Role of follicular fluid leptin hormone in women with polycystic ovarian syndrome in assisted reproductive technology

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Background/aim
Granulosa cells have the capacity for producing and storing leptin, proposing that this hormone is locally involved in regulating follicular growth. The aim of this work was to evaluate the levels of follicular fluid (FF) leptin hormone and its correlation with pregnancy outcome in polycystic ovarian syndrome (PCOS) cases undergoing intracytoplasmic sperm injection cycles using both long gonadotrophin-producing hormone (GnRH) agonist and fixed GnRH antagonist protocols.
Patients and methods
This was a randomized controlled trial that included 100 patients with PCOS from the reproductive unit of Alexandria University Hospitals, Egypt, who were randomly allocated in two groups (50 patients ech): group 1 included patients with PCOS undergoing long GnRH agonist protocol, and group 2 included patients with PCOS undergoing fixed GnRH antagonist protocol. Anthropometric measurements were done, in addition to assays of FF leptin, serum Anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone, estradiol, and progesterone levels, assessed by enzyme-linked immunosorbent assay techniques.
Results
FF leptin had a highly significant increase in nonpregnant patients when compared with pregnant patients in both groups. In addition, there were significant increases in BMI and weight, whereas insingnificant difference regarding height between nonagonist and antagonist groups.
Conclusion
Leptin can negatively influence in-vitro fertilizations outcomes via weakening several stages of endometrial and ovarian physiology. The exact function of leptin in the poorer outcomes commonly found in obese cases throughout controlled ovarian stimulation needs to be explained, and there is a need to assess the correlation between free bioactive leptin and in-vitro fertilizations cycle parameters.

Keywords:
fertility, follicular fluid, intracytoplasmic sperm injection, leptin, polycystic ovarian

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Introduction

Leptin is a protein formed particularly via adipose tissues and has an essential function in regulating the body mass and intake of the food [1,2]. Leptin suppresses appetite, increases the metabolic rates, and is connected with fat mass. It was suggested to have a role in controlling the reproductive feature and the pathophysiology of reproductive dysfunction, anorexia nervosa, and polycystic ovary syndrome (PCOS) [1,3].

In PCO cases undergoing in-vitro fertilization (IVF) treatments, high mean follicular phase serum luteinizing hormone (LH) levels had a negative effect on the fertilization and cleavage rates and gestation outcomes. To lessen LH concentration during the follicular phase and to avoid a premature LH surge, controlled ovarian stimulation (COS) with gonadotrophin, followed by downregulation with gonadotrophin-producing hormone (GnRH) agonist should be done. The GnRH agonist long protocol is the most commonly used procedure for PCOS cases [2].

Many investigations have recommended that the period of GnRH agonist administrations required to acquire pituitary suppressions for PCOS cases is commonly long relative to that for ordinary ovulatory cases [2]. PCOS cases undergoing IVF have an excessive hazard of growing ovarian hyperstimulation syndrome (OHSS), an acute iatrogenic complication of ovarian stimulations

triggered via exogenous and/or endogenous human chorionic gonadotrophine (hCG) [2].

The introduction of GnRH antagonists in late-years with a reduction in the occurrence of OHSS relative to GnRH agonists in the general populace may pose a novel safer treatment option for these cases [2]. GnRH antagonist has been progressively employed in COS for IVF from the late 1990s [2,4].

Throughout IVF/intracytoplasmic sperm injections (ICSI), an elevated comparative leptin boom is accompanied with adiposity and a discounted ovarian response (OR) [3].

These remarks assist the opportunity that excessive leptin concentration may decrease ovarian receptiveness to gonadotropins. Leptin may provide an explanation for in part why obese people need elevated quantities of gonadotropins than lean participants to acquire OHSS; thus, leptin is taken into consideration as a probable link among nutrients and reproductions [3,5]

Several research studies have confirmed adverse results of leptin on IVF results, involving reserve of ovarian follicular improvement and steroid genesis [6]. Therefore, the aim of the present study was to study the level of follicular fluid (FF) leptin hormone and its relation to pregnancy outcome in patients with PCO undergoing ICSI cycles using both long GnRH agonist and fixed GnRH antagonist protocols.

Patients and methods

Patients

This was a randomized controlled study that included 100 PCOS cases visiting the reproductive unit in Alexandria University Hospitals. Ages ranging from 20 to 40 years, normogonadotrophic women, PCOS cases (satisfying Rotterdam PCOS criteria), and BMI lower than 35 kg/m^2 were the inclusion criteria.

