ALIVE

ART LEARNING INITIATIVES FOR EXPERTS

progesterone rise in ART

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abbreviations

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ART	Assisted reproductive technology
COS	Controlled ovarian stimulation
ET	Embryo transfer
FSH	Follicle stimulating hormone
3β-HSD	3 β-hydroxysteroid dehydrogenase
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
Hp-hMG	Highly purified human menopausal gonadotropin
HR	Hazard ratio
IVF	*In-vitro* fertilization
ICSI	Intracytoplasmic sperm injection
LH	Luteinizing hormone
LBR	Live birth rate
LC-MS	Liquid chromatography tandem mass spectrometry
OPR	On-going pregnancy rate
OPU	Ovum pick-up
OHSS	Ovarian hyperstimulation syndrome
PE	Progesterone elevation
ROC	Receiver operating characteristic
rFSH	Recombinant follicle stimulating hormone
RR	Relative risk

expert insights

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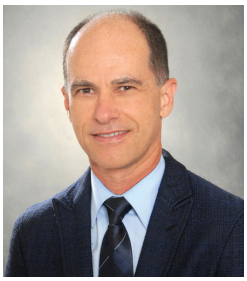

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Premature rise of progesterone during the late-follicular-phase in stimulated IVF cycles is a frequent event and its effect on the endometrial receptivity and on the assisted reproductive technique (ART) outcome has become a matter of intense debate and research.

An emerging body of evidence demonstrates that premature progesterone rise does have a negative impact on the outcome of the ART-success. Progesterone elevation (PE) prematurely opens the window of implantation, modifies endometrium receptivity and is therefore associated with a defective implantation. As demonstrated by the oocyte donation model, PE does not have an impact on oocyte quality.

Furthermore, PE is not a universal phenomenon and do not affect all patient populations equally. Therefore, it is highly recommended to closely monitor ovarian stimulation cycles by measuring serum progesterone, at least at the time of human chorionic gonadotropin (hCG) triggering. Therefore, progesterone threshold should be individually defined in each centre through a strict assessment of the local assay.

The existing evidence indicates that late-follicular-phase progesterone rise in gonadotropin releasing analog cycles is mainly caused by the supraphysiological stimulation of granulosa cells with exogenous follicle-stimulating hormone. Yet, the type of gonadotropin used for stimulation seems to play no significant role on progesterone levels at the end of stimulation. Patients with high ovarian response to control ovarian stimulation are more prone to exhibit PE at the late-follicular-phase.

Currently, caution should be applied to adopt specific cut-off values above which the effect of progesterone rise could be considered detrimental and recommends "freeze-all" based strategy solely on pre-defined cut-off points.

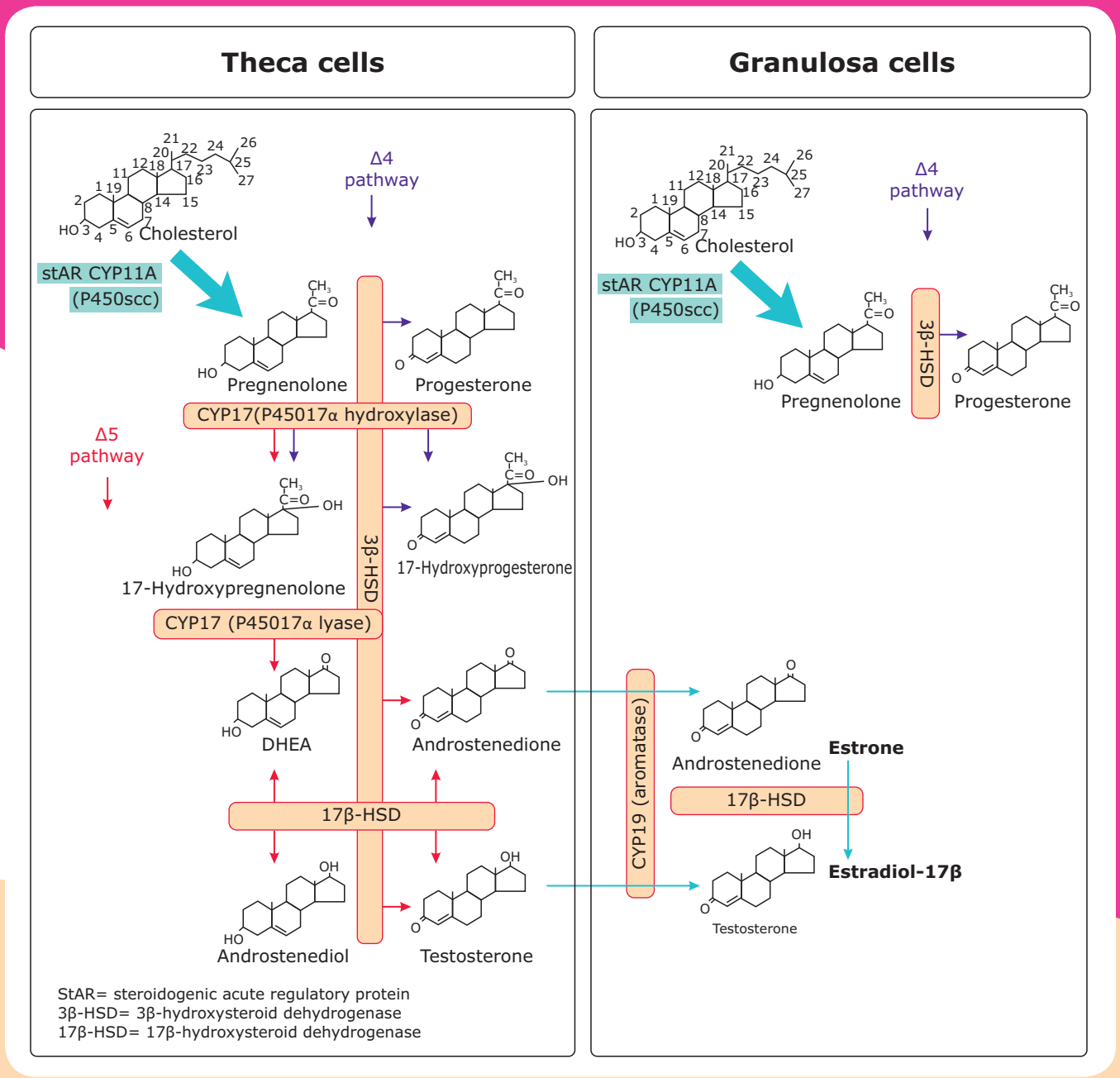
clinical corner

The steroidogenic pathway

1. *Leão Rde B, Esteves SC. Gonadotropin therapy in assisted reproduction: An evolutionary perspective from biologics to biotech. Clinics (Sao Paulo). 2014;69(4):279-93.*
2. *Ezcurra D and Humaidan P. A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. Reprod Biol Endocrinol. 2014;12:95.*
3. *De Ziegler D, Ayoubi JM, Frydman R, et al. Luteal phase support in assisted reproductive technologies: from here to there. Fertil Steril. 2018;109(1):57-58.*

- The starting point for steroid biosynthesis is the conversion of cholesterol in pregnenolone by cholesterol side-chain cleavage cytochrome P450 enzyme (P450scc, Figure 1).[1]
- One route for pregnenolone metabolism is the delta-5 pathway (red arrows) through CYP17 (P450c17). Pregnenolone hydroxylation at the C17α position forms 17-hydroxypregnenolone, and subsequent removal of the acetyl group forms the androgen precursor dehydroepiandrosterone (DHEA).[1]
- An additional route for pregnenolone metabolism is the delta-4 pathway (purple arrows), in which pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD) (an irreversible conversion). Progesterone is converted to 17- hydroxyprogesterone by CYP17.[1]
- CYP17 is exclusively located in thecal and interstitial cells in the extrafollicular ovary compartment, whereas CYP19 (aromatase), which converts androgens to estrogens, is expressed exclusively in granulosa cells, which are in the intrafollicular compartment.[1]
- Progesterone is the major secretory product of the growing follicle produced in the intrafollicular compartment by the granulosa cells, of which significant quantities reach the general circulation.[1]

