J Gynecol Obstet Hum Reprod 48 (2019) 51–54

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

Original Article

Premature progesterone rise on day of hCG negatively correlated with live birth rate in IVF cycles: An analysis of 1022 cycles

Julien Lepage [a,*], Gwenola Keromnes [d], Sylvie Epelboin [a], Dominique Luton [a,b], Chadi Yazbeck [c]

[a] Bichat Claude Bernard Hospital, AP–HP, 46, rue Henri-Huchard, 75018 Paris, France
[b] Paris 7 Denis Diderot University Paris, France
[c] Cherest Fertility Center, 5, rue Pierre-Cherest, 92200 Neuilly-Sur-Seine, France
[d] Groupe hospitalier Diaconesses Croix Saint-Simon, 12–18, rue du Sergent-Bauchat, 75012 Paris, France

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form 15 May 2018
Accepted 17 May 2018
Available online 18 May 2018

Keywords:
Progesterone
IVF
Pregnancy
Implantation
Live birth rates

ABSTRACT

Objective. – To investigate the relationship between serum *P* levels on the day of hCG administration and pregnancy outcomes in patients undergoing IVF.
Design. – Retrospective study.
Setting. – Teaching hospital.
Patients. – A total of 1022 IVF-ICSI cycles, frozen embryo transfer excluded.
Intervention(s). – Patients-all types of responder – underwent IVF with agonist or antagonist protocols. Clinical outcomes of IVF were analyzed according to plasma *P* levels.
Main outcome measure(s). – Ongoing pregnancy rates.
Results. – We proposed a serum *P* level of 1.57 ng/ml on day of hCG as a threshold for all types of responders and all protocols combined. Ongoing implantation rates were not affected by elevated progesterone. Live birth rate was inversely associated with serum *P* levels on day of hCG and more miscarriages were associated with $P > 1.57$ ng/ml. We have not found the progesterone > 1.57 ng/ml on the day of hCG as a prognostic factor for pregnancy.
Conclusion(s). – Elevated *P* level on the day of hCG administration negatively influence live birth rate and is correlated to an increase of miscarriage. The detrimental effect of *P* elevation on pregnancy seems not to be related substantially to endometrium receptivity. Thus, despite a comparable clinical pregnancy rate and an initial implantation rate, we demonstrate more spontaneous abortion and it would seem that the effect of progesterone is later.

1. Introduction

Progesterone is a steroid hormone secreted by granulosa and theca cells substantially under the influence of FSH (Follicle-stimulating Hormone) in the second half of the menstrual cycle. This hormone allows adequate preparation of the endometrium for embryo implantation; its secretion corresponds to the implantation window. The significance of this increase and its impact on pregnancy rates in late follicular phase of ovarian hyperstimulation IVF-ICSI (In Vitro Fertilization – Intra Cytoplasmic sperm injection) cycles is very controversial. There is no consensus. This increase in stimulated cycles is attributed in part to the number of follicles recruited [1,2], but also to exogenous FSH doses administrated [3,4] and to a lack of LH (Luteization Hormone) activity that induces the conversion of progesterone to estrogen and androgen. It would impact accelerated endometrial maturation leading to impaired responsiveness by advancing the implantation window [5,6]. Some authors talk about early luteinization of granulosa cells but this term seems unjustified in agonist cycles because the LH rates are low, whereas early luteinization is supposed to occur after the LH peak [7]. This problem is very variable according to authors, representing 2–38% of IVF cycles [8,9].

The aim of our study was to evaluate the prognosis of this elevated rate and to define a threshold of progesterone so as to purpose a course of action in such situations of elevated progesterone in late follicular phase.

2. Methods

We retrospectively included at Bichat Hospital Center (Paris 7 University) from January 2010 to December 2011, 1022 consecu-

* Corresponding author.
E-mail address: julien.lepage@aphp.fr (J. Lepage).

https://doi.org/10.1016/j.jogoh.2018.05.005
2468-7847/

tive IVF – ICSI cycles. All etiologies of infertility were considered: tubal, male, idiopathic or associated to endometriosis. Cycles inclusion criteria's were: all ranks of IVF - ICSI cycles (4 attempts permitted in France and reimbursed by Health Care Insurance), age less than 43 years old and all types of responders, a desensitizing early follicular phase serum progesterone before stimulation $\leq$1 ng/ml and a plasma level of progesterone in the 36 h preceding ovulation induction identified. Frozen embryo transfer cycles were excluded.

