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Clinical Outcomes in One of Four Freeze to Thaw Embryos Transfer Conventions were Increased by Administering GnRH Agonist in Luteum Phase: Retrospective Analysis

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OBJECTIVES

To determine if GnRH agonist (triptorelin) administration supporting luteum phase in transferring freeze to thaw embryos (F.E.T) improves clinical outcomes.

Methods and Designs: I have carried out analysis of a Retrospective Cohort and analyzed 3515 cycles of receiving FET at department of reproductive medicine of our hospital from February 2018 through December 2021. The Patients were divided into GnRH-a (triptorelin +existing treatment) group and No GnRH-a (existing treatment without Triptorelin) group. There were 1033 and 2485 cases in above groups respectively. Live births rates (L.B.R) and clinical pregnancies rates (C.P.R) were contrasted in two groups.

Results: We have found greater C.P.R (58.00% versus. 48.40%, P-value= 0.003) and L.B.R (52.70% versus. 45.60%, P-value = 0.001) for H.R.T-F.E.T cycles, and found no clinical significance for NC-FET(58.20% versus 52.90%,P-value=0.364) and (54.40% versus 47.00%, P-value=0.211), GnRH-a+HRT-FET(53.00%versus53.00%,P-value=0.176) and (46.20%versus47.30%, P-value=0.794), and Stimulation-FET (59.30% versus 52.90% P-value=00.566) and (59.30% versus 47.10% , P-value=00.247) in terms of C.P.R and L.B.R in two groups.

There was 47% increment of C.P.R in GnRH agonist group and there was 33% increment of L.B.R in same group.

Conclusions: During H.R.T-F.E.T cycles, administering of Triptorelin 3-4 times in the existing luteal support can improve C.P.R and L.B.R. Administering Triptorelin during Luteal phase can prove new option for luteal support. Success rate of IVF in women of older age will increase significantly

Keywords: Luteum Phase Support (L.P.S); Hormone Replacement Therapy(H.R.T); freeze to thaw embryos transfer (F.E.T); Natural cycle(N.C); Stimulation assisted Cycle, GnRH-a assisted HRT cycle

Abbreviations: CPR: Clinical Pregnancies Rates; LBR: Live Births Rates, LH: Luteinizing Hormone, L.P: Luteal Phase; AFC: Antral Follicle Count; NC: Naturally Cycles

Introduction

There are many protocols of endometrium preparation before FET: natural Cycles, normone replacement therapy (H.R.T) cycles, GnRHa assisted HRT cycles, Stimulated assisted cycles [1,2]. Each protocol has its advantages and limitations. FET cycles have gained significance, accounting for upto one-third of all American babies, ART technology is used [3]. Currently, there are many medications for luteal support in clinical practice, including progesterone, human chorionic gonadotropin (HCG) and estrogen [4]. Some studies have found that GnRHa is used for luteal support therapy, GnRHa stimulation makes

the pituitary gland increases the secretion of Luteinizing hormone (LH) for luteal support [5]. While other researches have shown that There is expression of GnRHa receptors on both sides of placentas, normal endometrium, Myometrium, ovaries and testes [6]. It is believed that GnRHa can affect the endometrium local GnRHa receptors exert a direct effect and can improve endometrial receptivity [7]. At present, GnRHa supplementation in Luteal phase promotes luteal function, embryonic development potential and embryo development, but the mechanism of endometrial receptivity is still unclear. The study we are presenting is retrospective analysis of F.E.T cycles of patients who are taking treatment at our reproductive center. The aim was to assess the effects of triptorelin in luteal phase (L.P) during FET Cycles for C.P.R and L.B.R and provide a basis for clinical application.

Materials and Methods

Research Objective

Our hospital's ethical committee gave approval to this investigation with protocol number 2023105 dated of 24.04.2023. From January 2019 to December 2021, we did analysis. We covered all FET-assisted pregnancy protocols. They are natural cycles, HRT, GnRHa +HRT Cycle, and Stimulation Cycle Protocols of FET assisted pregnancy. Retrospective data was obtained. Women›s records ranging in age from 20 to 52 were included. The range of their BMIs was 15 to 41.6 kg/m2. AMH ranged from 0 to 59. They experienced infertility for 0.2 to 22 years. They had their own oocytes and embryos. We excluded fresh cycle protocols for assisted pregnancy, oocyte donation cycles, donated embryos and uterine malformations. Before FET, all patients signed the necessary informed consent forms. We divide the patients into two groups: one that receives GnRH-a (Triptorelin) during the luteal phase, and the other that does not.