Exclusion criteria were low response in preceding intra-ICSI cycles and history of preceding ovarian surgeries, uterine factor sterility, endometriosis, and severe male factor sterility.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study was approved by the local Ethics Committee of Faculty of Medicine, Alexandria University Hospitals, with approval number 0105177, and a written informed consent was provided by each participant before their inclusion in the study.

Study design

This was a randomized controlled study that included 100 PCOS cases at Alexandria University Hospitals. Patients were randomly allocated into two groups (50 patients each) as follows:

(1) Group I: it included cases involved in the agonist group. All cases were given subcutaneous injections of GnRH agonist 0.1 mg triptorelin [decapeptyl (Ferring)] 6 days earlier to discontinuation of combined oral contraceptions (COCs). When desensitization occurred, daily intramuscular injection of gonadotrophin started. At that day the dose of GnRH agonist decreased to 0.05 mg and continued until the day of hCG.
(2) Group II: it included cases involved in the antagonist group they started the gonadotrophin on cycle day three. Then, GnRH antagonist 0.25 mg cetrorelix; cetrotide (Serono) was given daily on stimulation day sixth and continued till the day of hCG. The initial dosage of gonadotrophin was 150–225 IU/day for all cases in the two groups and was adapted in accordance with cases response to hCG. Choriomon (IBSA) was given in the two groups in a dosage of 10 000 IU intramuscular once after three or more follicles have mean diameters of greater than or equal to 1.8 cm in the agonist and greater than or equal to 1.7 cm in antagonist protocols.

Methods

A permission was obtained from the local ethics committee. A clear knowledgeable agreement was acquired from all of the cases. They were subjected to full history taking, including personal history, such as name, age, weight, height, occupation, residence, and assessment of BMI, and menstrual history; obstetric history, such as gravidity, parity, and mode of previous delivery in cases of secondary infertility; past history: medical disease, especially diabetes, thyroid dysfunction, and surgical and gynecological history; husband history, such as name, age, occupation, residence, special habits like smoking, and related surgical history like testicular varices; and previous exposure to radiation or chemotherapy for the couple. Abdominal and vaginal ultrasound scan was performed in early follicular phase. Hysterosalpingography was performed for each case for exclusion of tubal or uterine factor infertility. All patients received oral contraceptive pills starting on day 4 of

spontaneous menses of the cycle prior to the treatment cycle for 21 days. Which suppress gonadal function, and ovulation that occurred by LH surge. Cases have been randomization and subdivided to two groups.

Oocyte retrieval

Overall, 10 ml of FF specimens was taken in tubes at the ovum pickup day from 08 : 00–10 : 00 morning. The follicular fluid (FF) primary given via the embryologist for oocyte pickup and let the remaining fluid at minimum 15 min at temperature of the room earlier to centrifuge for 10 min for measurements of leptin hormone via sensitive enzyme-linked immunosorbent assay method (ELISA).

Sperm preparation

Semen specimens were gathered using masturbation after 3–5 days of ejaculatory asceticism, and then the specimens were prepared for swim up technique, in which 1 ml medium (Quinn Advantage medium, ART-1020; Sage) added with HAS (Quinn advantage SPS serum protein supernumerary, ART-3010; Sage) (A protein supplement that provides the beneficial growth-promoting activities of albumin and α- and β-globulins. This product is available in the USA) was placed in a 15 ml centrifugation tube, and the 1 ml of dissolved semen was sensibly layered beneath the medium. This was then raised for 1 h at 37°, and the highest 0.5 ml of intermediate was gathered for more usage.

Oocyte fertilization

Following incubation for 3 h, the cumulus cells were stripped using hyaluronidase liquid using a stripper. After final denudation, only MII oocytes (recognized by swelling of first polar body) were injected with the selected sperm with the best morphology under an inverted microscope.

Embryo culture and transfer: fertilized oocytes were kept in a cleavage culture medium (Quinn Advantage medium, ART-1026) added with HSA and incubated in a triple gas incubator at 37°C waiting for cleavage to occur. On day 5, two best scoring blastocysts were transferred using Labotect embryo transfer catheter under abdominal US guidance. The grades of the blastocyst were classed in accordance with the Gardener system for grading.

Luteal phase support protocol

It was started one day after or using intensive luteal support protocol as follows: daily IM progesterone injection 100 mg/d was given. Vaginal progesterone was initiated at 400 mg×2/d (Cyclogest 400 mg pessaries; Actavis Inc., Eidson, New Jersey, USA). Estradiol valerate orally 8 mg/d (Cyclopregnova 2 mg tablets; Bayer Inc., Whippany, New Jersey, USA) was given. Then, the two studied groups were compared in terms of their primary and secondary outcomes as follows.