Infertility workup included a hormonal blood test achieved between the 2nd and the 5th day of the cycle, comprising the plasma level of 17 beta estradiol, follicle stimulating hormone (FSH) and Anti-Mullerian Hormone (AMH), coupled to an intravaginal ultrasound with antral follicle count (AFC) on the third day, hysterosalpingography between the 7th and the 14th day, and a semen analysis performed in our spermiology laboratory. Other tests could be required depending on the clinical context.

Patients were stimulated according to standard protocols: long and short agonists or antagonists. The doses of gonadotropins used varied from 75 to 450 IU/day of stimulation (International Unity). These doses were defined according to age, body mass index (BMI), ovarian reserve and history of past stimulations. Ovulation triggering criteria were: at least two follicles over than 17 mm matching to the plasma estradiol rate. Oocyte retrieval took place 36 h after ovulation induction with hCG (hormone chorionic gonadotropin) and embryo transfer was performed at day 2 or day 3. The number of embryos transferred was discussed individually depending on the clinical aspects but our team promotes single embryo transfer according to the recommendations of the ESHRE 2003 (European Society of Human Reproduction and Embryology). Patients received luteal phase support after oocytes retrieval by vaginal micronized progesterone: 200 mg $\times$ 2 per day. Natural progesterone was continued until weeks 10 if pregnancy. Clinical pregnancy was defined by the presence of a gestational vesicle on transvaginal ultrasound at 7 weeks of amenorrhea. Spontaneous miscarriage was defined as an empty gestational vesicle or no cardiac activity before 12 weeks of gestation.

Progesterone assays were carried out at Bichat Hospital laboratory on autoanalyser Cobas 6000 (Roche, Mannheim), detection was made by electrochemiluminescence. The detection limits were 0.03 ng/ml with intra- and interassay coefficients of variability of 2.9% and 4.8%, respectively.

A statistical analysis was performed; the level of progesterone was defined as corresponding to the 95th percentile of progesterone serum level distribution in the patient's cohort.

A multivariate analysis was conducted to determine the pregnancy's prognostic factors in our population.

3. Results

3.1. Population's characteristics

The average patient's age was 34.3 $\pm$ 4.6 years. Regarding ovarian reserve, the averages were for FSH, AMH and antral follicular count (AFC) respectively: 6.91 $\pm$ 2.19, 3.63 $\pm$ 3.66 and 13.62 $\pm$ 8.94. We included all types of responders (Fig. 1a and b).

Patients received in most cases agonist protocols (69.21%), the antagonist protocol represented 30.79% of cycles.

The threshold of plasma progesterone on day of hCG was 1.57 ng/ml and corresponded to the 95th percentile of the distribution of plasma progesterone in the patient's cohort (Fig. 1).

3.2. IVF-ICSI results compared to the threshold of progesterone

5% of patients had a progesterone higher than 1.57 ng/ml.

Estradiol rates on day of hCG in patients with elevated progesterone were significantly higher: 2103.5 pg/ml vs. 1651.8 pg/ml (P = 0.002). The number of day 2 embryos obtained was greater in patients with progesterone >1.57 ng/ml: 10.55 vs. 8.66 (P = 0.04). We have not found increased FSH dose or longer stimulations needed in the high progesterone group. Among pregnant patients, there were statistically more miscarriages in patients with increased progesterone: 60% vs. 29% (P = 0.038). The live birth rate was higher in patients with progesterone <1.57 ng/ml: 70% vs. 40% (P = 0.048). Otherwise, implantation rates were not affected by progesterone (pregnancy rate per transfer: 33% vs. 34% P = 0.9) (Table 1).

3.3. Prognostic factors for pregnancy

Age appears – as expected – a pejorative prognostic factor for pregnancy, and, particularly from the age of 30 (OR = 0.62 (CI95 0.41–0.94)). Conclusions are the same for AMH (OR = 1.68 (CI95 1.04–2.70)).

