Effects of adding oestradiol supplementation in luteal phase in patients undergoing ICSI long agonist fresh embryo transfer cycles

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Abstract

Background: Luteal phase support (LPS) is a crucial step in ICSI/IVF cycles for embryo implantation. It has been agreed that progesterone supplementation is an integral part of luteal phase support and implantation. Different additional supplementations have been proposed in addition to progesterone; of which estradiol was one. This study was done to compare the additive role of ostradiol supplementation to progesterone for luteal phase support compared to progesterone alone in ICSI cycles.

Objectives: to assess the effect of oestradiol supplementation in addition to progesterone during luteal phase on the implantation rate in patients undergoing long agonist ICSI/IVF cycles.

Methods: A prospective randomized controlled double blinded study, two-hundred and thirty six patients undergoing their first ICSI cycle using the long agonist protocol, were enrolled in this study. Participants were then randomized into two equal groups of 118 patients each; **Group A:** received a dose of 400 mg progesterone twice daily in the form of vaginal or rectal suppositories, in addition to (2x2) placebo oral tablets (similar to estrogen tablets). **Group B:** received 400 mg progesterone twice daily in the form of vaginal or rectal suppositories, in addition to oestradiol valerate oral tablets in a dose of 4mg/day (2x2). In both groups, medications were started from the day of ovum pickup and for 14 days after embryo transfer. Participants were further divided in to two groups, according to their oestradiol levels. Implantation rate was set as the primary outcome, secondary outcome included chemical, clinical pregnancy and miscarriage rate per cycle.

Results: The implantation rate was significantly higher in the progesterone only group (Group A) compared to oestradiol and progesterone group (GroupB) (12.88% vs 7.98% respectively)

Conclusion: Supplementation of oestradiol to progesterone in luteal phase support confers no additional benefit to progesterone alone.

Further studies are required to elucidate the role of oestradiol in the luteal phase support in ICSI cycles.

Key words: Oestradiol, Progesterone, ICSI, luteal phase support, implantation rate .

Introduction

Adequate luteal phase function is a crucial part of embryo implantation and both pregnancy development and maintenance. In normal ovulatory cycles, progesterone secreted by the corpus luteum helps in pregnancy maintenance until the placenta takes over at 7 weeks. Therefore, a defective progesterone secretion, and hence a defect in the luteal phase would hamper the development and maintenance of the pregnancy process (1,2).

In controlled ovarian stimulation cycles, the multifollicular development and supra physiological levels of oestradiol and progesterone induce negative feedback on luteinizing hormone (3,4); thus resulting in luteal phase dysfunction, which in turn affects ICSI/IVF outcome negatively (5). This dysfunction has been observed in both GnRH agonist and antagonist protocols, hence luteal phase support became an integral step in ICSI/IVF cycles (6).

Progesterone plays an important role in the implantation process through different mechanisms at both cellular and humoral levels. At cellular level, progesterone reduces intracellular calcium concentration, and at humoral level it plays as an immunomodulatory. Progesterone prepares the endometrium and improves its receptivity for embryo implantation (7). Successful implantation requires the synchronization of the endometrium receptivity with the embryo, which is effectively achieved by progesterone supplementation (8).

In natural ovarian cycles, estrogen is secreted in addition to progesterone by the corpus luteum, thus suggesting that estrogen supplementation may play a role in luteal phase support in IVF/ICSI cycles (7). A met analysis in 2015, concluded that there was no role to oestradiol supplementation in addition to progesterone in IVF/ICSI implantation and pregnancy rates (9). Two further studies also concluded that adding estradiol conferred no additional benefit (10,11).

The use of oestradiol has been a matter of controversy over the past decade (12), we have designed this study to assess the role of additional oestradiol supplementation to progesterone in long agonist ICSI cycles.

Sample size

According to Lukaszuk et al. (2005) (13), a group sample size of 112 in each group achieves 80% power and 0.05 significance level to detect a difference between the group proportions of 0.1405. The proportion in the treatment group is assumed to be 0.0980 under the null hypothesis and 0.2385 under the alternative hypothesis. The proportion in the control group is 0.0980. The statistical test used is the two-sided Z test with pooled variance. The sample size increased by 5 % to be 118 in each group for dropout.

