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Effect of growth hormone on female infertility

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Abstract: One in six couples suffers from infertility, which is a common problem. It is defined as the failure to conceive following a reasonable period of sexual intercourse without the use of contraception. Growth hormone (GH) regulates male and female fertility and has been used to treat both male and female infertility. In addition to the pituitary gland, the ovary produces GH. Monofollicular growth is aided by GH. GH therapy is a treatment that is used as an adjuvant in ovarian stimulation and Assisted Reproductive Techniques (ART). GH supplementation has been shown to increase pregnancy rate in women with poor ovarian response. Growth hormone therapy plays a role in ovarian stimulation and has beneficial efficacy in carefully selected cases. The study's main goal was to look at the influence of growth hormone on the implantation process in women who had a poor ovarian response after intracytoplasmic sperm injection. The Baghdad IVF Center in Baghdad, Iraq, was home to 90 infertile women with poor ovarian response (POR). The women's ages ranged from 35 to 45. The BMI (body mass index) was calculated. On the day of ova collection, growth hormone levels were measured in both the follicular fluid and the serum. ICSI procedure was used and recorded the pregnancy status. Growth hormone levels were statistically insignificant between pregnant and non-pregnant cases on the day of ova pick up and embryo transfer.

keywords: Infertility, Assisted reproductive technology, *In vitro* fertilization, Growth hormone.

1. Introduction

Infertility is a major issue for millions of couples [1]**.** Infertility (clinical definition) is described as one year of unwanted non-conception with unprotected intercourse in women under the age of 35, during the fertile period of the menstrual cycle, or within six months in women over the age of 35 [2]. Infertility is a serious clinical issue that affects 8–12% of couples globally (estimates range from 48 to 180 million) [3]**.** Female infertility is on the rise, with rates ranging from 10% to 20% 6 [4].

Several factors have been claimed to influence women's fertility; in particular, lifestyle-related factors have drawn considerable attention in the last decade [5]. Maintaining a healthy weight is important because people who are either overweight or underweight are more likely to experience fertility failure, including a lower chance of success with fertility procedures [6]. The method of treatment is determined by the cause, severity, and duration of infertility; the patient's age; Tolerance to side effects; a background of responsiveness and side effects in previous treatments; and the specific treatment preferences of physician and facility associated with the case [7].

Pharmacological treatment, surgical intervention (mostly endoscopy), and assisted reproductive techniques (ART) are the three main therapeutic strategies [8]**.** Growth hormone (GH) has been used to treat female infertility for over twenty-five years [9]. The liver is the primary GH activity's endpoint, where GH makes insulin-like growth factor 1

(IGF-1), which is then the main way that GH works. IGF-1 receptors can be found in the oocytes, Ovarian granulosa, and theca cells [10]. Women over the age of forty who were treated with intracytoplasmic sperm injections (ICSI) were found to have higher rates of delivery and live births when given GH injections [11]. Since then, GH has primarily been used in older women. Recent research indicates, however, that GH may improve the success rate of IVF in some younger females with previous unexplained IVF failures[12]. GH is likely to play an important role as an adjuvant treatment in infertile women with POR undergoing IVF and embryo transfer. [13]. Human growth hormone has gained popularity as a co-gonadotrophin in ART, especially in poor responders [14]. Inappropriately selected cases, GH co-therapy has an effective role in ovarian stimulation [15]. The study's main purpose was to determine the impact of growth hormone on the implantation process in Women with a poor ovarian response after intracytoplasmic sperm injection.

2. Subjects and methods

2.1. Sample collection

The sample size was 90 infertile women undergoing intracytoplasmic sperm injection. All females were assessed in the Females' Infertility Clinic to treat infertility. The women ages enrolled were ranged from (30-43) years old and complained of primary and secondary infertility for a period ranging from more than one year to 2 -18 years. The 90 women chosen for this study were purposefully divided into two groups:

1-Control group: 39 women who were free of signs and symptoms of poor ovarian response, had regular cycles, and no endocrine abnormalities, and whose infertility was determined to be due to malefactors by the Clinic's male infertility specialist in charge. They were undergoing an ICSI cycle, 39 women were divided into two subgroups according to the cause of infertility, subgroup A: 24 infertile couples with primary infertility, and subgroup B: 15 infertile couples with secondary infertility.

