The Correlations Between Progesterone (P₄) on the Day of Trigger, (P₄ and LH) Values on the Retrieval Day on Endometrial Thickness and Top Quality Embryos

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Abstract

Purpose: There are limited studies about the impact of some hormones (including LH, P_4) on the number of top quality embryos, which in turn very important to expect the implantation potentials and IVF success. The purpose of our study is to explore the correlations between progesterone (P_4) hormone's values on the day of trigger, (P_4 , LH) hormones' values on the retrieval day, and how these hormones affect top quality embryos. In addition, this study reveal if endometrial thickness on the retrieval day does effected by or affects those hormones values.

Design and Methods: This study is prospective, single-center study conducted in the department of British Syrian IVF and Fetal Medicine, AL Rasheed Hospital, Syria. This study included 112 Women aged 23 to 45 years old. This study group had IVF according to one of two controlled ovarian stimulation protocols: GnRH antagonist protocol or GnRH agonist suppression protocol (long luteal phase). All the patients used either recombinant, highly purified FSH (GONAL-f[®], Merck KGaA Darmstadt, Germany) alone or in combination with purified urinary hMG (Menotropin, Bharat Serums and Vaccines Ltd, India) for ovarian stimulation. No special considerations were taken for the administration of ovulation stimulation drugs; however, the availability of these drugs in the local market during that period was the controlling factor, particularly considering the local sanctions. The causes of IVF varied between male factor, advanced women age, low ovarian reserve, Tubal factor, intermittent sexual relationship, polycystic ovarian syndrome, preimplantation genetic diagnosis (PGD), or combination of multiple factors. It is worth to mention that donation is prohibited according to our health ministry's laws.

Conclusion: In view of the fact that Top quality embryos plays an important role in IVF outcome, our study showed that elevation of number of retrieval oocytes, fertilized ovum, and value of P_4 on retrieval day led to raise of top quality embryos' number. LH level on retrieval day has no relation with Top quality embryos number. P_4 values raise on trigger day led to raise of the number of embryos but not top quality embryos. Endometrial thickness has no relation with (LH, P_4) on retrieval day, P_4 on trigger day, or top quality embryos.

Keywords: Progesterone Hormone; LH Hormone; Endometrial Thickness; Top Quality Embryos; Trigger Day; Retrieval Day

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Introduction

Luteinizing hormone (LH) is crucial to the emergence of a single dominant follicle in each ovulatory menstrual cycle [1]. In the first part of the follicular phase, LH stimulates androgen production in thecal cells [2], which synergistically increases estrogen production [3]. In the late follicular phase, LH helps to produce small amounts of progesterone, thereby promoting positive estrogen feedback, which is necessary for follicular development and maturation [4]. Ovulation-inducing LH surge is discharged by the pituitary gland. LH then reprograms granulosa cell function, leading to terminal differentiation (luteinization) rupture of the follicle wall, and release of the fertilizable egg [1]. In the luteal phase, LH promotes progesterone production, supporting development of the corpus luteum [2,5]. Therefore, LH is essential for normal follicular development and oocyte maturation [2,6]. The negative corner stone of gonadotropin stimulation is premature LH surge because of the positive feedback signal from estradiol to the pituitary gland, consequences are premature ovulation, a reduction in oocyte and embryo quality, and thus a reduced pregnancy rate [6,7]. Progesterone (P₄) is essential for support of the endometrium and implantation of an embryo in the normal menstrual cycle [8,9]. The corpus luteum is a transient endocrine organ that predominantly secretes progesterone, and its primary function is to prepare the estrogen primed endometrium for implantation of the fertilized ovum [10]. The endometrium is the site of implantation and pregnancy. Preparation for this important biological event relies primarily on progesterone, which takes the estrogen-primed endometrium toward a state of receptivity [11]. Regarding top quality embryo, embryo quality is currently widely considered the best available prediction model for pregnancy chances [12]. As embryo grading systems are useful in the prediction of embryo implantation [13]. Evaluation of cleavage-stage embryos should include cell number, size and symmetry, percentage of fragmentation, granulation, vacuoles and nuclear status (e.g. multinucleation). Blastocyst scoring should include expansion grade, blastocoel cavity size and morphology of the inner cell mass (ICM) and trophectoderm (TE). Assessment should be performed at standardized times post insemination [14]. In this study the classification of embryo made according to the criteria of atlas of human embryology [15] and ESHRE IVF labs guideline [14], as it is very important that the features related to implantation potential are assessed accurately and similarly [15]. Moreover, the number and the quality of transferred embryos are crucial for a successful pregnancy [16]. Therefore, large numbers of studies discuss various factors effect quality of embryos like [6,17].