Patients and Methods

We have conducted a prospective randomized controlled double blinded study, two hundred and thirty six patients undergoing GnRH long agonist protocol, with fresh embryo transfer were recruited at the Assisted Reproduction Treatment unit, Obstetrics and Gynecology department, Cairo University hospitals. The participants were recruited in the period between May 2019 and May 2022.

The study was conducted after the approval of

the the ethical committee of the Gynecology and Obstetrics; and was registered at the Clinical trial.gov (registration no.NCT03832894).

The included women had signed written informed consent before participating in this study after being informed of the purpose, interventions, outcome, and possible complications.

Inclusion criteria were female patients between 20- and 38-years old, undergoing GnRH long agonist protocol, with fresh embryo transfer on day 3, or day 5. Grade 1 and Grade 3, quality embryos were transferred under ultrasound guidance. Women were excluded if they had karyotypic abnormalities in either partner, uterine abnormalities, grade 3 / grade 4 (G3-G4) quality embryos, estradiol level 10,000 or more at time of trigger, egg /sperm donation/embryo donors, polycystic ovary syndrome (PCOS) patients, poor responders and patients with severe male factor.

All women in both groups were subjected to detailed history and clinical examination to ensure adherence to inclusion criteria. Trans-vaginal ultrasound (TVS) [vaginal probe 6.5MHz, Mindray, China] was done on day 2 to 5 to assess antral follicular count, uterus and adnexa then long agonist protocol was started.

Participants were then randomized into two main groups: Group A: Received 400 mg progesterone twice daily in the form of suppositories (Prontogest 400mg) either through the vagina or rectum, in addition to 2x2 placebo oral tablets (similar to estrogen tablets) for luteal phase support,. This was started on the day of oocyte retrieval and for 14 days after embryo transfer. Group B: Received a dose of 400 mg progesterone twice daily in the form of suppositories either vaginally or rectally, in addition to 2x2 oestradiol valerate oral tablets (Progynova 2mg, Bayer) in a dose of 4mg/day (2x2), for luteal phase support, from the day of ovum pickup and continued for 14 days following transfer of embryos.

In both groups, the participants were further subdivided into two subgroups, according to their estradiol levels: Subgroup A: patients with oestradiol levels less than 5000 pg/ml on the day of human Chorinic Gonadotropin (hCG) trigger. Subgroup B: patients with oestradiol level between 5000 -10,000 pg/ml on the day of hCG trigger. Randomization was done by withdrawing closed envelopes for each patient. Double blinding was applied (both patient and health administrator).

For both groups quantitative ß- HCG in serum was done after 14 days of embryo transfer and also TVS to detect clinical pregnancy at 6-7 weeks of gestation.

In the event of pregnancy in either group, same luteal phase support for group A and B was continued till 12 weeks gestation.

Statistical analysis

Pre-coded data was entered on the computer using Microsoft Office Excel Software Program 2018. Pre-coded data was then transferred and entered into the Statistical Package of Social Science Software program, version 25 (SPSS), to be statistically analyzed. Quantitative variables were described as mean ±SD, median, and range, while qualitative variables were described as frequency and percentage. For quantitative data, the Independent Sample t-test was used to compare normally distributed variables, and the Mann-Whitney U test was used to compare non-normally distributed variables. On the other hand, the Chi-square test/Fisher Exact test was used to compare qualitative variables. P-value was considered significant if less than 0.05.

Results

The results are illustrated in Tables 1, 2 and 3.

Table 1 displays different patient characteristics as age, BMI, type and duration of infertility and sociodemographic characteristics. As

shown both groups were properly matched regarding the aforementioned characteristics.

Table 2 shows the cycle characteristics as AFC, number of days of stimulation, dose of gonadotrophins, endometrial thickness on the day of embryo transfer and number of embryos transferred. Both groups were comparable except for the endometrial thickness which was significantly higher in the progesterone only group (groupA), compared to group the oestradiol and progesterone group (group B) [11 vs 10.5], with a p value 0.01.

Table 3 shows the implantation, chemical and clinical pregnancy rates and miscarriage rate among the study groups. The implantation rate was significantly higher in group A compared to group B (12.88% vs 7.98%), with a P value: 0.029.

The progesterone only subgroup with serum E2 levels higher 5000 pg.ml, showed significantly higher implantation rate than the oestradiol and progesterone subgroup with serum E2 levels greater than 5000 pg/ml; (13.38% vs 7.32%), p value 0.012.

The clinical and chemical pregnancy and miscarriage rates did not show any significant difference between both groups.