Primary outcomes

It included whole oocytes number, the mature oocytes number/cycle of induction, serum estradiol and progesterone levels on the hCG administrating day, the grading of embryos obtained, fertilizations rates (known as the amount of collected oocytes that developed 2 pronuclei), and implantation rate (which was defined as the proportion of pregnancy sacs observed at US screening at 6 weeks of pregnancy per transferred embryos number.

Secondary outcomes

Biochemical pregnancy was detected by serum B-hCG assessed 14 days after transfer of embryo. Clinically gestation was approved by the observation fetal cardiac pulsation 2 weeks after positive gestation testing by transvaginal US-screening.

Abortion rate was known as the proportion of cases whose pregnancy was terminated before 20 weeks of gestation.

Laboratory investigation

Serum Anti-Müllerian hormone (AMH) was assessed using kits of Eagle Bioscience Co. (USA) [7]. Follicle-stimulating hormone (FSH) and LH were assessed using kits of Eagle Bioscience Co. (USA) [8]. Estradiol and progesterone level assessments were done using kits of Abcam Company (Cambridge, UK) [9,10] respectively. FF leptin hormone was assayed using kits of Enzo Co (New York, NY, USA) [11]. All methods were performed by ELISA techniques, according to instructions of each kit.

Statistical analysis

The collected data were organized, tabulated, and statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc., Chicago, USA). For quantitative data, the mean and SD were calculated. Qualitative data were presented as number and percentages. χ^2-test, Student's *t*-test, and Mann–Whitney test were used as a test of significance. For interpretation of results of tests of significance, significance was adopted at *P* less than 0.05.

Results

The data presented in Table 1 showed insignificant changes among agonist and antagonist groups in terms

of patients' age, serum AMH level, serum FSH level, and serum LH level, as well as in AFC (antral follicle count) of women under investigation.

Table 2 showed that comparison between the two studied groups regarding anthropometric measurements. There were statistically significant differences ($P<0.05$) between agonist and antagonist groups regarding weight and BMI, whereas there were insignificant differences between agonist and antagonist groups regarding height.

Table 3 shows that a significant change was found among the studied groups in accordance to the number of stimulation days, estrogen level at hCG day, progesterone level at hCG day, and the number of oocytes retrieved, whereas an insignificant change was found among agonist and antagonist groups regarding the whole dose of FSH and endometrial width.

Regarding chemical pregnancy agonist group, 23 (46%) cases were gravid and 27 (54%) were nongravid, whereas in antagonist group, 20 (40%) cases were pregnant and 30 (60%) were nonpregnant. A nonsignificant change was found among them. Regarding clinical pregnancy, agonist group had 21 (42%) cases that were pregnant and 29 (58%) were nonpregnant, whereas in antagonist group, 19 (38%) cases were pregnant and 31 (62%) were nonpregnant. A nonsignificant change was found among them. Regarding abortion, in the agonist group, two (4%) cases had abortion, whereas in antagonist group, one (2%) case had abortion. A nonsignificant change was found among them, as shown in Table 4.

Table 1 Clinical and hormonal assays of agonist and antagonist groups of women under investigation

	Agonist: group 1	Antagonist: group 2	Test of significance	*P* value
Age (years)				
Minimum–maximum	20.0–39.0	21.0–40.00		
Mean±SD	30.94±6.025	31.12±5.858	*U*=1227.5	0.877
Median	32.0	31.50		
AMH (ng/ml)				
Minimum–maximum	2.90–10.90	3.0–11.90		
Mean±SD	7.36±2.600	7.63±2.598	*U*=1155.50	0.515
Median	7.60	7.65		
AFC (antral follicle count)				
Minimum–maximum	19.0–39.0	21.0–40.0		
Mean±SD	30.40±6.440	30.20±6.094	*U*=1227.0	0.874
Median	29.50	31.0		
FSH (IU/l)				
Minimum–maximum	4.90–7.50	5.0–7.50		
Mean±SD.	6.228±0.826	6.10±0.688	*t*=0.842	0.402
Median	6.15	6.10		
LH (IU/l)				
Minimum–maximum	8.50–16.40	8.20–16.50		
Mean±SD	12.704±2.532	12.010±2.496	*U*=1050.0	0.168
Median	12.45	11.65		

AMH, Anti-Müllerian hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; *t*, Student's *t*-test; *U*, Mann–Whitney test.

