

Liraglutide increases IVF pregnancy rates in obese PCOS women with poor response to first-line reproductive treatments: a pilot randomized study

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Abstract

Objective: Glucagon-like peptide-1 (GLP-1) has been investigated in regulation of reproductive system in animal models. The potential impact of short-term preconception intervention with liraglutide on fertility potential in polycystic ovary syndrome (PCOS) has not been evaluated yet.

Design: A prospective randomized open-label study was conducted in 28 infertile obese PCOS patients (age: 31.07 ± 4.75 , BMI: 36.7 ± 3.5 kg/m², mean \pm s.d.). They were assigned to metformin (MET) 1000 mg BID or to MET 1000 mg BID combined with low-dose liraglutide 1.2 mg QD s.c. (COMBI) for 12 weeks. Ovarian stimulation protocol was started after a 4-week medication-free period.

Methods: The *in vitro* fertilization pregnancy rate (PR) was defined as the number of clinical pregnancies confirmed by ultrasound visualization of the fetal cardiac activity, divided by the total number of cycles performed or embryo transfers (ET). The spontaneous PR was followed for 12 months.

Results: Patients in the MET group on average lost 7.0 ± 6.0 kg ($P=0.001$) compared with 7.5 ± 3.9 kg in the COMBI group ($P<0.001$) with no significant between-treatment difference ($P=0.246$). The PR per ET was significantly higher in the COMBI (85.7%) compared with the MET (28.6%) group ($P=0.03$). The cumulative PR in the time frame of 12 months was 69.2% in the COMBI group compared to 35.7% in the MET group.

Conclusions: Preconception intervention with low-dose liraglutide added to metformin is superior to metformin alone in increasing PRs per ET and cumulative PRs in infertile obese women with PCOS, despite comparable weight reduction in both groups. A potential impact of liraglutide on the reproductive system needs further exploration, in particular the GLP-1 impact on endometrial quality and receptivity.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility and affects up to 20% of women of reproductive age according to the Rotterdam criteria (1, 2). Nearly half of the women with PCOS are overweight or obese (3). The impact of obesity on *in vitro*

fertilization (IVF) outcomes is controversial, but most studies have shown lower implantation, clinical pregnancy and birth respective rates, and a higher miscarriage rate. Fewer oocytes are collected and fertilized, cycles are longer and require higher amounts of gonadotropins (4, 5, 6).

Weight loss prior to IVF procedures has been associated with significantly improved pregnancy rates (PR) and live birth rates (7, 8, 9, 10). Furthermore, a decreased number of IVF cycles required to achieve a pregnancy has also been reported after weight loss interventions (11). Based on the principles of fetal programming, improving a lifestyle before conception might lead to improved long-term health of the offspring (12, 13).

Lifestyle modification is recommended as the first-line treatment in obese infertile PCOS women (14, 15), whereas clomiphene citrate (CC) remains the second-line treatment for ovulation induction. Compared to CC, metformin is inferior in improving the respective rates of ovulation, pregnancy, live birth, miscarriage and multiple pregnancy (16). A potential benefit of metformin has been found in women with BMI greater than 35 kg/m² when combined with CC (17).

Long-acting glucagon-like peptide-1 (GLP-1) receptor agonist (RA) liraglutide has provided positive effects on weight reduction and glucose homeostasis in obese PCOS women (18, 19). It provides meaningful weight loss when combined with metformin compared to metformin only and lifestyle modification (18, 20). The mechanism of body weight reduction appears to be due to decreased food intake, mainly by a direct hypothalamic effect. It also delays gastric emptying partly due to a central action mediated via the autonomous nervous system (21, 22).

The potentially beneficial effects of liraglutide on IVF outcomes of PCOS obese patients have not been addressed yet. Since liraglutide is an effective insulin-sensitizing agent there is a theoretical assumption that weight loss will improve the reproductive outcomes by weight loss. Moreover, new animal data suggest that GLP-1 agonist plays a role in the regulation of hypothalamus-pituitary-ovary axis by neuroendocrine modulation of gonadotropin-releasing hormone release (23).

