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ARTICLE

Serum progesterone concentration and live birth rate in frozen–thawed embryo transfers with hormonally prepared endometrium

BIOGRAPHY

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KEY MESSAGE

Serum progesterone concentration <10 ng/ml on the day of FET is associated with significantly decreased pregnancy and live birth rates. A daily dose of 600 mg vaginal progesterone leads to low progesterone levels in over one-third of patients. Therefore, serum progesterone should be monitored in hormonal replacement therapy.

ABSTRACT

Research question: Is serum progesterone measurement on the day of embryo transfer associated with outcome of frozen–thawed embryo transfer (FET) in cycles using hormonal replacement therapy (HRT) for endometrium preparation?

Design: This single-centre retrospective study assessed the relationship between serum progesterone on embryo transfer day and live birth rates in 227 FET cycles. Endometrial preparation was performed by sequential administration of vaginal oestradiol until endometrial thickness was >7 mm, followed by transdermal oestradiol combined with 600 mg vaginal micronized progesterone.

Results: Mean serum embryo transfer day progesterone was 11.4 ng/ml. Serum progesterone <10 ng/ml was observed in 37% of cycles and was associated with significantly lower pregnancy (34% versus 48%, $P = 0.04$) and live birth rates (17% versus 31%, $P = 0.01$). Multivariate logistic regression analysis identified serum embryo transfer day progesterone as a significant prognostic factor for live birth rate (odds ratio [OR]: 2.75, 95% confidence interval [CI]: 1.40–5.43]). Receiver operator curve analysis for live birth rates by serum progesterone levels on embryo transfer day gave an area under the curve of 0.62 (95% CI: 0.53–0.72).

Conclusions: The data show that serum progesterone concentration is associated with live birth rate. This outlines the importance of measuring serum progesterone in FET with HRT although progesterone monitoring is not usually performed in routine practice. However, the optimal timing for measurement and further adaptive management in the presence of low values remain to be determined.

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KEYWORDS

Frozen–thawed embryo transfer
Hormonal replacement therapy
Live birth
Serum progesterone

INTRODUCTION

Over the past decade, the use of frozen–thawed embryo transfer (FET) has dramatically increased worldwide for several reasons: the implementation of ovarian stimulation, the great improvement in embryo survival rates related to the development of vitrification; the increased use of elective single embryo transfer policies; and the development of freeze-all strategies to prevent ovarian hyperstimulation syndrome in women at risk, or more commonly, to avoid the negative effect of supraphysiologic levels of steroids induced by controlled ovarian stimulation on embryo implantation. However, there is no consensus on the most effective method of endometrium preparation prior to FET in normo-ovulatory patients (*Mackens et al., 2017*). Indeed, no difference in terms of clinical pregnancy or live birth rates has been reported (*Groenewoud et al., 2018; Yarali et al., 2016*) among patients having undergone endometrial preparation during a natural or softly stimulated cycle or with hormonal replacement therapy (HRT). This latter protocol has become more and more popular because it makes it possible to spread out embryo transfers over a 5-day week. Sequential oestrogen administration is usually started at the beginning of the cycle to prevent FSH intercycle rise and follicular growth while enabling endometrial growth. Once endometrial thickness has reached at least 7 mm, progesterone is administered 2, 3 or 5 days before the scheduled embryo transfer, depending on the stage of embryo cryopreservation. Both oestradiol and progesterone administration have to be continued up to the luteo-placental shift at about 3 months of pregnancy. Although the corpus luteum secretes hormones other than oestradiol and progesterone (*Paulson, 2011*), this protocol gives excellent results, particularly in egg donation programmes.

However, there is still room for improvement in the administration route of steroids and the ideal dose, duration and schedule, which are usually not individualized to a patient's characteristics. The vaginal route of oestradiol and progesterone administration appears to be the most appropriate as it not only prevents the first pass in the liver, a source of thrombo-embolic adverse events, but also provides higher and more sustained serum steroid levels than the oral route (*Nahoul et al., 1993*) and achieves higher tissue levels than the intramuscular or subcutaneous routes (*Miles et al., 1994*) due to the first pass in the uterus. Nevertheless, great inter-individual variability is constantly reported, which might be the consequence of variations in the vaginal absorption rate or bioavailability (*Nahoul et al., 1993*). Progesterone levels are not usually routinely measured because uterine concentrations have been reported to be 10-fold higher than serum concentrations (*De Ziegler et al., 1997*). However, it has been reported recently that a range of serum progesterone concentrations during the implantation period was associated with optimal live birth rates (*Yovich et al., 2015*).