Discussion

There is no debate that progesterone supplementation is fundamental for luteal phase support. The question was there any beneficial role to adding oestradiol to progesterone on implantation and pregnancy rates in IVF/ICSI outcomes. This study was designed to compare the role of adding estradiol tablets in a dose of 4mg to vaginal progesterone in infertile patients with good prognostic factors (i.e good ovarian reserve indicated by their basal FSH and AMH levels ; undergoing their first ICSI/IVF cycle using the GnRh agonist long protocol.

In this randomized controlled trial, computer randomization together with double blinding of the two groups to estradiol and placebo, eliminated any element of bias that could be related to patient selection. Both groups were comparable regarding the age, basal hormone levels, BMI and smoking, thus making both groups comparable. Also both groups were comparable regarding the AFC, total dose of gonadotrophins, days of stimulation and number of embryos transferred, thus eliminating any bias in the implantation rate that could be due to number of embryos transferred.

The implantation rate was higher in group A (progesterone alone) 12.88%, whereas that for group B (progesterone and oestradiol valerate 4mg) was 7.98%, the p value 0.029% which makes it significantly different. The endometrial thickness was significantly greater in the progesterone only group 10.99 ± 1.24 Vs the oestradiol and progesterone group 10.31 ± 1.90, which was statistically significant p value 0.001%. The difference in endometrial thickness between both groups could explain the significantly higher implantation rate in the progesterone only group.

The chemical and clinical pregnancy rates were higher in the progesterone only group compared to the oestradiol and progesterone group (29.66% Vs 26.27%, p value 0.56), (27.12% Vs 22.03%, p value 0.364). However, this was not statistically significant. Pregnancy loss was higher in group B compared to group A 16.13% Vs 5.71%, again this was statistically insignificant.

Both groups had comparable serum E2 levels on the day of hcg trigger (2890.71 ± 1956.58 Vs 2843.89 ± 1834.91), with p value 0.850. This negates that the difference in implantation rates could be attributed to difference in serum E2 levels between the progesterone only group (group A) and the progesterone and oestradiol group (group B).However, serum E2 levels higher than 5000 pg/ml in progesterone only group was associated with significantly higher implantation rate (13.38%) compared to serum E2 levels of more than 5000 pg/ml

in the progesterone and oestradiol group (7.32%); p value 0.012. The significant difference between the major groups A and B in relation to serum E2 levels > 5000 pg/ml, could not be explained.

In a former systematic review of 4 articles from 2000 till 2016, Pinherio et al concluded that oestradiol addition to progesterone was not superior to progesterone alone in GnRH antagonist cycles. The patients included in different papers showed similar patient characteristics to our study and good prognostic factors, thus mitigating the bias that could be due to poor ovarian reserve. However, those studies used the GnRH antagonist protocol and not the long GnRH agonist protocol used in our study (10).

In a retrospective observational study that included 150 patients with 75 in each group, the pregnancy rate was 41% Vs 36 % in the oestradiol Vs control group. Again in this study, the GnRH antagonist protocol was the used protocol (11). It is believed that antagonist cycles show lower estradiol levels compared to agonist cycles. This marked decrease in serum pestradiol levels is secondary to increased serum progesterone levels seen in antagonist cycles (12). Despite that, addition of estrogen to progesterone in antagonist cycles conferred no superior results over progesterone alone in the aforementioned studies.

Another similar study to ours, but was open label; that included 160 patients divided equally in to two groups. The control group received progesterone suppositories 200 mg twice daily, and the intervention group, received oestradiol in the form of patch 100mcg/day in addition to progesterone. They concluded that supplementing oestradiol did not add any extra benefit, as the implantation rate did not differ between both groups (34.9% [51 of 146] vs. 28.9% [41 of 142], the ongoing pregnancy rate was the same in patients receiving oestradiol in addition to progesterone compared to progesterone alone (14).

Another meta analysis of different studies that was conducted in 2019 concluded that oestradiol supplementation in luteal phase in IVF/ICSI was beneficial compared to progesterone alone. However, they concluded that this benefit was only observed in GnRH agonist cycles only but its supplementation in antagonist cycles conferred no additional value on clinical pregnancy and implantation rates. This was contradictory to the fore mentioned studies which mainly evaluated its effect on antagonist cycles, and ours in which GnRh agonist protocol was the study protocol (15).