The aim of this pilot study was to evaluate the impact of low-dose liraglutide in combination with metformin compared to metformin alone on IVF PRs and cumulative PRs (IVF and spontaneous pregnancies) in infertile obese women with PCOS who had been previously poor responders to weight reduction with lifestyle modification, and resistant to the first-line reproductive treatments with CC or aromatase inhibitors. The secondary outcomes were to evaluate the impact of therapy on oocyte and embryo quality, weight loss and metabolic and endocrine parameters.

Subjects and methods

Subjects

Twenty-eight obese PCOS patients diagnosed according to the revised Rotterdam criteria (2) participated in a 12-week open-label prospective randomized outpatient clinical study between September 2014 and May 2015. Eleven women receiving the combined therapy and 8 receiving metformin had the classic PHO phenotype (PCO morphology (P), hyperandrogenic features (H) and oligo-amenorrhea (O)). Two women receiving metformin had the PH phenotype, and one had the PO phenotype. Two women receiving the combined therapy and 3 receiving metformin had the HO phenotype (24). The inclusion criteria were as follows: PCOS diagnosed according to the revised Rotterdam criteria, body mass index (BMI) ≥ 30 kg/m², age ≤ 38 , first or second IVF attempt; no severe male infertility. The exclusion criteria were type 1 or type 2 diabetes mellitus, history of carcinoma, personal or family history of MEN2, significant cardiovascular, kidney, or liver disease, use of medications other than metformin known or suspected to affect reproductive or metabolic functions or statins within 90 days before study entry, and no coexisting ovarian pathology.

All patients were informed of the study aims and signed the written consent form before entering the study, which was conducted in accordance with the Declaration of Helsinki and approved by the National Medical Ethics Committee (approval number: 161/08/14). The study is registered with ClinicalTrials.gov identifier: NCT03353948.

Study protocol

Patients were randomized to one of the two treatment arms: metformin (MET) 1000mg BID or metformin 1000mg BID combined with 1.2mg liraglutide QD s.c. (COMBI). Metformin was initiated with a dose of 500mg once per day and increased by 500mg every 3 days up to 1000mg BID. In the COMBI arm there was a run-in period of 12 days to titrate metformin up to 1000mg BID before liraglutide was added. Liraglutide was initiated with a dose of 0.6mg injected s.c. once per day and increased to 1.2mg after 3 days. Medical treatment in both groups lasted 12 weeks.

At the beginning of the study, lifestyle intervention was actively promoted in both groups. A reducing diet of 500–800kcal/day consisting of up to 50% of carbohydrates preferably with low glycemic index, 20% of proteins and

30% of fat, mostly mono- and poly-unsaturated, with the amount of saturated fat less than 10%, was advised. The participants were encouraged to increase consumption of fibers, whole grains, cereals, fruits and vegetables along with at least 30 min of moderate-intensity physical activity daily. The IVF procedure was offered to all the patients that completed the medical treatment after 1 month of medicine-free period. At baseline and at study end fasting blood was drawn for determination of glucose, insulin, androstenedione, total and free testosterone, sex hormone-binding globulin (SHBG) and safety parameters (complete blood count, markers of hepatic and renal functions). The standard 75-g oral glucose tolerance test (OGTT) was performed (25). We informed the women about the eventual unknown effects of liraglutide, and recommended barrier contraceptives according to the personal preferences of the women.

Assessment of anthropometric parameters

All patients underwent clinical, anthropometric and biochemical assessment at the baseline and at study endpoint. Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. BMI was calculated as the weight in kilograms divided by square of height in meters. Whole-body composition was carried out on a Hologic dual energy X-ray absorptiometry (DXA) (Discovery A) with the software provided by the manufacturer (QDR for Windows, Version 12.5) (26).

