these genes remains unclear.

Clomiphene citrate (CC) is a selective estrogen receptor modulator, historically used for the induction of ovulation in women with anovulation. CC binds to the hypothalamic estrogen receptors stimulating the release of gonadotropins from the anterior pituitary [20]. CC has been also been used in the past to evaluate protocols with reduced doses of gonadotropins in cases of male infertility and normal ovulatory function [21] or for a milder stimulation in cases of previous OHSS [21,22]. D'Amato et al. first, reported the use of CC in a novel protocol for ovarian stimulation in POR. Women in the control group were stimulated according to a standard long luteal phase stimulation protocol. In a more recent study, Oride et al. evaluated the efficacy of CC in 13 poor responders by comparing a short antagonist stimulation protocol with CC to a long luteal phase protocol without CC (46 *vs*. 20 cycles respectively). Four of the 13 patients achieved pregnancy with the CC + hMG cycles, suggesting that the addition of CC in the stimulation protocol may be of benefit in women with POR [23]. In contrast, in a study by Lin et al., there was no difference in pregnancy rates between patients treated with CC + hMG and those treated with the conventional long protocol (Lin Hwang et al., 2006). To add in the previous body of literature, in this study we evaluated whether the addition of CC to a high gonadotropin dose /GnRH antagonist regimen for women with history of prior POR, can improve the outcome of the stimulation as measured with the rate of successful pregnancies.

Materials and methods

This is prospective cohort trial that was conducted from June 2015 to September 2017 and involved patients diagnosed with POR in a previous stimulation according to the Bologna criteria. At least two out of the following criteria had to be met: Age over 40 years, Day 2 FSH > 12 mIU/mL, anti-Mullerian hormone (AMH) < 2 ng/mL, at least one previous cycle of ovarian stimulation with three or less oocytes or cancelled attempt due to poor response and serum estradiol levels < 500 pg/mL on the day of the human Chorionic Gonadotropin (hCG) administration. Twelve patients that met the inclusion requirements underwent 27 cycles of ovarian stimulation in total: 13 cycles under the human menopausal gonadotropin (hMG, hMG-group) antagonist protocol and 14 cycles under the CC-hMG antagonist protocol (CC-hMG-group). Each patient served as her own control for the two treatments. If a patient had two or more cycles under the same stimulation protocol each cycle was included separately. Furthermore, we tried to combine biological and clinical data sourced through the literature review to cross-compare genetic targets of clomiphene treatment with genes associated with "POR" obtained from the Drug-Gene Interaction database (DGIdb) [24,25] (Fig. 1). The study was approved by the ethics committee of the Aretaieion University Hospital and the Leto Maternity Hospital of Athens. The study was also reported at the clinical trials registry (clinicalTrials.gov) with registration number NCT02237755.

Treatment schedule

All patients had serum Anti-Mullerian hormone levels measured within a 3 months period prior to stimulation. A fixed hMG-antagonist protocol was used for all patients. Briefly, women were evaluated on the 2nd day of their menstrual cycle when a transvaginal ultrasound examination was performed and serum levels of FSH and estradiol were measured. Providing that serum FSH < 16 mIU/mL and estradiol < 70 pg/mL, ovarian stimulation was initiated on day 3 with 300 IU of hMG (Menopur; Ferring Pharmaceutical Hellas AE). All patients were re-evaluated on day 5 of stimulation (day 8 of the cycle), when a transvaginal sonogram was performed to evaluate follicular growth and serum estradiol levels were measured again. On day 6 of stimulation or when a leading follicle reached a diameter of 14 mm, a commercially available GnRH antagonist, Cetrorelix acetate 0.25 mg/day, was initiated (Cetrotide; Merck Serono Hellas AE). Patients had serial evaluations as needed. When at least two follicles reached an average diameter of 18–20 mm, final oocyte maturation was triggered with 10,000 IU of hCG (Pregnyl; Organon Greece Inc). Oocyte Pick Up (OPU) was carried out 34–36 h later. All patients

