MedCrave
Step into the World of Research

Obstet Gynecol Int J

Research Article

Open Access

Factors that may interfere in estradiol-progesterone plasmatic concentration in the mid-luteal phase of hyperstimulated cycles for IVF/ICSI

Abstract

Research question: We have demonstrated that plasmatic estradiol in mid-luteal phasel has prognostic value for clinical and ongoing pregnancy rate. This study investigated the possible factors that could interfere in the value of estradiol-progesterone in the mid luteal phase of cycles of IVF/ICSI

Design: Retrospective study, including patients ≤39years old and with dosage of estradiol-progesterone and beta hCG 6-7 days after fresh embryo transfer and the influence of several factors or variables upon these hormones.

Results: Of 189 cycles of IVF/ICSI with complete hormonal evaluation in mid-luteal phase, we studied the probably influence of ten factors upon plasmatic concentration of estradiol-progesterone. Only four factors had significant influence. Of them, the most important variable was beta hCG concentration (consequence of trophoblastic mass and number of implanted embryos), followed for number collected oocytes, and of litlle importance, scheme of final maturation with luteal phase correction (did not reach statistical difference), and day of transfer.

Conclusion: The estradiol-progesterone in the mid-luteal phase, in this research, were influenced by hCG, number of collected oocytes, scheme for final maturation, but the main influence factor was the beta hCG concentration, although not had been a good correlation coefficient between these hormones, due to a large difference in the their concentrations, as seen in the high values observed on the Standard Deviation. The high percentages of clinical and ongoing pregnancies in the ≥500g/ml group, were the result of higher beta hCG levels, due to the larger number of twin pregnancy in this group.

Keywords: IVF, luteal phase, estradiol and progesterone, ongoing pregnancy, chorionic gonadotropin

Volume 11 Issue 6 - 2020

Rodopiano De Souza Florencio
Associate Director Humana medicina reprodutiva, Goiania, Goyaz, Brazil

Correspondence: Rodopiano de Souza Florêncio, Associate Director, Humana Medicina Reprodutiva. Street 1129, number 751, Sector Marista, Goiania, Goiás, Brazil., CEP: 74175-140, Tel +55 62 99971-2152/55 62 3946-9070,
Email drodopanoflorencio@gmail.com

Received: November 23, 2020 | **Published:** November 30, 2020

Introduction

Many authors have studied the variation and average of the estradiol (E2) in the luteal phase in natural cycles,[1,2] and induced cycles on assisted reproduction, with hypophyseal blocking.[3–6] Such authors demonstrated that the serum estradiol levels in luteal phase are higher in the cycles with conception. Greb et al, 2004[7] showed that the estradiol levels behave differently from the embryo transfer day +4(D4) day after D2-3 embryo-tranferring between conceptive and non-conceptive cycles and, the average, was higher up to the D14. In the cycles with pregnancy, estradiol increased in an evident way from D6, whereas on the non-preganant, it decreased. They also observed that, in suplemented cycles in the luteal phase with chorionic gonadotropin (hCG), the estradiol values from the D6, were corrected and there were no case of dropping of such. Most of your patients were given hCG reinforcement in the luteal phase. The same group[8] carried out a prospective study with use of progesterone solely in the luteal phase and observed significant higer levels of estradiol in clinical conception (P) so early as the transfer day D7 with D2-D3 transferring, corresponding to the ovum pickup day +10(OP10). Ganesh et al.,[6] compared the E2 in pregnant and non-pregnant women on the 0, +7(D7) and +14(D14), in relation to embryonary transferring (ET), in in vitro fertilization or intracytoplasmatic injection (IVF/ICSI). Similar values were found on the day 0 significantly higher on the D7 and D14 pregnant patients. Some meta-analyses[9,10] and Pinheiro LMA et al, 2017,[11] did not detect any evidence of benefit of the estradiol use in the luteal phase, in order to increase the preganancy chance. Other authors,[12–16] noticed an increase on the chance of clinical pregnancy in prospective studies with the E2 use in the luteal phase. Kwon et al.,[17] also found statistical difference with an increase of chance in clinical pregnancy, with the addition of E2 to progesterone (PRG).

Fatemi et al.,[18] found higher results of estradiol with statistical significance on the 10th day after final maturation with hCG, in the group which utilized estradiol suplementation to progesterone in the luteal phase (760 pg/ml versus 580pg/ml, p=0.004), having used the 10th day post hCG (OP+8=pickup day +8), because on that day the hCG influence on the pregnant women was not present yet. Fujimoto et al.,[19] observed an increase of pregnancy chance with estradiol >500pg/ml in the mid-luteal phase (MLP); besides this, when patients

showed E2<100pg/ml in MLP did not get pregnant (NP), the same suggested a new cycle and adjusted the luteal phase by utilizing hCG and progesterone obtaining alike chances of preganancy with the estradiol >500pg/ml group. Recently, our group demonstrated that the serum estradiol in the luteal phase, pós transfer (D6-7), was a potent chemical, clinical and ongoing pregnancy chance prognostic factor from 500pg/ml.[20] Due to the fact of possible influence of various factors on the estradiol levels in MLP in addition to the aparent scarcity of data in literature on the specific theme, we were driven to identify the factors involved in the MLP estradiol serum concentrations in our material at disposal. All of the patients signed an informed consent form authorizing research on the data collected, retrospective, anonymously.

Objective

To determine the variables that interferes in the estradiol-progesterone concentration in the luteal phase of hyperstimulated cycles for FIV/ICSI.

Material and methods

A hundred eighty-nine of FIV/ICSI cycles with fresh embryo-transfer of 162 patients were evaluated in this study in the period of January 2010, to december 2012, and in the period of January to December 2017, only on the patients followed by the author, corresponding to approximately 18% of the cycles that were carried out in our clinic in those periods; in such periods, the hormonal dosages were performed 6 to 7 days after embryo-transfer.