Assessment of metabolic parameters

Glucose levels were determined using the standard glucose oxidase method (Beckman Coulter Glucose Analyzer, Beckman Coulter Inc., CA, USA). Insulin was determined by immunoradiometric assay (Biosource Europe S.A., Nivelles, Belgium). Insulin resistance (IR) was calculated by the homeostasis model assessment for IR (HOMA-IR): fasting serum insulin (U/L) × fasting plasma glucose (mmol/L)/22.5 (27). Impaired glucose tolerance (IGT) was identified by 2-hour glucose levels between 7.8 and 11.0 mmol/L, as defined by the American Diabetes Association criteria (25).

Assessment of endocrine parameters

Androstenedione was measured by specific double antibody RIA using 125 I-labeled hormones (Diagnostic

Systems Laboratories, Webster, TX). Total and free testosterone levels were measured by coated tube RIA (DiaSorin, S. p. A., Salluggia, Italy and Diagnostic Products Corporation, LA respectively). Intraassay variations ranged from 1.6 to 6.3%, and interassay variations ranged from 5.8 to 9.6% for the applied methods. Pre- and post-treatment samples for each patient were assayed in the same assay run.

Ovarian stimulation protocol

The short protocol of GnRH antagonist cetrorelix was used for ovarian stimulation in all the patients. The protocol consisted of daily stimulation with 225 IU of follicle stimulating hormone (Puregon; Schering-Plough, Kenilworth, New Jersey, USA) from day 2 of the menstrual cycle. GnRH antagonist cetrorelix acetate (Cetrotide; Merck Serono) in a dose of 0.25 mg s.c. was administered when the dominant follicle measured 14 mm in diameter. In all the patients buserelin acetate 0.6 mg s.c. (Suprefact; Hoechst AG, Frankfurt/Main, Germany) was administered for oocyte maturation when at least three dominant follicles measured ≥ 17 mm in diameter.

Oocyte retrieval, fertilization, embryo culture and transfer, luteal support

Transvaginal ultrasound-guided oocyte retrieval was performed under local anesthesia 36 h after buserelin administration. Oocytes were fertilized by intracytoplasmic sperm injection (ICSI). Embryos were cultured to the blastocyst stage in sequential media M1 and M2 (Origio; MediCult, Jyllinge, Denmark) until day 5. One or 2 best quality blastocysts were transferred into the uterus on day 5. If fewer than 3 oocytes were retrieved, the embryos were transferred on day 3. In case of supernumerary blastocysts or at increased risk of ovarian hyperstimulation syndrome blastocysts were frozen on day 5 or day 6 using a modified two-step Menezo method (28) and stored in liquid nitrogen at -196°C .

Luteal phase support consisted of 1500 IU of human chorionic gonadotropin (hCG) (Pregnyl; N.V. Organon, Oss, The Netherlands) administered after oocyte retrieval if fewer than 21 oocytes were retrieved. Progesterone (Estima Ge; Laboratoires EFFIK, Bruxelles, Belgium) was given intravaginally in a daily dose of 3×200 mg until a gestational sac with fetal cardiac activity was visible on day 30 after the ET.

Assessment of reproductive parameters

Oocyte maturity (MII stage) was defined as germinal vesicle break down (GVBD) with the presence of a polar body. Oocytes with a germinal vesicle or GVBD without a polar body (MI stage) were considered immature. Fertilization rate was defined as the ratio between the number of fertilized and mature oocytes. Oocyte degeneration rate was defined as the ratio between the number of degenerated and all retrieved oocytes. Immaturity rate was defined as the ratio between the number of immature and all retrieved oocytes. Blastulation rate was defined as the ratio between the number of blastocysts and mature oocytes. The blastocyst grading system introduced by Gardner *et al.* was used to evaluate the blastocyst quality (29).

Pregnancies were diagnosed by beta hCG serum determination 14 days after hCG administration and by a transvaginal ultrasound examination 4 weeks after the ET. PR was defined as the number of clinical pregnancies (ultrasound visualization of fetal cardiac activity) divided by the total number of cycles performed or embryo transfers. Cumulative PR was defined as the number of all pregnancies (spontaneous and IVF) divided by the number of patients. The spontaneous pregnancy rate was followed for 12 months after the end of antiobesity intervention.