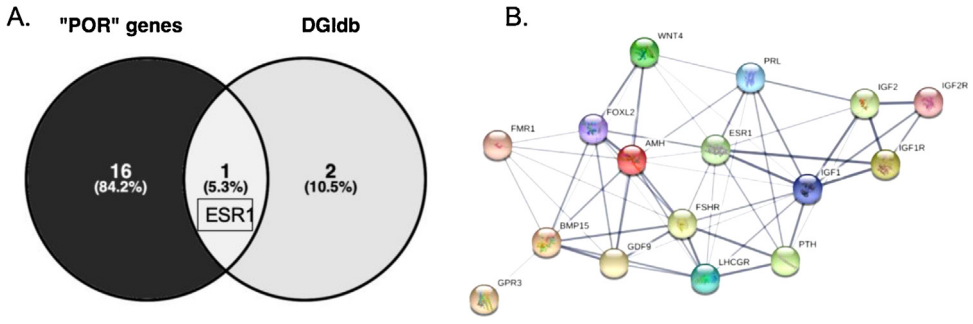

Fig. 1. Genes associated with Poor ovarian response and Clomiphene targets. (A) Genes associated with "POR" sourced through literature review. (B) STRING protein interaction database. DGIdb: Drug-Gene Interaction database. ESR1: Estrogen Receptor 1.

underwent routine ICSI based on the limited number of available oocytes regardless of the semen parameters. Patients with successful fertilization underwent embryo transfer (ET) under sonographic guidance on day 3 post OPU. Embryos were evaluated by the scoring system established by the Istanbul consensus workshop on embryo assessment [26]. Micronized progesterone (Utrogestan; Angelini Pharma ABEE) was used for supplementation of the luteal phase (200 mg four times daily vaginally). Serum beta-hCG was measured 12 days after the OPU and clinical pregnancy was confirmed by ultrasound visualization of fetal heart rate 2 weeks later. Patients who failed to respond to this protocol or those that did not get pregnant were offered the option of an additional cycle with the same stimulation protocol (300 IU/day of hMG) with the addition of 100 mg CC per day from day 3 to day 7 of the cycle (5 days) (CC-hMG-group) or oocyte donation.

Outcome measures: The primary outcome measured was clinical pregnancy rates. Secondary outcomes were the duration of the stimulation, number of follicles, number of oocytes retrieved, number of mature oocytes (Metaphase II-MII), fertilization rates, embryo quality, total number of embryos in each group and biochemical pregnancy rates.

Endocrine assays: Circulating serum levels of AMH, as well as FSH and estradiol were analyzed in samples collected on day 2 of the menstrual cycle. A commercially available kit (Gen 2 ELISA; Beckmann-Coulter) was used to measure AMH and electrochemiluminese immunoassay kit (ECLIA; Roche Diagnostics) were used to measure FSH and estradiol. The intra-assay and inter-assay coefficient of variation for AMH, FSH and estradiol were 5.7 and 4.6. 3.0 and 3.8, 3.9 and 5.6, respectively.

Statistical evaluation: A commercially available statistical program (IBM SPSS Statistics for Windows, Version 21.0. IBM Corp. Released 2012. Armonk, NY: IBM Corp) was used for the statistical analysis. Continuous data are expressed as mean values and standard error of the mean and were compared using the Mann Whitney-U test or paired samples t-test, where appropriate. Chi-square test was applied for the comparison of nominal variables between the two groups. A p-value <0.05 was considered statistically significant

Results

Twelve patients underwent 27 cycles of ovarian stimulation for IVF. These patients had 13 cycles using the standard gonadotropin antagonist protocol (hMG-group) as described previously and 14 cycles where the above-mentioned protocol was supplemented daily with 100 mg of CC from days 3–7 of stimulation. The time interval between the two stimulations was 1–3 months. The demographics characteristics for all patients are shown in Table 1. Comparative cumulative data for the two stimulation protocols are shown in Table 2.