Table 2 Anthropometric measurements of agonist and antagonist groups of women under investigation

	Agonist: group 1	Antagonist: group 2	Test of significance	*P*
Height				
Minimum–maximum	157–168	159–169		
Mean±SD	163.38±3.016	163.94±2.736	*U*=1143.00	0.458
Median	164.00	164.00		
Weight				
Minimum–maximum	54–88	60–92		
Mean±SD	68.86±10.100	74.58±10.065	*t*=2.341	0.021*
Median	69.00	73.00		
BMI				
Minimum–maximum	21.9–31.1	23.7–32.2		
Mean±SD	26.34±2.709	28.08±2.538	*t*=3.314	0.001*
Median	26.35	27.85		

P, *P* value for comparing between the two groups; *t*, Student's *t*-test; *U*, Mann–Whitney test. *Statistically significant at $P\leq0.05$.

Table 3 Hormonal data and ovarian stimulating features on the hCG day of agonist and antagonist groups of women under investigation

	Agonist: group 1	Antagonist: group 2	Test of significance	*P* value
Total dose of FSH (units)				
Minimum–maximum	1000–2600	1200–2700		
Mean±SD	1835±484.478	1829±395.135	t=0.068	0.946
Median	1900.00	1800.00		
Number of stimulation days				
Minimum–maximum	10–13	10–12		
Mean±SD	11.90±1.035	11.00±0.833	U=651.50	<0.001*
Median	12.00	11.00		
Estrogen level (pg/ml)				
Minimum–maximum	2200–5180	2500–7000		
Mean±SD	3746±917	4655±1315	t=4.010	<0.001*
Median	3943.50	4598.50		
Progesterone level (ng/ml)				
Minimum–maximum	0.38–2.80	0.15–2.80		
Mean±SD	1.614±0.675	0.899±0.555	t=5.486	<0.001*
Median	1.57	0.90		
Endometrial thickness (mm)				
Minimum–maximum	8–12	8–12		
Mean±SD	10.06±1.504	9.70±1.389	U=1071.50	0.208
Median	10.00	10.00		
Number of oocyte retrieved				
Minimum–maximum	15–35	12–28		
Mean±SD	23.06±5.640	19.00±4.634	U=748.00	0.001*
Median	22.50	18.50		

hCG, human chorionic gonadotrophine; *t*, Student's *t*-test; *U*, Mann–Whitney test. *Statistical significance at $P \leq 0.05$.

Table 4 Chemical and clinical pregnancy of agonist and antagonist groups of women under investigation

	Agonist: group 1 [*n* (%)]	Antagonist: group 2 [*n* (%)]	Test of significance	*P* value
Chemical pregnancy (86%)				
Pregnancy	23 (46.0)	20 (40.0)	χ^2=0.367	0.686
No-pregnancy	27 (54.0)	30 (60.0)		
Clinical pregnancy (80%)				
Pregnancy	21 (42.0)	19 (38.0)	χ^2=0.167	0.838
No-pregnancy	29 (58.0)	31 (62.0)		
Abortion	2 (4)	1 (2)	χ^2=0.344	1.000

χ^2 and *P* values for χ^2 testing. *t*, Student's *t*-test; *U*, Mann–Whitney test.

Table 5 revealed that insignificant change was found among agonist and antagonist groups regarding leptin; in addition, follicular fluid leptin had a highly significant increase in nonpregnant patients when compared with pregnant patients in both groups, revealing that a nonsignificant change was found among agonist and antagonist groups regarding leptin.

Discussion

The main objective of this study was to investigate the FF leptin hormone level and its correlation with fertilization rates (FR) and gestation outcomes in PCO cases undergoing ICSI cycles using both long-agonist and fixed-antagonist protocols. A randomized controlled trial was conducted where 100 PCOS cases were randomized and divided in two groups: group 1 included patients with PCOS undergoing long GnRH agonist protocol (n=50), whereas group 2 included patients with PCOS undergoing fixed GnRH antagonist protocol (n=50). The duration of the study ranged from 6 to 12 months.

