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Article

A prospective randomized study of the role of hormonal monitoring versus ultrasound only in predicting pregnancy in patients with programmed thawed embryo transfer cycles

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Abstract

Background: Hormonal monitoring impact on overall pregnancy rate in FET cycles and hence progesterone supplement adjustments remain debatable in current literature.
Objective: In this study we investigated the effect of monitoring and follow-up of serum progesterone, estradiol & luteinizing hormone levels and progesterone supplement adjustments on pregnancy outcomes for FET in programmed HRT cycles in comparison with ultrasound only in non-intervention group.
Methods: Six Hundred FETs were performed in a randomized prospective manner in an infertility center in Alexandria starting from 2019 till 2021.These were further subdivided based on computer randomization into 2 groups. **Group I (Non-intervention)** including 300 patients with only ultrasound monitoring & **Group II (intervention)** including 300 patients with monitoring of serum level

of progesterone, estrogen & luteinizing hormone as well as progesterone supplement adjustments which are further subdivided into three groups depending on progesterone level in patient's serum early in the morning prior to embryo transfer. Group II A: P4 levels < 5ng/dl, Group II B: P4 levels 5-10ng/dl, Group II C: P4 levels >10ng. The primary outcomes were clinical and ongoing pregnancy rate.

Results: Live birth and overall pregnancy rate showed no significant difference among group monitored with ultrasound only compared to the hormonal monitored group. However, on the day of embryo transfer, serum P4 > 11.83 ng/ml represented a cut-off value above which there is marked increase in OPR/LBR and a sensitivity of 53.7% & specificity of 51.2% in predicting pregnancy. Also, serum E2 value more than 292 pg/ml at day 18 demonstrated an increase in OPR/LBR**.**

Conclusion: Monitoring and follow-up of different hormones in patient's serum in programmed frozen embryo transfer cycles didn't have an impact on live birth, overall pregnancy & abortion rate when compared to ultrasound only in non-intervention group.

Keywords: Hormonal monitoring; Frozen embryos; Progesterone hormone; estradiol hormone

Introduction

Frozen Embryo transfer treatment rapidly expanded in recent years. FET can effectively prevent IVF associated complications, such as ovarian hyperstimulation syndrome and multiple pregnancy. In addition, FET serves as a safe & cost-effective way to increase cumulative pregnancy rate. (1)

The pregnancy outcomes after the FET is known to be dependent on multiple clinical factors, including the age of the woman, method of oocyte fertilization (i.e. In Vitro Fertilization (IVF or intra-cytoplasmic sperm injection (ICSI)), duration of infertility, FSH serum level, reasons for embryo cryopreservation, type of infertility (primary or secondary), and endometrial thickness on the day of embryo transfer.(2) Female fecundity was found to be affected dramatically by the elevated body mass index (BMI).(2,3)

It is generally accepted that an endometrial thickness below a minimum value of 6 to 8 mm showed negative predictive value for IVF outcomes, clinical pregnancy as well as live birth rates are significantly higher in patients with an endometrial thickness >9 to 10 mm. (4) Still, implantation can sometimes occur despite a thin endometrium. Also, the endometrial thickness of 8mm has been widely used as the threshold of endometrial receptivity in FET cycles. (5)

Women undergoing FET in programmed cycle require progesterone supplementation to initiate and maintain the secretory endometrium and pregnancy. There has been previous research into luteal phase support in frozen cycles, which has demonstrated that supplementation of progesterone does impact outcome in FET. (6)

Despite this evidence for the role of progesterone, there is surprisingly little data on the optimal values for serum P4 during the luteal phase and specifically on the day of embryo transfer in frozen cycles. (7) Lower pregnancy rates have been associated with low as well as high serum progesterone levels on the day of embryo transfer according to data from two separate studies. (8)

Though progesterone levels on the day of transfer have not yet been studied extensively in humans, tailoring the time of transferring a frozen embryo based on serial P4 values rather than cycle day number alone results in higher pregnancy rates. (8)
The value of measuring the serum E2 levels on the day of embryo transfer as an indicator for clinical pregnancy is still doubtful. The effect of serum E2 levels on the day of embryo transfer is poorly defined in the literature and needs further evaluation. (9)
Supplementation with estrogen (E2) from the early follicular phase without pituitary suppression in an artificial endometrium preparation cycle for frozen-thawed embryo transfer can lead to a rise in LH level, similar to that observed before ovulation. (10) By direct action on the endometrium, however, such a rise of LH might interfere with endometrial receptivity during a frozen embryo transfer cycle, in which no pituitary suppression is used. The significance of LH level on the day before addition of progesterone is not yet well defined. (11)

Objective

The present study was conducted to investigate the effect of monitoring and follow-up of serum progesterone, estradiol & luteinizing hormone levels and progesterone supplement adjustments on pregnancy outcomes for FET in programmed HRT cycles in comparison with ultrasound only in non-intervention group.