Methodology and Results

Study sample

112 female patients were included in our study sample, ages between 23 to 45 years, had IVF according to one of two controlled ovarian stimulation protocols: GnRH antagonist protocol or GnRH agonist suppression protocol (long luteal phase). All the patients used either recombinant, highly purified FSH (GONAL-f[®], Merck KGaA Darmstadt, Germany) alone or in combination with purified urinary hMG (Menotropin, Bharat Serums and Vaccines Ltd, India) for ovarian stimulation. No special considerations were taken for the administration of ovulation stimulation drugs; however, the availability of these drugs in the local market during that period was the controlling factor, particularly considering the local sanctions. The causes of IVF varied between male factor, advanced women age, low ovarian reserve, tubal factor, intermittent sexual relationship, polycystic ovarian syndrome, preimplantation genetic diagnosis (PGD), or combination of multiple factors. No specific criteria were applied in selecting patients, and there is no excluded criteria. The measurement of progesterone hormone levels was performed twice on oocyte retrieval day and on trigger day, while LH hormone level were measured only on oocyte retrieval day. Endometrial thickness is measured on retrieval day by transvaginal ultrasound.

Statistical analysis

The researcher used the following statistical methods, according to the statistical package for the social science (SPSS) version 25 for data analysis:

• **Descriptive statistics**: To know the mean and the standard deviation of quantitative variables entered into tests, in additionally to the percentage and frequencies for qualitative variables.

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- A normality test: Using the Kolmogorov-Smirnov test, to know the right test for the stud when the sample size is more than 50.
- **Correlation study**: Using the Spearman test to study the strength and side of the relationship between variables in a quantitative study.

Descriptive statistics

The following table shows the basic information about the sample and the data that was collected to introduce the study sample, as follows.

According to the result, male factor (MF) alone was the most frequent infertility factor, resulting in a ratio of 32.1%. Antagonist protocol for COS is the protocol that is most widely used protocol with a ratio of 85.7%. Menotropin is the first medicine most commonly used with a ratio of 94.6%. and Gonal-f was the second most commonly used medicine, with a percentage of 73.2%.

The Variable	Category	Number of cases	The ratio
The factor	Male Factor + AGE + low ovarian reserve	2	1.7
	РСО	2	1.7
	Intermittent sex	2	1.7
	Tubal factor	2	1.7
	Male factor + low ovarian reserve	4	3.8
	Male factor + Age	8	7.1
	Age	6	8
	Male factor + PCO	6	8
	Unexplained	10	8.9
	low ovarian reserve	10	8.9
	Age+ low ovarian reserve	10	8.9
	PGD (Sex selection)	14	12.5
	Male factor	36	32.1
COS protocol	Agonist/LLP	16	14.3
	Anta	96	85.7
First medicine	Menotropin	106	94.6
Second medicine	Gonal f	82	73.2

Table 1: Describes the study sample for categorical variables.

The following charts show the distribution of the study sample by factor, COS protocol, and medicine's kind.

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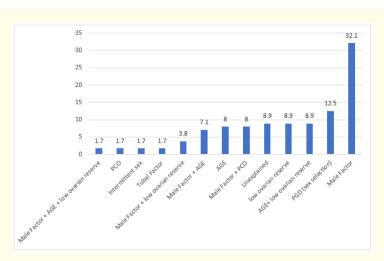


Chart 1: Distribution of the study sample according to the factor.

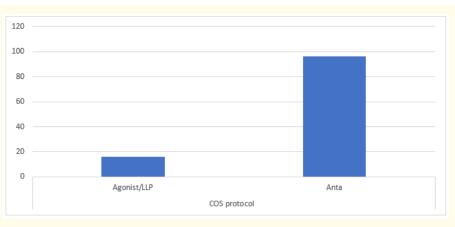


Chart 2: Distribution of the study sample according to the COS protocol.

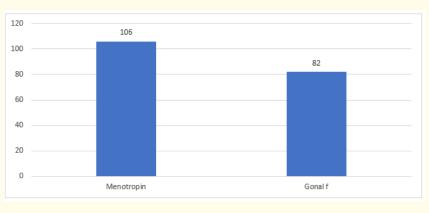


Chart 3: Distribution of the study sample according to the types of medicine.