ICSI/in vitro fertilization technique, in a brief description

The patients prepared for FIV/ICSI, with rare exceptions made use of oral contraceptive pills routinely in the pre-induction period, during 12 to 21 days. Basal-ultrasound was done on the last day of the pill use or the on the induction onset. For the agonist group we utilized 0.1 ml of leoprolide acetate subcutaneously (Lupron Kit®), daily from the 4th day before the pill use was discontinued. After 7 days, the dosage was cutdown to half. For the antagonist group, we made use of 1 ampoule of cetrorelix (Cetrotide®), subcutaneously, flexible scheme, starting with 12 to 13mm diameter follicles on the average. When the follicles reached an average diameter of 19-20mm, we administered 250mcg of recombinant chorionic gonadotropin (Ovidrel®). The pickup was done 35-36 hours, in most of the cases manually and, in a few cases, with a suction pump. The oocytes obtained were injected 2 to 3 hours after the pickup or inseminated in some cases of excellent semen. The fertilization was evaluated after 17 to 20 hours. The embryos formed were transferred on the 2-6 day, preferably two (2) and maximum three (3), in some special situations. The exceeding viable embryos, were frozen. In the case of ovarian hyperstimulation syndrome (OHSS) risk, the estradiol and progesterone were dosed in the morning, on the final maturation day. Patients with OHSS risk were administered 0.4ml of leuprolide acetate kit or 0,2ml of Gonapeptil®. The patients with embryo-transfer utilized 2mg of estradiol valerate (EV), orally, 8/8 hours' time span, and 200 mg of micronized progesterone, vaginal, 8/8 hours' time span, right from the pickup day, for the patients who utilized hCG for final maturation. In the case of the use of agonist for final maturation, we used oral estradiol valerate (EV), 6mg/day+Estradot®100, and daily of progesterone injection 50mg/day, starting on the pickup day. Only for the embryo-transfer patients we studied routinely the estradiol, progesterone and chorionic gonadotropin (bHCG) on the 6-7 days post transferring (D6-7) and, once again progesterone and bHCG, D14 to evaluate the chemical pregnancy. In the positive cases, endovaginal ultrasound was made on the 10th and 20th days after this date.

The estradiol, progesterone and beta hCG dosages were realized on electrochemiluminescence Modular Roche device (Cobas 60000).

We assessed retrospectively the estradiol serum, progesterone and chorionic gonadotropin dosages, D6-7 and D14 in IVF and ICSI cycles, but only the first dosage (D6-7) was object of investigation. The data was extract from the Excel spreadsheet. All of the cycles were induced, monitored, with pickup and embryo-transfer by the author. All the patients authorized retrospective research with their data under privacy. Study period of the dosages: From January 2010 to December 2012, and January to December 2017, in patients who had undergone embryo-transfer post IVF/ICSI, ≤39years and who lived in Goiânia, or nearby.

Total number of cycles evaluated: 189. The following variables were analyzed: Table 2, showed in the pregnant(P) and not pregnant(NP), the following averages: antral follicles number, collected oocytes number, number of days of oral contraceptive use (OC) in the cycle prior to induction, embryo-transfer day, embryo-transfer type classification Humana 3 (detailed in Table 1), E2, progesterone, beta HCG dosages and the percent of patients who made use of agonist in the final maturation with luteal phase adjustment as described previously. In Table 3, we evaluated in the P and NP groups two groups of collected oocytes (≤10 e ≥10) and, in these groups, we evaluated the three hormones average concentration as well as the percent of patients that had made use of agonist in the final maturation. Figure 2 showed us the evaluation of the oocytes per group, which enabled us to better understand this variable. In Figure 1, was evaluated the E2 concentration in patients with trigger with Ovidrel® and agonist with luteal phase adjusment for P and NP. In Table 4, (evaluation of the number of days use of OC in 4 groups) evaluating the results of the three hormones studied within the P and NP groups. In Table 5 (influence of the embryo-transfer day on the three hormones studied in P and NP). In Table 6, the variables in the estradiol groups <500 e ≥500picograms/ml of P and NP were evaluated, with the attempt of clearly identifying if some of these variables could determine a higher chance of pregnancy as seen in the ≥500pg/ml group, previously. In the ≤500pg/ml and ≥500pg/ml E2 concentration groups, dosages which were accomplished on the D6-7, we evaluated: the average age in each estradiol group, basal FSH dosages administered up to 6 cycles pre-treatment, antral follicles counting on the induction onset, number of days of contraceptive use on the previous cycle, total dose of gonadotropin (TGD) used in the cycle, percent of the use of the agonist for final maturation and luteal phase adjustment scheme as cited previously, oocytes picked up, transfer type (see embryo-transfer quality classification in Table 1), transfer day and the hormonoal levels in the luteal phase. In Table 7, estradiol (<500pg/ml, ≥500pg/ml, and > 900pg/ml groups), was evaluated a possible interference of the number of gestational sacs on the beta hCG concentration. In Table 8, we studied the relationship of the two E2 concentrations with the early gestational sacs, the percent of the 2 gestational sacs and the percent of ongoing pregnancy.

Finally, we evaluated the estradiol, progesterone and chorionic gonadotropin correlation coefficients.

Table 1 Embryo transfer classification, fresh (number of embryos, number of blastomeres, transfer day) Humana 3 – 2017

	Very Good (1) #	**Good (2)***	**Regular (3)****	**Bad (4)****
D2	(≥) 4bl (2)	≥4bl (1) or 3bl**	(≤) 2bl.	PN
D3	(≥) 8bl (2)	≥8bl (1) or 6-7bl**	4-5bl.	2-3bl
D4	≥80/% Compacted Morula (2)-M4	≥80% compacted Morula (1) or partial compacted (50-80%) **M3	morula compacted <50% (≥1), M2; M1 or clivate Embryo ≥6bl.**	(<) 6bl
D5	Blastocyst G1(2)	Blastocyst G1(1) or	Any Morula	(<) Morula

*At least 1 top embryo	Blastocyst G1; ≥3BB(gardner); G2 <3BB
**Any number of embryos transferred	Classification 1 and 2: compatible cleavage day
(<) morphology less than…	Classification 3: Delay of 24 hours in cleavage, 1 or +
# Minimal of two top embryos	Classification 4: Delay of 48 hours in cleavage, 1 or +
bl: blastomeres,	( ) n transferred
PN: pronucleus	M4 (grade 4 morula), M3… M2..., M1...