Methods

Study design, setting and participants

Patient allocation*: patients were allocated to the intervention and non-intervention groups (after being eligible by meeting both inclusion and exclusion criteria described later) in a serial randomization pattern using the computer. One patient is allocated to the intervention group and the next one to the non-intervention group in repeated sequence until completing sample size studied.*
The present study was a prospective study where randomization of patients was computer based in a serial manner. Six Hundred FETs were performed in a private IVF center in Alexandria starting from 2019 till 2021 were further subdivided into 2 groups. The institutional review board and ethics committee, faculty of medicine, Alexandria University approved the study protocol (serial number #0201269; IRB No:00012098) and informed written consent was obtained from all participants after discussing the nature of the study.

Group I (Non-intervention) including 300 patients with only ultrasound monitoring & **Group II (intervention)** including 300 patients with monitoring of serum level of progesterone, estrogen & luteinizing hormone as well as progesterone supplement adjustments which are further subdivided into three groups depending on progesterone level in patient's serum early in the morning prior to embryo transfer. Group II A: P4 levels < 5ng/dl, Group II B: P4 levels 5-10ng/dl, Group II C: P4 levels >10ng. Our inclusion criteria were age of female patient < 42 years, normal intrauterine factor, all embryos are day 5 or day 6 frozen blastocysts. Our exclusion Criteria were recurrent implantation failure,

previously known major thrombophilia factors, non-compliance to given protocol, endometrial thickness < 7mm, pre-existing metabolic diseases (Diabetes Mellitus & Hypertension).

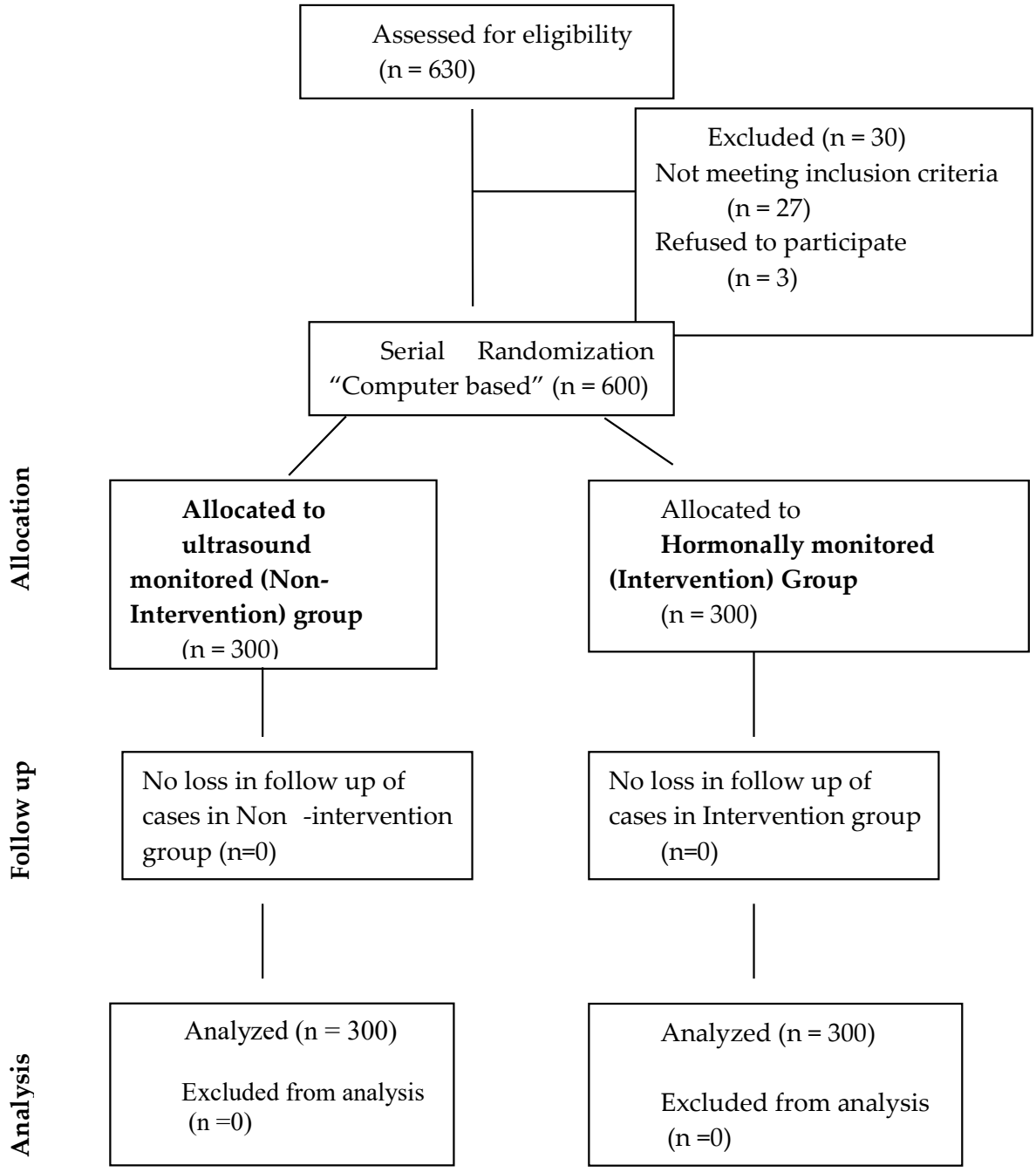
Protocol of hormonally prepared endometrium in thawed embryo transfer cycles