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The factor	Minimum	Maximum	Mean	Standard deviation
Age	23	45	32.2	5.986
Number of oocytes retrieved	0	29	10.89	7.55
Number of expected oocytes	0	18	7.34	5.181
Number of fertilized oocytes	0	18	5.3	4.293
Number of first-degree embryos (top quality embryos)	0	11	2.75	2.914
Number of transfer embryos	0	6	2.34	1.621
Endometrial thickness	6	17.6	10.087	2.2315
Progesterone levels on trigger day	0.2	2.96	2.01	3.82712
LH level on the day of oocyte retrieval	0.11	21.23	4.152	4.24657
Progesterone levels on the day of oocytes retrieval	1.4	47.09	15.2896	11.9378

The following table shows a description of the variables of the quantitative study sample.

Table 2: Description of the study sample for quantitative variable.

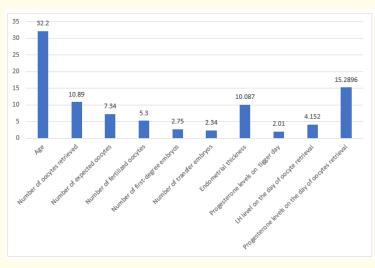


Chart 4: Description of the study sample for quantitative variable.

The age of the study ranged from 23 - 45 years, on average 32 years. Between 0 - 29 oocytes were retrieved, with an average of 10 oocytes. The number of expected oocytes ranged from 0 to 18, with an average of 7. The total number of fertilized oocytes was between 0 - 18, with an average of 5 fertilized oocytes. Number of top quality embryo (first-degree embryos) ranged between 0 - 11, on average of 2 first-degree embryos. The number of transferred embryos ranged between 0 - 6, on average 2 transferred embryos. The endometrial thickness was measured between 6 - 17 mm, with an average of 10 mm. Progesterone levels on the day of trigger day ranged from 0.2 - 2.96, with an average 2. LH levels on the retrieval day ranged from 0.11 - 21.23, with an average of 4.1520, and progesterone levels on the retrieval day ranged from 1.40 - 47.09, with an average of 15.2896.

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Nature of data

The Kolmogorov-Smirnov test was used to determine the distribution of quantitative variables when the sample size was over 100.

When the p-value was less than 0.05, some variables were not distributed normally, and when it was over 0.05, some other variables were distributed normally.

		Proges- terone levels on the day of oocytes retrieval	LH level on the day of oocyte retriev- al	Pro- ges- terone levels on tigger day	Endo- me- trial thick- ness	Num- ber of transfer embryos	Number of first-degree embryos (Top quality Embryos)	Num- ber of fertil- ized oocytes	Numb- er of ex- pected oo- cytes	Number of oo- cytes re- trieved	Age
Age	R										1
	p- value										
Numb-	R									1	-0.505**
er of oocytes re- trieved	p- value										0.00
Number	R								1	0.858**	-0.454**
of ex- pected oocytes	p- value									0.00	0.00
Number	R							1	0.911**	0.769**	-0.369**
of fertil- ized oocytes	p- value								0.00	0.00	0.005
Numb-	R						1	0.750**	0.682**	0.554**	-0.328*
er of first- degree embryos (Top quality embry- os)	p- value						-	0.00	0.00	0.00	0.014
Num-	R					1	0.605**	0.752**	0.711**	0.611**	-0.368**
ber of transfer embryos	p- value						0.00	0.00	0.00	0.00	0.005

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n	7	
υ	1	

Endo- metrial thick- ness	R				1	-0.075	-0.048	-0.056	-0.015	-0.020	0.036
	p- value				•	0.584	0.726	0.683	0.915	0.881	0.790
Proges- terone	R			1	0.123	0.221	0.241	0.376**	0.408**	0.357**	-0.423**
levels on tigger day	p- value				0.367	0.101	0.077	0.004	0.002	0.007	0.001
LH level on the	R		1	-0.279*	0.197	-0.226	-0.137	-0.303*	-0.351**	-0.335*	0.179
day of oocyte retrieval	p- value			0.041	0.153	0.101	0.327	0.026	0.009	0.013	0.196
Proges- terone levels on the day of oocytes retrieval	R	1	-0.112	0.513**	-0.037	0.336*	0.464**	0.458**	0.470**	0.547**	-0.379**
	p- value		0.419	0.00	0.787	0.011	0.00	0.00	0.00	.000	0.004
					*Signific	cant at the 0.0	5 level.		<u> </u>		
					R: 5	Spearman's rl	10.				

Table

Study the relationship among study variables

The Spearman test used to analyse the relationship between quantitative variables in this section, and the chart below shows its results.