Table 2 Variables that may interfere in the estradiol concentration in MLP in patients ≤39years in pregnants (P) and non pregnants (NP)

	P (89)	**NP (100)**	**P**
Average Age (M)	32.47±4.30	33.20±3.71	0.2136
E2 (MLP)	856.10±948.80	361.50±203.40	<0.0001
Progesterone (MLP)	44.53±66.26	13.84±14.58	<0.0001
βHCG (MLP)	23.97±37.12	0.9±1.60	<0.0001
A.F. Antral Follicle	14.91±7.02	13.15±7.40	0.1267
Collected Oocytes (M)	12.76±8.98	12.33±7.31	0.7174
Days of use of OC in the previous cycle (M)	18.33±7.02	17.28±6.72	0.1457
Embryo-Transfer Day (M)	3.52±0.88	3.45±0.85	0.3932
Type of Transfer (Humana 3)	1.36±0,63	1.56±0.87	0.0896
% Agonist (final maturation)	17/89=19.10%	20/100=20%	0.9898

MLP, mid-luteal phase; E_2, estradiol; A.F, antral follicle; OC, oral contraceptive

Table 3 FIV/ICSI – Influence of the oocytes collected on E2, Prg e βHCG in MLP in pregnants (P) and non pregnants (NP)

	P			**NP**		
	≤10	**>10**	**P**	**≤10**	**>10**	**P**
Oo. Col. (M)	6.56±2.37	20.20±8.19	-	6.43±2.74	17.92±5.86	-
E_2 (MLP)	900±966.2	807.00±902.30	0.6508	374.42±181.50	349.20±223.50	0.537
Progesterone (MLP)	53.62±72.18	33.46±57.29	0.1717	15.42±20.13	12.30±4.79	0.2889
βH.C. G (MLP)	22.40±42.07	25.82±30.74	0.6716	1.28±1.84	0.54±1.24	0.021
% Agonist (final maturation)	Jan-48	15/41	0.0003	Jan-49	19/51	0.0001
	-2.80%	-36.58%		-2.04%	-37.25%	

Oo. Col, oocytes collected; MLP, mid-luteal phase; E_2, estradiol; Prg, progesterone

Citation: Florencio RDS. Factors that may interfere in estradiol-progesterone plasmatic concentration in the mid-luteal phase of hyperstimulated cycles for IVF/ICSI. *Obstet Gynecol Int J.* 2020;11(6):355–364. DOI: 10.15406/ogij.2020.11.00537

Table 4 FIV/ICSI – Influence of the number of days of the OC use in the previous cycle on the E2, Prg and βH.C. G concentrations in the MLP in pregnants (P) and non pregnants (NP)

	P			NP		
	E_2	Prg	βHCG	E_2	Prg	βHCG
Not Utilized	793±481.80 (a)	87.35±100.5 (c)	27.49±25.67 (e)	292.2±186.60 (g)	13.05±5.88 (i)	1.56±2.21 (k)
≤13	680.30±723.90	32.03±35.32	8.76±7.18	367±218.20	16.27±12.63	0.45±0.68
14-18	986.70±1039	46.89±84.28	16.09±20.83	312.80±148.50	11.74±7.16	0.90±1.70
>18 dias	862.3±1068 (b)	36.69±46.16 (d)	27.18±31.48 (f)	399.30±224.80 (h)	14.71±21.47 (j)	0.85±1.57 (l)
P	(a)/(b)=0.8430	(c)/(d)=0.024	(e)/(f)=0.9831	(g)/(h)=0.1420	(i)/(j)=0.8020	(k)/(l)=0.2487

OC, oral contraceptive; E_2, estradiol; Prg, progesterone

Table 5 FIV/ICSI – Influence of the embryo-transfer day on E_2, Prg and βHCG in the MLP in pregnants (P) and non pregnants (NP)

	P			NP		
Embryo-transfer day	E_2	Prg.	βH.C. G	E_2	*Prg.	βHCG
D2	595±416.2 (a)	22.22±11.69 (c)	3.10±3.74 (e)	396.70 (g)	17.25±8.98 (i)	0.86±0.69
D3	742.5±893.9	28.00±30.08	9.81±9.45	336.3±165.2	13.10±9.70	0.53±0.93
D4	1088±1064	70.47±94.71	39.20±51.56	335.6±209.4	11.32±5.53	0.66±1.52
D5	893.4±1055 (b)	55.49±75.14 (d)	48.21±41.39 (f)	382.3±213.70 (h)	11.70±5.32 (j)	0.32±0.35
P	(a)/(b)=0.4307	(c)/(d)=0.1823	(e)/(f)=0.0041	(g)/(h)=0.8680	(i)/(f)=0.1510	(k)/(l)=0.0701

E_2, estradiol; Prg, progesterone; MLP, mid-luteal phase

Table 6 Factors which may interfere with estradiol (pg/ml) in the luteal phase, patients ≤39years, FIV/ICSI, two levels of estradiol

	P			NP		
Estradiol	**<500**	**≥500**	**P**	**<500**	**≥500**	**P**
Age	33.01±3.73	33.96±3.60	0.2542	32.97±4.73	32.57±3.92	0.7607
FSH	6.80±2.87	6.17±2.23	0,366	7.82±2.69	7.07±2.75	0.436
A.F.	13.06±7.54	13.60±7.43	0.7593	14.22±8.75	15.45±7.46	0.4776
OC	17.07±6.91	16.88±7.82	0.2227	17.46±5.54	20.07±8.01	0.096
TGD	2277.1±599.27	2015±592.26	0.06	2296±537.51	2029.59±629.70	0.04155
AGON%	20.77	25.92	0.6829*	21.95	16.32	0.7756*
Oo.C.	12.06±7.05	12.23±8.21	0.9211	14.15±10,84	11.60±6.99	0.1746
T.Type	1.57±0.93	1.55±0.69	0.9282	1.40±0.67	1.33±0.59	0.574
T.D.	3.45±0.81	3.77±0.96	0.6624	3.41±0.83	3.63±0.90	0.216
Prg	13.56±7.25	68.52±80.57	<0.0001	12.02±6.87	12.54±26.54	0.0233
βH.C. G	12.55±19.49	33.09±45.39	0.0065	0.75±1.38	1.35±2.05	0.1004
E_2 (MLP)	293.90±119.60	1310±1080	NR	270.90±112.30	692.2±162.30	NR

A.F, antral follicle; OC, oral contraceptive; G.T.D, gonadotropin total dose; AGON%, percent of agonist use in the final maturation; Oo. C, oocytes collected; T. Type, type of transfer humana 2 – figure1; TD, transfer day; E_2 (MLP), estradiol on DT+6-7; Prg, progesterone on DT+6-7; βH.C. G, chorionic gonadotropin on DT+6-7