We have not found elevated progesterone levels (>1.57 ng/ml) on the day of hCG as prognostic factor for pregnancy (OR 0.85 (CI95 0.39–1.85 95)).

We did not find any impact of stimulation protocols on pregnancy rates (Table 2).

4. Discussion

This retrospective study on over than 1000 cycles of IVF - ICSI found that high and higher than 1.57 ng/ml progesterone serum levels on day of hCG were associated with higher estradiol levels (P = 0.002) and more embryo on day 2 (P = 0.04). We did not find

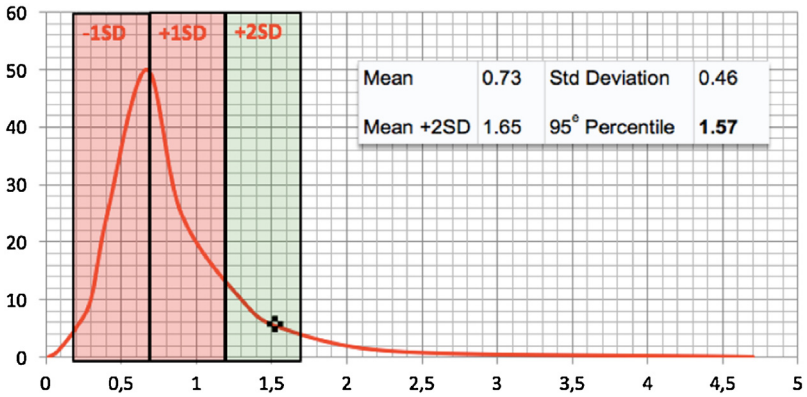

Fig. 1. Threshold of progesterone on day of hCG (ng/ml).

Table 1
IVF-ICSI results compared to the threshold of progesterone.

	P4 < 1.57 ng/ml	P4 ≥ 1.57 ng/ml	*P*
Age (years)	34.46 (4.70)	34.00 (4.61)	0.53
AMH (ng/ml)	3.63 (3.75)	4.09 (4.66)	0.55
FSH total Dose (UI)	2294.2 (948.3)	2351.2 (979.0)	0.70
Stimulation duration (days)	9.91 (1.87)	9.91 (1.85)	0.99
E2 on hCG day(pg/ml)	**1651.8 (941.2)**	**2103.5 (958.2)**	**0.002**
LH on hCG day (UI/L)	2.34 (2.01)	2.92 (3.88)	0.34
Nb oocytes retrieved	8.66 (5.08)	10.55 (7.34)	0.09
Nb Embryos obtained (day 2)	**4.45 (3.31)**	**6.05 (4.85)**	**0.04**
Nb embryos transferred	1.44 (0.82)	1.39 (0.84)	0.70
Rank of attempt (first attempt)	53.12%	53.13%	0.19
Antagonist protocol	30.49%	28.21%	0.76
Transfer cancelation	16.91%	18.18%	0.71
Pregnancy rate/cycle	28.43%	27.27%	0.87
Pregnancy rate/transfer	34.22%	33.33%	0.91
Abortion ≤ 12SA	**29.15%**	**60.00%**	**0.038**
Baby born live rate	**69.85%**	**40.00%**	**0.048**

Bolded lines represent statistically significant results.

any impact of elevated progesterone levels on day of hCG on pregnancy rates per cycle and pregnancy rates per transfer, and we did not demonstrate either that progesterone is a prognostic factor for pregnancy in IVF – ICSI cycles all protocols combined. However, we showed in this situation more spontaneous miscarriages (60% vs. 22.95%) $P = 0.016$ and fewer live births rates (40% vs. 69.85%) $P = 0.048$ and therefore a possible delayed implantation alteration linked to an early endometrial maturation and a disturbance of the implantation window.

In the literature, Bosch et al. [4] in a retrospective analysis of 4000 agonists and antagonists cycles found a threshold of progesterone of 1.5 ng/ml by trend analysis. They highlight more ongoing pregnancies beyond weeks 22 and more deliveries (31% vs. 19%), $P = 0.00006$. These results are comparable to ours.