As expected, women in the CC-hMG group were slightly older (39.23 *vs*. 40.43, p: 0.004) and there was a statistically significant difference in AMH levels between the two groups (2.10 *vs*. 2.00, p: 0.028). Overall, no differences were found in day 2 FSH (10.21 *vs* 10.98, p:0.214) or day 2 estradiol levels (59.38 *vs*. 45.71, p: 0.078 for the hMG-group and the CC-hMG-group respectively). Interestingly, women in the CC/hMG group required one additional day of medication (7.38 *vs*. 9.14, p: 0.022). Consequently, the amount of gonadotropin required was increased in the CC-hMG group (3323.08 *vs*. 4087.50, p: 0.014). Peak estradiol levels were significantly higher in the CC-hMG group, as compared to the hMG group (1522.57 *vs*. 614.73, p:0.001), in accordance to the higher number of large follicles (5.29 *vs*. 2.23, p: 0.001). As a result, the number of oocytes retrieved was higher in the CC/hMG group (4.5 *vs*. 2 p: 0.012). Subsequently, significant differences were also noted in the total number of fertilized oocytes (3.23 *vs*. 1.83, p: 0.028), although the fertilization rates between the two groups were similar (0.59 % *vs*. 0.72 %, p: 0.249). As a result, there was a significant difference in the total number of embryos available for ET or cryopreservation (2.40 *vs*. 3.64, p: 0.027) and, consequently, in the overall number of embryos transferred (2.25 *vs*. 2.7, p: 0.014). There was a borderline significant difference in the number of high-quality embryos between two groups (those with 7 or 8 cells on day 3 and grades 1 or 2) [26].

Furthermore, more cycles were cancelled in the hMG group due to no response to the stimulation [61.5 % (8 out of 13) *vs*. 7.1 % (1 out of 14), p: 0.034] in the CC-hMG-group. Looking at the primary outcome (clinical pregnancy rates), there were no clinical pregnancies in the hMG-group, whereas in the CC-hMG group there were two confirmed clinical pregnancies out of the twelve embryo transfers (ET) that were carried out (16.67 %) and two live births, but this difference did not reach statistical significance (p-value: 0.423). On the contrary there was a significant difference in the rate of biochemical pregnancies, as there were 5 positive β-hCG tests out of the 12 ET in the CC-hMG group (41 %) in contrast to all negative in the hMG-group (p: 0.014).

Finally, analyzing bio-clinical data sourced in literature, we identified a common gene, ESR1 (Estrogen Receptor 1), between the clomiphene genetic targets and POR-associated genes. Further analysis through the STRING protein interaction database [27], indicated the centrality of ESR1 to the network (Fig. 1).

Table 1
Clinical, hormonal and embryologic data of the two groups.

	hMG group n = 13	CC-hMG group n = 14	p-value
Age ± SE	39.23 ± 1.15	40.43 ± 1.00	0.004
Day 2 FSH ± SE	10.21 ± 0.86	10.89 ± 0.89	0.214
AMH ± SE	2.10 ± 0.56	2.00 ± 0.52	0.028
Day 2 estradiol ± SE	59.38 ± 8.90	45.71 ± 4.11	0.078

Discussion

Our study demonstrated that the addition of CC in a stimulation regimen with hMG/antagonist for poor responders may reduce cancelation rates, improve follicular response resulting in a significant increase in the number of oocytes retrieved and the embryos available for transfer. In addition, there was a significant increase in biochemical pregnancies. However, we could not demonstrate any benefit in terms of clinical pregnancies and live births. Furthermore, the addition of CC 100 mg to the stimulation regimen increased the miscarriage rate (3 out of 5 biochemical pregnancies). Several stimulation strategies have been proposed for the management of those patients with varying and often conflicting results. In a study by Bosa et al., 244 patients were analyzed for their response to a GnRH microdose agonist protocol (50 μg SC of leuprolide acetate twice daily on day 2 of the cycle) (Group 1) and to hMG/ GnRH antagonist protocol (Group 2). Both groups received at least 375 IU of gonadotropins per day. There was a significant increase in the number of oocytes retrieved in the micro-dose flare up protocol group (3.6 ± 2.4 *vs*. 2.8 ± 1.9 p: 0.005), as well as significantly higher implantation rate (27.8 % *vs*. 18.8 % p: 0.04). Despite this, there was no significant difference in the clinical pregnancy rates between the two groups (19.8 % *vs*. 14.4 % p: 0.13) [28].

Regarding the administration of androgens and especially DHEA prior to ovarian stimulation data has also been controversial, with some investigators supporting their use [29,30]. While others, failed to confirm these findings [31,32].