Statistical analysis

The results are presented as means \pm s.d. or percentages as appropriate. Normal data distribution was checked with the Shapiro–Wilk test. For the normally distributed

data, *t*-test was used (paired samples *t*-test for comparison of before/after parameters, independent samples *t*-test for comparison between the arms). For non-normally distributed data Mann-Whitney *U* test and Wilcoxon signed ranks test were used. For categorical data we used chi-square test. *P* value of 0.05 was considered statistically significant. Statistical analysis was performed using IBM SPSS Statistics, version 24 (IBM Corp, Armonk, NY).

Results

Population characteristics

Twenty-eight patients started the study, and 27 (14 on MET, 13 on COMBI) completed the treatment according to the protocol and were included in the analysis (Fig. 1). One patient in the COMBI group discontinued the study because of protocol violation, 2 patients in the MET group refused IVF. One patient in the COMBI and 1 in the MET group conceived spontaneously immediately after the completed treatment. The remaining 23 patients (11 in the MET and 12 in the COMBI group) attended an IVF after the completed treatment.

Baseline results

There were no significant differences in patients' characteristics of infertility history between the treatment groups (Table 1). There were no significant differences at baseline in any of the anthropometric, metabolic and endocrine parameters between the groups except in 120 min overload insulin levels (Table 2).

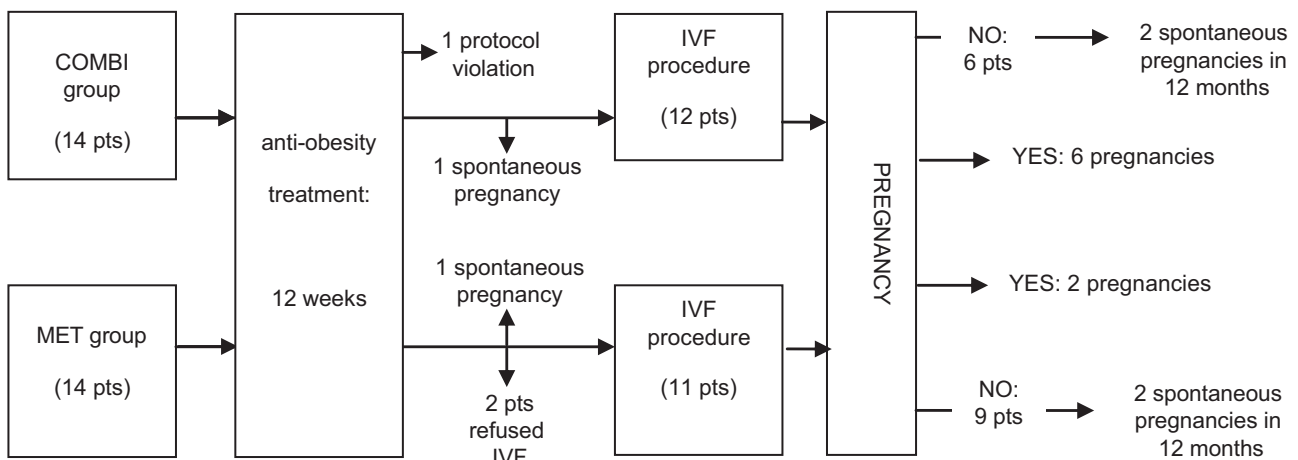


Figure 1

Flowchart of the study with included pregnancies during the observation time of 1 year. COMBI, combination of metformin and liraglutide treatment arm; IVF, *in vitro* fertilization; MET, metformin treatment arm; pts, patients.

Table 1 Data of infertility history. Values are mean \pm s.d. or %.