Method

In our reproductive center, we mostly use the N.C, H.R.T-F.E.T, G.n.R.H.a-H.R.T , and stimulated cycles to get the endometrium ready for transferring frozen embryos [8]. To this investigation, we want to assess GnRH-a›s efficiency in supporting luteum phase in each of these four FET regimens. To support luteum phase in F.E.T protocols, GnRH-a was observed improving clinical outcomes during ART treatments at our reproductive center, but there is no published data to support this. For this reason, we gathered data, conducted a retrospective analysis, and split these protocols into two groups. During the LPS stage in the study group, we employed GnRH-a in addition to other conventional therapies, On the other hand, we didn›t administer GnRH-a and instead employed standard LPS methods. The full procedure we followed for this investigation was as follows: We assessed women›s ovulation for candidates with Natural Cycles based on each of their menstrual cycles. Transvaginal ultrasounds were performed on women between the ninth and tenth days of their menstrual cycle. Transvaginal ultrasonography, serum estradiol (E2), and serum luteinizing hormone (LH) were used to track follicular growth. We daily did transvaginal ultrasound examinations until ovulation when the LH level was greater than 20 IU/L [9]. hCG in the amount of 5000 international units was given to initiate oocyte ovulation when the dominating follicle›s average diameter was larger than 17 millimeters, and LH was less than 20 IU/L.An embryo transfer was performed on the third day, during the cleavage phase [10].

On days 2 or3 of monthlies, oral estradiol valerate (Progynova, Bayer, Berlin, Germany) at a dose of 6–8 mg had been given daily for the H.R.T–F.E.T cycle [11]. Transvaginal ultrasonography and serum progesterone levels were assessed after 10 to 12 days. when the thickness of endometrium was at least 7 millimeters, progesterone dose of 200 mg was given vaginally thrice a day. When the Serum progesterone was 1.5 ng/mL, and 20 mg of dydrogesterone was administered orally twice daily for 2-5 days [12-14]. 3.75 mg of GnRHa was given into patients as part of the GnRHa-HRT regimen in early days (2/3 d) of menstruation for the early Follicular phase. Regardless of their treatment condition, following 28 days, we required them to go back hospital [15]. The patient›s ultrasound results and hormone levels were then used to determine if the patient had reached a state of pituitary downregulation. When levels of estrogen (E2) reached 183.5 pmol/L, follicle stimulating hormone (FSH) reached 5 U/L, luteinizing hormone (LH) reached 5 U/L, endometrial thickness reached 5 mm, and no significant follicle or cyst was seen, the standard criteria for defining down-regulation status was applied [16]. Drugs like clomiphene citrate and letrozole with or without human menopausal gonadotropin (HMG) were used in the Stimulated Cycle Protocol of F.E.T to stimulate ovulation. Endogenous estrogen and progesterone helped to get ready the endometrium [17].

Embryo Thawing Transfer: We defrosted D3 embryos using customary methods for vitrification, and we performed transplanting when more than 50% of the blastomeres survived following thawing.

Luteal Support Method: Frozen Thawed Embryo Transfer: We began providing dydrogesterone on the second day following ovulation, depending on the needs of each patient. Some patients preferred oral drugs, some requested injections, and yet others used vaginal suppository, Up until 14 days following transplantation, the dosage was as follows: 20 mg/d orally and 60–80 mg/day of progesterone by injection, or 200 mg twice daily via vaginal suppository. For the GnRH-a group, triptorelin acetate (France), 0.1 mg/dose, was injected subcutaneously once on the fourth or sixth day following oocyte retrieval (after ovulation) in the basic addition of progesterone and dydrogesterone14 days after transplantation, Triptorelin was terminated while other LPS treatments were continued after being administered four times every three days in addition to the patient›s ongoing treatment. In the triptorelin group, after administering 3-4time triptorelin, administration of existing luteal support medications continues till 12th week of pregnancy, while in non-GnRHa group, only existing luteal support medications

without addition of triptorelin were continued to take during the same period of pregnancy. On days 35, 55, and 75, the second, third, and fourth pregnancy tests were performed. All luteal support drugs were ceased being administered once ectopic pregnancy was determined to be present or when the pregnancy wasn›t found during the test.

Observation Indicators and Follow-Up: After 14, 35, 55 or 75 days following transplantation, patients successfully completed an HCG blood serum pregnancy test. They followed up to the delivery. We looked at their live birth rates and clinical pregnancy rates.

Statistical Analysis

For statistical analysis, we used the SPSS program. Continuous data were reported as means SD. To establish the statistical significance of percentages and odd ratios, we compared the averages using cross-tabs, performed Chi-square test, and calculated risk estimates. We defined significance of statistics as $P < 0.05$, and an odd ratio greater than 1.