On comparing pregnancy rates in relation to serum E2 levels in the major groups A and B, , we could not establish any correlation between serum E2 levels and pregnancy rates. Previous studies showed such positive correlation (16, 17); other studies failed to establish any correlation of serum E2 levels with pregnancy rate (18). Further studies and met analysis are needed to evaluate the role of serum estradiol levels on IVF/ICSI outcomes.

The strength of our study is that it was double blinded which makes it unbiased. Furthermore, it involved one protocol and the patients were comparable regarding characteristics and IVF/ICSI prognostic factors, thus making the results more reliable. The limitation in our study was sample size, larger sample size might be more informative.

In conclusion, adding estradiol did not improve implantation rates, to the contrary, it compromised the outcome. More studies have to be done to evaluate if there is any role to adding oestradiol in luteal phase support in fresh embryo transfer cycles, and till then its use should not be recommended outside the scope of research.

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Results

Table 1: Characteristics of the study groups

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
Age (years)	29.66 ± 5.37 30 (19 - 39)	28.94 ± 5.21 28 (18 - 40)	0.297
BMI	28.51 ± 5.10 29 (19 - 42)	28.75 ± 4.62 29 (20 - 44)	0.698
Gravidity	0.69 ± 1.32 0 (0 - 8)	0.58 ± 1.02 0 (0 - 5)	0.826
Parity	0.25 ± 0.57 0 (0 - 4)	0.19 ± 0.45 0 (0 - 2)	0.411
Previous abortions	0.44 ± 1.14 0 (0 - 8)	0.39 ± 0.92 0 (0 - 5)	0.967
Occupation • Housewife • Employer	 101 (85.59%) 17 (14.41%)	 114 (96.61%) 4 (3.39%)	**0.005***
Residence • Urban • Rural	 73 (61.86%) 45 (38.14%)	 74 (62.71%) 44 (37.29%)	0.893
Type of infertility • Primary • Secondary	 76 (64.96%) 41 (35.04%)	 77 (65.25%) 41 (34.75%)	0.962
Infertility Duration (years)	4.62 ± 2.72 4 (1 - 15)	4.76 ± 2.97 4 (1 - 14)	0.961

Table 2: ICSI cycle characteristics of the study groups

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
AFC	12.60 ± 4.13 12.5 (3 - 23)	11.64 ± 3.43 12 (5 - 22)	0.052
Number of Days of Stimulation	12.63 ± 2.75 13 (4 - 21)	12.02 ± 2.61 12 (6 - 20)	0.082
Number of GN Ampoules	43.41 ± 14.54 39.5 (12 - 90)	42.99 ± 13.83 40 (18 - 90)	0.822
Endometrial thickness	10.99 ± 1.24 11 (8 - 14)	10.31 ± 1.90 10.5 (3 - 15)	**0.001***
Serum E2 Level	2890.71 ± 1956.58 2300 (305 - 10263)	2843.89 ± 1834.91 2468 (355 - 9550)	0.850
Day of ET	3.16 ± 0.82 3 (2 - 5)	3.06 ± 0.72 3 (2 - 5)	0.376
Number of Embryos Transferred	3.09 ± 0.83 3 (1 - 4)	3.19 ± 0.82 3 (1 - 4)	0.407
Number of gestational sacs	1.47 ± 0.76 1 (1 - 4)	1.15 ± 0.46 1 (1 - 3)	0.058

Table 3: Analysis of the study outcome

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
Implantation Rate	47/365 (12.88%)	30/376 (7.98%)	**0.029***
Implantation Rate • <5000 pg/ml • >5000 pg/ml	 5/51 (9.80%) 42/314 (13.38%)	 6/48 (12.50%) 24/328 (7.32%)	**0.670** **0.012***
Chemical Pregnancy	35/118 (29.66%)	31/118 (26.27%)	0.562
Chemical Pregnancy • <5000 pg/ml • >5000 pg/ml	 31/102 (30.39%) 4/16 (25.00%)	 24/103 (23.30%) 7/15 (46.67%)	0.252 0.208
Clinical Pregnancy	32/118 (27.12%)	26/118 (22.03%)	0.364
Clinical Pregnancy • <5000 pg/ml • >5000 pg/ml	 28/102 (27.45%) 4 /16 (25.00%)	 20/103 (19.42%) 6/15 (40.00%)	0.174 0.306
• Pregnancy loss	2/35 (5.71%)	5/31 (16.13%)	0.240