	MET (n=14)	COMBI (n=13)	P value
Age (years)	31.1 \pm 4.7	30.1 \pm 3.6	NS
Infertility duration (month)	32.7 \pm 20.1	56.2 \pm 33.3	NS
Primary type of infertility (%)	50.0 (7/14)	75.0 (9/13)	NS
Unsuccessful attempt to reduce weight (%)	78.6 (11/14)	75.0 (9/13)	NS
Previous therapy: CC (%)	50.0 (7/14)	61.5 (8/13)	NS
Previous therapy: AI (%)	21.4 (3/14)	30.8 (4/13)	NS
LEKO (%)	28.6 (4/14)	53.8 (7/13)	NS

AI, aromatase inhibitors; CC, clomiphene citrate; COMBI, combination of metformin and liraglutide treatment arm; LEKO, laparoscopic electrocoagulation of ovary; MET, metformin treatment arm; NS, not significant.

Changes in measures of obesity

Body mass decreased significantly in both treatment groups ($P=0.001$ in the MET, $P<0.001$ in the COMBI group). Weight decreased similarly in the MET and the COMBI group ($P=0.246$). Of the 27 patients, 17 (63.0%) were high responders who lost more than 5% of body weight in 3 months (69.2% (9/13) in the COMBI vs 57.1% (8/14) in the MET group). Compared to the baseline, the patients in the MET and the COMBI group lost on average 6.99 ± 6.02 kg and 7.51 ± 3.89 kg respectively. No patient experienced a net weight gain. The data of the post treatment body mass is presented in [Table 2](#).

Likewise, a significant BMI reduction of 2.5 ± 2.0 kg/m² in the MET, and 2.7 ± 1.3 kg/m² in the COMBI group was noted after treatment compared to the baseline ($P<0.001$ in the MET and the COMBI treatment) with no difference in the treatment employed ($P=0.791$).

Comparable results were obtained for the body waist circumference with a significant reduction in the treatment/time effect in both therapeutic groups. The patients treated with MET lost on average 11.3 ± 9.2 cm and the patients on COMBI lost 11.7 ± 9.0 cm ($P=0.001$ in the MET and COMBI group) of waistline. All treatment interventions resulted in significant reduction of visceral adipose tissue mass, volume and area as assessed by DXA. At 3 months, the average visceral adipose tissue (VAT) area in both groups decreased below 160 cm², the threshold associated with a high risk of cardiovascular disease in women (30).

Metabolic changes

Seven (2 in the MET, 5 in the COMBI group) patients had IGT at the beginning of the study. After the treatment, 3 patients in the COMBI and none in the MET group normalized their response to the glucose upload.

HOMA-IR score values were reduced after both interventions, the reduction being significant only in the COMBI group ($P=0.04$). Fasting and post OGTT decreased significantly also in the COMBI group only ($P=0.004$ and $P=0.05$ respectively) ([Table 2](#)).

Endocrine changes

There was a significant increase in SHBG noted in the MET and COMBI treatment arm compared to the baseline (MET: $P=0.019$, COMBI: $P=0.03$) ([Table 2](#)).

Pregnancies and IVF parameters

In the COMBI group, 9 of the 13 (69.2%) patients became pregnant in one year. One patient conceived spontaneously immediately after treatment, 6 pregnancies occurred after an IVF procedure and 2 spontaneous after an unsuccessful IVF ([Fig. 1](#)). In the MET group 5 of the 14 (35.7%) patients became pregnant in one year: 1 immediately after the treatment, 2 after an IVF procedure and 2 spontaneous after an unsuccessful IVF. Two patients in the MET group declined IVF for personal reasons ([Fig. 1](#)).

The PR per ET was significantly higher in the COMBI (85.7%) compared to the MET group (28.6%) ($P=0.03$). There was no statistically significant difference between the groups by the dose of stimulation, number of retrieved oocytes, respective number of mature, fertilized and degenerated oocytes, number of embryos and number of blastocysts on day 5 ([Table 3](#)).

Adverse events

The adverse events were transient and mild. The patients in the MET group reported nausea (1/14) and diarrhea (1/14). The patients in the COMBI group reported nausea (5/13) and headache (2/13). Low-dose and gradual titration reduced the GI side effects associated with liraglutide.

Table 3 IVF outcomes in the MET and the COMBI group.