Results

We looked at a total of 3518 cycles, 1033 of which were in the research group and were given Triptorelin until 10–12 weeks following embryo transfer. Of these 1033 cycles, 587 were noted for C.P.R, and 531 for L.B.R. The Non-GnRH-a group had a total of 2483 cycles; 1277 of those cycles had clinical pregnancies, while 1129 of those cycles had live birth rates reported. They were all treated using the standard practice of luteal phase support after embryo transfers. (Table 1) lists women›s basic characteristics for the study: age, BMI, duration of infertility, AMH, and antral follicle count (AFC) had no important difference in two groups. As shown in (Table 2), there had no important differences between the two groups in terms of endometrial thickness. However, total number of transferred embryos was found lower in GnRHa group than in non-GnRHa group. (Table 3) presents the outcomes after embryo transfer. For HRT-FET cycles, we discovered important differences in frequencies to clinical pregnancy (58.00% versus 48.40%, P-value= 0.003) and live births (52.7% vs. 45.6%, P = 0.003) between two groups.

Table 1: Contrast of basic indicators in two groupings.

Items	GnRHa (n=1033)	NonGnRHa (n=2485)	P-Value
Age(years)	33.32±5.62	33.46±5.56	0.518
BMI (kg/m^2)	23.64±3.59	23.68±23.68	0.76
Duration of infertility (years)	4.49±3.46	4.47±3.51	0.864
AMH	4.36±4.34	4.36±4.27	0.955
AFC	20.61±12.32	21.56±44.53	0.468

Table 2: Comparison of transfer of embryos in two groupings.

Items	GnRHa (N=1033)	NonGnRH (N=2485)	P value
Endometrial thickness(mm)	9.84±1.92	9.8±2.03	0.598
Total number of transferred embryos	1.73±.444	1.78±0.414	0.001

Table 3: Comparison of pregnancy outcomes in the two groupings.

Items	GnRHa group (n=1033)	NoGnRH group (n=2485	P value	OR	95% CI	Increment / decrement
CPR(all-FET)	56.8% (n= 587)	51.4% (n=1277)	0.003	1.24	1.08,1.44	24%
LBR(all-FET)	51.4% (n=531)	45.4% (n=1129)	0.001	1.27	1.10,1.47	27%
NC-FET						
CPR	58.2% (n=46)	52.9% (n=1818)	0.364	1.24	0.791.95	24%
LBR	54.4% (n=43)	47.0% (n=1617)	0.211	1.35	0.86,2.11	35%
HRT-FET						
CPR	58.0% (n=391)	48.4% (n=1338)	0.003	1.47	1.24,1.75	47%
LBR	52.7% (n=355)	45.6% (n=1262)	0.001	1.33	1.12,1.57	33%
GnRH-a+HRT-FET						
CPR	53.0% (n=134)	53.0% (n=1730)	0.176	1	0.77,1.29	0.0%
LBR	46.2% (n=117)	47.3% (n=1543)	0.794	0.96	0.74,1.24	-4%
Stimulation-FET						
CPR	59.3% (n=16)	52.9% (n=1848)	0.566	1.30	0.6,2.79	30%
LBR	59.3% (n=16)	47.1% (n=1644)	0.247	1.64	0.76,3.53	64%

C.P.R for the NC-FET, GnRH-a+HRT-FET, and Stimulation-FET cycles had no important difference in above two groups (58.20% versus 52.94%, P-value=0.364), (53.00% versus 53.00%, P-value= 0.176), and (59.30% versus 52.90%, P-value= 0.566) respectively. L.B.R for these two groups had no important differences for NC-FET, G.n.R.H-a+HRT-FET, or Stimulation-FET cycles (54.40% versus 47.00, P-value=0.211), (46.20% vs 47.30%, and 59.30% vs 47.10%, P-value=0.247) respectively. In first group, the odds ratio for clinical pregnancy following H.R.T-F.E.T cycles had 1.47, CI 95%: 1.24, 1.75, and it had great significant (P-value=0.003). C.P.R increased by 47% in the G.n.R.Ha-H.R.T group. In the same group, the odds ratio for live birth during H.R.T-F.E.T cycles had 1.33, CI 95%: 1.12, 1.57, and it had important significant (P-value= 0.001). L.B.R increased by 33% in the GnRHa-H.R.T group.