Figure 1. Human steroidogenesis



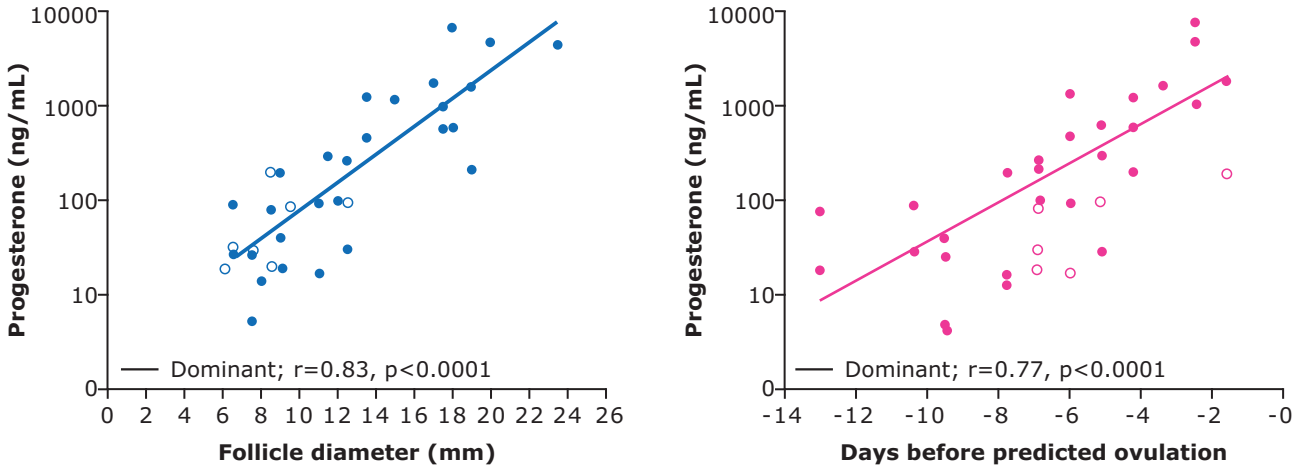
- ***In humans, intrafollicular progesterone is a terminal product that cannot be converted to estradiol by luteinizing hormone or human chorionic gonadotropin (hCG) due to lack of P450-17α.[2]***
- ***In practice, this implies that once either progesterone or 17-OH-progesterone has been produced it will not further convert into androgens and subsequently estradiol (E2) in the ovary.[3]***

Local and systemic intrafollicular progesterone concentrations during normal cycles

Schneyer AL, Fujiwara T, Fox J, et al. Dynamic changes in the intrafollicular inhibin/activin/follistatin axis during human follicular development: relationship to circulating hormone concentrations. J Clin Endocrinol Metab 2000;85:3319–3330.

The intrafollicular concentration of progesterone of women during the normal menstrual cycle was described by Schneyer and colleagues who showed clearly the relationship between the production of progesterone and follicle diameter and the number of days before ovulation (Figure 2). Progesterone was detectable even in the smallest follicles and increased linearly with both follicle size (r=0.83; p<0.0001) and maturity (r=0.77; p<0.0001).

Figure 2. Steroid hormone concentrations in aspirated follicles



Filled circles show the dominant follicles and open circles show the non-dominant follicles.

Clearly, there is a tremendous output of progesterone by the developing follicle during normal cycles.

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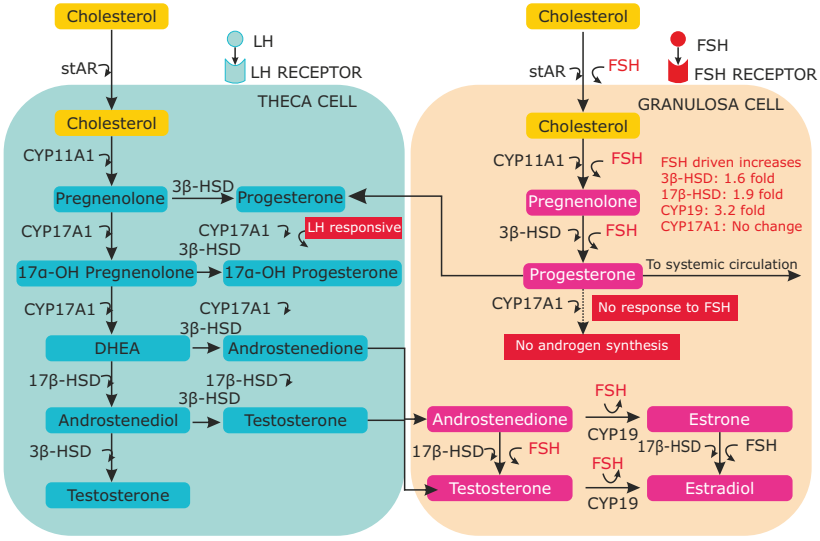
Granulosa cells produce progesterone in response to follicle stimulating hormone (FSH) stimulation

Oktem O, Akin N, Bildik G, et al. FSH Stimulation promotes progesterone synthesis and output from human granulosa cells without luteinization. Hum Reprod. 2017;32(3):643-652.

- This study shows that FSH has a direct stimulatory effect on '3β-HSD' and progesterone production in human granulosa cells, in addition to its stimulatory effect on the expression of other steroidogenic enzymes required for estrogen synthesis. As a result of this, progesterone output from the samples stimulated with FSH was increased along with E2 in a dose-dependent fashion.

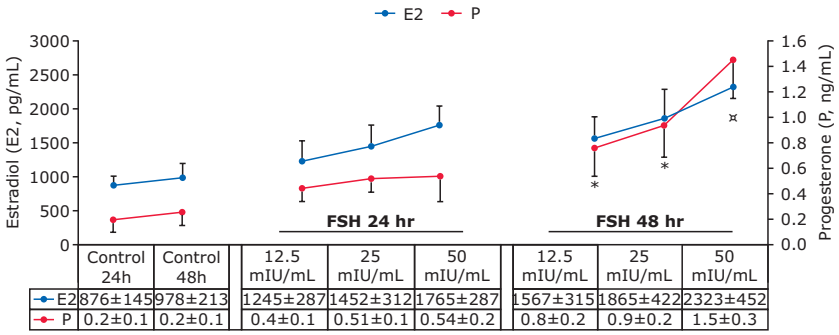
- Rise in progesterone preceding hCG administration is not associated with premature luteinizing hormone (LH) surge it does not reflect a true luteinization event. It is typically seen in stimulated *in-vitro* fertilization (IVF) cycles and is significantly correlated with the intensity of ovarian stimulation; hence patients with more follicles and oocytes have higher progesterone levels.

