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ORIGINAL ARTICLE

GnRh Agonist Treatment Improves Implantation and Pregnancy Rates of Frozen–Thawed Embryos Transfer

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About the Author

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Abstract

Objective To study the effect of GnRh agonist administration prior to estrogen–progesterone preparation of the endometrium on the implantation rate in frozen–thawed embryo transfer (FET) cycles in infertile patients treated with IVF/ICSI.

Design Prospective controlled study.

Setting Private center in Alexandria, Egypt.

Patients Patients undergoing frozen–thawed embryo transfer FET.

Intervention(s) Patients were divided into two groups, A and B. Group A patients consisted of 110 patients (110 cycles) who received daily subcutaneous injections of 0.1 mg of the GnRh agonist triptorelin starting from the mid-luteal phase of the cycle preceding the actual FET cycle. The dose was reduced to 0.05 mg from the second day of the cycle when daily oral estradiol valerate 6 mg was also started. Daily vaginal supplementation of micronized progesterone 400 mg b.d. was started after 12 days when the GnRh agonist was also stopped. Frozen–thawed embryos were transferred on day + 1 of their chronological age and when the endometrium reached 12 mm in thickness. Group B consisted of 100 patients (100 cycles)

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who started daily estradiol valerate 6 mg administration from the second day of the FET cycle and followed the same regimen but without prior treatment with triptorelin. *Main Outcome Measures* Implantation and pregnancy rates were compared among the two groups.
Results There was a significant increase in implantation rate in the GnRh agonist group (group A) compared to the estrogen and progesterone only group (group B) (44.1 vs. 21.1 %; $P = 0.002$*). The pregnancy rate was also significantly higher in group A compared to group B (65.5 vs. 42 %, $P = 0.013$*).
Conclusions GnRh agonist administration during endometrial preparation for FET increases the implantation and pregnancy rates.

Keywords ICSI · Vitrification · FET · GnRH agonist

Introduction

Frozen–thawed embryo transfer (FET) enables the excess embryos generated by IVF/ICSI to be stored and utilized at a later date. This reduces wastage after IVF and increases the chance of conceiving after one cycle of ovarian stimulation and oocyte retrieval [1].

In contrast to the complex stimulation protocols employed to stimulate multiple follicular growth for IVF, FET protocols are simpler, with the primary aim limited to adequate preparation of the endometrium to receive the thawed, transferred embryo(s) [2].

A crucial factor for implantation in frozen–thawed embryo transfer is exact synchronization between endometrial maturation and embryo development. Frozen–thawed embryo transfer has been successfully performed during a natural cycle after spontaneous ovulation, stimulated cycles [3] or hormone replacement cycles [4].

Natural cycle protocol does not require exogenous hormones administration. However, problems often occur when this protocol is used as accurate monitoring of the cycle required to determine ovulation entails higher costs and discomfort. The exact timing of ovulation is often difficult to determine, particularly in women with irregular cycles. Urinary LH stick tests allow women to monitor ovulation at home, but they are not always easy to interpret [5].

To overcome the disadvantages of LH monitoring, hCG triggering of ovulation is often employed in NC-FET in what is termed "modified NC-FET." This approach does not require LH monitoring, but regular ultrasound evaluation of the dominant follicle is required to ensure appropriate timing of hCG administration. As soon as the dominant follicle is of sufficient size (17–18 mm), hCG is administered as a surrogate to the endogenous LH surge to trigger final oocyte maturation and ovulation, which takes place 36–38 h later [6].In both true and modified NC cycles, thawing and transfer of the embryo should be performed 3–5 days after ovulation depending on the stage of the embryo when frozen [7].

Planning NC-FET carries the risk of unexpected ovulation and difficulty in ensuring timely thawing and transfer of the embryo. In the event of an early unexpected ovulation, treatment is usually canceled; hence, the risk of cycle cancelation rate is high [8].

Follicular stimulation protocols were used to achieve endometrial preparation through follicular development and estrogen production [9]. Endometrial exposure to drugs, the high cost of medications, the need for frequent monitoring and their limited flexibility have rendered all follicular stimulation protocols the least cost effective option for FET. Currently, these protocols are rarely used in routine clinical practice [10].

Many different hormonal replacement protocols have been described for endometrial preparation, varying with respect to the types of drugs used, routes of administration, dosage, and whether or not GnRh analogues were given [11].

Recently, the most popular protocol involves pituitary down-regulation with a GnRh agonist to avoid spontaneous ovulation before sequential administration of estradiol valerate orally and progesterone (as intramuscular injections or vaginal suppositories) [12].

The current study was conducted to study the effect of GnRh agonist administration prior to estrogen–progesterone preparation of the endometrium on the implantation rate in frozen–thawed embryo transfer (FET) cycles in infertile patients treated with IVF/ICSI.

Patients and Methods

Design

Prospective controlled study.

Two hundred and ten women were recruited for the study among the patients prepared for ICSI/FET in a private center in Alexandria, Egypt between January 2014 and January 2015. The study was explained to them, and written and informed consent for participation was obtained.

Inclusion criteria included IVF/ICSI patients undergoing frozen (vitrified)–thawed embryo transfer.