2- Poor ovarian response group: 51 females were split into two subgroups based on type and duration of infertility, which ranged from >2 to 18 years, into subgroup A: 39 infertile couples had primary infertility, and subgroup B: 12 couples had secondary infertility.

2.1.1. Inclusion criteria: Females with POR (diminished ovarian reserves) and non-POR.

2.1.2. Exclusion criteria: Patients who have endometriosis, polycystic ovarian syndrome, and a chromosomal problem.

Non-POR (Male factor) was used as a control group.

The current study was approved by the Medical Ethics Committee at Mansoura University (MS.21.09.1652). All patients were given a thorough explanation of the study's purpose before signing the consent form.

2.2. History and physical examination

Each couple underwent a complete history with physical examination in an attempt to find the factors that could be the cause of infertility. Early follicular phase FSH and LH levels were measured in women taking part in the current study on the second or third day of a spontaneous or induced cycle. It was necessary to measure anti-mullerian hormone (AMH) in women over the age of thirty-five to ensure that the patient's ovarian reserve was normal. In addition, serum prolactin, testosterone, and thyroid function tests (FreeT3, FreeT4, and TSH) were performed on days 2-3 of the cycle (CD2) for assessment of the hypothalamus-pituitary function. Also, serum E2 (on CD2) and progesterone (on CD21) were measured for assessment of ovarian function. Activin A hormone, AR factor, and GH hormone were measured on the FF and serum on the day of ovarian pickup. *For Male partner evaluation:* included complete reproductive history and at least two semen analyses. In this study, both partners were evaluated together.

2.3. Ovarian stimulation medicine

2.3.1. GNRH antagonist protocol

It began on D2 of the menstruation with the administration of Recombinant FSH subcutaneously once daily. The Cetrorelix (GnRH antagonist) was usually given, as a multiple doses regime. Doses were adjusted according to age, BMI, FSH and E_2 Two protocols were used for GnRH antagonist:

a. ***Fixed protocol:*** From stimulation day 6 or 7, the antagonist was administered daily.

b. Flexible protocol: Once the follicle diameter reached less than 14 mm, GnRH antagonist injections were administered.

The antagonist was continued, along with the recombinant FSH stimulation, until an adequate response was obtained, after which hCG injection was used for ovulation triggering.

2.4. Sampling of blood on the day of oocyte retrieval

On the day of oocyte retrieval, venous blood samples (5ml) were collected for GH hormone determination using a disposable syringe into a serum separating tube (gel and clot activator) and allowed to clot for 30 minutes. Sera were obtained after centrifugation at a rate of 3000 rpm for 10 minutes, and the clear sera were stored at -20 °C until assayed.

2.5. Assisted reproductive technologies (ICSI)

2.5.1. Oocyte retrieval

After 34-36 hours of hCG administration, aspiration of oocytes was done by transvaginal ultrasound-guided oocyte aspiration under general anesthesia. The woman was placed in the lithotomy position, vagina washed by normal saline irrigation. Follicles from both ovaries were aspirated through the Wallace oocyte recovery system (single lumen needle). The suction apparatus (120 mm Hg) was ideal. Starting with the right ovary, moving on to the left ovary, and finally, the FF was given to the embryologist to determine the quantity and quality of cumulus-oocyte complexes aspirated. After collecting the oocyte-cumulus complexes, they were washed with flushing media to remove any blood residue from the aspirated follicles, graded, and transferred into drops of universal IVF media overlaid by mineral oil in an incubator at 5% CO2, 37°C, and 95% air humidity. Following oocyte retrieval, the women were given antibiotics, analgesics, and luteal phase support.

2.5.2. Follicular fluid sampling on the day of ova pick up

GH hormone levels determination within FF that aspirated on the day of oocyte retrieval from follicles under transvaginal ultrasound guidance. The FF that was obtained pooled in the plain tube for each woman after the isolation of oocytes by an embryologist (any FF without cumulus-oocyte complexes or contaminated with blood were discarded), then it was centrifuged at a rate of 3000 rpm for 10 minutes, to separate cellular contents and debris. Finally resulting FF supernatant was transferred to new plain tubes and stored at −20 °C until assayed, as it has previously done by another investigator.