	MET (n=11)	COMBI (n=12)	P value
Total dosage GNT (IU)	2250 ± 275	2172 ± 320	NS
No. of retrieved oocytes/patient	9.7 ± 5.9	12.8 ± 9.4	NS
No. of mature (MII) oocytes/patient	6.8 ± 5.7	8.8 ± 8.0	NS
Fertilization rate (%)	78.7 (59/75)	73.6 (78/106)	NS
Oocyte degeneration rate (%)	11.2 (12/107)	10.4 (16/154)	NS
Immaturity rate (%)	14.0 (15/107)	13.0 (20/154)	NS
No. of embryos on day 5/patient	4.5 ± 5.8	5.5 ± 5.5	NS
No. of blastocysts/patient	2.4 ± 4.7	3.2 ± 4.3	NS
Blastulation rate (%)	36.0 (27/75)	35.8 (38/106)	NS
No. of transferred embryos	1.4 ± 0.5	1.3 ± 0.5	NS
Pregnancy rate per cycle (%)	18.18 (2/11)	50.0 (6/12)	NS
Pregnancy rate per ET (%)	28.57 (2/7)	85.7 (6/7)	0.03
Implantation rate (%)	3/10	6/13	NS
No. of cancelled fresh ET because of hyperstimulation risk	2	4	NS
Cryopreservation (%)	45.5 (5/11)	58.3 (7/12)	NS
No. of cryopreserved embryos/patient	4.8 ± 6.3	5.0 ± 4.7	NS

Values are mean ± S.D. unless otherwise stated.

ET, embryo transfer; MII, metaphase II; NS, not significant.

Discussion

This is the first report demonstrating that short-term preconception treatment with liraglutide in combination with metformin is superior to metformin alone in increasing PRs per ET and cumulative PRs in obese PCOS women who had been previously resistant to weight reduction with lifestyle modification and first-line reproductive treatments. The observed superiority is challenging, particularly since both treatment interventions resulted in a comparable reduction of body weight and visceral adipose tissue.

Weight loss prior to either spontaneous conception or conception by IVF in obese women is recommended by international guidelines (15), but the data on optimal magnitude and timing are limited. Until now, short-term weight reduction directly before IVF has been assessed in a few prospective randomized controlled studies only (7, 8, 9, 10, 31). The results about reproductive outcomes of IVF are inconclusive. Higher PRs and DRs were achieved in weight loss group (48 vs 14%; $P=0.007$; 44 vs 14%; $P=0.02$) with diet, exercise and behavioral support (8). A recent Spanish study showed a significantly higher cumulative live birth rate (61.9 vs 30%) (9). In the study of obese no-PCOS Australian patients who lost their weight of 3.8 ± 3.0 kg by diet and physical activity in 5–9 weeks there were no differences in PRs and DRs between the intervention and the control group, the increased odds ratio of pregnancy was associated with a waist circumference reduction (7). By contrast, the largest multicentric study including 290 obese women showed

no improvement in reproductive outcomes due to weight reduction prior to IVF (31).

In the present study, 69% of women in the COMBI and 57% in the MET group lost more than 5% of body weight within a 12-week period of pharmacological intervention along with promoted lifestyle modification. Women in the MET group lost on average 7.0 ± 6.0 kg, whereas women in the COMBI group lost 7.5 ± 3.9 kg. Similarly, a significant reduction in BMI, visceral adipose tissue and waist circumference was noted in both treatment groups compared to baseline values/measurements with no differences regarding the type of treatment. The magnitude of weight loss in the COMBI arm was expected and in line with previous reports (18, 19, 32). On the other hand, the magnitude of weight loss in the MET arm was beyond expectation since the greatest documented absolute weight reduction with metformin in obese women with PCOS is about 2.7 kg representing less than 5% of weight reduction (33). Greater weight loss in our cohort might be at least partly explained by the extremely high motivation for lifestyle modification encouraged by a multidisciplinary team of endocrinologists and gynecologists mainly through education about a higher incidence of pregnancy and fetal risks associated with maternal obesity (13).