All patients were given 8 mg estradiol valerate orally on daily basis for 13 days beginning with the first day of either a spontaneously or induced menstrual cycle.

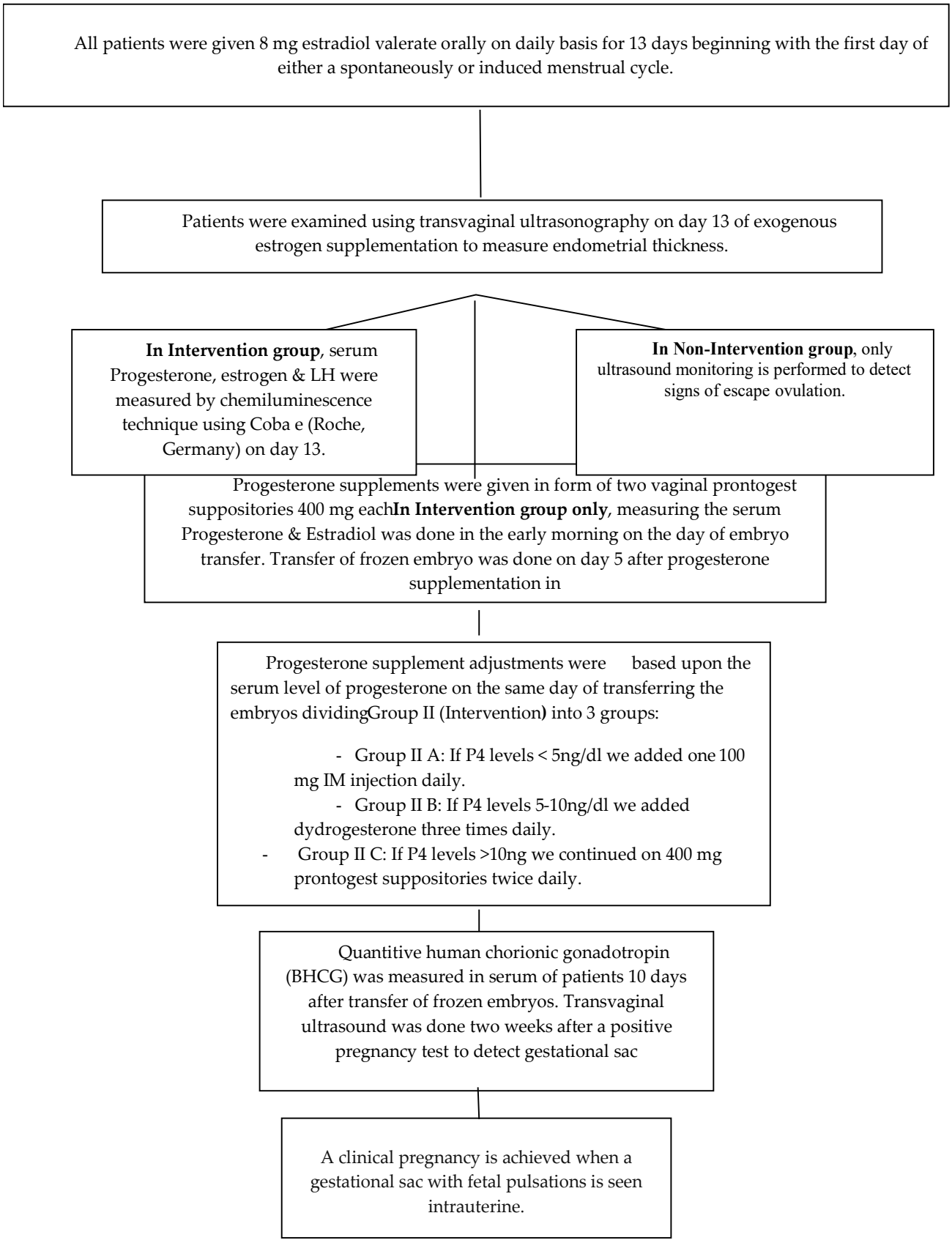
Patients were examined using transvaginal ultrasonography on day 13 of exogenous estrogen supplementation to measure endometrial thickness and to detect signs of escape ovulation. Progesterone supplements were given in form of two vaginal prontogest suppositories (Marcyrl Pharmaceuticals) 400 mg each. Transfer of frozen embryo was done on day 5 after progesterone supplementation. Quantitive human chorionic gonadotropin (BHCG) was measured in serum of patients 10 days after transfer of frozen embryos. Transvaginal ultrasound was done two weeks after a positive pregnancy test to detect gestational sac. A clinical pregnancy is achieved when a gestational sac with fetal pulsations is seen intrauterine.