In our IVF unit, an HRT protocol for FET was historically reserved for specific indications such as anovulation, severe endometriosis and oocyte donation. It led to 10% higher pregnancy losses than those obtained in FET without HRT (unpublished data) and this difference was attributed to patient characteristics. To improve scheduling of FET, HRT was introduced for normo-ovulatory patients in 2015. Surprisingly, the analysis of our results (unpublished data) for these patients in 516 FET cycles (299 HRT and 217 slightly stimulated or only triggered cycles) showed similar pregnancy (36% versus 33%) but lower delivery rate by starting pregnancy (61% versus 78%, $P = 0.019$). Furthermore, lower progesterone levels on the day of pregnancy test were observed in women having undergone an HRT protocol (13 ± 8 versus 42 ± 25 ng/ml, $P < 0.001$). Therefore, it was postulated that serum progesterone levels might influence FET outcome when HRT is used. The present study aimed to measure progesterone levels on the embryo transfer day and further assessed the effect of an adjustment of the administered progesterone dose.

MATERIALS AND METHODS

Patients and study method

From 8 March 2016 to 8 March 2017, all patients undergoing a FET with HRT for endometrial preparation were advised to provide a blood sample for hormonal measurement on embryo transfer day in order to adjust the administered progesterone dose as described in the following paragraph. Patient characteristics such as birth date, body mass index (BMI), smoking habits, indication for assisted reproductive techniques (ART) (male, tubal, endometriosis, unexplained, ovulatory or mixed), diminished ovarian reserve (DOR) defined by anti-Müllerian hormone (AMH) <1 ng/ml or antral follicle count <7 were registered as routine practice in the electronic patient files in our database. A retrospective analysis of the data was performed. The primary endpoint was the relationship between progesterone levels on the embryo transfer day and the live birth rate. Secondary endpoints included biochemical pregnancy (positive β-HCG test) rate, clinical pregnancy (ultrasound visualization of fetal heartbeat) rate, ongoing pregnancy rate at 12 weeks of amenorrhoea and first trimester pregnancy loss rate.

Protocol of endometrial preparation

Micronized oestradiol (Provames®; Merus Labs Luxco, Luxembourg) was started vaginally at the dose of 1 mg twice a day from the first day of a natural menstrual cycle without previous down-regulation with gonadotrophin-releasing hormone (GnRH) agonist. After 10–12 days of oestrogen therapy, a blood sample and a vaginal ultrasound were performed for measurement of oestradiol, LH, progesterone and endometrial thickness. If endometrium thickness was <7 mm, oestrogen therapy was prolonged and/or dose increased if required according to oestradiol measurement. If a triple-line endometrium of ≥7 mm thickness was observed with serum progesterone concentrations <1.5 ng/ml, administration of vaginal micronized progesterone (Progestan®; Besins International, Montrouge, France) 200 mg three times a day was initiated in the evening (referred to as day 0 of progesterone administration), and oestradiol administration was switched from the vaginal to transdermal route (Vivelledot®; Novartis Pharma, Rueil-Malmaison, France); 100 µg patch × 2 every 3 days. Embryo transfer was performed after the morning administration of progesterone, on day 2 of progesterone administration for Day 2 embryos, on day 3 for Day 3 embryos and on day 5 for blastocysts. A blood sample was taken just before embryo transfer for serum oestradiol

and progesterone measurement. If progesterone concentrations were <10 ng/ml, patients were advised in the afternoon to increase the progesterone dose to 400 mg three times a day and a new blood sample was performed 2 days later to check serum progesterone levels. The supplementation was continued at the same dose until the pregnancy test on day 15 of adjusted progesterone administration. This luteal support was continued until gestational weeks 10–12 in viable pregnancies and discontinued in patients who were not pregnant. A vaginal ultrasound scan was performed during the sixth week of amenorrhoea to assess the presence and the number of gestational sacs with heartbeat embryo. First trimester pregnancy losses were calculated by the difference between the number of patients with a positive pregnancy test and the number of patients with an ongoing pregnancy at 12 weeks of amenorrhoea.

Embryo transfer

Embryos were issued from either IVF or intracytoplasmic sperm injection cycles and were vitrified and warmed as previously described (*Sifer et al., 2012*) on Day 2, Day 3 or at the blastocyst stage. All embryo transfers were performed under ultrasound guidance. The number of transferred embryos, the stage of embryos and the quality of embryos were recorded. If at least one good-quality embryo was transferred, the quality was graded Q+. Criteria for Q+ quality were for Day 2 embryos: 3 to 5 cells without fragmentation, for Day 3 embryos: 6 to 10 cells with less than 20% fragmentation according to the Holte classification (*Holte et al., 2007*) and for blastocysts: fully expanded to hatched blastocysts with inner cell mass and trophectoderm A or B quality (from 4BB upwards) according to the Gardner classification (*Gardner and Schoolcraft, 1999*).