Within 12 months after a short-term pharmacological intervention prior to IVF, 9 of the 13 (69.2%) patients in the COMBI and 5 of the 14 (35.7%) patients in the MET group became pregnant. In the COMBI group, 1 pregnancy was spontaneous immediately after treatment, 6 pregnancies were achieved following IVF, and 2 spontaneous after an

unsuccessful IVF. In the MET group, 1 pregnancy occurred immediately after treatment, 2 following IVF, and 2 spontaneously after an unsuccessful IVF. Improvement in the cumulative PR did not reach statistical significance, but the tendency toward improved fertility potential in the COMBI arm is apparent, particularly if severely resistant infertility of the study population is taken into account. We may speculate that a larger study population would result in a significant improvement in the cumulative PR. The results of our study cannot be directly compared with other prospective randomized controlled studies assessing the impact of short-term weight reduction directly before IVF because there are some substantial differences in outcome measures, study populations and treatment protocols.

The original choice of preconception treatment protocol resulted in a very interesting yet highly challenging observation that the PR per ET was significantly higher in the COMBI (85.7%) compared to the MET group (28.6%) despite a comparable weight reduction in both arms. In agreement with the expected PR in PCOS patients attending IVF (around 30%) we could not prove any additional beneficial effect of metformin on PR per ET (34). By contrast, we noted a highly beneficial effect of liraglutide that increased the PR per ET from the expected 30% to 85.7%. Furthermore, cumulatively twice as many patients became pregnant using the combination of liraglutide and metformin (70%) compared to 36% using only metformin in the observation period of 1 year.

Although the current data on GLP-1 analogs and reproduction are scarce, liraglutide might affect the reproductive system through several different mechanisms. Weight reduction could be one of important mechanisms which was proven in a 24-week open-label prospective randomized clinical study of 176 overweight/obese women with PCOS that received short-acting GLP-1RA exenatide (Exe) compared to MET. The short-term Exe therapy was linked to significant weight loss (4.29 ± 1.29 kg vs 2.28 ± 0.55 kg, $P < 0.001$), total fat percentage reduction, improved IR and increased menstrual frequency ratio. The rate of natural pregnancy in the time frame of 12 weeks in the Exe-treated patients was significantly higher than in the MET-treated patients (43.60 vs 18.70%) (35). In general, the proposed mechanism by which weight loss improves menstrual cyclicity and ovulation has been explained mainly by a reduction of visceral fat and improved insulin sensitivity (36, 37). Improvement in menstrual frequency addressed as a primary outcome was confirmed in the first study that had used GLP-RA Exe in the PCOS population (38).

The second mechanism of action of liraglutide could be explained by improvement of IR that is associated with weight reduction. The role of IR and hyperinsulinemia in the development of PCOS has been thoroughly explored. It is generally accepted that they play an important role in the pathophysiology of the PCOS and anovulation. Insulin may augment the effects of luteinizing hormone (LH) by increasing the secretion of ovarian androgens (39). Insulin also suppresses the secretion of SHBG by the liver leading to increased levels of free circulating testosterone (39). The improved insulin sensitivity might have the pivotal role in restoring ovarian function. In our study IR as assessed by HOMA-IR score was significantly reduced in the COMBI group. An increase in SHBG that decreased the bioavailability of androgens was also noted in the MET and the COMBI arm. The majority of androgens decreased in both arms, although this decrease did not reach a statistically significant difference.

However, the superior effect of COMBI on the PR per ET despite the comparable reductions of body weight, visceral adipose tissue and androgen bioavailability in both arms imply some unknown effects of liraglutide on fertility potential that is beyond weight reduction and improved IR. Some important animal studies have shown the involvement of GLP-1 in the regulation of the reproductive system through the hypothalamus-pituitary system. Interestingly, intracerebroventricular injection of GLP-1 increases the levels of plasma LH in rats (40). Recently, a study in rats has found that the expression of the GLP receptor varies during the estrous cycle in the hypothalamus, pituitary and ovary. Acute treatment with native GLP-1 during the proestrus phase doubled the amplitude of the LH surge and resulted in increased progesterone in the luteal phase. These changes increased the number of mature graafian follicles resulting in increased fertility. However, treatment with EXE had the opposite effect to GLP-1 reducing the LH surge by 50% (41). The hypothalamic-pituitary axis is a regulatory system that provides regular menstrual cycles with consequential ovulations, and regulates the feeling of hunger and eating habits. We assume that GLP-1 takes a part in coupling these two activities in the hypothalamus-pituitary system and acts as a metabolic signal to the reproductive system (42, 43). In response to 48 h of fasting, the rats' hypothalamic GLP-1 content was significantly reduced when there was a rapid reduction in pituitary LH. Upon re-feeding there was a trend for GLP-1 to return to normal, perhaps aiding the recovery of LH secretion (44).