Obesity was common in more than the half of the women with PCO. The central fat distribution (android obesity) exacerbated the risks of diabetes mellitus and cardiovascular disease. In addition to this fact that obesity involved the peripheral tissue, the intra-abdominal fat improved in PCOS females, which is independent of obesity [6]. The present study shows that a significant change was found among the

Table 5 Comparison between follicular fluid leptin and each of chemical and clinical pregnancy

	Agonist: group 1	Antagonist: group 2	*P* value
Follicular fluid leptin (ng/ml)			
Minimum–maximum	7.8–18.8	8.2–20.8	
Mean±SD	13.52±3.313	14.38±3.547	0.213
Median	13.85	14.45	
Chemical pregnancy			
Pregnancy	10.48±1.839	10.85±1.624	0.500
No-pregnancy	16.11±±1.639	16.74±2.271	0.240
P value	<0.001*	<0.001*	
Clinical pregnancy			
Pregnancy	10.20±1.668	10.73±1.577	0.317
No-pregnancy	15.92±1.728	16.62±2.326	0.194
P value	<0.001*	<0.001*	

All data are expressed as mean±SD. *Significant difference at $P<0.05$, using Student's *t*-test.

studied groups regarding weight ($P=0.021$) and BMI ($P=0.001$), whereas a nonsignificant change was found regarding height ($P=0.458$) between agonist and antagonist groups. This is in contrary to the study of Llaneza-Suarez *et al*. [12] as they reported that a nonsignificant change was found among the groups regarding weight and BMI. According to Garruti *et al*. [13] there was no significant difference among their groups regarding BMI.

Leptin decreases appetite, increases energy expenditure, and decreases the neuropeptide Y productions from hypothalamus. Neuropeptide Y raises nutrition intakes, and after a long-term management, it leads to obesity. Leptin can as well play a job in reproduction, with complex interaction at several stages of the hypothalamic-pituitary-ovarian axis. Circulating leptin level was positively connected to body fat independent of PCOS in accordance to some investigations [14].

Some researchers have straightly evaluated fat gene expressions of leptin in females with PCOS. Primarily, Carmina *et al*. [15] concluded a reduced leptin gene expression in subcutaneous fat in women with PCOS versus weight-matching control group. Lately, however, their group found that subcutaneous leptin mRNA was high in overweight/obese females with PCOS relative to ordinary controls, with the two groups graded by BMI ($<$ or $\geq 25\ kg/m^2$). By means of analogous gene expression analysis, Svendsen *et al*. [16] got remarkable identical findings, revealing that although subcutaneous leptin mRNA was high in obese in comparison with in ordinary controls. As each of these participants was from a dissimilar ethnical background, the difference among the early study by Carmina *et al*. [15] and the two next investigations may only incompletely explained by ethnical changes.

In the study in our hands, a statistically significant change was found among agonist and antagonist groups in accordance to total dose of FSH, the number of stimulation days, estrogen level at hCG day, progesterone level at hCG day, and the number of oocyte retrieved. A nonsignificant change was found among agonist and antagonist groups regarding endometrial thickness. Our results are supported by the results of Khalifa *et al*. [17], as they concluded that in accordance to the stimulation period in the studied groups, it was high significant in the agonist group. As GnRH antagonist protocol attains fast and rescindable suppressions of LH with no any flare-up result that removes the necessity for extended managements to accomplish pituitary suppressions, so lesser days of stimulation scan are needed to ensure improved case compliance. The estrogen levels in hCG day were high significant in agonist group (4912.40 ±2934.571 pmol/l) relative to antagonist group (2926.75±2110.384 pmol/l). This is because of high number of mature oocytes in agonist group. High level of E2 in hCG day could be association with OHSS, but in their report, no cases was present with OHSS. This can be simply considered by modification the dosage of gonadotropins according to follicular growing with modification of dosage of gonadotropin. This agrees with the work of Lainas *et al*. [18]. This can be because of smaller sample size and dissimilar antagonist protocol employed. In a report made by Orvieto *et al*. [19] matching GnRH agonist with GnRH antagonist (226 cases in the agonist group and 261 in the antagonist group), it was revealed that estrogen levels on hCG day were elevated in agonist group.

On the contrary, Kaur *et al*. [20] reported the comparison of the extended agonist protocols with flexible antagonist protocols in women with PCO (60 patients given GnRH agonist extended protocols and 40 given flexible GnRH antagonist protocols) and

found a change in stimulation days between both groups.