Figure 3. FSH stimulation promotes the synthesis of progesterone in granulosa cells



StAR= steroidogenic acute regulatory protein, 3β-HSD= 3β-hydroxysteroid dehydrogenase, 3/17β-HSD= 17β-hydroxysteroid dehydrogenase, LH=luteinizing hormone
DHEA=dehydroepiandrosterone, CYP17A1 gene= Cytochrome P450 17A1, CYP19=aromatase

Figure 4. *In vitro* E2 and progesterone (P) production of the samples stimulated with FSH



	Control 24h	Control 48h	12.5 mIU/mL	25 mIU/mL	50 mIU/mL	12.5 mIU/mL	25 mIU/mL	50 mIU/mL
E2	876±145	978±213	1245±287	1452±312	1765±287	1567±315	1865±422	2323±452
P	0.2±0.1	0.2±0.1	0.4±0.1	0.51±0.1	0.54±0.2	0.8±0.2	0.9±0.2	1.5±0.3

E2
p<0.05
Control 24h *vs.* FSH subgroups 24h
p<0.01
Control 48h *vs.* FSH subgroups 48h

P
p>0.05
Control 24h *vs.* FSH subgroups 24h
* p<0.05
Control 48h *vs.* FSH 12.5 and 25 mIUL/mL 48h
¤ p<0.01
Control 48h *vs.* FSH 50 mIUL/mL 48h

These findings suggest that gonadotropin stimulation and/or the degree of ovarian stimulation might play a pivotal role in premature rise of serum progesterone level before ovulation trigger at the late-follicular-phase during multi-follicular development in stimulated IVF cycles.

progesterone elevation: definition

Lawrenz B and Fatemi HM. Effect of progesterone elevation in the follicular phase of IVF-cycles on the endometrial receptivity. Reprod Biomed Online. 2017;34(4):422–428.

Progesterone elevation (PE) is defined as a threshold of more than 0.9 ng/mL. Meanwhile, evidence mounts that progesterone levels above **1.5 ng/mL** on the day of final oocyte maturation may lead to reduced pregnancy rates when the embryo transfer is carried out in the same cycle.

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cut-off values of progesterone for cycle cancellation

1. Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

2. 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(6):1321–7.e1–4.

3.Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: A cohort analysis of 3296 IVF cycles. Hum Reprod. 2015;30(3):684-91.

4. Griesinger G, Mannaerts B, Andersen CY, et al. Progesterone elevation does not compromise pregnancy rates in high responders: a pooled analysis of in vitro fertilization patients treated with recombinant follicle-stimulating hormone/gonadotropin-releasing hormone antagonist in six trials. Fertil Steril. 2013;100(6):1622–8.e1–3.

In the literature, the relationship between progesterone elevation and pregnancy rate has been analyzed by using different thresholds of serum progesterone on the day of hCG. The thresholds have been arbitrarily chosen and lie between 0.4 ng/mL and 3 ng/mL. Nevertheless, following the analysis of a large series the optimal progesterone threshold over which a detrimental effect on *in-vitro* fertilization (IVF) outcome might be observed has been estimated at 1.5 ng/mL.[1]

Progesterone thresholds and impact on IVF outcome

- Some researchers have reported that raised progesterone levels (>1.5 ng/mL) would be detrimental and thus a "freeze-all" embryos policy should be adopted.
- **The progesterone cut-off points associated with decreased pregnancy outcomes in fresh embryo transfer cycles were[2]**

1.5 ng/mL Poor responders	1.75 ng/mL Intermediate responders	2.75 ng/mL High responders

- **In this article it was observed that the progesterone mean values differed according to ovarian response[3]**

1.18 ng/mL Poor responders	1.47 ng/mL Intermediate responders	1.89 ng/mL High responders

- PE is more common in the high ovarian response group than intermediate and poor ovarian response groups.
- A PE does not uniformly mean a failed implantation, because there are still clinical pregnancies recorded in cycles with high progesterone levels. Hence, there is a need to identify the subgroup of patients who have a good chance of conception despite elevated progesterone levels.
- Griesinger G, *et al.,* has suggested that there is a subgroup of high responders in whom elevated progesterone does not negatively affect the outcome.[4]
- Although PE has been associated with decreased pregnancy rates in several studies, PE does not seem to affect all patient populations equally with high responders with PE achieving similar pregnancy success than counterparts without PE.[1]

PE is not a universal phenomenon with evidence indicating that its detrimental consequences on pregnancy outcomes do not affect all patient populations equally.

progesterone cut-off levels for high-responders

to prevent detrimental effects on clinical outcomes

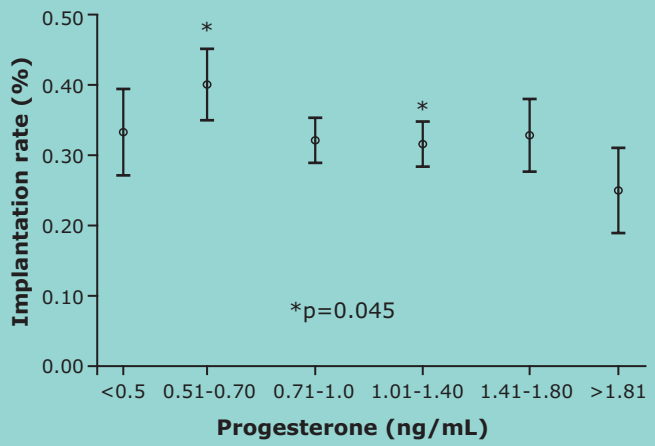
Requena A, Cruz M, Bosch E, Meseguer M, et al. High progesterone levels in women with high ovarian response do not affect clinical outcomes: A retrospective cohort study. Reproductive Biology and Endocrinology : RB&E. 2014;12:69.

A retrospective cohort study included 2850 women classified as high responders. Women were classified on basis of progesterone level into following groups: <0.5 ng/mL, 0.50–0.70 ng/mL, 0.71–1.00 ng/mL, 1.01–1.40 ng/mL, 1.41–1.80 ng/mL, and >1.81 ng/mL.

Implantation rates according to progesterone levels

- ✓ In fresh cycles, a marginal significant difference (p=0.045) was observed in implantation rates between patients within the progesterone interval 0.51-0.70 ng/mL and patients with progesterone levels ≥1.80 ng/mL (Figure 5).
- ✓ The odd-ratio associated with the effect of progesterone on the implantation rate was −0.056.

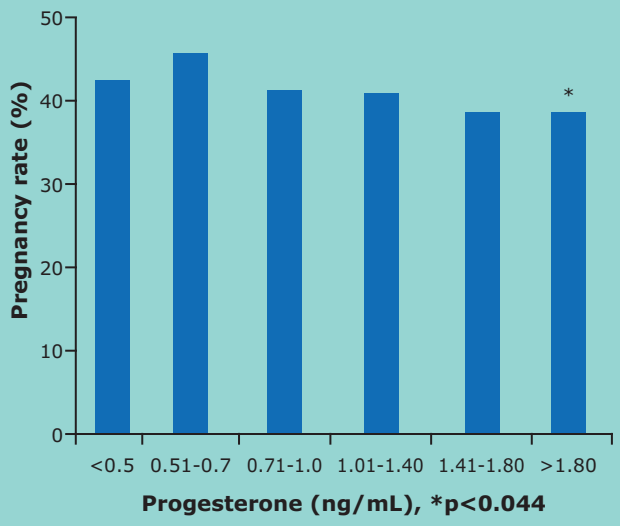
Figure 5. Implantation rates according to progesterone levels



Pregnancy rates according to progesterone levels

The pregnancy rate decreased (p=0.048) when progesterone levels were >1.8 ng/mL compared to patients with progesterone levels below this value (Figure 6).