It is confusing in the different studies on the outcomes exposed between implantation rates, ongoing pregnancy rates and live birth rates. Indeed, we found no impact on implantation rates but one on miscarriages and live birth rates but those results are correlated with the Venetis and al meta-analysis which found no significant association of elevated progesterone on pregnancy rates and implantation rates. However, this study is discussed because of the inclusion using arbitrarily low progesterone thresholds of 0.9 ng/ml determined by ROC curves [1]. Nevertheless, the same team of Venetis, in 2013, published an update of this meta-analysis which reviewed 60,000 agonist cycles and found a progesterone threshold ≥0.8 ng/ml on the day of HCG associated with decreased probability of pregnancy achievement in case of fresh embryo transfer including live birth rates.

Others works focused on the implantation rates. A recent large retrospective study [10] included 11,055 long agonist cycles in all types of responders and found three different progesterone cut-off depending on the type of ovarian response to stimulation: low responder P4 > 1.5 ng/ml, intermediate responders P4 > 1.75 ng/ml and high response P4 > 2.2 ng/ml. For the three groups, implantation and clinical pregnancy rates were statistically reduced. In our study, 8.6–10.5 oocytes are retrieved, which puts it in the intermediate group of Xu, where the threshold of progesterone is not 1.57 but 1.75 ng/ml. Once again the comparison is difficult because they are only focused on agonists cycles. Similar results were published by Wu et al. in 2012 on 2921 IVF agonists cycles with a lower ongoing pregnancy and live birth rate [11].

These studies exposed an impact of elevated progesterone only in agonists IVF cycles. We included all type of protocols, but in subgroup analysis we did not find any difference in results. Bosch and al, 2010, found in all protocol types a threshold of P4 > 1.5 ng/ml deleterious on results, with levels of progesterone recovered higher if agonist protocol than antagonist [4]. Huang and al 2012, meanwhile, have analyzed in subgroups according to the type of long or short agonist protocol and found different threshold. Live births rates were statistically reduced in long protocol if P4 > 1.2 ng/ml in contrast to the short protocol where this results were not shown for a cut off P4 > 2 ng/ml [12]. A recent study on 2244 cycles compared the types of protocols (agonist or antagonist) on pregnancy outcomes. They concluded that in the event of an increase in progesterone level during an antagonist cycle, it is worse prognosis than in agonist cycles and it is better to promote cryopreservation and to cancel the cycle [13].

If there is no impact of this high progesterone on deferred transfers, this would imply that there is no impact on oocyte or either embryo and that everything is related to the endometrium and the implantation. Two hypothesis: the first, our cohort is too weak to demonstrate this impact on implantation, but in this case our rate of increased miscarriage and our altered live birth rate is only the reflection of this implanting problem that we can not prove? The second hypothesis is that the endometrial impact is not the only cause attributable and that the high progesterone would have an oocyte and embryo impact? We defend the first hypothesis. Indeed, no study has demonstrated the deleterious effect on frozen embryo transfer [10]. Thus, we may wonder to freeze the embryos in case of P4 > 1.5 ng/ml [14].

Moreover, other studies have focused on the effect of the progesterone depending on the embryonic stage, Elgindy and al and Papanikolaou and al have demonstrated when progesterone > 1.5 ng/ml a clinical pregnancy rates decreased but these results were not found if transfer was done at blastocyst stage [15,16]. Thus, a transfer after prolonged culture may be an alternative to the freezing of embryos in these situations with relatively conserved pregnancy rates [17], although recent studies affirm the contrary and privilege freezing [18,19]. Furthermore, along the lines of egg donation, high levels of progesterone in donor does not affect oocyte quality or endometrial receptivity of the receivers which exclude the role of progesterone on the oocyte [20]. Similarly, Xu and al, found only an impact on the endometrium [10]. But these results are discussed by others with a deleterious effect on both endometrium receptivity and oocytes [3,21]. Indeed, in the embryo-endometrial dialog, the quality of the embryo seems as important as the endometrial receptivity. Another possibility is that high progesterone has negative effects on the quality of the oocyte or embryo resulting from fertilization and which could explain our results showing more miscarriages despite initially identical implantation rates. For this hypothesis,

Table 2
Prognostic factors for pregnancy.