2.5.3 Sperm preparation

After 2-5 days of abstinence, Masturbation was used to collect the husband's sperm into a dry, clean, and sterile plastic dish on the day of ova pickup, and the sample was immediately transported to the laboratory and placed in an incubator at 37℃ for 30 minutes to allow liquefaction. If the husband has azoospermia, the sperm was extracted surgically from the testis, epididymis, or vas deferens. The swim-up procedure is used for sperm preparation and the seminal plasma can be overlaid immediately with the culture medium so the sperm is allowed to swim from the seminal plasma into the culture medium. Then the sperm suspension was washed adequately to remove the seminal plasma constituents. Alternatively, the semen sample was diluted and centrifuged and the pellet loosened and overlaid.

2.5.4. Oocyte preparation

Following oocyte retrieval, the cumulus corona cells were denuded using the enzyme hyaluronidase and mechanically, and oocyte maturity was determined. The oocyte maturation was assessed by the presence or absence of the germinal vesicle, or the first polar body, then grading oocytes into a germinal vesicle (GV), metaphase one (MI), metaphase two (MII), furthermore classified into the normal or abnormal oocyte. MII oocytes that have extruded the first polar body with normal morphology and were suitable for microinjection were carefully evaluated, and

giant oocytes or oocytes with large polar bodies were not injected.

2.5.5. Intracytoplasmic sperm injection technique

ICSI was performed in the laboratory by a clinical embryologist 4–6 hours after oocyte aspiration. Denudation was performed after 1-2 hours by exposing the cells to a buffered medium containing (80 IU/ml) hyaluronidase for enzymatic removal of corona cells and cumulus. The oocytes were aspirated in and out of a Pasteur pipette, rinsed several times then incubated till ICSI. The denuded oocytes were examined for nuclear maturation.

The ICSI procedure is done by using a microscope, using multiple micro-manipulation devices, which were (micromanipulators, micro-injectors, and micro-pipettes), and the procedure is done by the clinical embryologist. After the ICSI procedure (16-17) hours, fertilization could be assessed for evidence of normal fertilization which was defined as the existence of two pronuclei and two polar bodies.

2.5.6. Embryo transfer

Embryo transfer was done without anesthesia on day two (four cells embryos), day three (six-eight cell embryos), and post-ICSI depending on the women's age, embryo quality, and the number of embryos available. Embryo transfer was usually done by using trans-abdominal ultrasound, through a filled bladder, using a flexible catheter (Cook-Ireland Ltd), which passes through the vagina and the cervix into the uterine cavity about (10-15 mm) from the uterine fundus, where the embryos were placed to implant**.**

2.5.7. Luteal phase support

Progesterone therapy was given to all women for the luteal phase support, in form of Cyclogest (Actavis, Barnstable, UK) ® 200- 400 mg twice/day, or (Crinone, ® 8% progesterone gel, Merk), transvaginal, treatment initiated from the day of ova pick up until pregnancy test was performed. The pregnancy test is done between 12 and 14 days after the ET., and luteal phase support was continued up to 12 weeks of gestation. A vaginal ultrasound examination was done (6 - 7) weeks, after the embryo transfer for confirmation of pregnancy.

2.6. Measurement of GH hormone

The obtained FF and serum were used to measure the levels of GH hormone, by using the enzyme-linked immunosorbent assay (ELISA) technique (Human red, Germany), by using a diagnostic kit (My biosource USA), which provides a quantitative determination of the human activin A and LIF factor concentration in the follicular fluid and serum.

2.7. Procedures of hormonal assay of HGH hormone

The solid phase enzyme-linked immunosorbent assay principle underpinned the HGH Elisa kit. Sheep anti-HGH antibody was used for solid-phase immobilization (micrometer wells), and mouse monoclonal anti-HGH antibody was used in the antibody-enzyme (horseradish peroxidase) conjugate solution. This means that both antibodies are used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react with the antibodies simultaneously. Thus, HGH molecules are trapped between the solid phase and enzyme-linked antibodies, resulting in a sandwich of HGH molecules. Wells were cleaned with water following a 45-minute incubation at room temperature to get rid of antibodies that haven't been bound yet. It was then mixed with a TMB reagent solution and incubated for 20 minutes. This results in a blue color being formed. Stopping the color development with a stop solution caused the color to turn yellow and to be measured at 45nm. The more intense the color of the test sample, the more HGH there was in it.