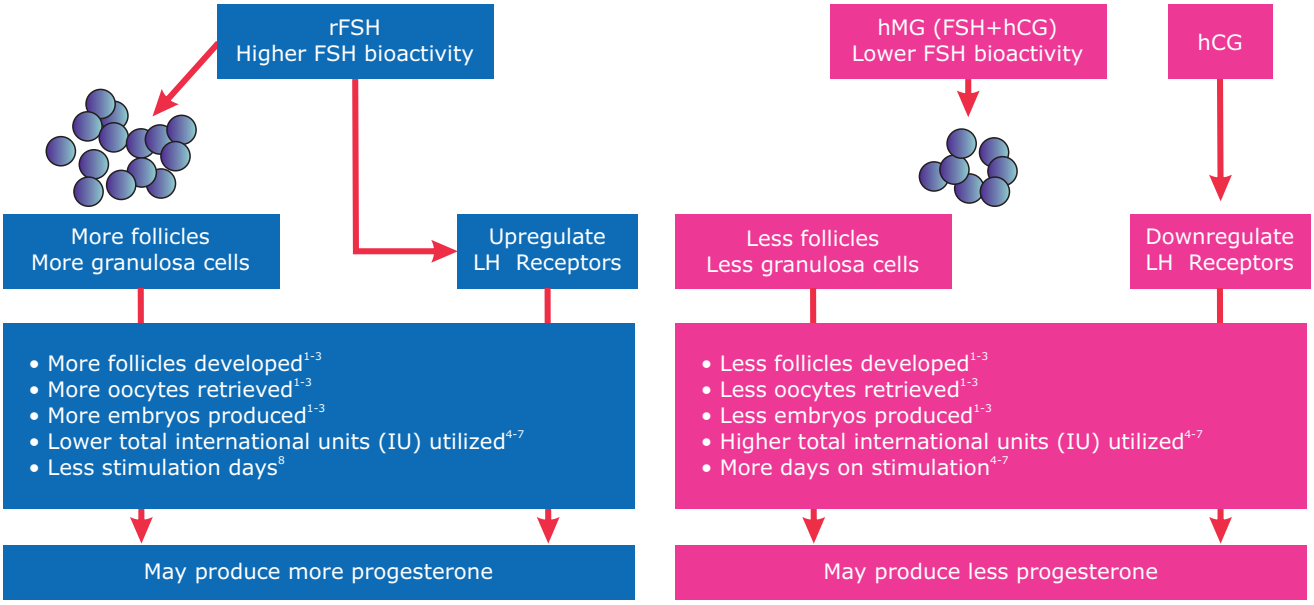
Figure 6. Pregnancy rates according to progesterone levels



The risk of pre-ovulatory rise in progesterone levels increases with ovarian response. A direct comparison of clinical outcomes among high responders, revealed a gradually decreasing trend when progesterone levels were more than 1.8 ng/mL, however, this decline has no clinical significance. Thus, the clinical outcomes are not affected in high responders and receiver operating characteristic (ROC) curve values failed to find any prediction suggesting that clinical outcomes may be barely significant.

Role of gonadotropins

Recombinant follicle stimulating hormone (rFSH) *vs.* human menopausal gonadotropin (hMG)



1. *Bergh C, et al. Hum. Reprod.1997; 12(10): 2133-9. Bergh C, Howles CM, Borg K, et al. Recombinant human follicle stimulating hormone (r-hFSH; Gonal-F) versus highly purified urinary FSH (Metrodin HP): results of a randomized comparative study in women undergoing assisted reproductive techniques. Hum Reprod. 1997;12(10):2133–2139.*
2. *Frydman R, et al. Hum. Reprod. 2000; 15(3): 520-5 Frydman R, Howles CM, Truong F, et al. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. Hum Reprod. 2000;15(3):520–525.*
3. *Andersen AN, Devroey P and Arce JC. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum. Reprod. 2006; 21(12): 3217–27.*
4. *Trew GH, et al. Reprod Biol Endocrinol. 2010; 8: 137. Trew GH, Brown AP, Gillard S, et al. In vitro fertilisation with recombinant follicle stimulating hormone requires less IU usage compared with highly purified human menopausal gonadotrophin: results from a European retrospective observational chart review. Reprod Biol Endocrinol. 2010;8:137.*
5. *Nyboe Andersen A, et al. Hum Reprod. 2006; 21(12):3217-3227 Nyboe Andersen A, Devroey P, Arce J C, et al. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Hum Reprod. 2006;21(12):3217–3227*
6. *Devroey P, et al. Fertil Ster. 2012; 97(3): 561-571. Devroey P, Pellicer A, Nyboe Andersen A, et al. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. Fertil Steril. 2012;97(3):561–571.*
7. *Frydman R, Howles CM, and Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. The French Multicentre Trialists. Hum. Reprod. 2000; 15(3):520–5*
8. *EMEA Final decision July 30 2009 – SmPC approved amendments of marketing authorization for Gonal-f. available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000071/WC500023744.pdf.Accessed on July 24, 2018.*

MEGASET study antagonist cycles

Devroey P, Pellicer A, Nyboe AA, et al. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with the compulsory single-blastocyst transfer. Fertil Steril. 2012;97(3):561–571.

Devroey P, *et al*., Compared the efficacy and safety of highly purified menotropin (hphMG) and recombinant FSH (rFSH) for controlled ovarian stimulation in a GnRH antagonist cycle with the compulsory single-blastocyst transfer. In this trial, COS with hphMG or rFSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer on day 5 in one fresh or subsequently frozen blastocyst replacement in natural cycles initiated within 1 year of each patient's start of treatment (Table 1).

Number of oocytes retrieved with hphMG and rFSH was 9.1 and 10.7 (p<0.001), respectively.

Table 1. Clinical parameters from the stimulation phase to embryo transfer

	hphMG (n=374)	rFSH (n=375)	p value
Day 6 of stimulation			
Follicles ≥12 mm	3.6 ±2.8	4.2±3.1	0.011[a]
E2 (pmol/L)	2,626±1,405	2,973±1,702	0.003[b]
Progesterone (nmol/L)	2.2±1.9	2.8 ±10.8	0.025[b]
Progesterone (ng/mL)	**0.69**	**0.88**	**0.025[b]**
Day of oocyte retrieval			
Number of oocytes retrieved	9.1±5.2	10.7 ± 5.8	**<0.001**
Metaphase II oocytes/oocytes retrieved	77±23%	78 ±19%	-

Note: Numbers are mean±SD unless otherwise indicated. [a]Wilcoxon test. [b]Test for treatment difference based on log-transformed values.

The average serum progesterone level and the proportion of patients with serum progesterone concentrations above 1.25 ng/mL at the end of stimulation (16% in the hphMG group and 14% in the rFSH group) were similar between the treatment groups. In this study, the threshold value for defining serum PE was 1.0 ng/mL.

comparison between rFSH and hMG in progesterone rise in ART cycles

Bosch E, Vidal C, Labarta E, et al. Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists—a randomized study. Human Reproduction 2008;23(10):346–351.

Highly purified human menopausal gonadotropin (hp-hMG) and recombinant FSH (rFSH) have been used for ovarian stimulation in infertile women undergoing treatment for *in-vitro* fertilization or intracytoplasmic sperm injection (IVF/ICSI) and embryo transfer.

Number of oocytes retrieved with hphMG and rFSH was 11.3 and 14.4 ($p<0.001$), respectively.

Table 2. Ovarian stimulation outcome

	hphMG (n =122)	rFSH (n =126)	p value
Total Gn dose (IU)	2481±994	2624±801	0.22
Serum progesterone (P) on day of hCG (ng/mL)	**0.73±0.42**	**0.99±0.48**	**<0.001**
Number of COCs collected	11.3±6.0	14.4±8.1	0.001
Number of Metaphase II (ICSI)	7.8±4.0	9.7±6.0	0.004
Fertilization rate	69.8±26.4	68.9±22.3	0.765

Values are expressed by mean±SD. Gn, gonadotrophin; COC, cumulus-oocyte complexes

A similar outcome was observed for hp-hMG and rFSH when used for stimulation in GnRH antagonist cycles. Increased progesterone levels have been already related to FSH administration, in either GnRH antagonist cycles or GnRH agonist long protocol cycles.

The significance of progesterone rise from the above studies

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles.Front Endocrinol (Lausanne). 2018;9:201.

Progesterone elevation (PE) is not a universal phenomenon with evidence indicating that its detrimental consequences on pregnancy outcomes do not affect all patient populations equally. Late-follicular-phase PE, commonly defined as progesterone levels of 1.5 ng/mL or greater at the day of hCG trigger, has been reported in 6–30% of controlled ovarian stimulation (COS) cycles.