Clomiphene citrate historically has been used to induce ovulation in women with non or oligo-ovulatory cycles or luteal phase deficiency [20]. Jovanovic et al. examined efficacy of adding

Table 2
Stimulation results (clinical, hormonal and embryologic data) of the two groups.

	hMG group n = 13	CC-hMG group n = 14	p-value
Peak estradiol ±SE	614.73 ± 111.38	1522.57 ± 250.33	0.001
Average Total hMG dose ±SE	3323.08 ± 272.20	4087.50 ± 86.85	0.014
Cancellation rates	8/13 (61.5 %)	1/14 (7.1 %)	0.022
Duration of stimulation (days) ± SE	7.38 ± 0.60	9.14 ± 0.18	0.022
Number of follicles ± SE	2.23 ± 0.58	5.29 ± 0.63	0.001
Number of Oocytes ± SE	2 ± 0.60	4.5 ± 0.64	0.012
Number of Embryos ± SE	2.40 ± 0.51	3.64 ± 0.49	0.027
Biochemical pregnancy rates	0/4 (0.0 %)	5/12 (35.7 %)	0.014
Clinical pregnancy rates (Live birth rates)	0/4 (0.0 %)	2/12 (14 %)	0.423

CC in a GnRH antagonist protocol to the same patients (n: 48). They concluded that the combined protocol could be more suitable in POR cases [33]. Our findings are in agreement with the study by D'Amato et al. [34], as well as with the study by [33]. In contrast, we contradicted the findings of Oride et al. [23]. Unfortunately, we were unable to demonstrate statistically significant difference in live-birth rate.

Our findings supported that supplementation of CC in the stimulation protocol led to a significant reduction in cancellation rates in comparison to hMG group (7.1 % *vs.* 61.5 % respectively, p: 0.022). Peak estradiol levels were significantly higher in the CC-hMG group at the end of the stimulation, reflecting on the increased number of follicles. In average 4.5 oocytes were retrieved in the CC-hMG group in contrast to the 2 in hMG group. Subsequently, more embryos were available for transfer in the CC-hMG group (3.64) than in hMG group (2.40), as presented in Table 2b.

There were 5 patients in the CC supplemented group with positive β-hCG test in comparison to none in the hMG group, a statistically significant difference (p: 0.014). On the other hand, only 2 out of 5 positive β-hCG test ended in clinical pregnancies and live births in the CC-hMG group without statistically significant difference. These findings were in agreement with the study of Jovanovic et al. [33] as well as the D'Amato et al. [34], who did not find any difference in clinical pregnancy rate. It could be explained by the well-known adverse effect of CC on endometrial receptivity as it has been associated with endometrial thinning [35,36], which, as presented by Richter et al., can affect the implantation rate irrespective of maternal age and embryo quality [37].

Unlike the previously published data and according to our results, the CC-hMG protocol required 1.7 additional days of stimulation (7.38 *vs*. 9.14, p: 0.022). While this prolongation may require an added amount of gonadotropins, it seems that may have a beneficial effect in follicular and oocyte maturity. Furthermore, based on our literature review, this study, for the first time, combined biological and clinical data in support of clomiphene efficacy during the ovarian stimulation of PORs. Genes associated with "POR" were sourced through literature review [18] and cross-compared with genetic targets of clomiphene treatment as published in the DGIdb [24] through Venn diagram analysis [25] (Fig. 1). Currently, DGIdb contains over 14,144 drug-gene interactions by 2611 genes and 6307 drugs and in addition it includes 6761 genes belonging to one or more of 39 potentially druggable gene categories. A common gene between the two lists was identified as the ESR1 (Estrogen Receptor 1), a ligand-activated transcription factor. Additional research through the STRING protein interaction database [27], showed that ESR1 play the central role at functional interaction networks of proteins (Fig. 1). The existence of this common gene might explain the effect of clomiphene in the poor responder group.

In conclusion, as it has been demonstrated by this study; a combined CC-hMG ovarian stimulation protocol in women who diagnosed with POR and previous failed IVF cycles could improve stimulation results without, however, increasing the pregnancy and live-birth rates. For patients unwilling to proceed to oocyte donation, the possible technic of "freeze all embryos" and embryo transfer in subsequent cycle may be of some benefit. Further studies preferably randomized with sufficient power are required to clarify further our findings.