	Adjusted OR[a]	CI 95%
Age (years)		
<30	1.00	–
30–38	**0.62**	**0.41–0.94**
>38	**0.42**	**0.25–0.70**
AMH (log) J3	**1.68**	**1.04–2.70**
Stimulation protocol		
Agonist	1.00	–
Antagonist	0.83	0.58–1.19
Progesterone (log) on day of hCG**	0.84	0.47–1.48
Progesterone on day of hCG[b]		
<1.57 ng/ml	1.00	–
≥1.57 ng/ml	0.85	0.39–1.85
***N* = 765**		

[a] Adjusted on female age, rank of attempt, basal AMH level, stimulation protocol, progesterone level on day of HCG, and number of transferred embryos; bolded lines represent statistically significant results.
[b] Analysis of progesterone was done on two different models according to quantitative and qualitative variables used.

there is no consensus. Huang et al., 2016, retrospectively analyzed 4236 cycles and found that a progesterone level higher than 2 ng/ml resulted in a decreased number of "Top Quality embryos" [22]. These results could be supported by animal models showing that oocyte competence was regulated by progesterone-sensitive genes [23].

The main strength of our study is to highlight that each progesterone assay is dependent on the method used, from each published work emerges the variability of progesterone thresholds and partly related to these assay mismatches. The innovative point of our work is therefore to propose a simple method of determining for each ART center its own progesterone threshold according to the dosage method used and its own practices.

5. Conclusion

The significance of increased *P* serum levels and its impact on pregnancy rates in late follicular phase of ovarian hyperstimulation IVF-ICSI cycles is controversial but seems to be deleterious in late phases of implantation and impact negatively live birth rates.

Effort should be made by each ART center to define its own threshold according to progesterone bioassays used and to consider to differ embryo transfer according to the cutoff established.

Disclosure of interests

The authors declare no conflicts of interest.