Serum hormonal measurement

Hormonal measurements were carried out using commercially available chemo-luminescence immunoassays with an automated Elecsys immunoanalyser (ECLIA, Roche Diagnostics, Meylan, France). The sensitivity of the assay was 5 pg/ml for oestradiol, 0.03 ng/ml for progesterone and 0.07 IU/l for LH. Intra- and inter-assay coefficients of variation were respectively 5 and 10% for oestradiol, 3% and 5% for progesterone and 2.3% and 2.6% for LH.

Statistical analysis

Outcomes of FET were compared between patients below or above the threshold of serum progesterone level currently used to define adequate corpus luteum, i.e. 10 ng/ml (*Hull et al., 1982; Jordan et al., 1994*) and after breakdown into four groups covering the range of observed *P*-values. Results from the descriptive analysis are expressed as mean ± SD in tables. Univariate analyses were performed using the Pearson chi-squared test for nominal variables and the Student *t*-test for continuous variables. The explanatory factors significantly associated with the outcome on univariate analysis were included in a multivariate model by logistic regression with a stepwise enter. A *P*-value <0.05 was considered as statistically significant. StatView (Abacus Concepts, Berkeley, CA, USA) and Statistical Analysis System Version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA) were used for analyses.

Ethics

This retrospective study received ethical approval from a review board committee (Centre Hospitalier Intercommunal de Créteil) dated 9 February 2018.

RESULTS

During the study period, 250 cycles of FET with HRT were performed in 218 patients. Six cycles (six patients in an oocyte donation programme) were excluded from the analysis and the hormonal dosage was missing in 17 cycles (17 patients). Therefore, the final analysis included 227 cycles with hormonal dosage on embryo transfer day in 195 patients. Following administration of 600 mg daily micronized vaginal progesterone, mean serum progesterone level was 11.4 ± 4 ng/ml with a normal distribution but a large range (2.3–33.0 ng/ml), as shown in FIGURE 1. Progesterone levels were below the 10 ng/ml threshold in 37% of cycles.

Characteristics of patients in cycles with progesterone levels on embryo transfer day <10 ng/ml were not significantly different from those of cycles with progesterone ≥10 ng/ml (TABLE 1). However, patients in cycles with progesterone <10 ng/ml also had lower oestradiol levels, following both vaginal administration of 2 mg daily micronized oestradiol (prior to introduction of progesterone, $P = 0.002$) or transdermal administration of a 200 µg oestradiol patch every 3 days (embryo transfer day, $P = 0.01$). In cycles with progesterone <10 ng/ml, although FET was more frequently performed at the blastocyst stage, live birth rate was significantly reduced ($P = 0.02$). This was related to a significant decrease in both biochemical pregnancy ($P = 0.04$), clinical pregnancy ($P = 0.008$) and ongoing pregnancy ($P = 0.01$) rates. Nevertheless, the difference in first trimester pregnancy losses between cycles with or without progesterone levels on embryo transfer day <10 ng/ml did not reach significance (48% versus 31%).

An increase in progesterone administration to 1200 mg daily was performed in all 85 patients with progesterone <10 ng/ml on embryo transfer day. A subsequent progesterone measurement 2 days post-embryo transfer was available for 80 out of 85 patients (five missing dosages, one pregnancy ending in live birth for these cycles). The progesterone dose increase allowed a progesterone level ≥10 ng/ml to be reached in 69% of patients who were below the threshold on embryo transfer day. Outcome of the patients whose progesterone levels were corrected by increased doses was no different to those whose progesterone levels remained <10 ng/ml (TABLE 2). Despite doubling the progesterone dose, the frequency distribution histogram of progesterone levels remained in a similar range (FIGURE 2). Furthermore, in patients reaching progesterone levels ≥10 ng/ml 2 days post-embryo transfer with 1200 mg, mean progesterone levels were no different from those observed on embryo transfer day with 600 mg.

In the univariate analysis, factors significantly associated with live birth rate were: age ($P = 0.004$), DOR ($P = 0.02$), presence of at least one Q+ embryo at transfer ($P = 0.002$) and progesterone level on embryo transfer day ($P = 0.003$). In the stepwise multivariate logistic regression analysis (TABLE 3), serum progesterone level on embryo transfer day is an independent prognostic factor for live birth rate with OR 2.75 (95% CI: 1.40–5.43) after adjustment for the embryo stage at transfer and significant variables for live birth in univariate analysis.