Citation: Florencio RDS. Factors that may interfere in estradiol-progesterone plasmatic concentration in the mid-luteal phase of hyperstimulated cycles for IVF/ICSI. *Obstet Gynecol Int J.* 2020;11(6):355–364. DOI: 10.15406/ogij.2020.11.00537

Table 7 Pregnant Patients: E_2, PRG and Beta concentrations, and the number of early gestational sacs in two Estradiol groups

	E_2 <500pg/ml			E_2 >500pg/ml		
	1GS	2GS	p	1GS	2GS	p
E_2	305.43±128.47	259.36±84.11	0.2976	1106.03±971.52	1508.62±1060.34	0.3226
PRG	12.56±6.06	15.93±9.43	0.2014	52.57±63.52	85.31±78.72	0.2644
Beta	13.50±22.2	9.75±6.8	0.6056	18.90±23.15	49.90±32.20	0.0313

GS, gestational sac

Table 8 Relationship of estradiol concentration in MLP with the number of early gestational sacs, percent of two gestational sacs and percent of twin pregnancy ongoing

	<500pg/ml (41*)		>500pg/ml (51)	
N° of GS	1GS (27)	2GS (11)	1GS (27)	2GS (24)
5-6 weeks				
% 2 Gestational Sacs	-	11/41[a]	-	24/51[A]
/Pregnant		-26.82%		-47.05%
% Ongoing/pregnant	21/27[b]	09/11[B]	18/27c	24/24[C]
≥12 weeks	-77.77%	-81.81%	-66.66%	-100%
% Twin pregnancy ongoing	0	06/11[d]	0	11/24[D]
≥12 weeks		-54.54%		-45.84%

*3 patients did not show gestational sacs and presented early bleeding with abortion

pa/A=0.0470 - pb/B=0.3026 - pc/C=0.0020 - pd/D=0.6321

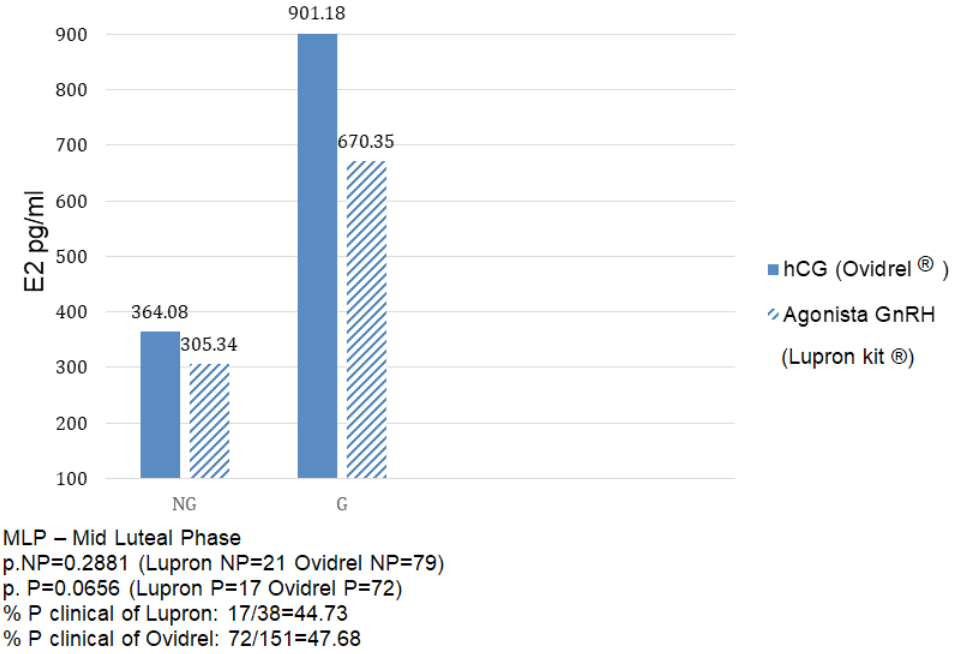
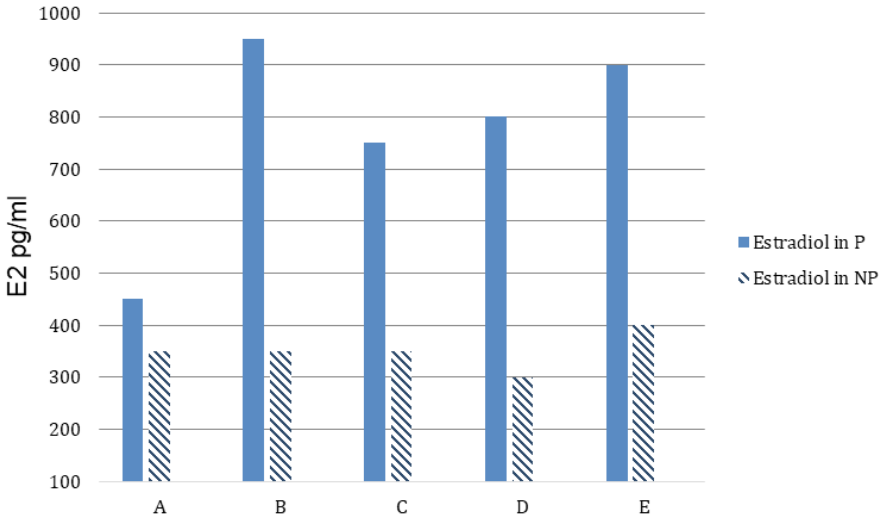


Figure 1 MLP Estradiol Concentration of Estradiol in patients with trigger of Ovidrel® versus Agonist with Luteal Phase Adjustment.



P	Average	E_2 avg	BhCG avg
A=1-4[a]	2.92±0.86	489±299,10	17.6±24.28
B=5-9[b]	7.19±1.47	990,8±969,20	14.21±16.49
C=10-13[c]	11.05±1.19	725,50±562,10	21.74±21.65
D=14-17[d]	15.20±1.03	789,70±492	34.87±40.59
E=>17[e]	25.36±7.75	910±1139	25.81±29.87
NP	**Average**	**E_2 avg**	**BhCG avg**
A=1-4	3±1.17	366±141.50	1.21±0.86
B=5-9	7.19±1.41	369.60±185	1.32±1.32
C=10-13	11.21±1.12	365.20±216	1.07±1.93
D=14-17	15.28±1.13	307.4±217.90	0.91±1.91
E=>17	23.13±4.53	379±241.10	0.29±0.50
p.a/b=0.0334	p.a/c=0.1840	p.a/d=0.1104	p.a/e=0.1109

Figure 2 Influence of the n° oocytes collected on estradiol concentrations in P and NP.