The results are shown in the following table:

- Significance test for studying the relationship between the age and each of (the number of oocytes retrieved, the number of expected oocytes, the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on both retrieval and trigger days) was less than 0,05 which means that there is a real, statistically significant relationship among the age and each of (the number of expected oocytes, the number of oocytes retrieved, the number of fertilized oocytes, the number of first-degree embryos, progesterone levels on both retrieval and trigger days) was less than 0,05 which means that there is a real, statistically significant relationship among the age and each of (the number of expected oocytes, the number of oocytes retrieved, the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on both trigger day and retrieval day) with 95 percent confidence.
- Significance test for studying the relationship among the age and each of (Measure of endometrial thickness, and LH levels on retrieval day) was more than 0.05 which leads us to know that there is no real, statistically significant relationship among the age and each of (endometrial thickness, and LH level on the retrieval day) with 95 percent confidence.

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- Significance test for studying the relationship among the number of oocytes retrieved and each of (the number of expected oocytes, the number of first-degree embryos, the number of transferred embryos, LH level on retrieval day, progesterone levels on trigger day, and progesterone levels on retrieval day) was less than 0.05 which means that there is a real, statistically significant relationship among the number of oocytes retrieved and each of (the number of mature oocyte, the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on trigger day, and progesterone levels on retrieved and each of (the number of mature oocyte, the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on trigger day, progesterone levels on retrieval day and LH level on retrieval day) with 95 percent confidence.
- Significance test for studying the relationship among the number of oocytes retrieved and the endometrial thickness was more than 0.05 which means there is no real, statistically significant relationship among the number of oocytes retrieved and endometrial thickness, with 95 percent confidence.
- Significance test for studying the relationship among the number of mature oocytes and each (the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on both days of oocyte retrieval and trigger day, and LH level on the day of oocyte retrieval) was less than 0,05 which means that there is a true, statistically significant relationship among the number of mature oocytes and each of (the number of fertilized oocytes, the number of first-degree embryos, the number of mature oocytes and each of (the number of fertilized oocytes, the number of first-degree embryos, the number of transferred embryos, progesterone levels on both days of oocyte retrieval and trigger, and LH level on the day of oocyte retrieval) with 95 percent confidence.
- The significance test for studying the relationship between the number of mature oocytes and the endometrial thickness, was more than 0.05 which means that there is no real, statistically significant relationship between the number of mature oocytes and the endometrial thickness, with 95 percent confidence.
- Significance test for studying the relationship among the number of fertilized oocytes and each of (the number of first-degree embryos, the number of transferred embryos, progesterone levels on both days of oocyte retrieval and trigger, and LH level on the day of oocyte retrieval) was less than 0.05 which means that there is real, statistically significant relationship among the number of fertilized oocytes and each of (the number of first-degree embryos, the number of transferred embryos, progesterone levels on both retrieval day and trigger day, and LH level on the day of oocyte retrieval) with 95 percent confidence.
- Significance test for studying the relationship between the number of fertilized oocytes the endometrial thickness was more than 0,05 which means that there is no real, statistically significant relationship between the number of fertilized oocytes and endometrial thickness with 95 percent confidence.
- Significance test for studying the relationship among the first-degree embryos and each of (the number of transferred embryos, and progesterone levels on the day of oocyte retrieval) was less than 0.05 which means that there is a real, statistically significant relationship among the first-degree embryos and each of (the number of transferred embryos, and progesterone levels on the day of oocyte retrieval) with 95 percent confidence.
- Significance test for studying the relationship among the first-degree embryos and each of (endometrial thickness, progesterone levels on trigger day and LH level on the day of oocyte retrieval) was more than 0.05 which tell us that there is no real, statistically significant relationship among the first-degree embryos and each of (the measure of endometrial thickness, progesterone levels on trigger day and LH level on the day of oocyte retrieval) with 95 percent confidence.
- Significance test for studying the relationship between the number of transferred embryos and progesterone levels on the day of oocyte retrieval was less than 0,05 which means that there is a real, statistically significant relationship between the number of oocytes retrieved and progesterone levels on the day of oocyte retrieval with 95 percent confidence.
- Significance test for studying the relationship among transferred embryos and each of (endometrial thickness, progesterone levels on trigger day and LH level on the day of oocyte retrieval) was more than 0.05, which means that there is no real, statistically significant relationship among transferred embryos and each of (the measure of endometrial thickness, progesterone levels on trigger day and LH level on the day of oocyte retrieval) with 95 percent confidence.