When serum progesterone levels on embryo transfer day were split into

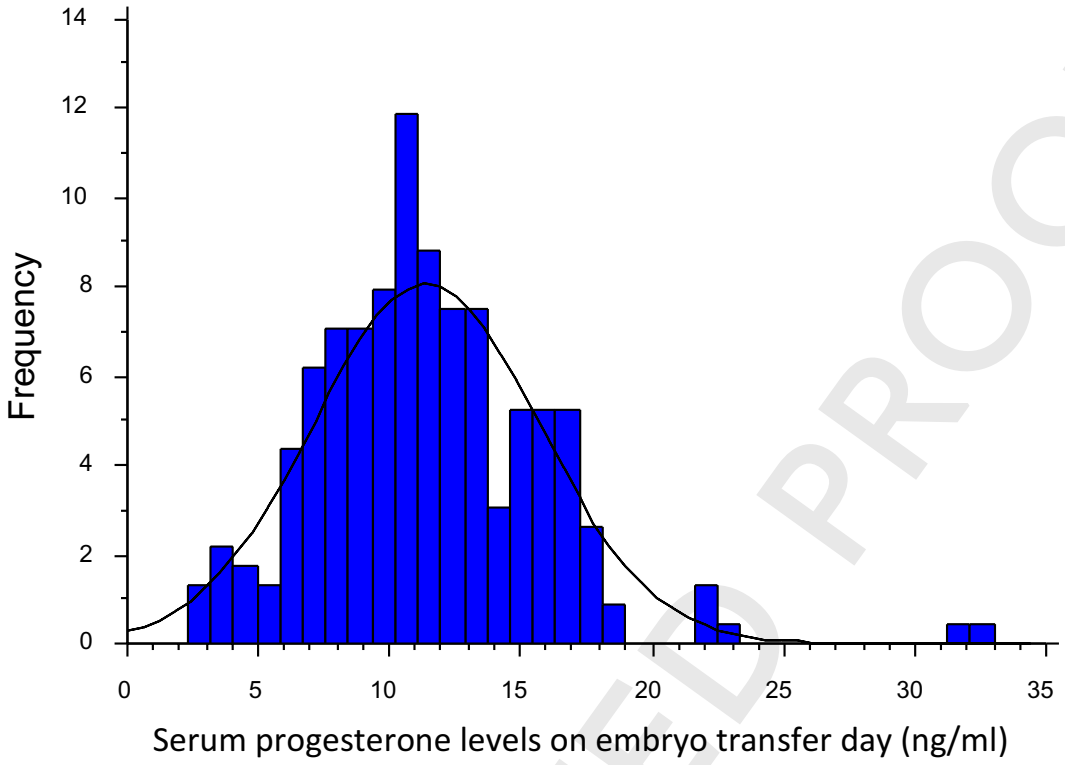

FIGURE 1 Frequency distribution histogram of serum progesterone levels on embryo transfer day in frozen–thawed embryo transfer (FET) cycles with hormonal replacement therapy (600 mg daily micronized vaginal progesterone) for endometrial preparation.

four groups (FIGURE 3): progesterone <5 ng/ml, progesterone 5 to <10 ng/ml, progesterone 10 to <15 ng/ml, and progesterone ≥15 ng/ml, outcomes appeared to be better with a higher threshold than 10 ng/ml, although only clinical pregnancy was significantly different (P = 0.03) between groups. The receiver operating characteristic (ROC) curve showed a significant predictive value of serum progesterone levels on the embryo transfer day for live birth with an AUC = 0.62 (95% CI: 0.53–0.72; P = 0.01; FIGURE 4). The optimal serum progesterone threshold for live birth was 13.5 ng/ml (44.1% sensitivity, 79.2% specificity, 43% positive predictive value, 80% negative predictive value, accuracy 0.7). Serum progesterone thresholds in which both sensitivity and specificity were >50% should be between 10.7 ng/ml (70% sensitivity, 53% specificity) and 12.3 ng/ml (51% sensitivity, 70% specificity).