Statistical calculation

The T-test was used to evaluate the differences between the averages and, the X-squared test, the Fisher exact test, to compare the ratios. The statistical programs used were from Excel statistics tests, Quick Calcs of Graph Pad. Tests significance for $p<0.05$.

Results

We studied 189 IVF/ICSI cycles with fresh transferring, which presented hormonal dosages evaluation in MLP and age ≤39years. The clinical pregnancy rates in this material, which includes low, normal, and high ovarian reserve were:

a. Group < 500pg/ml (34.74%),
b. Group ≥ 500pg/ml (64.47%),
c. Group ≥900pg/ml (86.20%,).

The clinical pregnancy index in the group that used Ovidrel® was 47.68% and 44.73% in the agonist group for final maturation (Lupron® 0.4ml) with luteal phase adjustment (Figure 1), but it should be pointed out that for around half of the agonist patients we utilized "freeze all"and they were excluded. All of our cycles used E2 in the luteal phase, and when was used Ovidrel® for final maturation, was utilized estradiol valerate 6mg/day and in the agonist final maturation group we added to valerate the use of the transdermal (Estradot® 100) daily, in addition to intramuscular progesterone injection 50mg/day. The average dosages of E2 on the D6-7, which corresponds to OP9-11, in the Ovidrel ® group, showed 901.18pg/ml on the P and 364.08 on the NP and the Lupron®Kit 0,4ml group, 670.35 on the P and 305.54 NP (Figure 1) and for the total of P and NP (856.10 versus 361.50 p=<0.0001. Upon comparing the P and NP groups in Table 2, we did not see any difference in the following averages: age, antral follicles, picked up oocytes, days of use of OC, embryo-transfer day, type of transfer, percent of agonist use in the final maturation, though E2, PRG, and beta hCG presented remarkable differences (p<0,0001). In Table 3, The P and NP oocytes collected were studied, sorted out into two groups ≤10 oocytes collected and >10 oocytes. In the P group as expected, there was difference in relation to the percent of patients who made use of agonist in the final maturation (2.08%versus 36.58%, p=0.0003), in the group of NP we found similar results (2.04% versus

37.25%, p=0,0001), and the level of beta hCG was higher in the group ≤10 oocytes collected (1.28mui/ml versus 0.54mui/ml), p=0.0210. When we sorted out the oocytes picked up into more groups (Figure 2), to better evaluate this variable, we noticed a similarity of E2 in the NP; however, in the P group, we observed an influence of the number of oocytes collected over the average of E2, having occurred an increase of E2 (group A 489+/-299.10 versus 990pg/ml +/-969.20 in group B,p), even with the beta hCG average decrease in group B and, from group C onwards there was a slow progressive increase of the E2 average. The influence of the number of days and use of OC on the previous cycle on the E2, PRG and beta hCG was assessed in Table 4. Only for PRG (87.35ng/ml in the group which did not use OC versus 36.69 ng/ml, in group with >18 days of use (p.c/d 0.024) was found statistical difference. In Table 5, which evaluated the influence of the embryo-transfer day on the hormones, we found statistical difference only for beta hCG. In the group P, we found 3.10mui/ml with transfer on D2 and 48.21mui/ml with transfer on D5, p=0.0041 and, a tendency to an inverted difference between D2/D5 in the NP group (0,86mui/ml on D2 and 0.32 on D5, p=0.0701). In Table 6, we observed a tendency to a lower use of gonadotropin in the group with higher concentrations of E2 >500pg/ml, p=0.060 and 0.0415 in NP. In the group >500pg/ml in P, there was a remarkable difference on the PRG (p=0.0002) and beta hCG (p=0.0065) concentrations. In the NP group, the PRG was higher in the subgroup ≥500pg/ml, (p=0.0233). The other variables did not show any difference. In Table 7, the importance of the trophoblastic tissue in the production of beta hCG is shown, through evaluation of the number of gestational sacs observed in each estradiol subgroup, where in the group of E2 <500 pg/ml, similar results were observed for the 3 hormones in the patients with 1 and 2 gestational sacs, and for the ≥500pg/ml group, a higher quantity in beta hCG in the 2 gestational sacs group (p.0.0313). Table 8 shows a higher percent of 2 gestational sacs in the E2 >500pg/ml, p.a/A=0.0470, 100% of ongoing pregnancy in the >500 pg/ml group, even having 45.84% of twin pregnancy ≥12 weeks in this group. By analyzing the correlation coefficient (Pearson) among the hormones, in the P group, we observed: 0.74 between E2 and PRG, 0.19 between beta hCG and E2; 0.29 between beta hCG and PRG. In the NP group: 0.37 between E2 and PRG; 0.20 between beta hCG and E2; 0.19 between beta hCG and PRG.

Discussion

Estradiol and progesterone in the luteal phase of induced cycles may be influenced by many factors:

i. Induction scheme (natural cycle, hyperstimulated, different final maturation schemes). Our work encompassed the study of hyperstimulated cycles and two final maturation schemes (hCG versus agonist).

ii. Different luteal phase maturation schemes, cycles with agonist doses to increase the LH and stimulate the corpus luteum, or the use of hCG in the luteal phase at different schemes. Our work evaluated micronized progesterone and estradiol with fixed scheme, in the cases of the patients that made use of Ovidrel®, and luteal phase adjustment scheme on a model close to the one suggested by Engmann et al.,[21] for patients using agonist in the final maturation. We did not perform any dual trigger and, rarely, employed hCG on the pickup day as advised by Humaidan et al.,[22,23]

iii. Number of oocytes picked up of any maturation level (indirectly dependent on the number of follicles of ≥12mm) has correlation with the estradiol level up to the final maturation, but it also has influence on the level of estradiol in the mid-luteal phase? We will see it ahead on the discussion of this topic.

iv. The number of days of contraceptive use in previous cycles exerts any influence on the estradiol levels in the MLP of the hyperstimulated cycle? Apparently not, as we will see further.

v. Different days of transferring and and hormonal collecting fixed post-transferring, quality of the embryos trasferred (here evaluated as transfer type, by simple morphological evaluation), may have influence on such evaluations? Will see it further in the discussion section.

vi. By studying the two levels of estradiol in P and NP, is it possible to detect influencing variables? number of gestational sacs, etc. Apparently, the levels of beta hCG, determined by the trophoblastic mass, such as in the case of multiple pregnancy, was the most important variable, as we will see in the discussion section.

vii. What is the level of negative influence in the mid-luteal phase of the agonist use for final maturation and whether the scheme used to rescue the luteal phase was efficient or not?