- Researchers showed that the incidence of PE (>1.5 ng/mL) was 13.3%, but on-going pregnancy rate (OPRs) were not significantly different between patients with normal progesterone levels and PE (27.0% *vs.* 19.0%).
- Progesterone concentration was strongly associated with the number of follicles and retrieved oocytes. There was no significant association between the late-follicular-phase progesterone concentration and clinical pregnancy rate.
- Progesterone levels neither had a negative impact on the oocyte quality and endometrial receptivity nor did it affect pregnancy success.

It is important to note that PE values were <1.5 ng/mL in hphMG and rFSH groups, implying statistically significant values could be clinically irrelevant.

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Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: A cohort analysis of 3296 IVF cycles

Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. Hum Reprod. 2015;30(3):684–691.

The potential association of serum progesterone on the day of hCG administration with the outcome of an IVF cycle has been one of the major controversies in the endocrinology of ovarian stimulation. This study aimed to estimate the net effect of PE on the day of hCG on live birth rates (LBR) by quantifying the effect of the most important known confounders. Moreover, a secondary aim was the exploration of the potential moderating effect of ovarian response on the effect of PE on LBR.

PE>1.5 ng/mL on the day of hCG was observed in 243 cycles (7.4, 95% CI: 6.5–8.3) and no significant difference in the incidence of PE was observed between gonadotropin-releasing hormone (GnRH) agonist and antagonist cycles (8.3% *vs.* 6.8%, respectively; p=0.117).

In this study, the association of progesterone elevation (>1.5 ng/mL) with LBR was not significant [OR: 0.78,95% CI:0.56–1.09].

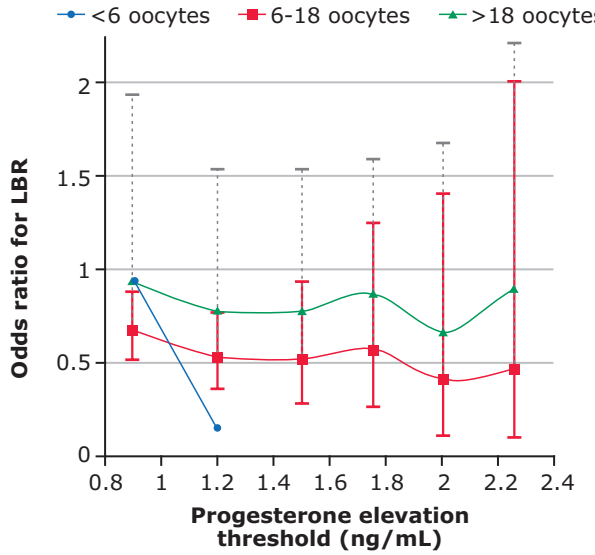
Table 3. Association of PE with LBR using a bivariate and multivariable method of analysis (per different thresholds)

Outcome	PE thresholds (ng/mL) OR (95% CI)					
Threshold (ng/mL)	0.9	1.2	1.5	1.75	2.0	2.25
Bivariate						
Live birth (per OPU)	1.03 (0.88–1.22)	0.85 (0.69–1.06)	0.78 (0.56–1.09)	0.87 (0.56–1.33)	0.60 (0.30–1.21)	0.65 (0.28–1.51)
Live birth (per ET)	1.02 (0.86–1.20)	0.85 (0.68–1.05)	0.78 (0.56–1.10)	0.88 (0.57–1.36)	0.64 (0.31–1.30)	0.71 (0.30–1.68)

OPU: Ovum pick–up; ET: Embryo transfer.

✓ **In a multivariable model, NO significant effect between PE and the probability of LBR detected in cycles with low (<6 oocytes) and high (>18 oocytes) ovarian response**

Figure 7. Odd ratio for LBR after multivariable analysis according to the ovarian response group



Serum progesterone values on the day of hCG did not affect the management of these cycles (e.g., no embryo transfers were cancelled due to elevated progesterone on the day of hCG).

Progesterone concentration associates significantly to follicle number and luteinizing hormone concentration but not to pregnancy rate in patients undergoing *in-vitro* fertilization or intracytoplasmic sperm injection

Andersen CY, Bungum L, Andersen AN. Preovulatory progesterone concentration associates significantly to follicle number and LH concentration but not to pregnancy rate. Reproductive BioMedicine Online. 2011;23:187–195.

In this study, the concentration of progesterone on the day of ovulation induction, irrespective of whether or not rLH was added, showed a strong positive association with the number of oocytes retrieved and the number of follicles observed on sonography.

Table 4. Progesterone concentration on the day of ovulation induction about a number of oocytes retrieved, number of follicles >10 mm and total FSH consumption.

Variable	Progesterone concentration (ng/mL)						ANOVA
	<0.31	0.31-0.62	0.62-0.94	0.94-1.25	1.25-1.57	>1.57	
Patients[a]	2	34	108	116	66	113	
Oocytes retrieved	6.5 ± 0.5	6.8 ± 0.6	7.6 ± 0.4	9.2 ± 0.5	9.7 ± 0.6	10.3 ± 0.5	p<0.0001
Follicles>10 mm	10.0 ± 4.0	11.3 ± 1.1	13.9 ± 0.8	15.1 ± 0.9	15.6 ± 0.8	17.8 ± 0.8	p=0.0007
FSH consumption (IU)	2432 ± 324	1995 ± 144	2226 ± 101	2192 ± 85	2147 ± 124	2264 ± 90	NS

Values are mean ± SEM unless otherwise stated. [a]In 36 cycles the number of oocytes and a number of follicles were not recorded properly. NS = not statistically significant.

Table 5. The biochemical and clinical pregnancy rates about late-follicular-phase progesterone concentration.

Variable	Total	Late-follicular-phase serum progesterone concentration (ng/mL)						
		≥0.31	≥0.62	≥0.94	≥1.25	≥1.57	≥1.88	≥2.20
Per patient included								
Patients	475	472	432	306	187	124	80	45
Biochemical pregnancy rate (%)	40	40	40	43	47	41	39	44
Clinical pregnancy rate (%)	31	31	30	32	34	28	30	36
Per embryo transfer								
Patients	419	416	379	276	171	112	72	39
Biochemical pregnancy rate (%)	45	45	45	47	51	45	42	49
Clinical pregnancy rate (%)	35	35	34	36	37	31	33	41

Values are n or n (%).

✓ As per this study the highest pregnancy rate was found in the group of patients who had the highest late-follicular-phase progesterone concentrations (i.e., >2.2 ng/mL) and thus developed many follicles.

- ✓ ***A study conducted by the same author in 2006, reported that elevated progesterone concentrations above 1.25 ng/mL were associated with a poorer pregnancy outcome. However, in 2011, the present study does not support an association between progesterone concentration on the day of HCG administration and the probability of clinical pregnancy. This may indicate an evolution of treatment strategies across this period.***

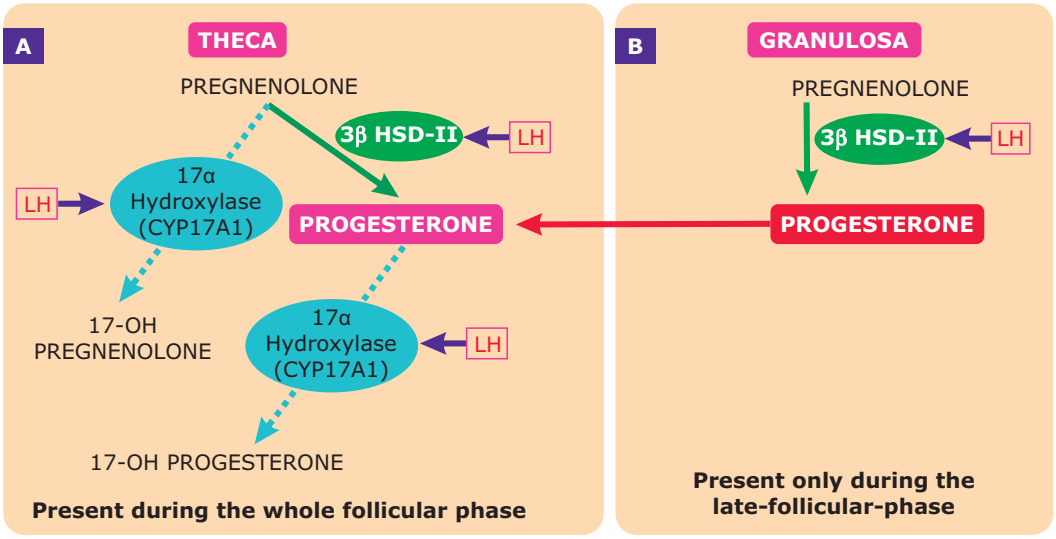
The present study does not support an association between progesterone concentration on the day of hCG administration and the probability of clinical pregnancy in women undergoing ovarian stimulation with GnRH agonists and gonadotrophins for ART.