DISCUSSION

These results outline the crucial role of progesterone for the establishment and maintenance of pregnancy in FET with HRT for endometrial preparation, because serum progesterone level on the day of embryo transfer is an independent prognostic factor for live birth rate. The results confirm previous data reported with the vaginal route of progesterone administration in ART (*Yovich et al., 2015*) and more recently in oocyte donation (*Labarta et al., 2017*), as well as those obtained with parenteral administration of progesterone in similar contexts (*Brady et al., 2014; Kofinas et al., 2015*). As a consequence, contrary to common practice, it may be mandatory to monitor serum progesterone levels in HRT because low progesterone levels were observed in over one-third of patients (progesterone <10 ng/ml) with 600 mg daily vaginal micronized progesterone and in 25% of women in the study by *Labarta et al. (2017)* (progesterone <9.7 ng/ml) with 800 mg daily vaginal micronized progesterone. Another strategy could be to provide higher progesterone doses in all patients because it has been reported as a means of optimizing pregnancy rates while decreasing the risk of miscarriages (*Alsbjerg et al., 2013*). However, in this series, progesterone levels remained below the threshold in about 10% of patients, regardless of progesterone dose (600 mg or increase to 1200 mg). Furthermore, measurement of progesterone levels in HRT cycles does not present the same drawback as the dosage in the luteal phase, which leads to a huge variation because of pulsatile progesterone secretion. Indeed, progesterone levels have been shown to be steady from 6 h after vaginal introduction up to 24 h (*Nahoul et al., 1993*). Our data also confirm, as previously reported, that oestradiol levels either before progesterone introduction (*Bocca et al., 2015; Remohí et al., 1997*) or at the time of embryo transfer (*Yovich et al., 2015*) are not a factor associated with live birth rates, provided the endometrium has reached a thickness >7 mm.

The mechanisms that could explain the wide range in serum progesterone levels despite administration of the same dose of progesterone in all patients remain unclear. Our observation of lower oestradiol levels after both vaginal and transdermal oestradiol administration in those patients with low progesterone levels advocates for variability of absorption through the cutaneous epithelium. However, sexual intercourse has been shown to reduce progesterone levels after vaginal administration

TABLE 1 CHARACTERISTICS OF PATIENTS, FET CYCLES AND OUTCOME ACCORDING TO SERUM PROGESTERONE LEVELS ON EMBRYO TRANSFER DAY

	Progesterone ≥10 ng/ml *n* = 142 cycles[a] (123 patients)	Progesterone <10 ng/ml *n* = 85 cycles[a] (79 patients)	*P*-value
Age (years)	33.8 ± 4.6	34.7 ± 3.9	NS
BMI (kg/m^2)	24 ± 4	25 ± 4.5	NS
Smokers (%)	15	14	NS
Indication (%)			NS
Male	43	28	
Tubal	18	22	
Endometriosis	10	13	
Unexplained	10	13	
Ovulatory	10	14	
Mixed	9	10	
DOR (%)	16.9	15.3	NS
Oestradiol prior to progesterone introduction (pg/ml)	1341 ± 870	1017 ± 614	0.002
LH prior to progesterone introduction (IU/l)	4.6 ± 4.4	6.4 ± 7.0	NS
Progesterone prior to progesterone introduction (ng/ml)	0.2 ± 0.2	0.3 ± 0.2	NS
Endometrial thickness (mm)	9.7 ± 2.3	9.7 ± 2.5	NS
Oestradiol on embryo transfer day (pg/ml)	245 ± 187	185 ± 149	0.01
Progesterone on embryo transfer day (ng/ml)	13.8 ± 3.5	7.4 ± 1.9	<0.001
Mean number of transferred embryos	1.5 ± 0.6	1.5 ± 0.7	NS
Stage at transfer Day 2/Day 3/blastocyst (%)	11/43/46	6/29/65	0.01
Transfer with at least one Q+ embryo (%)	74	71	NS
Biochemical pregnancy (%)	48	34	0.04
Clinical pregnancy (%)	35	19	0.008
Ongoing pregnancy at 12 weeks (%)	33	17	0.01
Live birth (%)	31	17	0.02

BMI = body mass index; DOR = diminished ovarian response; FET = frozen embryo transfer; NS = non-significant.

[a] The sum of the number of patients in each group is higher than the total number of patients (*n* = 195) as seven patients had one cycle with progesterone >10 ng/ml and another cycle with progesterone <10 ng/ml.

(*Merriam et al., 2015*). Finally, these variations are also observed following the parenteral route of progesterone administration (*Brady et al., 2014; Kofinas et al., 2015*) and could be related to variations in metabolism.

The parenteral route of progesterone administration has been shown to be more effective than the vaginal route in some retrospective studies (*Haddad et al., 2007; Kaser et al., 2012*) but not all (*Shapiro et al., 2014*).