Many authors have demonstrated higher levels of estradiol in MLP (D6-7) of patients who got pregnant after IVF/ICSI, in natural cycles,[1,2] as well as in hyperstimulated cycles.[3–6] Balasch et al.,[24] Csemiczky et al.,[25] observed the prognostic strength for clinical and ongoing pregnancy of estradiol and progesterone in the MLP in IVF cycles in which were employed clomiphene and gonadotropin. Fatemi et al.,[26] demonstrated that the patients who had used E2 in the luteal phase presented concentrations statistically higher of this hormone in relation to the ones who utilized progesterone only, up to 10 days after hCG, such a period without the endogenous hCG influence on the P yet. Different authors have demonstrated clinical pregnancy chances higher with higher levels of estradiol in the MLP, mainly ≥500pg/ml.[19] These authors, differently from our experience,[20] have already noticed remarkable differences on the pregnancy chance in the 100-500pg/ml group, whereas our group noticed difference only in the >500pg/ml group. Besides this, our group demonstrated very high chances of clinical and ongojng pregnancy with estradiol concentrations ≥900pg/ml, in the MLP.

In this work, we assessed the possibility of detecting variables, which could have influence on these E2 and PRG levels in the MLP. In Table 2, which evaluated in the P and NP groups the age averages, antral follicles, picked up oocytes, OC days of use in the previous cycle, embryo-transfer day, transfer type, and percent of the use of agonist for final maturation, among others. There were statistical differences only for E2, PRG, beta-hCG. In Table 3 we tried studying in P and NP, the average of two groups of oocytes collected (≤10 e >10) and we observed a trend to higher level of progesterone without statistical difference, in the ≤10 oocytes group in P. Additionally, we observed statistical difference only in the group that had used agonist for final maturation in the >10 oocytes in P and NP, as expected. This apparent result of lack of relationship with the levels of E2 and the average of oocytes collected, was better evaluated in Figure 2, which grouped the oocytes collected in smaller subgroups, enabling us to understand the importance of the number of ovarian follicles sucked on the E2 and progesterone levels. In the P patients, the difference between groups A and B, with E2 (489+/-299.10 versus 990.8+/-969.20, p=0.0334) was observed, showing no difference on the beta hCG levels in these two groups. These two groups were made up of patients who had utilized hCG solely for the final maturation. Right from the C group the use of agonist for the final maturation, began to

alter the E2 and PRG averages in the luteal phase due to the luteolysis more precocious.[27,28] The percent of agonist use for the final maturation in the E group (Figure 2) was 68%. Humaidan et al.,[29] assessed the chances of pregnancy in patients who had made use of agonist for the final maturation and the use of vaginal progesterone, plus 4mg/day of oral estradiol versus hCG. They came to the conclusion that this scheme (oral E2+ vaginal PRG) did not avoid the luteolysis caused by the agonist, which led to lower indexes of clinical pregnancy and a higher chance of abortion. The same group,[22] carried out prospective study where they compared hCG 10000IU versus agonist for the final maturation with the addition of 1500IU of hCG, on the pickup day. The pregnancy results in this study were apparently similar, but a difference not significant of 7% of born alive, compelled the group to carry out additional studies. Once more Humaidan et al.,[23] performed a multicentric study and observed low risk of OHSS with the agonist + 1500IU hCG on the pickup day; however, when they added another dose of 1500IU hCG on D5, post-pickup day, there was an increase on the OHSS risk chance, which took them to set aside this last scheme. Figure 1, which evaluated the groups that made use of Ovidrel® and agonists of GnRH in the final maturation for P and NP, showed us that the E2 difference in the NP was small without any statistical difference (364.08pg/ml versus 305.34, p.0.2881), evidencing that the hormonal replacement in our material was efficient for the agonist group, as described in the material and methods section. Nonetheless, in the P group, it was more evident, but still did not reach the statistical difference (901.8 /670.35, p.0.0656), probably due to the size of our sample, and also for the efficient hormonal replacement as suggested by the authors.[21,30,31] Although the E2 levels had been lower in the group which made use of agonist in the luteal phase in our material, the clinical pregnancy chances with embryo-transfer at fresh in the two groups, did not show statistical difference (agonist 44.73% and hCG 47.68%, p. 0,8561), confirming the findings of Engman et al.,[30] and Di Luigi 2010.[31] In this material, in very few cases hCG was used in low dosages, on the pickup day to adjust the luteal phase as suggested by the authors,[22] due to the risk of OHSS, even with low doses of hCG in our trial. The inconvenience of this luteal phase replacement was the use of injectable progesterone because of local reactions to the injection. This inconvenience may be ruled out with the use of subcutaneous progesterone.[32] The number of days of contraceptive use, in the previous cycle (Table 4), did not have any influence on the E2 levels; however, where OC was not used, there were higher levels of PRG in the MLP in P (no use 87.35+/-100.50 and ≥18 days of use 36.69+/-46.16, p.0.024), such difference was apparently without any importance for the pregnancy chances in this material.

The influence of the transfer day on the hormonal levels studied (Table 5), should show differences on the D2 vs. D5 days, once the dosages were administered on fixed days post-transfer and, this, was really observed in the P (beta hCG D2 3.10+/-3.74 versus D5, 48.21+/-41.39, p=0.0041). Despite this, the differences between E2 and PRG of D2 and D5, did not present statistical difference. The change of reference from the hormonal collecting to the pickup day, and not from the last year's transfer, solved this variable.