2.8. ICSI Outcome

Total oocytes, metaphase II oocytes, total embryos, and implantation rates were counted (calculated by dividing the number of gestation sacs with fetal heart seen on ultrasound scan by the total number of transferred embryos), fertilization rates, blastocyst rates, pregnancy rates, and embryo quality rates were among the ICSI outcome measures.

2.9. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 was used to analyze the data. To describe the data, descriptive statistics such as frequency, range, mean, and standard

deviation were calculated. The t-test for independent samples was used to compare the groups (unpaired t-test between two groups) and the chi-square (for non-continuous or percentage data) and the results were considered statistically significant when the p-value was less than 0.05.

3. Results and Discussion:

3.1. Demographic features of the patients enrolled in the current study

Ninety infertile females diagnosed as poor ovarian responders were involved in the current study. The demographic features of patients were illustrated in Table (1) and according to the results, the mean patient's age was 35.73 ± 4.29 years (range 30 - 44 years).

The results in **Table (1)** showed that 63 patients (70 percent) had primary infertility and 27 (30 percent) had secondary infertility, with a mean duration of infertility of 6.8 years (ranging from 2 to 18 years).

Table (1): Demographic features of patients in the current study

Parameter	Range	Mean ± SD
Age (years)	30 - 44	35.73 ± 4.29
Duration of infertilityyears	2.0 - 18	6.80 ± 4.22
Type of infertilityn.(%)	Primary infertility	63 (70 %)
	Secondary infertility	27 (30 %)

n.: Number of patients; SD: Standard deviation.

3.2. The pregnancy rate of patients involved in the current study

Thirty-nine patients out of ninety infertile females with poor ovarian response became pregnant with a pregnancy rate of 43%, **figure (1).**

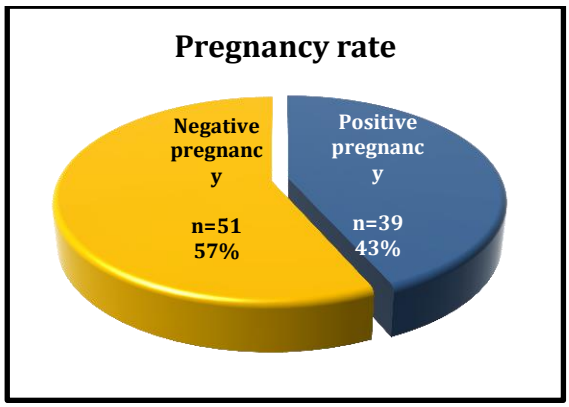

Fig (1): Pregnancy rate of poor ovarian responder patients

3.3. Comparison of demographic features between pregnant and nonpregnant patients

Figure (2) shows a comparison of the mean age, type, and duration of infertility between pregnant and non-pregnant females. The age and duration of infertility were expressed as means plus standard deviations, while the type of infertility was expressed as frequencies and percentages.

There was no significant difference in mean patient age (P=0.867), type of infertility (P=0.400), or duration of infertility (P=0.126) between pregnant and non-pregnant women, according to the findings.

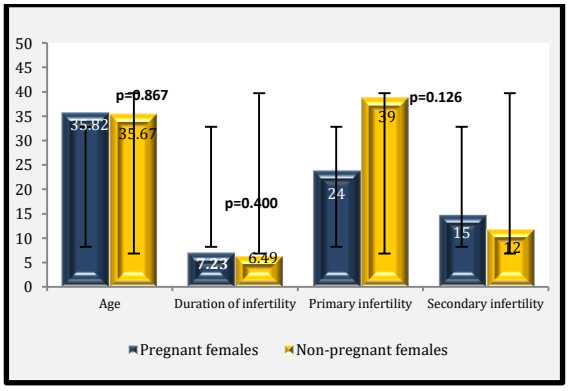

Fig (2): Comparison of demographic features between pregnant and non-pregnant women

3.4. Comparison of hormonal levels between pregnant and non-pregnant females

In figure (3) the comparison of hormonal concentrations between pregnant and non-pregnant patients was demonstrated, there were also no significant differences in FSH, LH, prolactin, E2, and AMH levels between pregnant and non-pregnant females (P > 0.05).