The measurement of serum progesterone on embryo transfer day seems to come too late because the increase in progesterone doses immediately after transfer, although effective in rescuing most cases with low progesterone

TABLE 2 OUTCOME ACCORDING TO 2 DAYS POST-EMBRYO TRANSFER MEASUREMENT OF PROGESTERONE LEVELS FOLLOWING INCREASE TO 1200 MG/DAY VAGINAL MICRONIZED PROGESTERONE IN PATIENTS WITH PROGESTERONE <10 NG/ML ON THE EMBRYO TRANSFER DAY

	Progesterone ≥10 ng/ml *n* = 55	Progesterone <10 ng/ml *n* = 25	*P*-value
Mean progesterone level on post-embryo transfer control (ng/ml)	12.6 ± 2.7	8.0 ± 1.5	<0.001
Biochemical pregnancy (%)	36	32	NS
Clinical pregnancy (%)	22	16	NS
Ongoing pregnancy at 12 weeks (%)	22	12	NS
Live birth (%)	20	12	NS

NS = non-significant.

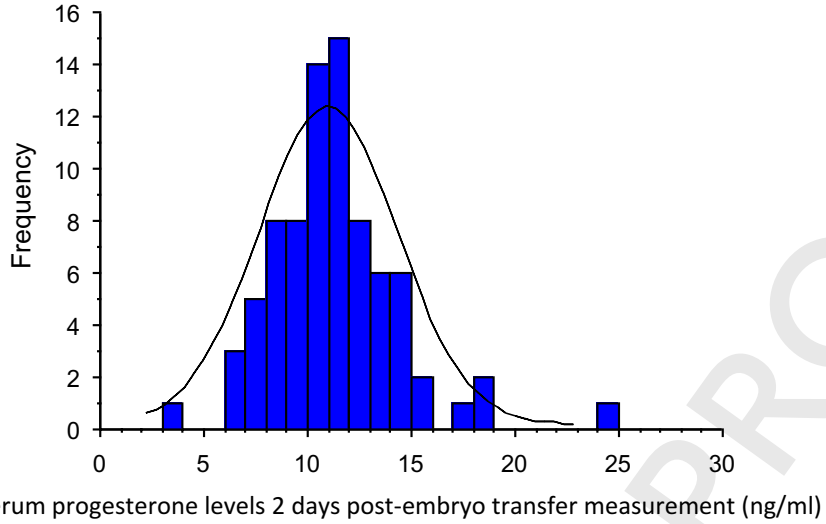

FIGURE 2 Frequency distribution histogram of serum progesterone levels on 2 days post-embryo transfer measurement in FET cycles with double dose of progesterone (1200 mg daily micronized vaginal progesterone).

levels, seemed ineffective for improving outcomes. Nevertheless, the design and sample size of this study do not allow us to firmly conclude on the inefficacy of this strategy, but similar results were also shown with intramuscular progesterone in oocyte donation cycles (*Brady et al., 2014*). Consequently, monitoring should be done before transfer and soon enough to allow adjustment of progesterone doses and probably postponement of transfer. According to the previously described pharmacokinetic study (*Nahoul et al., 1993*), monitoring could be done as early as the second day of progesterone administration. However, the effectiveness of this strategy is still to be demonstrated.

Finally, what is the minimal threshold of progesterone serum level that should be chosen to optimize live birth rate? It probably depends on the route of administration. Oral or parenteral routes of administration probably require a higher progesterone threshold than the vaginal route, due to the absence of first pass in the uterus. We have retained

TABLE 3 A STEPWISE MULTIVARIATE LOGISTIC REGRESSION ANALYSIS OF FACTORS RELATED TO LIVE BIRTH RATE AFTER ADJUSTMENT FOR EMBRYO STAGE AT TRANSFER, AGE, DIMINISHED OVARIAN RESPONSE AND EMBRYO QUALITY. NS: NON-SIGNIFICANT

	Adjusted OR (95% CI)	*P*-value
Embryo stage at transfer		NS
Day 2	1	
Day 3	1.35 [0.23; 7.91]	
Blastocyst	2.07 [0.36; 11.92]	
Age (years)		NS
≥35	1	
≥30 to <35	1.52 [0.74; 3.15]	
<30	2.72 [1.14; 6.47]	
Diminished ovarian reserve		NS
No	1	
Yes	0.41 [0.13; 1.30]	
Transfer with at least one Q+ embryo		0.04
No	1	
Yes	3.05 [1.06; 8.77]	
Progesterone >10 ng/ml on embryo transfer day		0.02
No	1	
Yes	2.75 [1.40; 5.43]	

CI = confidence interval; NS = non-significant; OR = odds ratio.