In Intervention group, serum Progesterone, estrogen & LH were measured by chemiluminescence technique using Coba e (Roche, Germany) on day 13 after priming endometrium with 8mg estradiol valerate on a daily basis beginning as early as the first day of menstrual cycle. Measuring the serum Progesterone & Estradiol was done in the early morning on the day of embryo transfer. Progesterone supplement adjustments were based upon the serum level of progesterone on the same day of transferring the embryos dividing Group II (Intervention group**)** into 3 groups: Group II A: If P4 levels < 5ng/dl we added one 100 mg intramuscular injection daily (Marcyrl Pharmaceuticals). Group II B: If P4 levels 5-10ng/dl we added dydrogesterone (duphaston; Abbott Pharmaceuticals) three times daily. Group II C: If P4 levels >10ng we continued on 400 mg prontogest suppositories twice daily.

CONSORT diagram of the Design: Randomized Control trial (Figure 1)



Flow Chart of the study (Figure 2)



Statistical analysis of the data

Data were fed to the computer and analyzed using The IBM SPSS software package version 20.0**.** **(**Armonk, NY: IBM Corp**)** is used to introduce the data to the computer and interpret it as well**.** Illustrating the qualitative data was performed by adopting number and percent. Establishment of the normality of distribution was done by using the Kolmogorov-Smirnov test. The range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR) were applied to represent the quantitive data. Significance of the obtained results was judged at the 5% level. To compare between different groups, Chi-square test was applied. Student t-test was used to compare between two studied groups and for normally distributed quantitative variables. The F-test (ANOVA) was used to compare between more than two groups & for normally distributed quantitative variables. Receiver operator characteristic (ROC) curves were also constructed where appropriate.

Results

For each cycle of frozen embryo transfer, data from the primary ICSI cycle was collected. We found that there was a slightly significant difference between ultrasound monitored **(Group I)** and hormonally monitored (**Group II)** significant regarding BMI, female age, male semen parameters. On the other hand, there was an insignificant difference between 2 groups regarding age of male patients & endometrial thickness measured. (**Table 1 & 2**)