Upon evaluating the variables in the two E 2 groups in P and NP (Table 6), we observed higher levels of PRG in the ≥500pg/ml group, with statistical difference on the progesterone levels in P and NP, confirming a good correlation with E2. The beta hCG also was higher in the ≥500pg/ml for P and, in this table, a smaller use of gonadotropin took place in the ≥500pg/ml group for P and NP, probably for referring to a group of a better prognostic. The Pearson's correlation coefficient showed that the E2 and PRG values have an increasing relation more harmonious in the case of the P patients (0.74), though NP patients do not have the same characteristics (0.37). The relation between the concentrations of beta hCG /PRG and beta hCG/E2 were weak for the P (0.29 and 0.19) and NP (0.19 and 0.20), as a result of the great concentrations variablility as evidenced by the values seen in the concentrations standard deviation of these hormones. This broad variation could also be explained by the second decrease of E2 and PRG post hCG of the final maturation that begins on the 6-7 day and intensifies on the 10th day in NP, being slower the variation in the group that uses estradiol in the luteal phase, while the decrease of beta in this date is more prominent, as demonstrated by Fatemi et al.,[18] The new increase of estradiol and progesterone in the group P, begins on OP7 and, in an evident way on the OP10 (D7) as demonstrated by Sonntag et al.[8]: even though, there is still hCG low concentrations. The early signal of implantation as showed by Grebb et al.,[7] on DT4, corresponding to OP6-7, could be a result of the beta hCG brought forth by the adhering blastocyst, and consequent precocious production of the isoform B152 hCG,[33] or of the hCG production itself in the secretionary phase of the endometrium.[34] Our hormonal collection did not study this very early phase, but just from the D6-7 on, corresponding to OP8-11, such a period, which already demonstrated the E2 statistical difference in P/NP. In the P, our material showed in the E2 <500pg/ml group, 14% had beta <2mui/ml, and in the ≥500 group, 12%. It is worth pointing out that these hCG levels were all with transfer on D2 or D3, which corresponds to OP8-9, conversely to D5-6(OP11-12) transfers. This shows us clearly that if we utilize these hormones (beta hCG, E2, PRG) as a criterion for pregnancy diagnostics and prognostics, we have to collect blood sample routinely on OP11. The fact of having blood sample collected on different dates with regards to oocytes pickup, it did not alter the E2 and PRG prognostic strength, as seen in Table 4, though it was made evident that the ideal would be blood sample collecting on the OP11, for all the patients. As for the ongoing pregnancy, Grebb at al.[7] observed higher E2 levels right from D12, corresponding to the OP14, differently from our results, which showed higher levels of E2 from the OP9-10, but in an evident way on OP10-11. These different findings probably are attributed to the fact that in Grebb et al.,[7] data, >50% of the patients had made use of hCG doses in the luteal phase, which increased the levels of E2 during a longer period. Why do patients with E2 levels in the MLP over 500pg/ml, have higher levels of clinical and ongoing pregancies and had it increased even more over 900pg/ml.?

One of the explanation of this finding is ascribed to the fact that the E2 levels are accompanied by higher levels of beta hCG, mainly in the 2 gestational sacs group (Table 7), which would be more compatible with a higher trophoblastic mass; in Table 8, the P group with <500pg/ml levels, had a percent of 26.82% 2 gestational sacs presence, whereas for the ≥500pg/ml group, the percent was 47.5%, p=0.0470. It can be inferred that the high indexes for clinical and ongoing pregnancies in our material accompanied by higher levels of E2 and PRG, were the consequence of the higher levels of beta hCG, observed in this group, as a result of higher trophoblastic mass, confirmed some days later with a higher percent of 2 gestational sacs visible. Besides this, the apparent lower index of abortion cited in our previous work,[20] occurred only as a result of the fact that the pregnancy continued evolving, even after having one of the gestational sacs been aborted, that is, the abort/gestational sacs indexes were similar for the two groups; however, the fact of having a higher number of embryos implanted, was conducive to occurring around 100% of ongoing in the ≥500pg/ml group in the 2 gestational sacs group (Table 8). We still highlight 100% of ongoing pregancy with E2 >900pg/ml levels (1 or 2 gestational sacs) and over 60% of 2 gestational sacs presence

in this group (not shown), but upon calculating the abortion index per gestational sac, we observed 26% (chemical+clinical), similar to the abortion data in our clinic for this age group. In addition, two cases of good prognostic and high levels of E2 and PRG in the 1 gestational sac group, may have happened only by the fact of the presence of a higher trophoblastic mass (hard to gauge) and consequently higher levels of hCG.

Conclusion

The E2 and PRG in the MLP in this research, were influenced by the beta hCG, number of oocytes collected, dosage of total gonadotrophin adminsitration, number of gestational sacs, and agonist for final maturation althought it did not reach statistical difference due to luteal phase correction, but the main influence factor was the beta hCG concentration, although not had been a good correlation coefficient between these hormones, due to a large difference in the their concentrations, as seen in the high values observed on the standard deviation. The high percentages of clinical and ongoing pregnancies in the ≥500pg/ml group, were the result of higher beta hCG levels, due to the larger number of twin pregnancy in this group. The highest percentages of abortion/patient in this group, but identical percentage of abortion/early gestational sac, paved the way for a higher percentage of pregnancy in evolution in this group.

Acknowledgments

I appreciate the efficient work of Ellen Glaucia de Souza Lima and Naiane Nunes da Silva, in the construction of tables and graph; to Amanda de Amorim Araujo for reading and suggestions for corrections; to Carlos Eduardo de Oliveira for the correction of translate.

Funding

None.

Conflicts of interest

This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors.