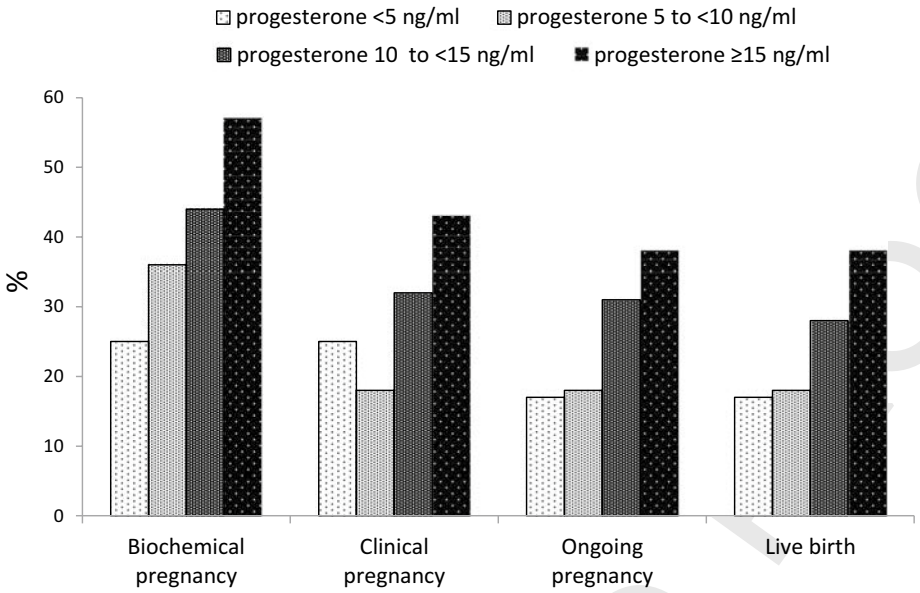

FIGURE 3 Outcome according to serum progesterone levels on embryo transfer day split into four groups: progesterone < 5 ng/ml (n = 12), progesterone 5 to <10 ng/ml (n = 73), progesterone 10 to <15 ng/ml (n = 98), progesterone ≥15 ng/ml (n = 44). Significant difference between groups was P = 0.03 for clinical pregnancy; not significant for biochemical pregnancy, ongoing pregnancy and for live birth.