Table 1: Comparison between the two studied groups according to personal history

	Group I (Non-intervention) (n = 300)		Group II (Intervention) (n = 300)		Test of Sig.	P
	No.	%	No.	%		
Previous pregnancy						
1ry infertility	237	79.0	206	68.7	χ^2= 8.290*	0.004*
2ry infertility	63	21.0	94	31.3		
BMI						
Normal	75	25.0	107	35.7	χ^2= 8.126*	0.017*
Overweight	211	70.3	182	60.7		
Obese	14	4.7	11	3.7		
Min. – Max.	21.0 – 34.0		19.0 – 38.0		t=2.140*	0.033*
Mean ± SD.	26.39 ± 2.16		25.81 ± 2.43			
Median (IQR)	27.0 (24.50–28.0)		26.0 (24.0–27.0)			
Duration of Infertility (years)						
Min. – Max.	1.0 – 21.0		1.0 – 19.0		U= 43598.50	0.505
Mean ± SD.	4.73 ± 3.17		a4.55 ± 2.97			
Median (IQR)	4.0 (2.0– 6.0)		4.0 (2.0– 6.0)			
Female age						
Min. – Max.	18.0 – 42.0		19.0 – 41.0		t=2.726*	0.007*
Mean ± SD.	28.91 ± 4.99		30.04 ± 5.19			
Median (IQR)	28.0 (25.0 – 32.0)		30.0 (26.0– 34.0)			
Male age						
Min. – Max.	20.0 – 70.0		24.0 – 64.0		t=1.573	0.116
Mean ± SD.	34.39 ± 6.68		35.23 ± 6.46			
Median (IQR)	33.0 (30.0– 38.0)		35.0 (30.0– 39.0)			

Table 2: Comparison between the two studied groups according to Male factor, Hormonal levels(E2&P4) in gonadotropin stimulation cycle & Endometrial thickness

General Examination	Group I (Non-intervention) (n = 300)		Group II (Intervention) (n = 300)		Test of Sig.	P
Semen count (m)						
Min. – Max.	0.0 – 222.0		0.0 – 340.0		U= 40073.5*	0.020*
Mean ± SD.	34.59 ± 39.28		39.76 ± 39.76			
Median (IQR)	20.50 (3.3–54.0)		32.0 (7.0–61.0)			
Motility A						
Min. – Max.	0.0 – 80.0		0.0 – 80.0		U= 38124.0*	0.001*
Mean ± SD.	24.24 ± 21.04		29.57 ± 21.48			
Median (IQR)	20.0 (7.5–40.0)		25.0 (10.0–45.5)			
Motility B%						
Min. – Max.	0.0 – 84.0		0.0 – 85.0		U= 41525.5	0.101
Mean ± SD.	20.04 ± 18.66		21.21 ± 16.84			
Median (IQR)	15.0 (5.0–31.5)		19.0 (7.0–33.0)			
Normal forms %						
Min. – Max.	0.0 – 85.0		0.0 – 85.0		U= 39782.50*	0.014*
Mean ± SD.	9.76 ± 17.83		12.32 ± 19.06			
Median (IQR)	3.0 (2.1–6.5)		4.0 (2.2–10.0)			
TESE (testicular sperm extraction)	**No.**	**%**	**No.**	**%**		
Negative	259	86.3	283	94.3	χ^2= 10.994*	0.001*
Positive	41	13.7	17	5.7		
Previous Transfer cycles						
Negative	258	86.0	211	70.3	χ^2= 21.573*	<0.001*
Positive	42	14.0	89	29.7		
E2 at triggering						
Min. – Max.	450.0 – 23089.0		692.0 – 10465.0		U= 38619.0*	0.003*
Mean ± SD.	3573.29 ± 2559.55		3791.13 ± 1810.79			
Median (IQR)	3035(1986.0– 4412)		3342(2359.0– 4706)			
p4at triggering						
Min. – Max.	0.04 – 4.30		0.13 – 7.09		U= 40788.0*	0.047*
Mean ± SD.	1.05 ± 0.62		1.18 ± 0.77			

Median (IQR)	0.93 (0.6– 1.4)	1.03 (0.7– 1.4)		
Endometrial thickness				
Min. – Max.	3.0 – 13.0	7.0 – 14.0	t=1.155	0.249
Mean ± SD.	9.12 ± 1.25	8.99 ± 1.38		
Median (IQR)	9.0 (8.0– 10.0)	9.0 (8.0– 10.0)		

Following transfer of frozen embryos, OPR was 53% in **Group I** vs. 58.3 in **Group II**, p value=0.189. Live Birth rate was 45.3% in **Group I** compared to 46% in **Group II**, p value=0.87. The rate of abortion in **Group I** and **Group II** was 7.7% & 12.3% respectively (**Fig. 3**).