Impact of luteinizing hormone activity supplementation on serum progesterone levels

Hugues JN. Impact of 'LH activity' supplementation on serum progesterone levels during controlled ovarian stimulation: a systematic review. Hum Reprod. 2012;27(1):232–243.

The purpose of the analysis was to assess the impact of supplementation with 'luteinizing hormone (LH) activity' products on serum progesterone changes before hCG administration in GnRH analog-treated women.

Figure 8. Effects of LH on progesterone production during the follicular phase



A) Stimulation 3β-hydroxysteroid dehydrogenase (HSD) type II induces a positive effect on progesterone production. Activation of CYP17 which converts progesterone and pregnenolone to 17-hydroxy derivates reduces progesterone synthesis

B) LH activates 3β-hydroxysteroid dehydrogenase (HSD) type-II enzymatic activity with a positive effect on progesterone production

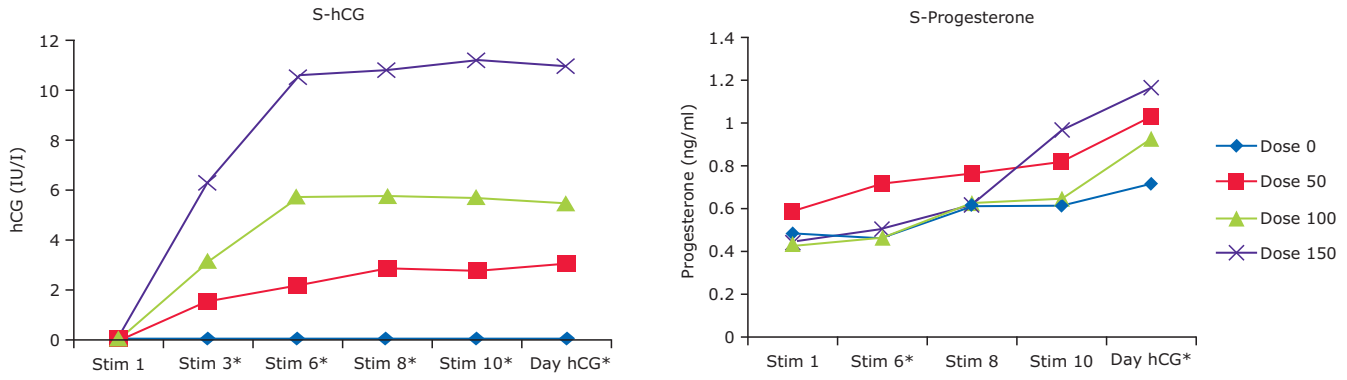
Analysis shows that a decrease in serum progesterone was mainly observed when 'LH activity' supply was administered from the beginning of COS. In contrast, patients who displayed an increase in serum progesterone received LH activity supplementation only during the late part of COS.

Does type of LH activity impact progesterone production?

Thuesen LL, Loft A, Egeberg A, et al. A randomized controlled dose-response pilot study of addition of hCG to recombinant FSH during controlled ovarian stimulation for in vitro fertilization. Hum Reprod. 2012;27(10):3074–3084.

A prospective randomized, controlled, open-label dose–response pilot study in 62 patients was conducted at Copenhagen University Hospital, Denmark to analyze the clinical, embryological and endocrine aspects of adding increasing doses of hCG (0, 50, 100 or 150 IU) to rFSH (150 IU/day) from the first day of stimulation for IVF. As a result the lowest serum progesterone was found in control arm (D0) group (0.72 ng/mL) and the highest (1.17 ng/mL) in the hCG high dose (D150) group. Supplementation of hCG induced a dose-related increase in the progesterone increments.

Figure 9. Daily serum concentrations (means) of hCG and progesterone during the stimulation



The key importance of this study was to show that within the hCG dose range of 0–150 IU/day, supplementation with hCG did not seem to reduce but rather to increase late-follicular-phase progesterone levels.

22

Strategies to minimize the potential adverse effects of elevated serum progesterone level on *in-vitro* fertilization pregnancy rates

Patton PE, Lim JY, Hickok LR, et al. Precision of progesterone measurements with the use of automated immunoassay analyzers and the impact on clinical decisions for in vitro fertilization. Fertil Steril. 2014;101(6):1629–36.

Two strategies have been offered to minimize the potential adverse effects of elevated serum P level on IVF pregnancy rates.

- The first approach proposes **altering the timing of hCG administration.** Instead of traditional criteria, in which hCG administration is based on follicular size and estrogen levels, hCG is given when a "subtle progesterone rise" is detected.
- A second strategy **promotes embryo cryopreservation** during an IVF cycle when progesterone levels exceed a predetermined threshold value. Cryopreserved embryos are then thawed and transferred into a prepared endometrium at a later date.

future prospects

Evaluation of progesterone:estradiol (P:E) ratio

Mascarenhas M, Kamath MS, Chandy A, Kunjummen AT. Progesterone/Estradiol ratio as a predictor in the ART cycles with premature progesterone elevation on the day of hCG trigger. Journal of Reproduction & Infertility. 2015;16(3): 155–161.

- Progesterone or estradiol was introduced as a novel marker by Younis *et al.,* in 1998, and it was calculated as progesterone (ng/mL)×1,000/estradiol (pg/mL).
- Alternative approaches including the determination of PE duration and measurement of follicle index ratio (PF) may prove useful to assess the role of follicular phase PE on pregnancy success, but further research is needed to confirm their clinical utility.
- As per Mascarenhas M, *et al.,* among the cycles with a PE (>1.5 ng/mL), the subgroup of cycles with an elevated P:E ratio of >1 had a poorer outcome. In the other subgroup with a P/E ratio of ≤1, the clinical pregnancy rates did not significantly differ from the cycles with a normal progesterone level of ≤1.5 ng/mL.

Hence, the deleterious effect of progesterone elevation is possibly confined to only those cycles which have an elevated P/E ratio as well. A subsequent study concluded that P/E ratio was a better predictor than serum progesterone alone for prediction of clinical pregnancy but P/E ratio has low sensitivity and positive predictive value to predict pregnancy rates.

Individualization of therapy-based on progesterone values in relation to fresh or frozen embryo transfer

Esteves SC, Khastgir G, Shah J. Association between progesterone elevation on the day of human chronic gonadotropin trigger and pregnancy outcomes after fresh embryo transfer in in vitro fertilization/intracytoplasmic sperm injection cycles. Front Endocrinol (Lausanne). 2018;9:201.