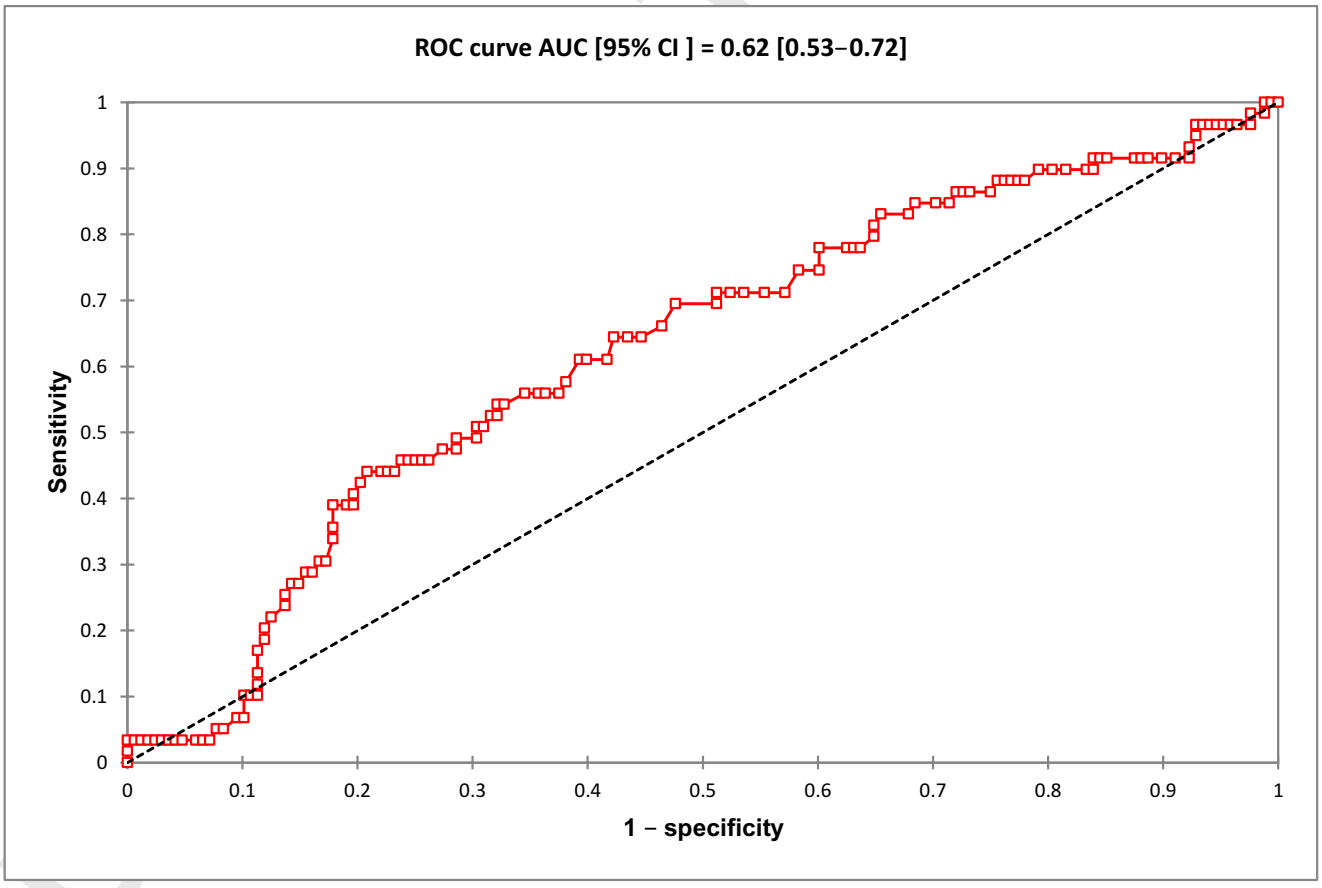

FIGURE 4 Receiver operating characteristic (ROC) curve for prediction of live birth rate by serum progesterone levels on the day of embryo transfer. AUC = area under the curve.

the threshold of 10 ng/ml, which is close to the value reported as an adequate progesterone production by the corpus luteum in natural cycles (*Hull et al., 1982*). Indeed, a progesterone level higher than 10 ng/ml in the mid-luteal phase is routinely used to assess an adequate corpus luteum after induction of ovulation. Furthermore, some authors retained it as a prerequisite before embryo transfer in substituted cycles for FET (*Kofinas et al., 2015*). This threshold is close to the value of 9.2 ng/ml reported recently by *Labarta et al. (2017)* as associated with significantly lower ongoing pregnancy rate in oocyte donation. Nevertheless, in all three series using vaginal progesterone (our data, *Labarta et al., 2017* and *Yovich et al., 2015*), higher concentrations close to 15 ng/ml were associated with better success rates. ROC curve analysis of our data shows a serum progesterone threshold of 13.5 ng/ml for the best positive and negative predictive values. Applying the same criteria of choice to define the threshold from the ROC curve as in the study by *Labarta et al. (2017)*, we obtain a threshold of 10.7 ng/ml, very close to the value of 11 ng/ml reported by this author. Studies with parenteral progesterone seem to show discrepancies in optimal progesterone threshold, with values below (*Kofinas et al., 2015*) or above 20 ng/ml (*Brady et al., 2014*).

The strength of our study is the real-world analysis of clinical practice with a follow-up to live birth. Its weakness is the retrospective nature of data but potential confounders are taken into account in the multivariate analysis. Another concern is the doubling of progesterone doses in patients with progesterone <10 ng/ml on embryo transfer day that could be considered a bias when analysing the impact of serum progesterone on embryo transfer day on live birth rates. Indeed, progesterone dose increase could impede the comparability of the two groups and be responsible *per se* for lower pregnancy rates, as too high progesterone levels following vaginal administration were also reported to be associated with lower pregnancy rates (*Yovich et al., 2015*). But in our study, mean progesterone levels with increased doses to 1200 mg were no different from those obtained at first measurement with 600 mg and maximal values of progesterone levels remained below the ceiling of about 30 ng/ml reported by *Yovich et al. (2015)*.

In conclusion, this study shows that serum progesterone measurement prior to embryo transfer might become a mandatory tool in the optimization of FET cycles, in case a protocol with HRT for endometrial preparation is used. However, the best way to proceed in the presence of low serum progesterone levels remains to be determined. We suggest that a low level of serum progesterone after vaginal administration could lead to either an increase in the vaginal administered dose, a switch to another route of progesterone administration or the cancellation of the HRT cycle and the continuation of FET with another protocol such as natural or slightly stimulated cycles.

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