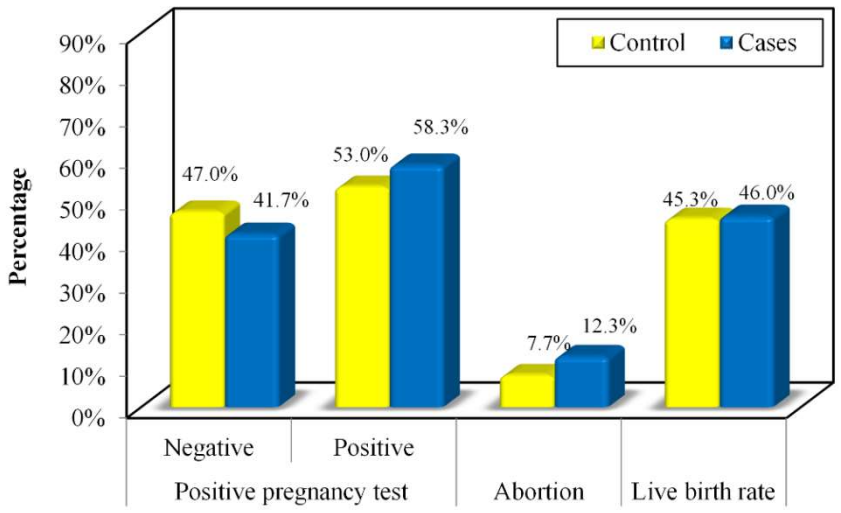


Figure (3). Comparison between the two studied groups according to Positive pregnancy test, Abortion and Life birth rate.

The value of P4 measured in nanogram per milliliter on day of embryo transfer was further subdivided into three subgroups (<5, 5–10, >10), and progesterone supplements were applied. There were similar OPR among three subgroups (53.6, 56.3, 60.7 %, respectively). The live birth rate (46.3, 39.4 & 48.6%) and abortion rate (7.1, 16.9 & 12.1 %) were statistically insignificant among three hormonal monitored subgroups (A, B & C) (**Fig. 4**).

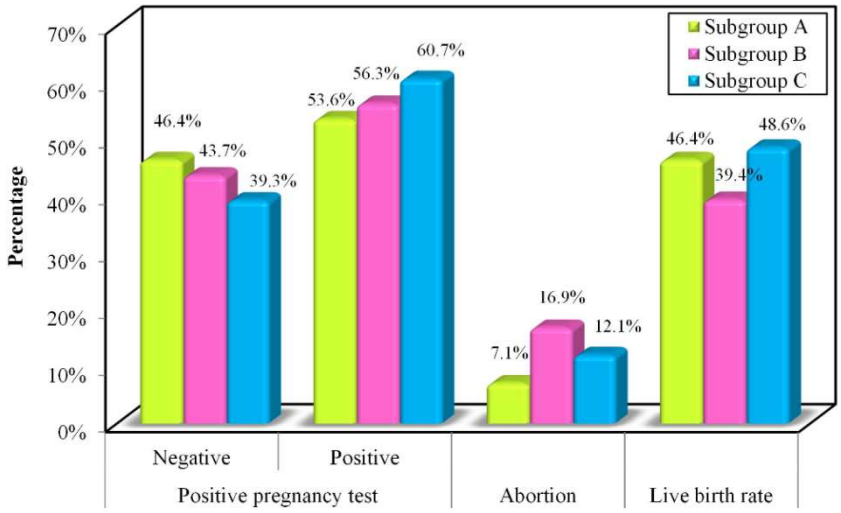


Figure (4). Comparison between the three studied groups according to Positive pregnancy test, Abortion and Live birth rate.

Among the hormonally monitored three subgroups, the hormonal levels of E2, LH, P4 at day 13 as well as E2 at day 18 were statistically insignificant. However, the P4 levels at day 18 were significantly difference between the three subgroups with a mean value of 3.52 ± 1.42, 7.59 ± 1.44 & 21.84 ± 20.04 ng/dl in groups A, B & C respectively (**Table 3**)

Table 3. Comparison between the three studied groups according to different hormonal parameters.