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Author contribution

Concept, analysis, format, revision, editing, literature search, extraction analysis, and drafted the manuscript: VN, TO, SG, SK; Literature search, extraction analysis, and drafted the manuscript: LG; All remaining authors contributed to this review equally each including their input on their respective expertise: BP, BE

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2020.05.026.

References

[1] Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotrophin stimulation. Br J Obstet Gynaecol 1997;104(5):521–7.
[2] Ubaldi FM, Rienzi L, Ferrero S, Baroni E, Sapienza F, Cobellis L, et al. Management of poor responders in IVF. Reprod Biomed Online 2005;10(2):235–46.
[3] Karimzadeh MA, Mashayekhy M, Mohammadian F, Moghaddam FM. Comparison of mild and microdose GnRH agonist flare protocols on IVF outcome in poor responders. Arch Gynecol Obstet 2011;283(5):1159–64.
[4] Kim CH, Jeon GH, Cheon YP, Jeon I, Kim SH, Chae HD, et al. Comparison of GnRH antagonist protocol with or without oral contraceptive pill pretreatment and GnRH agonist low-dose long protocol in low responders undergoing IVF/intracytoplasmic sperm injection. Fertil Steril 2009;92(5):1758–60.
[5] Kim CH, Kim SR, Cheon YP, Kim SH, Chae HD, Kang BM. Minimal stimulation using gonadotropin-releasing hormone (GnRH) antagonist and recombinant human follicle-stimulating hormone versus GnRH antagonist multiple-dose protocol in low responders undergoing in vitro fertilization/intracytoplasmic sperm injection. Fertil Steril 2009;92(6):2082–4.
[6] Kansal Kalra S, Ratcliffe S, Gracia CR, Martino L, Coutifaris C, Barnhart KT. Randomized controlled pilot trial of luteal phase recombinant FSH stimulation in poor responders. Reprod Biomed Online 2008;17(6):745–50.
[7] Marci R, Caserta D, Dolo V, Tatone C, Pavan A, Moscarini M. GnRH antagonist in IVF poor-responder patients: results of a randomized trial. Reprod Biomed Online 2005;11(2):189–93.
[8] Polyzos NP, Devroey P. A systematic review of randomized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? Fertil Steril 2011;96(5)1058–61 e1057.