- According to the largest systematic review and meta-analysis published to date, the odd ratio (OR) for pregnancy reduction associated with PE was 0.64 (95% CI: 0.54–0.76).
- Transformation of the OR mentioned above into absolute pregnancy rate reduction (APRR) translated in 10.1% APRR (95% CI: 6–14%). Using these assumptions, it is possible to estimate the net effect of performing fresh embryo transfer in cases of PE for a given IVF Program. In Table 6, the net effect of PE on pregnancy is estimated for an IVF center performing 1,000 cycles per year with an overall baseline pregnancy rate of 40% per fresh embryo transfer, considering three different scenarios of PE incidence as commonly reported in the literature.
- According to these estimations, the net effect on pregnancy reduction for the overall population subjected to fresh embryo transfer IVF/ICSI cycles in a given IVF center would range from 0.5 to 3.0% in the best and worst-case scenarios, respectively.

Table 6. Different scenarios to evaluate net effect of PE on pregnancy for an IVF center performing 1,000 cycles per year

Cycles	Scenario 1	Scenario 2	Scenario 3
Cycles with PE (%)	5	15	30
Cycles with PE[a] (N)	50	150	300
Expected pregnancies in the subgroup of PE[b] (N)	20	60	120
Achieved pregnancies corrected by APRR[c] (N)	18	54	108
Overall pregnancy reduction per 1,000 cycles; N (%)	2 (0.5%)[d]	6 (1.5%)[d]	12 (3.0%)[d]

[a]Per 1,000 cycles. [b]Considering 40% as the overall PR per fresh embryo transfer. [c]Considering 10% absolute pregnancy rate reduction (APRR). [d]400 pregnancies would be expected overall per 1,000 cycles.

- Clinicians would need to monitor progesterone levels in 1,000 cycles and intervene in 50–300 cycles with PE to potentially avoid 2–12 implantation failures by applying the freeze-all strategy. Notably, despite improvements in cryopreservation techniques and an overall favorable outcome with the transfer of frozen-thawed vitrified embryos, pregnancy rates reported by individual centers still vary, with a success rate of approximately 50%.

An individualized approach should be used in cases of PE, which could include fresh embryo transfers in hyper-responders with low-risk of ovarian hyperstimulation syndrome (OHSS) and in patients with supranumerary embryos undergoing blastocyst transfer. In normal responders with PE undergoing day 3 fresh embryo transfers, a "freeze-all" strategy might be considered. As for poor responders, the optimal strategy in the face of PE is yet to be determined.

Rescue strategies to prevent premature progesterone (P) rise and progesterone elevation

Lawrenz B, Labarta E, Fatemi H, et al. Premature progesterone elevation: targets and rescue strategies. Fertil Steril. 2018;109(4):577–582.

During ovarian stimulation, the addition of corticosteroids in patients with an initial higher P level has the ability to reduce the P levels during ovarian stimulation

Avoidance of enhanced ovarian stimulation towards the end of the follicular phase by performing a step-down approach reduces the incidence of P elevation on the day of final oocyte maturation.

Prolonging ovarian stimulation beyond the optimal stimulation result of ≥3 follicles of a size of ≥17 mm should be avoided, as it will increase the risk of P elevation.

Delay of embryo transfer from cleavage stage embryos to blastocyst embryos has to be seen critical in overcoming the effect of P elevation.

A freeze-all approach can be applied as ultima ratio, due to the cycle-segmentation with performance of the embryo transfer in a subsequent cycle.

The key element of ovarian stimulation treatment for IVF is individualization of ovarian stimulation treatment.

The precision of progesterone measurements with the use of automated immunoassay analyzers and the impact on clinical decisions for *in-vitro* fertilization

Patton PE, Lim JY, Hickok LR, et al. Precision of progesterone measurements with the use of automated immunoassay analyzers and the impact on clinical decisions for in vitro fertilization. Fertil Steril. 2014;101(6):1629–1636.

- The steroid hormone progesterone is predominantly produced by luteinized granulosa cells after exposure to the periovulatory surge of LH during spontaneous menstrual cycles or after the administration of exogenous hCG during COS.
- Most IVF centers use different automated analyzers to measure progesterone levels. Despite similarities in assay methodologies, reported progesterone thresholds from retrospective observational studies are highly variable, ranging from 0.9–2.5 ng/mL.

- Immulite 1000 (Siemens Healthcare Diagnostics): based on chemiluminescence technology; P range 0.2–40 ng/mL.
- Access II Immunoassay system (Beckman-Coulter): dioxetane-based chemiluminescence. P range 0.08–40 ng/mL.
- Tosoh AIA 600II (Tosoh Biosciences): based on chemiluminescence technology. P range 0.1–40 ng/mL.
- Roche Cobas e411 (Roche Diagnostics): based on chemiluminescence technology; P range 0.03–60 ng/mL.

Inter and intraassay coefficients of variation (CVs) of progesterone measurements were compared for each analyzer with liquid chromatography-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS is the most precise method to measure progesterone

Progesterone threshold measurements used for IVF clinical decisions should be interpreted cautiously and based on laboratory- and method-specific data.

A validated progesterone standard incorporated into daily immunoassays could improve medical decision accuracy.

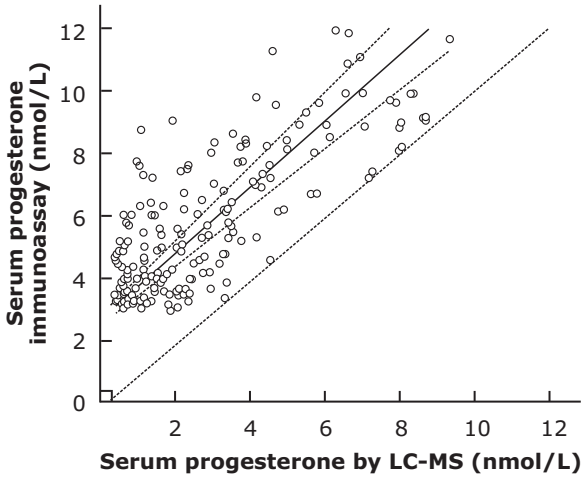
Minimal assay variability of the internal standard would assist the clinician in making accurate clinical care decisions, whereas the finding of imprecise assay conditions (e.g., CV >10%) would trigger a repeated assay and thereby reduce the potential for diagnostics errors.

The accuracy of a direct progesterone immunoassay

Shankara-Narayana N, Zawada S, Walters KA, et al. Accuracy of a direct progesterone immunoassay. JALM. 2016;1(3):294–299.

- Serum progesterone is routinely measured by direct immunoassay on multiplex platforms, which have a rapid turnaround, are relatively inexpensive, and are easily integrated into chemical pathology laboratory assay profiles.
- The most widely used and best-established indication for measurement of the serum progesterone is that a high midluteal concentration confirms recent ovulation. Also, more recently, there has been growing used to diagnose premature luteinization based on late-follicular-phase measurement of much lower levels of serum progesterone.
- A recent study demonstrates that a widely used progesterone immunoassay consistently overestimates serum progesterone levels when compared with the reference method of liquid chromatography-tandem mass spectrometry (LC-MS) measurement and is increasingly severe at lower serum progesterone concentrations.