The present study shows that a nonsignificant change was found between the studied groups regarding oocyte maturation rates, FRs, and number of moved embryos. Findings of our study were in line with the reports of Lin *et al*. [21] (nine RCTs, examinating PCOS cases experiencing IVF/ICSI, involving 588 females who experienced extended agonist protocol and 554 females who experienced antagonist protocol), which concluded that a nonsignificant change was found in number of retrieved oocytes among the studied groups. Furthermore, Cheung *et al*. [22] reported that a nonsignificant change was found between the study groups regarding the number of mature, immature and degenerate oocytes, FR, and entire embryos attained. In contrary with our results, the results obtained by Kaur *et al*. [20] can be simply considered to the high mature oocytes' number gotten in agonist group. Furthermore, Khalifa *et al*. [17] reported that in accordance to the retrieved oocytes number in the studied groups, it was significantly high in the extended agonist group.

Approximately 9–24% of infertile women experiencing supported reproductions have a meager response to ovarian stimulation. Most IVF programmers use long GnRH agonist protocols for ovarian stimulations. Via involving hypophyseal desensitization, GnRH agonist protocols avoid premature ovulations and luteinizations and decrease the cycle canceling rates significantly in comparison with cycles where gonadotropins are managed only [23]. Latest, GnRH antagonists were obtainable. GnRH antagonists relatively block pituitary GnRH receptors, involving a fast, reversible suppressions of gonadotrophin secretions [24].

GnRH antagonists may be employed to avoid a LH surge throughout controlled OHSS (COH) deprived of the hypoestrogenic adverse effects, flare-up, or long downregulating of the interval accompanied with agonists. The antagonists straightly and quickly constrain gonadotropin release in some hours via modest binding to pituitary GnRH receptors. This feature permits their usage at any time throughout the follicular stage [25].

The current study shows that a nonsignificant change was found between the studied groups regarding rates of implantations, biochemical gestation, clinical gestation, current gestation, live birth, abortions, and OHSS. Our results are supported by the studies of Hosseini *et al*. [26], who presented no change in the rate of gestation between the two regimens. Griesinger *et al*. [27] in 2006 made a comparison between agonist and antagonist protocols in 305 PCOS cases and involved four reports. Pregnancy rates were not different significantly in the studied groups, but the occurrence of severe OHSS was low significantly in the antagonist group. Pundir *et al*. [28] in a recent meta-analysis, which included nine RCTS with 966 women, tried to find whether GnRH antagonist protocols decrease the danger of OHSS in PCOS cases. No difference was found in severe OHSS rates, but when patients with moderate and severe OHSS were combined, there was a significant ($P<0.0001$) lower incidence in the antagonist group.

The findings were similar to the study of Lainas *et al*. [18]. They had two cases in agonist group and none in antagonist group with moderate OHSS, the difference being insignificant. Although this insignificant difference may be because of smaller number of patients in antagonist group, Bahçeci *et al*. [29] in their pilot study also found no difference in OHSS rate in the two regimens. So, in their opinion, cautious evaluation of patients before stimulation, low starting dose of gonadotropin, and cautious displaying of follicular growing with dosage modification of gonadotropin can reduce the incidence of OHSS in PCOS cases experiencing COH with extended agonist regimen.

Cheung *et al*. [22] reported that the rate of clinical gestation per cycle started (16.1 vs. 9.4%; $P=0.22$), per retrieval (26.3 vs. 14.3%; $P=0.20$), and per transfer (26.3 vs. 17.6%; $P=0.26$) were high as well in the antagonist group, but the changes were nonsignificant. Lecke *et al*. [30] investigations as well concluded the rate of ongoing gestation. Pooling the findings of these investigations revealed nonsignificant change among cases managed with the long-agonist protocols in comparison with the antagonist protocols (odds ratio: 1.05, 95% confidence interval: 0.81–1.37); the two reports concluded the precise live birth rate or rate of delivery.

Regarding the relation between follicular fluid leptin and each of chemical and clinical pregnancy show, the present study showed that follicular fluid leptin had highly significant increase in nonpregnant patients when compared with pregnant patients in both groups. In the study of Shaaban [31], the mean FF Activin A was 880.8±354.6 pg/ml, 29 out of 90 cases

(32.2%) attained gestation, and 25 cases (86.2%) have greater than 50% score A embryos on the second-day in contradiction to 33 cases (54.1%) in the nonpregnant group ($P<0.02$). This means that gestation was related positively to second-day embryo quality. FF Leptin and activin A did not correlate to the second-day embryo quality or gestation outcomes.