[9] Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of' poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011;26(7):1616–24.
[10] Goverde AJ, McDonnell J, Schats R, Vermeiden JP, Homburg R, Lambalk CB. Ovarian response to standard gonadotrophin stimulation for IVF is decreased not only in older but also in younger women in couples with idiopathic and male subfertility. Hum Reprod 2005;20(6):1573–7.
[11] De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. Lancet 2010;376 (9744):911–21.
[12] Keay SD, Barlow R, Eley A, Masson GM, Anthony FW, Jenkins JM. The relation between immunoglobulin G antibodies to Chlamydia trachomatis and poor ovarian response to gonadotropin stimulation before in vitro fertilization. Fertil Steril 1998;70(2):214–8.
[13] Garcia-Velasco JA, Somigliana E. Management of endometriomas in women requiring IVF: to touch or not to touch. Hum Reprod 2009;24(3):496–501.
[14] Oktem O, Oktay K. Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. Cancer 2007;110(10):2222–9.
[15] Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS Med 2011;8(1)e1000386.
[16] Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number), Alviggi C, Andersen CY, Buehler K, Conforti A, De Placido G, et al. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. Fertil Steril 2016;105(June (6)):1452–3, doi:http://dx.doi.org/10.1016/j.fertnstert.2016.02.005.
[17] Humaidan P, Alviggi C, Fischer R, Esteves SC. The novel POSEIDON stratification of' Low prognosis patients in Assisted Reproductive Technology' and its proposed marker of successful outcome. F1000 Res. 2016;23(5):2911, doi: http://dx.doi.org/10.12688/f1000research.10382.1.
[18] Greene A, Patounakis G, Segars J. Genetic associations with diminished ovarian reserve: a systematic review of the literature. J Assist Reprod Genet 2014;31 (8):935–46.
[19] Fusco F, Paciolla M, Chen E, Li X, Genesio R, Conti A, et al. Genetic and molecular analysis of a new unbalanced X;18 rearrangement: localization of the diminished ovarian reserve disease locus in the distal Xq POF1 region. Hum Reprod 2011;26(11):3186–96, doi:http://dx.doi.org/10.1093/humrep/der266.
[20] Dickey RP, Holtkamp DE. Development, pharmacology and clinical experience with clomiphene citrate. Hum Reprod Update 1996;2(6):483–506.
[21] Hwang JL, Huang LW, Hsieh BC, Tsai YL, Huang SC, Chen CY, et al. Ovarian stimulation by clomiphene citrate and hMG in combination with cetrorelix acetate for ICSI cycles. Hum Reprod 2003;18(1):45–9.
[22] Lin YH, Seow KM, Hsieh BC, Huang LW, Chen HJ, Huang SC, et al. Application of GnRH antagonist in combination with clomiphene citrate and hMG for patients with exaggerated ovarian response in previous IVF/ICSI cycles. J Assist Reprod Genet 2007;24(8):331–6.
[23] Oride A, Kanasaki H, Miyazaki K. Comparison of human menopausal gonadotropin stimulation with and without clomiphene for in-vitro fertilisation in poor-responders. J Obstet Gynaecol 2015;35(2):163–7.
[24] Griffith M, Griffith O, Coffman A, Weible J, McMichael J, Spies N, et al. DGIdb: mining the druggable genome. Nat Methods 2013;10(12):1209.
[25] Venny O. An interactive tool for comparing lists with Venn Diagrams. 2007.
[26] Alpha Scientists in. Reproductive, M, E. S. I. G. o. Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26(6):1270–83.
[27] Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 2010;39(suppl_1):D561–8.
[28] Boza A, Cakar E, Boza B, Api M, Kayatas S, Sofuoglu K. Microdose flare-up gonadotropin-releasing hormone (GnRH) agonist versus GnRH antagonist protocols in poor ovarian responders undergoing intracytoplasmic sperm injection. J Reprod Infertil 2016;17(3):163–8.
[29] Casson PR, Lindsay MS, Pisarska MD, Carson SA, Buster JE. Dehydroepiandrosterone supplementation augments ovarian stimulation in poor responders: a case series. Hum Reprod 2000;15(10):2129–32.
[30] Wiser A, Gonen O, Ghetler Y, Shavit T, Berkovitz A, Shulman A. Addition of dehydroepiandrosterone (DHEA) for poor-responder patients before and during IVF treatment improves the pregnancy rate: a randomized prospective study. Hum Reprod 2010;25(10):2496–500.
[31] Sunkara SK, Pundir J, Khalaf Y. Effect of androgen supplementation or modulation on ovarian stimulation outcome in poor responders: a meta-analysis. Reprod Biomed Online 2011;22(6):545–55.
[32] Vlahos N, Papalouka M, Triantafyllidou O, Vlachos A, Vakas P, Grimbizis G, et al. Dehydroepiandrosterone administration before IVF in poor responders: a prospective cohort study. Reprod Biomed Online 2015;30(2):191–6.
[33] Jovanovic VP, Kort DH, Guarnaccia MM, Sauer MV, Lobo RA. Does the addition of clomiphene citrate or letrazole to gonadotropin treatment enhance the oocyte yield in poor responders undergoing IVF? J Assist Reprod Genet 2011;28(11):1067–72.
[34] D'Amato G, Caroppo E, Pasquadibisceglie A, Carone D, Vitti A, Vizziello GM. A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. Fertil Steril 2004;81(6):1572–7.
[35] Haritha S, Rajagopalan G. Follicular growth, endometrial thickness, and serum estradiol levels in spontaneous and clomiphene citrate-induced cycles. Int J Gynaecol Obstet 2003;81(3):287–92.
[36] Takasaki A, Tamura H, Taketani T, Shimamura K, Morioka H, Sugino N. A pilot study to prevent a thin endometrium in patients undergoing clomiphene citrate treatment. J Ovarian Res 2013;6(1):94.
[37] Richter KS, Bugge KR, Bromer JG, Levy MJ. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. Fertil Steril 2007;87(1):53–9.