Moreover, from the previous studies we know that diabetes mellitus is associated with severe changes in the

structure and function of endometrium due to induced cellular toxicity in endometrial epithelial and stromal layers (45). The induced alterations in the endometrium may cause implantation failure, pregnancy loss and defective placentation which have been commonly observed in PCOS as well. In diabetic rats, increased endometrial stromal fibrosis and degeneration were demonstrated. Interestingly, a short acting GLP-1 analog Exe improved the endometrial function in this animal model. The authors suggested that the observed effect of Exe might be mainly due to antagonizing oxidative stress and decreasing transforming growth factor beta levels, thus decreasing fibrosis of the endometrium (46). Since in our study there were no between group differences in the stimulation process (duration, the dosage of gonadotropins), number of oocytes and their quality, number of embryos and their quality, we believe that further research should be focused on the yet unaddressed impact of GLP-1 on the quality and endometrial receptivity.

Regarding the safety issue, GLP-1 RA has a well established favorable 8-year risk-benefit balance in patients with type 2 diabetes and in obese individuals without diabetes (47). The knowledge regarding human exposure to GLP-1 RAs during pregnancy is extremely limited and based on a single reported case of liraglutide exposure up to 13th week of gestation of a woman with type 2 diabetes with a normal pregnancy outcome (48). A preconception intervention with short-term GLP-1 RA Exe has been reported in another case report of an overweight woman with PCOS and IR (49). No important safety issue has been reported in a recent large clinical study evaluating the effects of preconception intervention with Exe on reproductive and metabolic function in overweight/obese PCOS (35). Based on a well established short-term safety profile of liraglutide in patients with type 2 diabetes and obese subjects, and on some limited data on the use of preconception intervention with GLP-1 RAs, a short-term preconception treatment in PCOS subjects seems to be acceptable.

Our study has some limitations. It was conducted as a pilot project and the conclusions are very limited by a small sample size. Further larger studies should be powered using these preliminary results. Spontaneous conception in severely infertile women after weight reduction with liraglutide and metformin encourages the evaluation of potential effects of weight loss on spontaneous conception rates in larger studies with the protocols including delayed IVF procedures in order to avoid unnecessary IVF. Furthermore, the superior impact of COMBI on PRs per ET despite the comparable reduction

of body weight in both arms was unexpected, and implies a potential direct crosstalk between GLP-1 and reproductive system that needs further exploration. The results intend to be the basis of more thorough research and proof-of-concept designs. However, the main strength of this pilot study is the original concept of an efficient short-term weight-reducing intervention with liraglutide directly prior to IVF in infertile obese women with PCOS marked by population and protocol homogeneity.

In conclusion, this is the first pilot study providing preliminary evidence that a short-term preconception intervention with low-dose liraglutide added to metformin is superior to metformin alone in increasing PRs per ET and cumulative PRs in infertile obese women with PCOS despite comparable weight reduction in either treatment. Our observations are not definitive but do encourage further exploration of the efficacy and safety of this class of medications on reproductive outcomes in different obesity-related populations. The precise role of liraglutide in the relationship between the GLP-1 axis and female reproduction remains to be elucidated, in particular the GLP-1 impact on endometrial quality and receptivity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

V Š: protocol development and data collection, manuscript writing. M J: protocol development and data collection, manuscript writing and editing. A J: protocol development, project supervision and manuscript editing. E V B: protocol development, project supervision and manuscript editing.

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