Ahmeid [32] revealed that a nonsignificant change was found in FFs leptin at oocyte retrieval day among the gravid and nongravid group. There was a significant positive association between FF leptin and BMI in gravid cases, and a significant positive association among FFs leptin with BMI in nongravid females. In another study of Takikawa *et al*. [33], a nonsignificant association was found among levels of leptin, intrafollicular insulin, and adiponectin. A significant change was found in the concentrations of insulin (P=0.007) but not leptin or adiponectin, among gravid ($n=20$) and nongravid ($n=26$) cycles. Only two gestations were found in the 12 cycles in which the insulin concentrations were higher than 7<td:hsp sp="0.5"/>mU/l in FF, but 18 gestations was found in the 34 cycles in which the concentrations of insulin was lower than 7 mU/l ($P=0.043$). The considerably elevated concentrations of insulin in FF were found in nongravid cycles of PCOS cases.

Conclusion

Our results identified that follicular fluid leptin had a highly significant increase in nonpregnant patients when compared with pregnant patients in both groups. We recommended that leptin can negatively influence IVF outcomes by impairing several phases of ovarian and endometrial physiology. The accurate function of leptin in the poorer results generally observed in obese patients during COS needs to be explained, and it must be exciting to assess the association among free bioactive leptin and the IVF cycle parameters.

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Conflicts of interest

There are no conflicts of interest.

References

1 Martínez-Sánchez N. There and back again: leptin actions in white adipose tissue. Int J Mol Sci 2020; 21:6039.

2 Klein S. Adipose tissue leptin production and plasma leptin kinetics in humans. Diabetes 1996; 45:984–987.

3 Bjørbæk C. Expression of leptin receptor isoforms in rat brain microvessels. Endocrinology 1998; 139:3485–3491.

4 Behery M, Hasan E, Eman Ahmed Ali E, Eltabakh A. Comparative study between agonist and antagonist protocols in PCOS patients undergoing ICSI: a cross-sectional study. Middle East Fertil Soc 2019; 24:2.

5 Tamara T, Gomaa I, Mohamed N, El-Ganzoury H. The association between follicular fluid leptin, insulin resistance and ICSI outcome in women with unexplained infertility. Nat Sci 2018; 16:12.

6 Carmina E, Bucchieri S, Esposito A, Del Puente A, Mansueto P, Orio FG, Rini G. Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance. J Clin Endocrinol Metab 2007; 92:2500–2505.

7 Dumont A, Robin G, Catteau-Jonard S, Dewailly D. Role of anti-Müllerian hormone in pathophysiology, diagnosis and treatment of polycystic ovary syndrome: a review. Reprod Biol Endocrinol 2015; 13:1.

8 Lee Y, Yang H, Lee S, Kwon S, Hong E, Welsh H. Onion root (*Allium fistulosum*) restores ovarian functions from letrozole induced-polycystic ovary syndrome. Nutrients 2018; 10:1430.

9 Ben Hameid AS, Al-Sindi TA, Allow AK, Nafie EM, Alahmad BE, Faisal GG. Substantial effect of fenugreek seeds aqueous extract on serum estradiol level in ovarian hyperstimulation syndrome rat model. Oman Med J 2019; 34:238–243.

10 Erickson GF. The ovary: basic principles and concepts. Physiol Endocrinol Metab 3rd ed., 1995; 973–1015.

11 Rehman R, Jamil Z, Khalid A, Fatima SS. Cross talk between serum Kisspeptin-Leptin during assisted reproduction technique; Pak J Med Sci 2018; 34:342.

12 Llaneza-Suarez D, Llaneza P, González C, De-La-Fuente P, García-Ochoa C, Garrido P, *et al.* Assessment of follicular fluid leptin levels and insulin resistance as outcome predictors in women undergoing in vitro fertilization–intracytoplasmic sperm injection. Fertil Steril 2014; 102:1619–1625.

13 Garruti G, De Palo R, Rotelli MT, Nocera S, Totaro I, Nardelli C, *et al.* Association between follicular fluid leptin and serum insulin levels in nonoverweight women with polycystic ovary syndrome. Biomed Res Int 2014; 7.

14 Tan BK, Chen J, Hu J, Amar O, Mattu HS, Adya R, *et al.* Metformin increases the novel adipokine cartonectin/CTRP3 in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2013; 98:E1891–E1900.

15 Carmina E, Chu MC, Moran C, Tortoriello D. Subcutaneous and omental fat expression of adiponectin and leptin in women with polycystic ovary syndrome. Fertil Steril 2008; 89:642–648.

16 Svendsen PF, Christiansen M, Hedley PL, Nilas L. Adipose expression of adipocytokines in women with polycystic ovary syndrome. Fertil Steril 2012; 98:235–241.