	Subgroup A (n = 56)	**Subgroup B (n = 71)**	**Subgroup C (n = 173)**	**H**	***P***
E2 at D13					
Min.–Max.	108.0–500.0	88.0–569.0	56.0–613.0	1.123	0.570
Mean ± SD.	244.32 ± 100.78	257.86 ± 102.97	259.46 ± 103.28		
Median (IQR)	242.50 (153.0–322.5)	228.0 (180.0–327.0)	249.0 (181.0–315.0)		
LH at D13					
Min.–Max.	1.40–24.0	1.70–41.0	0.40–56.0	0.751	0.687
Mean ± SD.	11.25 ± 5.19	11.86 ± 7.89	11.11 ± 6.91		
Median (IQR)	9.98 (7.7–15.0)	11.0 (7.0–13.5)	9.90 (7.0–13.0)		
P4 at 13					
Min.–Max.	0.05–0.70	0.05–1.40	0.05–11.30	3.908	0.142
Mean ± SD.	0.19 ± 0.16	0.21 ± 0.19	0.34 ± 1.09		
Median (IQR)	0.14 (0.1–0.3)	0.19 (0.1–0.2)	0.20 (0.1–0.3)		
E2 at D18					
Min.–Max.	19.0–707.0	58.0–3132.0	9.40–740.0	4.401	0.111
Mean ± SD.	315.45 ± 134.40	372.01 ± 349.31	311.37 ± 112.19		
Median (IQR)	279.0 (233.0–376.0)	330.0 (269.0–385.5)	288.0 (237.0–365.0)		
P4 at D18					
Min.–Max.	0.07–4.99	5.10–9.9	10.0–231.0	234.962*	<0.001*
Mean ± SD.	3.52 ± 1.42	7.59 ± 1.44	21.84 ± 20.04		
Median (IQR)	3.95 (2.6–4.6)	7.70 (6.4–8.9)	16.32 (12.8–23.8)		

H: H for Kruskal Wallis test
p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$
IQR: Inter quartile range
SD: Standard deviation

On the day of embryo transfer, P4 > 11.83 ng/ml represented a cut-off value above which there is marked increase in OPR/LBR and a sensitivity of 53.7% & specificity of 51.2% in predicting pregnancy. Also, the E2 value more than 292 pg/ml at day 18 demonstrated an increase in OPR/LBR (**Table 4**).

Table 4. **Validity (AUC, sensitivity, specificity) for e2 at D13, LH at D13, P4 at 13, E2 at D18 and P4 at D18 to predict pregnancy.**

	AUC	*P*	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			L.L	U.L					
E2 at D13	0.537	0.274	0.471	0.604	>239	53.1	53.6	61.6	45.0
Lh at D13	0.532	0.350	0.465	0.598	≤10.8	58.3	50.4	62.2	46.3
P4 at D13	0.542	0.218	0.476	0.608	≤0.19	53.7	52.0	61.0	44.5
E2 at D18	0.502	0.957	0.435	0.569	>292	52.6	49.6	59.4	42.8
P4 at D18	0.529	0.394	0.462	0.595	>11.83	53.7	51.2	60.6	44.1

Discussion

Frozen embryo transfers (FET) are considered to be an essential component of *in vitro* fertilization (IVF) programs, and their utilization has dramatically expanded in the past few decades. In our study, we aimed to compare pregnancy and live birth rates between standard protocol of FET in non-intervention group (ultrasound guidance only) and hormonally monitored protocol in intervention group.

The median age of females in intervention group (**Group II)** was 30 years which was slightly higher when compared to 28 years in non-intervention group (**Group I)** but with similar live birth rate in both groups reaching 46% in case group compared to 45.3% in control group.

In our study, despite of the difference in BMI distribution where overweight patients in intervention group (70.3%) are higher than in intervention group (60.7%), it seems that BMI had no effect on the overall pregnancy group in our study. Our results were similar to Farhi et al. (12) who illustrated in his study that the detrimental effect of obesity was reduced by high-quality blastocyst transfer in patients undergoing IVF treatment.

The mean value for endometrial thickness monitored by ultrasound prior to frozen embryo transfer was 9.12 ± 1.25 & 8.99 ± 1.38 cm in ultrasound and hormonally monitored group respectively with comparable CPR & LBR.

The mean duration of infertility was 4.73 ± 3.17 years in **Group II** similar to **Group I** which was 4.55 ± 2.97 years. However, patients with primary infertility in **Group I** constituted 79 % compared to 68.7% in **Group II** with no influence on overall pregnancy & live birth rate between both groups.