Ovarian Stimulation Protocol

All patients underwent stimulation using the long agonist protocol starting from mid-luteal phase of previous cycle using decapeptyl 0.1 mg subcutaneously. Stimulation was

started using hMG starting from second day of menses with suppression confirmed by estradiol level <50 pg/ml. All cases were monitored as usual by using ultrasound examination and hormonal evaluation including E2 and P4 serum values. Serum estradiol and serum progesterone levels were measured on the day of hCG administration.

Embryo Culture and Vitrification

Oocyte retrieval was performed 34–36 h after 10,000 IU of hCG were given. ICSI procedure was completed as usual 4–6 h after oocyte retrieval; fertilization was confirmed 16–18 h after ICSI.

On day 3, embryos were scored followed by vitrification of surplus high quality ones using open technique.

Endometrial Preparation and Embryo Transfer

Patients were divided into two groups A and B.

Group A patients consisted of 110 patients (110 cycles) who received daily subcutaneous injections of 0.1 mg of the GnRh agonist (triptorelin) starting from the mid-luteal phase of the cycle preceding the actual FET cycle. The dose was reduced to 0.05 mg from the second day of the cycle when daily oral estradiol valerate 6 mg was also started. Daily vaginal supplementation of micronized progesterone 400 mg twice daily was started after 12 days when the GnRh agonist was also stopped. Frozen–thawed embryos were transferred on day + 1 of their chronological age and when the endometrium reached 12 mm in thickness.

Group B consisted of 100 patients (100 cycles) who started daily estradiol valerate 6 mg administration from the second day of the FET cycle and followed the same regimen but without prior treatment with triptorelin.

Serum B-hCG was measured 2 weeks after embryo transfer, and clinical pregnancy was confirmed by the presence of a fetal heartbeat identified on transvaginal ultrasound 28 days after embryo transfer.

Results

Base line data of the included women were comparable regarding age and body weight (Table 1).

There was a significant increase in implantation rate in the GnRh agonist group (group A) compared to the estrogen and progesterone only group (group B) (44.1 vs. 21.1 %; $P = 0.002$*) (Table 2).

The pregnancy rate was also significantly higher in group A compared to group B (65.5 vs. 42 %, $P = 0.013$*) (Table 3).

Table 1 Characteristic feature of the two studied groups

	GnRh analogue group A "$n = 110$"		No GnRh analogue group B "$n = 100$"		P
	No.	%	No.	%	
Age					0.325
<25	30	27.3	22	22.0	
25–35	44	40.0	55	55.0	
↑ 35	36	32.7	23	23.0	
Range mean ± SD					
Weight					0.275
Normal weight	42	74.5	78	78.0	
Overweight	18	16.4	15	15.0	
Obese	10	9.1	7	7.0	

Table 2 Comparison between the two studied groups regarding the total number of E.T and implantation rate

	GnRh analogue "$n = 110$"	No GnRh analogue "$n = 100$"	P
Total number of E.T	322	285	
No. of implantation	142	60	
Implantation rate	44.1	21.1	0.002*

* Significant P value

Table 3 Comparison between the two groups regarding the pregnancy rate

	GnRh analogue "$n = 110$"	No GnRh analogue "$n = 100$"	P
Total No. of cases	110	100	
Number of pregnancy cases	72	42	
Pregnancy rate	65.5	42.0	0.013*

* Significant P value

Discussion

Currently, ovarian stimulation protocols using GnRh analogues and gonadotropins yield large number of oocytes. With improved culture methods, a higher number of good quality embryos have become available for transfer or cryopreservation.

The characteristics of cryopreservation methods, such as exposure time of cells to the different cryoprotectant solutions and to their different concentrations, as well as the rate of formation of extra- and intracellular ice crystals, have critical effects on survival and viability of human embryos [13].

Vitrification is a cryopreservation technique that utilizes high cooling rates used to obtain an ice-free, glass-like

solidification of cells and tissues [14], thus improving post thaw survival rate of frozen embryos and consequently improving their implantation rate [15].

Several studies have been advocated to study other factors that may affect or improve both implantation and clinical pregnancy rate of thawed–frozen embryos.

It was found that exact synchronization between endometrial preparation and embryo development is the most important factor affecting success of thawed–frozen embryo transfer.

Such preparation may be spontaneous (natural cycle protocols), hormonal stimulation or induced by after artificial preparation of the endometrium with exogenous estrogen along with or without pituitary down-regulation with GnRh agonist [16].

In present study, we compared endometrial preparation for frozen–thawed embryo transfer with and without pituitary down-regulation by GnRh agonist.

The programmed cycle using a GnRh agonist before estrogens and progesterone administration aims at obtaining pituitary down-regulation, avoiding spontaneous ovulation and cycle cancelation which is the main drawback of natural cycle protocol [16].

Our current study observed a significant increase in the implantation and pregnancy rates when GnRh agonist were administrated prior to estrogen–progesterone preparation of the endometrium in thawed–frozen embryo transfer as the implantation rate was significantly higher in (group A) who received triptorelin starting from the mid-luteal phase of the cycle preceding the actual FET cycle compared to (group B) who did not have prior treatment with triptorelin (44.1 vs. 21.1 %; $P = 0.002$*). Pregnancy rate was also significantly higher in group A compared to group B (65.5 vs. 42 %, $P = 0.013$*).

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

Ethical Approval All procedures performed in the study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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