17 Khalifa EEAE, Orief YI, Said THM, Elmaksoud DAAA. Fixed gonadotropin-releasing hormone antagonist protocol versus gonadotropin-releasing hormone agonist long protocol in patients with polycystic ovary syndrome treated for intracytoplasmic sperm injection cycles. Int J Reprod Contracept Obstet Gynecol 2016; 5:1135.

18 Lainas TG, Sfontouris IA, Zorzovilis IZ, Petsas GK, Lainas GT, Alexopoulou E, *et al.* Flexible GnRH agonist long protocol in patients with polycysticovary syndrome treated for IVF: a prospective randomized controlled trial (RCT). Hum Reprod 2010; 25:683–689.

19 Orvieto R, Meltcer S, Homburg R, Nahum R, Rabinson J, Ashkenazi J. What is the preferred GnRH analogue for polycystic ovary syndrome patients undergoing controlled ovarian hyper-stimulation for in vitro fertilization? Fertil Steril 2009; 91:1466–1468.

20 Kaur H, Krishna D, Shetty N, Krishnan S, Srinivas M, Rao KA. A prospective study of GnRH long agonist versus flexible GnRH antagonist protocol in PCOS: Indian experience. J Hum Reprod Sci 2012; 5:181–186.

21 Lin H, Li Y, Li L, Wang W, Yang D, Zhang Q. Is GnRH antagonist protocol better in PCOS patients? A meta-analysis of RCTs. PLoS ONE 2014; 9: e91796.

22 Cheung LP, Lam PM, Lok IH, Chiu TTY, Yeung SY, Tjer CC, Haines CJ. GnRH antagonist versus long GnRH agonist protocol in poor responders undergoing IVF: a randomized controlled trial. Hum Reprod 2005; 20:616–621.

23 Faber BM, Mayer J, Cox B, Jones D, Toner JP, Oehninger S. Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. Fertil Steril 1998; 69:826–830.

24 Diedrich K, Diedrich C, Santos E, Zoll C, Al-Hasani S, Reissmann T, *et al.* Suppression of the endogenous lutenizing hormone surge by the gonadotrophin-releasing hormone antagonist Cetrorelix during ovarian stimulation. Hum Reprod 1994; 9:788–791.

25 Shavit T, Ellenbogen A, Michaeli M, Kartchovsky E, Ruzov O, Shalom-Paz E. In-vitro maturation of oocytes vs in-vitro fertilization with a gonadotropin-releasing hormone antagonist for women with polycystic ovarian syndrome: can superiority be defined?. Eur J Obstet Gynecol Reprod Biol 2014; 179:46–50.

26 Hosseini MA, Aleyasin A, Saeedi H, Mahdavi A. Comparison of gonadotropin-releasing hormone agonists and antagonists in assisted reproduction cycles of polycystic ovarian syndrome patients. J Obstet Gynaecol Res 2010; 36:605–610.

27 Griesinger G, Diedrich K, Tarlatzis BC, Kolibianakis EM. GnRH-antagonists in ovarian stimulation for IVF in patients with poor response to gonadotrophins, polycystic ovary syndrome, and risk of ovarian hyper-stimulation: a meta-analysis. Reprod Biomed Online 2006; 13:628–638.

28 Pundir J, Sunkara SK, El-Toukhy T, Khalaf Y. Meta-analysis of GnRH antagonist protocols: do they reduce the risk of OHSS in PCOS?. Reprod Biomed Online 2012; 24:6–22.

29 Bahçeci M, Ulug U, Ben-Shlomo I, Erden HF, Akman MA. Use of a GnRH antagonist in controlled ovarian hyper-stimulation for assisted conception in women with polycystic ovary disease: a randomized, prospective, pilot study. J Reprod Med 2005; 50:84–90.

30 Lecke SB, Morsch DM, Spritzer PM. Association between adipose tissue expression and serum levels of leptin and adiponectin in women with polycystic ovary syndrome. Genet Mol Res 2013; 12:4292–4296.

31 Shaaban MM. Follicular fluid activin A and leptin are not correlated with IVF outcome measures. Egypt J Fertil Steril 2013; 17:11.

32 Ahmeid MS. Correlation between follicular fluid leptin and the pregnancy rate in women who underwent ICSI. Med J Tikrit Univ 2017; 22:249–253.

33 Takikawa S, Iwase A, Goto M, Harata T, Umezu T, Nakahara T. Assessment of the predictive value of follicular fluid insulin, leptin and adiponectin in assisted reproductive cycles. Gynecol Endocrinol 2010; 26:494–499.