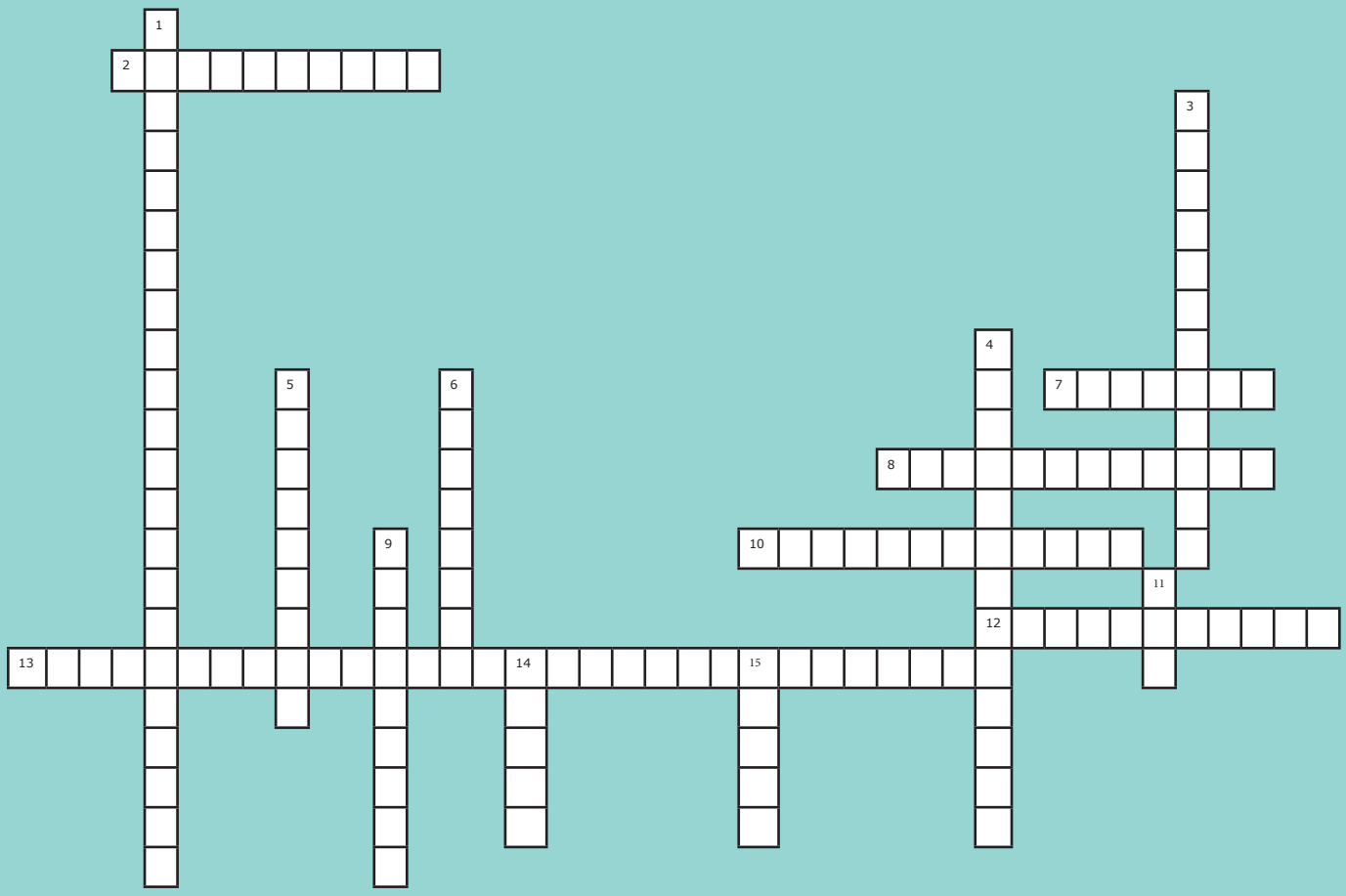
Figure 10. Passing–bablok regression of serum progesterone by immunoassay (y-axis) against LC-MS assay (x-axis) showing the regression (solid line) with the line of identity (dotted line) and 95% CIs for slope (dashed lines).



- Immunoassay overestimated serum progesterone in almost every sample with increasingly high variability and deviation at lower concentrations Figure 10 (immunoassay <5 nmol/L, equivalent to LC-MS <2 nmol/L).

The patients who may benefit from progesterone measurements are individuals undergoing controlled ovarian stimulation. Evidence presented allows better characterization of serum progesterone measurement at lower concentrations required for clinical diagnosis of premature elevation of progesterone during an IVF cycle. Direct (non-extraction) progesterone immunoassay consistently overestimates serum progesterone concentration, especially at lower levels.

28 crossword



Across

2. …… strategy to minimize the potential adverse effects of elevated serum P level on IVF pregnancy rates.
7. Different …….levels used to define premature progesterone elevation.
8. The elevated ……..during stimulated cycles, ranging from 0.8 to 2.0 ng/mL.
10. Progesterone plays a key role during the second phase of the menstrual cycle and is particularly important for ……..and progression of pregnancy.
12. The critical step in steroidogenesis is the initial conversion of ……. to pregnenolone (the primary C21 product), from which all other steroids are generated.
13. Controlled ovarian stimulation (COS) is an important component of ……

Down

1. A rise in serum progesterone levels on the day of hCG administration is known as ……
3. Notably, most circulating progesterone (~95%) is produced in the intrafollicular compartment by the granulosa cells *via* the action of 3β-HSD that catalyzes the conversion of ……..(delta-4 pathway) under LH influence.
4. A significant decrease in ……with serum progesterone levels above 1.5 ng/mL on the day of hCG administration.
5. Studies showed a negative influence on the pregnancy outcome in the case of ……..progesterone elevation.
6. Validated progesterone ……..incorporated into daily immunoassays could improve medical decision accuracy.
9. High progesterone levels correlate significantly with high …….levels.
11. The administration of relatively high doses of ……is required to achieve multi-follicular growth.
14…… is expressed only in the granulosa cells.
15. ……..is located exclusively in thecal/interstitial cells (i.e. the extrafollicular compartment of the ovary).

Across
2. Freeze-All
7. Cut-off
8. Progesterone
10. Implantation
12. Cholesterol
13. Assisted reproductive technology

Down
1. Premature luteinisation
3. Pregnenolone
4. Pregnancy rate
5. Premature
6. Standard
9. Estradiol
11. FSH
14. CYP19
15. CYP17

FEEDBACK FORM

Issue 2
August 2018

ALIVE

Thank you for going through the contents of **ALIVE Newsletter Issue 2.** To ensure that future issues will be of interest to you, we would greatly appreciate your feedback on the format and content of this issue.

Name (Optional): _____

Satisfaction Score for ALIVE Newsletter Issue 2

Progesterone rise in ART: Issue 2; August 2018

Rating Scale	**Poor ----------Excellent** (Please circle the appropriate rating)									
Scientific content	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Relevance of the topic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Impact on my daily practice	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Innovation	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Overall level of satisfaction	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**

Issue 2
August 2018

What aspects of the Newsletter issue 2 did you find particularly interesting and/or informative?

_____

_____

Please suggest topics/areas that you would like to be covered in future issues of the ALIVE Newsletter?

_____

_____

How can the subsequent Newsletter issues be improved?

_____

_____

Disclaimer: *This information is being collected for informational purpose only which will help us in evaluating the quality and content of the Newsletter and making improvements in future.*

THREE CENTURIES OF TRANSFORMATION

MERCK 350th ANNIVERSARY

2018

2015 — Acquisition of Sigma-Aldrich

2014 — Acquisition of AZ Electronic Materials

2011 — We take up the fight against schistosomiasis, aiming to eliminate this tropical disease in Africa

2010 — Acquisition of Millipore

2007 — Acquisition of Serono

1995 — Initial public offering

1969 — The Institute for Experimental Drug Research is established

1955 — We enter the field of bio-technology, assuming a pioneering role in Germany

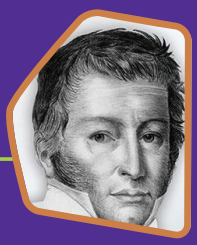
1950/60 — Our subsidiaries are founded or reestablished around the world

1945 — All foreign subsidiaries are lost and the company starts up again from the ruins in Darmstadt

1917 — Our U. S. subsidiary Merck & Co, is expropriated as a consequence of World War 1

1900 — Merck from Darmstadt, Germany is represented on all continents

1827 — Emaneul Merck begins production on an industrial scale



1668 — Friedrich Jacob Merck purchases the "Angel pharmacy" (Engel-Apotheke) in Darmstadt

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