References

1. Lenton EA, Sulaiman R, Sobowale, et al. The human menstrual cycle: plasma concentration of prolactin, LH, FSH, estradiol and progesterone in conceiving and non-conceiving women. *J Reprod Fertil*. 1982;65:131–139.
2. Baird DD, Wilcox AJ, Weinberg CR, et al. Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. *Hum Reprod*. 1997;12:2607–2613.
3. Hutchinson-Williams KA, Lunenfeld B, Diamond MP, et al. Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconception cycles in an in vitro fertilization program. *Fertil Steril*. 1989;52:441–445.
4. Aktan E, Bozkurt K, Ozer D, et al. The effect of mid-luteal estradiol level on the outcome of ICSI-ET cycles. *Arch Gyn Obst*. 2004;269:134–138.
5. Friedler S, Zimerman A, Schachter M, et al. The midluteal decline in serum estradiol levels is drastic but not deleterious for implantation after in vitro fertilization and embryo transfer in patients with normal or high responses. *Fertil Steril*. 2005;83:54–60.
6. Ganesh A, Sourendrakan G, Chattopadhyay R, et al. Luteal phase estradiol level: a potential predictive marker for successful pregnancy in in vitro fertilization/intracytoplasmatic sperm injection. *Fertil Steril*. 2009;91(4):1018–1022.
7. Greb RR, Lettmann N, Sonntag B, et al. Enhanced oestradiol secretion briefly after embryo transfer in conception cycles from IVF. *RBM Online*. 2004;9(3):271–279.
8. Sonntag B, Loebbecke KC, Nofer JR, et al. Serum estradiol and progesterone in the mid-luteal phase predict clinical pregnancy outcome in IVF/ICSI cycles. *Gynecol Endocrinol*. 2013;29(7):700–703.
9. Jee BC, Suh CS, Kim YB, et al. Effects of estradiol supplementation during the luteal phase of in vitro fertilization cycles: a meta-analysis. *Fertil Steril*. 2010;93(2):428–436.
10. Na Huang, Situ B, Chen X, et al. Meta –analysis of estradiol for luteal phase support in in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril*. 2015;103(2):367–373.
11. Pinheiro LMA, Candido PS, Moreto TS, et al. Estradiol use in luteal phase and its effects on pregnancy rates in IVF cycles with GnRH antagonist: a systematic review. *JBRA Assist Reprod*. 2017;21(3):247–250.
12. Gorkemil H, Ak D, Akyneck C, et al. Comparison of pregnancy outcomes of progesterone or progesterone +estradiol for luteal phase support in ICSI-ET cycles. *Gyn Obstet Invest*. 2004;58:140–144.
13. Lukaszuk K, Liss J, Lukaszuk M, et al. Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril*. 2005;83:1372–1376.
14. Drakakis P, Loutradis D, Vomvolaki E, et al. Luteal estrogen supplementation in stimulated cycles may improve the pregnancy rate in patients undergoing in vitro fertilization/intracytoplasmic sperm injection-embryo transfer. *Gynecol Endocrinol*. 2007;23(11):645–652.
15. Farhi J, Weissman A, Steinfeld Z, et al. Estradiol supplementation during the luteal phase may improve the pregnancy rate in patients undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril*. 2000;73:761–766.
16. Kutlusoy F, Guller I, Erdem M, et al. Luteal phase support with estrogen in addition to progesterone increase pregnancy rates in in vitro fertilization cycles with poor response to gonadotropins. *Gynecol Endocrinol*. 2014;3095:363–366.
17. Kwon SK, Kim CH, Lee KH, et al. Luteal estradiol supplementation in gonadotropin- releasing hormone antagonist cycles for infertile patients in vitro fertilization. *Clin Exp Reprod Med*. 2013;40(3):131–134.
18. Fatemi HM, Camus M, Kolibianakis EM, et al. The luteal phase of recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in vitro fertilization cycles during supplementation with progesterone or progesterone and estradiol. *Fertil Steril*. 2007;87:504–508.
19. Fujimoto A, Osuga Y, Fujiwara T, et al. Human chorionic gonadotropin combined with progesterone for luteal support improves pregnancy rate in patients with low late- midluteal estradiol levels in IVF cycles. *J Assist Reprod Genet*. 2002;19(2):550–554.
20. Florencio RS, Meira MSB, Cunha MV, et al. Plasmatic estradiol concentration in the mid-luteal phase is a good prognostic factor for clinical and ongoing pregnancies, during stimulated cycles of in vitro fertilization. *JBRA Assist Reprod*. 2018;22(1):8–14.
21. Engman L, Siano L, Schmidt D, et al. GnRH agonist to induce oocyte maturation during IVF in patients during IVF in patients at high risk of OHSS. *RBM Online*. 2006;13(5):639–644.
22. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, et al. 1500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. *Fertil Steril*. 2010;93(3):847–854.
23. Humaidan P, Polyzos NP, Alsbjerg B, et al. GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: two prospective controlled multi-centre studies in IVF patients. *Hum Reprod*. 2013;28(9):2511–2521.

24. Balasch J, Creus M, Fabregues F, et al. Hormonal profiles in successful and unsuccessful implantation in IVF-ET after combined GnRH agonist/gonadotropin treatment for superovulation and hCG luteal support. *Gynecol Endocrinol*. 1995;9(1):51–58.

25. Czeminsky G, Wramsby H, Landgrem BM. Luteal phase estradiol and progesterone levels are stronger predictors than follicular phase follicle stimulating hormone for the outcome of in-vitro fertilization treatment in women with tubal infertility. *Hum Reprod*. 1996;11(11):2396–2399.

26. Fatemi HM, Kolibianakis EM, Camus M, et al. Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: a randomized controlled trial. *Hum Reprod*. 2006;21(10):2628–2632.

27. Fauser BC, de Jong D, Olivennes F, et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. *J Clin Endocrinol Metab*. 2002;87(2):709–715.

28. Beckers NG, Macklon NS, Eijkemans MJ, et al. Non supplemented luteal phase characteristics after the administration of recombinant human chorinic gonadotropin, recombinant luteinizing hormone, or gonadropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in in victro fertilization patients after ovarian stimulation with recombinant follicle – stimulating hormone and GnRH antagonist co-treatmen. *J Clin Endocrinol Metab*. 2003;88(9):4186–4192.

29. Humaidan P, Ejdrup Bredkjaer H, Bungum L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. *Hum Reprod*. 2005;20(5):1213–1220.

30. Engman L, Di Luigi A, Schmidt D, et al. The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: a prospective study. *Fertil Steril*. 2008;89:554–556.

31. Di Luigi AJ, Engman L, Schimidt DW, et al. Gonadotropin-releasing hormone agonist to induce final maturation prevents the development of ovarian hyperstimulation syndrome in high-risk patients and leads to improved clinical outcomes compared with coasting. *Fertil Steril*. 2010;94:1111–1114.

32. Sator M, Radicioni M, Cometti B, et al. Pharmacokinets and safety profile of a novel progesterone aqueous formulations administered by the s.c. route. *Gynecol Endocrinol*. 2013;29(3):205–208.

33. Kovalevskaya G, Genbacev O, Fisher SJ, et al. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source of choriocarcinoma-like hCG. *Mol Cel Endocrinol*. 2002;194:147–155.

34. Alexander H, Zimmermann G, Wolkersdorfer GW, et al. Utero-ovarian interaction in the regulation of reproductive function. *Hum Reprod Update*. 1998:4(5):550–559.

Citation: Florencio RDS. Factors that may interfere in estradiol-progesterone plasmatic concentration in the mid-luteal phase of hyperstimulated cycles for IVF/ICSI. *Obstet Gynecol Int J.* 2020;11(6):355–364. DOI: 10.15406/ogij.2020.11.00537