Discussion

In four of our F.E.T cycles, H.R.T-F.E.T appears to be the most effective cycle protocol in terms of C.P.R and L.B.R when triptorelin doses administered during the luteum phase comparing to old luteum phase treatment [18]. Embryo's quality and endometrium's receptivity are key parameters that affect the success rate of a frozen-thawed embryo transfer [14] Qian Y,2023). Naturally cycles (N.C), hormones replacement therapies cycles (H.R.T), G.n.R.Ha+H.R.T cycles, and stimulation cycles can all be used to get ready the endometrium [14,19,20]. For embryo implantation and pregnancy maintenance, the corpus luteum must function normally. COS-related corpus luteum dysfunction can result in a low pregnancy rate, low embryo implantation rate, and a high rate of early miscarriage [21]. As a result, clinical research on the luteal support drugs used in ART treatment is becoming quite popular. Although LH secretion in the luteal phase can partially rebound after GnRHa was stopped, progesterone synthesis may not be raised. Endometrial biopsy evidence shows that once the endometrial development sheds off, the development of glandular cells slows down following the administration of GnRHa in the middle of the luteal phase. Progesterone levels falling will have an impact on both uterine contraction and endometrial growth. A high frequency of uterine contraction during transplantation can impair embryo placement, prevent implantation, and lower pregnancy rates, according to research using ultrasound to assess the frequency and direction of uterine contraction [22].

Some researchers reported administering 0.1 mg dose of GnRH agonist as luteum support during the sixth day direct after fertilization [23,24]. These results had shown that this treatment significantly enhanced clinical outcomes like I.R(implantations rates), P.R(pregnancies rates) and B.R(birth rates) when compared to placebo. This improvement may be explained by the combined effects of GnRHa at the embryos and corpus luteums [25]. some researchers employed GnRHa successfully as luteum support to IVF-ET treatments, and intrauterine artificial insemination, and they hypothesized that GnRHa would also be useful in ART [25,26]. GnRHa can boost other pregnancy-related peptides released by the corpus luteum, like relaxin, in addition to just raising progesterone and E2 levels in the blood. LH may directly affect the endometrium, causing it to release cytokines and angiogenic substances that are helpful for embryo implantation. Additionally, it may directly act on the embryo and encourage its development because trophoblastic cells contain GnRHa receptors [23]. The endogenous corpus luteum is at its lowest stage six days following egg retrieval. At this point, GnRHa is used as the corpus luteum›s primary support. It binds to the pituitary gland›s newly produced GnRHa receptor, generating a «flare up» effect that increases the secretion of the ovarian hormones FSH and LH. Increased LH causes granulocytes to secrete more progesterone, which improves ovarian luteal function and makes pregnancy more likely to develop and remain so [27].

Early investigations revealed GnRHa receptor expression in maternal endometrium and human embryonic trophoblast cells. According to one study, functional LH receptors had been identified in human uterine tissue, which raises the possibility that using GnRHa during mid of luteum phase will enhance likelihood of clinical pregnancy and facilitate embryo implantation [28]. A group of authors reported that a single injection of GnRHa in luteum phase increased C.P.R and embryo implantation compared to the standard luteal support group [29]. Human embryos and endometrial stromal cells both have GnRHa receptor mRNA, and giving GnRHa during mid of luteum phase may encourage early implantation embryos to secrete hCG. Studies from recent years have suggested using GnRHa as luteal support, however the sample size is relatively small. Future discussions will focus on how the luteal phase support differs from the fresh cycle and how the success rate in freeze to thaw embryo transfer cycles has enhanced because of advances in freeze-thaw technology [30]. Patients who underwent all four FET cycles were chosen for investigation. C.P.R and L.B.R of GnRHa(Triptorelin) group were 47% and 33% greater than those of the group without GnRHa addition, and had important differences statistically, based on the results of H.R.T-F.E.T cycles.

Many studies are interested in learning whether giving GnRHa in luteum phase increases the chance of abnormal fetal births In this study, additional monitoring of the mothers and fetuses had done to see if the use of GnRHa during the luteal phase raises the risk of fetal birth abnormalities.

Conclusion

C.P.R and L.B.R can rise when GnRHa is added during luteum phase, and it may also open up new possibilities for luteal support. In our center, this study is, however, only on a small scale. To further compare the variations in the use of GnRHa in various freeze-thaw schemes, the selection of treatment population, the use dose of GnRHa, the time and frequency of administration, and have obtained a unified standard for the effective luteal support of GnRHa, it is suggested that we conduct RCT on a large sample of the center. At the same time, we must consider how GnRHa use affects perinatal children.

Limitations

This study›s primary limitations were its retrospective design, small sample size and possible bias. To validate our findings, large-scale randomized controlled trials are required.

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Data Availability

Data is available on special request from corresponding author.

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