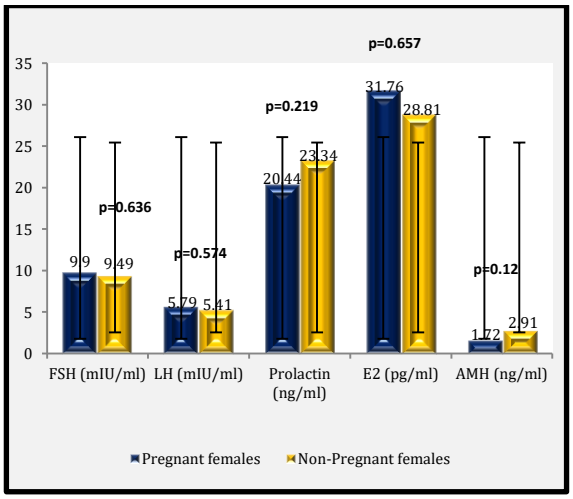

Fig (3): Comparison of hormonal levels (FSH, LH, prolactin, E2, and AMH)

3.5. Comparison of ICSI characteristics between pregnant and non-pregnant females

In figure (4) the comparison of oocytes and embryo characteristics between pregnant and non-pregnant women was illustrated.

in pregnant females, the counts of total oocytes, metaphase II oocytes, total embryos, and transferred embryos were significantly increased. There was also a significantly higher implantation rate in pregnant females 77.38 % versus 0 % in non-pregnant women.

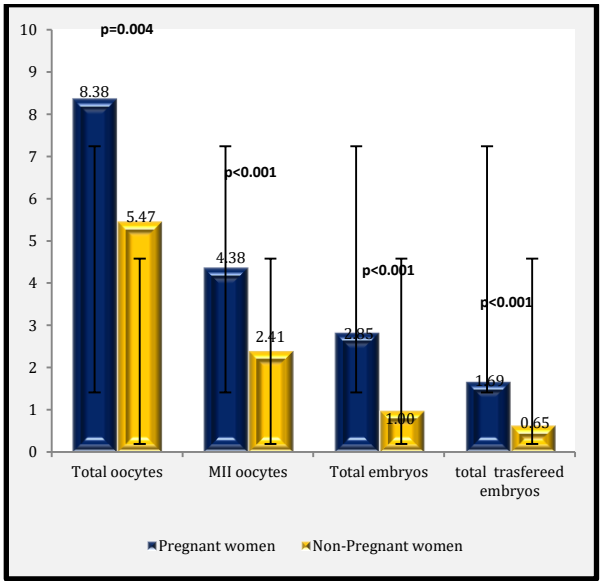

Fig (4): Comparison of ICSI characteristics (total oocytes counts, metaphase II oocytes, total embryos, and transferred embryos).

3.6. Growth hormone levels in serum and follicular fluid of pregnant and non-pregnant females

Growth hormone, activin, and amphiregulin growth factor had been measured three times, the first and the second measurement at oocytes pickup and in the follicular fluid while the third measurement was at the time of embryo transfer. These biomarkers revealed significantly higher levels of activin and amphiregulin in pregnant females at the time of oocyte pick up ($P < 0.001$), however, there was no significant difference between pregnant and non-pregnant patients ($P > 0.05$) in these two biomarkers in follicular fluid or at the time of embryo transfer. At all three measurements ($P > 0.05$), there was an insignificant difference in growth hormone levels between pregnant and non-pregnant patients , as shown in figure (5) and Table (2)

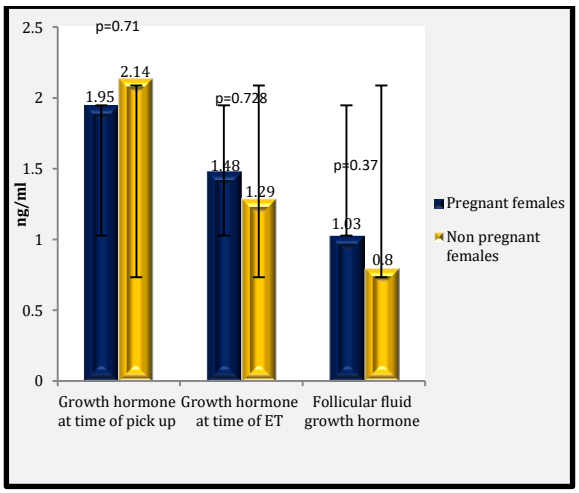

Fig (5): The levels of growth hormone in pregnant and non-pregnant women were compared