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- Significance test for studying the relationship among the measure of endometrial thickness and each of (progesterone levels on both days of oocyte retrieval and trigger day, and LH level on the day of oocyte retrieval) was more than 0.05, which means that there was no real, statistically significant relationship among the endometrial thickness and each of (progesterone levels on both days of oocyte retrieval and trigger, and LH level on the day of oocyte retrieval) with 95 percent confidence.
- Significance test for studying the relationship among progesterone levels on trigger day and each of (progesterone levels and LH hormone levels also on retrieval day) was less than 0.05 which means that there is a real, statistically significant relationship between progesterone level on trigger day and (progesterone level and LH hormone level on the day of oocyte retrieval) with 95 percent confidence.
- Significance test for studying the relationship among progesterone levels on the retrieval day and LH hormone levels also on the retrieval day) was more than 0.05 which means that there is no real, statistically significant relationship between progesterone levels and LH hormone levels on retrieval day with 95 percent confidence.

Discussion

There are many studies discussed the value of progesterone on the HCG day [17] and how its elevation associated with low pregnancy rate [18-21]. This negative relation is explained by; the effect of elevated peripheral progesterone levels in the late follicular phase seems to be on the endometrium and the window of implantation, which may lead to asynchrony between the endometrium and the developing embryo [19]. On the other hand the impact of P_4 elevation on the oocyte numbers is still controversial, some study showed positive statistical significance [17,22], while other studies found, that P_4 values on day of trigger do not affect the number of oocytes retrieved [23].

In this study, we found that as the progesterone value elevates on trigger day it reflects an increase in number of retrieval oocytes, mature oocytes, fertilized ovum, and subsequently the number of embryos but surprisingly the number of top quality embryos does not increase. This may refer to other factors (not included in this study) that play a crucial role in determining the quality of embryos such as paternal genome. So, there is no statistical significance between top quality embryos and P_4 on HCG day and it cannot be use to expect the top quality embryos but it is useful for expecting the number of embryos in general.

In regard of P_4 values on the oocytes retrieval day a significant positive correlation was found between higher P_4 level and number of retrieval oocytes, mature oocytes, fertilized ovum, and top quality embryos. This result may play a role in explanation the result of many studies that found a significant correlation between the levels of P_4 on the day of oocytes retrieval and a positive outcome for the ART procedure [24] as the top quality embryos are one of the essential factors for successful ART.

In regard of LH level on retrieval day, LH exerts different functions during the different stages of both natural and stimulated cycles, Physiological levels of LH are clearly important for follicle development. If the LH level is abnormal, it will lead to abnormal follicular development [25]. Number of studies mention that elevation of LH in the HCG day associated with reduction in oocyte retrieved [25] that can cause negative impact on ART. In this study, LH level on retrieval day has a negative statistical significant with only the number of mature oocyte retrieved regardless about the protocol used weather it is Antagonist or agonist. Hypothetically low mature oocytes' number affects the number and quality of embryos negatively, subsequently high LH value on retrieval day associated with low top quality embryos, nevertheless unforeseen in this study we found that LH values on retrieval day has no relation with the number of top quality embryos. In addition, there is a positive statistical significant between P_4 values on trigger day and LH values on retrieval day. This relation can situated clinically to figure out the cases with premature Luteinization based on LH value on retrieval day after determining a threshold of LH level in further studied. Moreover, there is no correlation between LH value and P_4 value on retrieval day.

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In regard of endometrial thickness, it was measured in a median longitudinal plane of the uterus as the maximum distance between the endometrial-myometrial interfaces of the anterior to the posterior wall of the uterus, on the day of oocytes retrieval without taking the pattern of endometrial (degree of Latinization) in concern. The endometrial thickness effects on IVF is controversial. It is one of the factors that determine the receptivity of endometrium which in turn very important in IVF success.

In this study, we found that the endometrial thickness does not affect or effect by any of (LH, p_4) hormones on retrieval day or P_4 hormone on HCG day. In addition, there is no statistical significant between endometrial thickness and top quality embryos. This result does not go with or against the studies that focused of the role of endometrial thickness in ART. It is simply focus on the correlations between the parameters in this study including top quality embryos and endometrial thickness regardless of anything else.

Conclusion

Researches has always been about obtaining top quality embryos (TQE) as it plays an important role in IVF outcome, our study showed that elevation of number of retrieval oocytes, fertilized ovum, and value of P_4 on retrieval day led to raise of top quality embryos' number. LH level on retrieval day has no relation with Top quality embryos number. P_4 values raise on trigger day led to raise of the number of embryos but not top quality embryos. Endometrial thickness has no relation with (LH, P_4) on retrieval day, P_4 on trigger day, or top quality embryos.

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