Despite the obvious superiority of the different semen parameters (sperm count, progressive motility & vitality) in intervention group compared to non-intervention group, this superiority didn't affect the overall CPR & LBR in FET cycles. Our findings showed high similarity with Aghajanova et al. (13) which stated that unfavorable outcomes of IVF treatment was not related to neither age of male patient or even sperms showing abnormal morphology. In addition, they concluded that the adverse effects of poor semen quality were restored by using IVF/ICSI programs.

An important factor that influences the implantation rate in FET cycles is the number of previous trials of transfer. So, we took this point in consideration when determining our inclusion criteria. Patients with their first transfer attempt constituted the majority of our patients accounting for 86.3% & 94.3% in ultrasound and hormonally monitored group respectively which had no impact in the overall CPR & LBR.

The median value of E2 at the day of the trigger in ICSI cycles in **Group II** was 3342pg/m which was higher than E2 levels in **Group I** which reached 3035pg/ml, however it didn't affect overall CPR & LBR in the subsequent FET cycles. Yu et al.(14) showed similar results in his study where elevated levels of estradiol in the main ICSI cycles didn't have a negative impact on success of the subsequent FET cycles in terms of both implantation and overall pregnancy rate.

This design showed that both non-intervention & intervention group showed similar pregnancy and live birth rate. Despite hormonal monitoring prior to frozen embryo transfer and progesterone supplement adjustments applied in intervention group, the overall pregnancy & live birth rate showed mild variation between different subgroups but still insignificant.

Regarding impact of serum estradiol levels (E2 at day13) measurement prior to progesterone initiation in programmed thawed embryo transfer cycles, Macken's et al.(15) result was similar to our designed study where serum E2 levels in late-proliferative stage didn't have an impact on rate of live birth in patients having thawed embryo transfer in programmed cycles.

In our study, the cut off value of LH level less than of 10.8 IU/L at day13 had a sensitivity of 58.3% & specificity of 50.4% in predicting pregnancy. Regarding pregnancy outcome, monitoring of LH levels didn't generate useful data with no added prognostic value. However, Ruiqiong et al showed that low values of LH one day before progesterone initiation in programmed thawed embryo transfer cycles of ovulatory women are associated with a lower LBR.(16)

Kofinas et al.(7) proposed in his study that in order to optimize pregnancy and implantation rate, P4 levels in FET cycles should be sustained between 10 and 20 ng/ml. This was in accordance to our study group where the cut off value of P4 on day of embryo transfer > 11.83 ng/ml carried a 53.7% sensitivity & 51.2 % specificity in predicting pregnancy.

However, there are major differences in the 2 designs when compared. Unlike our design study, Kofinas et al. (7) used euploid embryos which allowed assessing the precise role of P4 levels on implantation without worrying about the impact of embryos with genetic abnormality affecting the overall analysis which remains an advantage of their study. On the other hand, all cases with progesterone levels less than10 ng/ml were ruled out from their study and their impact on pregnancy rate couldn't be demonstrated. But in our study, we analyzed the effect of different P4 levels and their hormonal adjustment on the pregnancy rate with no added value regarding the CPR & LBR.

In another study, Alyasin et al (17) pointed out that serum P4 value of 32.5 ng/ml on the day of embryo transfer was linked to decrease rates of live birth. However, in this design, the serum P4

levels in the three subgroups on day of embryo transfer were similar regarding overall CPR, LBR and abortion rate.

We faced some limitations in our study regarding the non-intervention group. Patients in this group didn't have a hormonal pre-transfer assessment so it's not possible to determine if the progesterone levels are suboptimal or not when compared to intervention group.

Another point we wish to address in this study is the fact that we didn't have a post embryo transfer hormonal assessment in case group to determine whether the progesterone dose adjustment in subgroup A & B reached optimal desired levels or not and hence affects the pregnancy rate.

Conclusions

From this study we conclude that monitoring and follow-up of different hormonal serum levels (E2, P4 & LH) before initiation of progesterone in programmed FET cycles didn't have an impact on pregnancy and live birth rate in comparison with ultrasound only in non-intervention group. The progesterone supplements adjustments depending on values of progesterone on day of FET didn't affect the overall clinical pregnancy rate in comparison to the non-hormonally monitored group.

Due to the limited role for the value of serum progesterone levels in FET cycles in predicting the overall pregnancy and rate of live birth, studies on a larger scale are required to confirm this theory.

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Conflict of interest

The authors declare no conflict of interest.

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