Table (2): Biomarker comparisons in pregnant and non-pregnant women

	Pregnant females	Nonpregnant females	p-value
Growth hormone at the time of pickup (ng/ml)	1.95 ±2.52	2.14 ±2.34	0.713
Growth hormone at the time of ET (ng/ml)	1.48 ±2.95	1.29 ± 2.29	0.728
Follicular fluid growth hormone (ng/ml)	1.03 ±0.86	0.80 ±1.16	0.372

4. Discussion

Ninety infertile females diagnosed as poor ovarian responders were involved in the current study. The mean patient's ages were 35.73 ± 4.29 years (range 30 - 44 years). Sixty-three patients (70%) presented with primary infertility, while 27 (30%) presented with secondary infertility, with a mean duration of infertility of 6.8 years (ranging from 2 to 18 years). Thirty-nine patients out of ninety infertile females with poor ovarian response became pregnant with a pregnancy rate of 43%. Al-Murshidi et al. [16] discovered that the rate of pregnancy after ICSI was 28.89 percent, which is consistent with our findings. In women with unexplained cause, the pregnancy rate was (33.30%) followed by women with the explained cause which was (25.90%). Pregnant women's ICSI parameters were better than those of non-pregnant women. There was no significant difference in mean patient age (P=0.867), type of infertility (P=0.126), or duration of infertility (P=0.400) between

pregnant and non-pregnant patients, according to our findings. Asimakopoul et al. [17] discovered that Pregnant women had a higher mean age than non-pregnant women. (32.21 ± 6.68 vs. 31.80 ± 5.38 years), though There was no statistically significant difference (P > 0.05). This is similar to the findings of Faraj et al. [18] who found a non-significant difference in age (28.9 ± 4.7 vs. 27.2 ± 5.2) between pregnant and non-pregnant women. Furthermore, Ashrafi et al. [19]found that In primary infertility, pregnancy rates were lower than in secondary infertility (OR=1 vs. 1.005, 95 percent CI=0.774-1.30; p= 0.969), but this difference was not statistically significant. A previous study found no significant difference (P=0.302) in the duration of infertility (years) between the pregnant (6.4 ± 4.7) and non-pregnant (7.8 ± 4.5) groups Vural et al**.** [20]. The pregnant female's group showed a non-significant (p > 0.05) difference in the levels of FSH, LH, prolactin, and E2 when compared with the non-pregnant female's group, according to our findings when we compared hormonal levels between pregnant and non-pregnant patients. Also, Ahn et al. [21]**,** 2021 found no significant difference in LH levels (7.96 ± 4.76), (7.10 ± 5.28) (P=0.598), and E2 levels (62.11 ± 44) (60.03 ± 68.91) (P=0.915) between pregnant and non-pregnant females, while the pregnant group had lower serum FSH levels (7.14 ± 3.32) compared to non-pregnant (9.048.71), (P=0.008). Vural et al. [20] discovered no significant differences in prolactin (ng/ml) (P=0.193) between pregnant (10.4 ± 5.8) and non-pregnant (14.0 ± 8.2) females.

In the current study, we discovered that pregnant females had significantly higher total oocyte counts, metaphase II oocyte counts, total embryo counts, and transferred embryo counts. In addition, pregnant women had a significantly higher implantation rate of 77.38 percent compared to 0% in non-pregnant women. This finding is consistent with the findings of **Ashrafi et al.** [19] where the number of retrieved metaphase II (MII) oocytes was significantly higher in the pregnant group (8.3 ± 3.9) than in the non-pregnant group (7.2 ± 4.1) (OR= 1.06, 95 percent CI =1.04 - 1.09), (P < 0.0001) and the number of embryo transfers in the pregnant group (2.50 ± 0.66) in the non-pregnant group (2.36 ± 0.79) (OR=1.29, 95 percent CI=1.11 1.48. Yang et al. [22] reported that the total number of embryos (7.61 ± 3.99 vs. 6.67 ± 4.14) (P < 0.001) was significantly higher in the pregnant group compared to the non-pregnant group.

At all three measurements, the difference in growth hormone levels between pregnant and non-pregnant patients was statistically insignificant (P > 0.05), but in the study by Tarlatzis et al. [23] The opposite was discovered: GH levels in follicular fluid from pregnant women were significantly lower (P < 0.05) than in non-pregnant women (2.8 versus 3.5 ng/ml).

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