References

[1] Venetis CA, Kolibianakis EM, Papanikolaou E, Bontis J, Devroey P, Tarlatzis BC. Is progesterone elevation on the day of human chorionic gonadotrophin administration associated with the probability of pregnancy in in vitro fertilization? A systematic review and meta-analysis. Hum Reprod Update 2007;13(July (4)):343–55.
[2] Fanchin R, de Ziegler D, Castracane VD, Taieb J, Olivennes F, Frydman R. Physiopathology of premature progesterone elevation. Fertil Steril 1995;64 (October (4)):796–801.
[3] Smitz J, Andersen AN, Devroey P, Arce J-C. MERIT Group endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. Hum Reprod Oxf Engl 2007;22(March (3)):676–87.
[4] Bosch E, Labarta E, Crespo J, Simón C, Remohí J, Jenkins J, et al. Circulating progesterone levels and ongoing pregnancy rates in controlled ovarian stimulation cycles for in vitro fertilization: analysis of over 4000 cycles. Hum Reprod Oxf Engl 2010;25(August (8)):2092–100.
[5] Silverberg KM, Burns WN, Olive DL, Riehl RM, Schenken RS. Serum progesterone levels predict success of in vitro fertilization/embryo transfer in patients stimulated with leuprolide acetate and human menopausal gonadotropins. J Clin Endocrinol Metab 1991;73(October (4)):797–803.
[6] Melo MaB, Meseguer M, Garrido N, Bosch E, Pellicer A, Remohí J. The significance of premature luteinization in an oocyte-donation programme. Hum Reprod 2006;21(June (6)):1503–7.
[7] Hugues J, Massé-Laroche E, Reboul-Marty J, Boîko O, Meynant C, Cédrin-Durnerin I. Impact of endogenous luteinizing hormone serum levels on progesterone elevation on the day of human chorionic gonadotropin administration. Fertil Steril 2011;96(September (3)):600–4.
[8] Ubaldi F, Camus M, Smitz J, Bennink HC, Van Steirteghem A, Devroey P. Premature luteinization in in vitro fertilization cycles using gonadotropin-releasing hormone agonist (GnRH-a) and recombinant follicle-stimulating hormone (FSH) and GnRH-a and urinary FSH. Fertil Steril 1996;66(August (2)):275–80.
[9] Bosch E, Valencia I, Escudero E, Crespo J, Simón C, Remohí J, et al. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with in vitro fertilization outcome. Fertil Steril 2003;80(December (6)):1444–9.
[10] Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, et al. Serum progesterone level effects on the outcome of in vitro fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. Fertil Steril 2012;97(June (6)). 1321–7.e1-4.
[11] Wu Z, Li R, Ma Y, Deng B, Zhang X, Meng Y, et al. Effect of HCG-day serum progesterone and oestradiol concentrations on pregnancy outcomes in GnRH agonist cycles. Reprod Biomed Online 2012;24(May (5)):511–20.
[12] Huang R, Fang C, Xu S, Yi Y, Liang X. Premature progesterone rise negatively correlated with live birth rate in IVF cycles with GnRH agonist: an analysis of 2566 cycles. Fertil Steril 2012;98(September (3)). 664–70.e2.
[13] Orvieto R, Nahum R, Meltzer S, Liberty G, Anteby EY, Zohav E. GnRH agonist versus GnRH antagonist in ovarian stimulation: the role of elevated peak serum progesterone levels. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2013;29(September (9)):843–5.
[14] Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60,000 cycles. Hum Reprod Update 2013;19(October (5)):433–57.
[15] Elgindy EA. Progesterone level and progesterone/estradiol ratio on the day of hCG administration: detrimental cutoff levels and new treatment strategy. Fertil Steril 2011;95(April (5)):1639–44.
[16] Papanikolaou EG, Kolibianakis EM, Pozzobon C, Tank P, Tournaye H, Bourgain C, et al. Progesterone rise on the day of human chorionic gonadotropin administration impairs pregnancy outcome in day 3 single-embryo transfer, while has no effect on day 5 single blastocyst transfer. Fertil Steril 2009;91(March (3)):949–52.
[17] Huang P-C, Chen M-J, Guu H-F, Yi Y-C, Ho JY-P, Chen Y-F, et al. Effect of premature serum progesterone rise on embryo transfer outcomes and the role of blastocyst culture and transfer in assisted reproductive technology cycles with premature progesterone rise. Taiwan J Obstet Gynecol 2015;54(December (6)):641–6.
[18] Corti L, Papaleo E, Pagliardini L, Rabellotti E, Molgora M, La Marca A, et al. Fresh blastocyst transfer as a clinical approach to overcome the detrimental effect of progesterone elevation at hCG triggering: a strategy in the context of the Italian law. Eur J Obstet Gynecol Reprod Biol 2013;171(November (1)):73–7.
[19] Demirel LC, Aydogdu S, Ozdemir AI, Dönmez E, Benli H, Ferraretti AP, et al. Session 71: ovarian stimulation. Hum Reprod 2012;27(January (Suppl. 2)):ii109–11.
[20] Check JH, Wilson C, Choe JK, Amui J, Brasile D. Evidence that high serum progesterone (P) levels on day of human chorionic gonadotropin (hCG) injection have no adverse effect on the embryo itself as determined by pregnancy outcome following embryo transfer using donated eggs. Clin Exp Obstet Gynecol 2010;37(3):179–80.
[21] Huang B, Li Z, Zhu L, Hu D, Liu Q, Zhu G, et al. Progesterone elevation on the day of HCG administration may affect rescue ICSI. Reprod Biomed Online 2014;29(July (1)):88–93.
[22] Huang B, Ren X, Wu L, Zhu L, Xu B, Li Y, et al. Elevated progesterone levels on the day of oocyte maturation may affect top quality embryo IVF cycles. PLOS ONE 2016;11(January (1)):e0145895.
[23] Urrego R, Herrera-Puerta E, Chavarria NA, Camargo O, Wrenzycki C, Rodriguez-Osorio N. Follicular progesterone concentrations and messenger RNA expression of MATER and OCT-4 in immature bovine oocytes as predictors of developmental competence. Theriogenology 2015;83(April (7)):1179–87.