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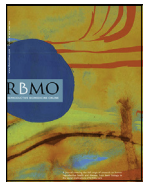

ARTICLE

Factors associated with serum progesterone concentrations the day before cryopreserved embryo transfer in artificial cycles

BIOGRAPHY

Dr Iñaki González-Foruria is an attending gynaecologist at Dexeus University Hospital, Barcelona, a Doctor in Medicine and Surgery with an international distinction from the University of Barcelona. His clinical practice focuses on infertility care and assisted reproduction, with a special interest in the endometriosis patient, ovarian stimulation for IVF and implantation.

Iñaki González-Foruria*[,1], Sofia Gaggiotti-Marre[1], Manuel Álvarez, Francisca Martínez, Sandra García, Ignacio Rodríguez, Buenaventura Coroleu, Nikolaos P. Polyzos

KEY MESSAGE

Serum progesterone concentrations the day before cryopreserved embryo transfer are independently associated with live birth rates. Body weight, age, time of blood sampling and previous history of low progesterone are determinants of progesterone concentrations when using hormone replacement therapy.

ABSTRACT

Research question: What factors determine serum progesterone concentrations the day before cryopreserved embryo transfer in artificially prepared cycles?

Design: Retrospective cohort study at a university-affiliated fertility centre including infertile women under 45 years old using own oocytes who underwent a total of 685 single cryopreserved blastocyst transfers under hormonal therapy. Determinants that affected live birth rate (LBR) were analysed using a multivariate logistic regression. Univariate analysis and multivariate linear regression were used to evaluate independent factors that affect serum progesterone concentrations.

Results: Age (odds ratio [OR] 0.93; 95% confidence interval [CI] 0.89–0.96), duration of oestradiol (OR 0.96; 95% CI 0.92–0.99), serum progesterone concentrations (OR 1.04; 95% CI 1.01–1.08) and patients who underwent preimplantation genetic testing for aneuploidies (PGT-A) (OR 2.17; 95% CI 1.55–3.03) were independently associated with LBR. After univariate analysis, determinants of progesterone concentrations were: age, weight, history of a previous cryopreserved embryo transfer with serum progesterone concentrations <10 ng/ml, and time of blood extraction. The multivariate linear regression showed that increasing age presented a positive correlation with progesterone concentrations (β = 0.11; 95% CI 0.01–0.20). On the contrary, significant negative correlations with progesterone concentrations were shown for a previous history of serum progesterone value <10 ng/ml (β = –3.13; 95% CI –4.45 to –1.81]), higher weight (β = –0.05; 95% CI –0.08 to –0.01) and the time of blood sampling during the day (β = –0.13; 95% CI –0.25 to –0.01).

Conclusions: This study adds more evidence regarding the importance of serum progesterone concentrations before frozen embryo transfer (FET). It also showed that body weight, age, time of blood sampling and a history of low progesterone are determinants associated with progesterone concentrations before blastocyst FET.

Dexeus Mujer, Department of Reproductive Medicine, Dexeus University Hospital, Barcelona, Spain
[1]These authors should be regarded as joint first authors.

*Corresponding author. E-mail address: inagon@dexeus.com (I González-Foruria). https://doi.org/10.1016/j.rbmo.2020.03.001 1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest.

KEYWORDS

Artificial preparation
Frozen embryo transfer
Hormone replacement therapy
Live birth rate
Progesterone

INTRODUCTION

The production and secretion of progesterone by the corpus luteum is essential for an adequate endometrial transformation in order to achieve and maintain a pregnancy. Improvements in the cryopreservation process have allowed good reproductive results to be achieved with fewer complications (*Devroey et al., 2011*; *Evans et al., 2014*) such as ovarian hyperstimulation syndrome and multiple pregnancies. In these cases, the secretory transformation of the endometrium can be achieved in a natural, natural-modified or artificial cycle by the exogenous administration of progesterone and oestradiol. Previous studies have demonstrated a detrimental effect of low serum progesterone concentrations around the day of embryo transfer in patients undergoing frozen embryo transfer (FET) with artificial endometrial preparation (*Brady et al., 2014*; *Gaggiotti-Marre et al., 2019*; *Labarta et al., 2017*). Other authors have compared different dosages or routes of progesterone supplementation for these patients (*Asoglu et al., 2019*; *Shapiro et al., 2014*), without finding significant differences in terms of reproductive results. However, while there is a significant correlation between low serum progesterone concentrations and lower live birth rates (LBR) and higher miscarriage rates (*Gaggiotti-Marre et al., 2019*), researchers have not found an explanation for this finding. Whether this correlation is due to intrinsic patient characteristics, cycle aspects or other factors, is yet to be elucidated. There is a lack of evidence to explain the great disparity in inter-patient serum progesterone values shown in these studies, given the exact same treatment is administered to all the women studied.

Given the growing belief that 'one treatment does not fit all', this study sought to find patient and/or cycle parameters that could help predict which women are at risk of having a low serum progesterone value around the time of FET.

MATERIALS AND METHODS

Study design

A retrospective cohort study of 685 single blastocyst transfers was performed at a private university clinic between March 2016 and February 2018.

Study population

The study included 578 infertile women under 45 years old, who underwent an IVF cycle using their own oocytes during the described period. Only FET cycles were included. Patients underwent single-embryo transfer (SET) in the blastocyst stage under artificial endometrial preparation. Some patients underwent more than one FET and each attempt was included in the analysis as an independent event.

Study protocol

Ovarian stimulation and embryology procedures

All IVF cycles were performed under ovarian stimulation with gonadotrophins and pituitary suppression with gonadotrophin-releasing hormone (GnRH) analogues (agonists or antagonists) according to established standard protocols (*Martínez et al., 2016*). After oocyte retrieval, conventional IVF was performed. In cases of male factor and those cycles undergoing preimplantation genetic testing for aneuploidies (PGT-A), mature oocytes were microinjected 40 h after ovulation triggering with human chorionic gonadotrophin or with triptorelin acetate in those cases at risk of ovarian hyperstimulation syndrome. Embryos were cultured in a time-lapse incubator using single-step culture media (LifeGlobal®, USA). Embryos that reached the blastocyst stage (D5–D6) were either immediately cryopreserved, or biopsied for PGT-A and cryopreserved afterwards using the standard vitrification method (*Solé et al., 2013*).

Endometrial preparation

Starting on the second or third day after menstrual bleeding, patients received either 2 mg/8 h oral oestradiol valerate (Progynova®, Schering, Spain) or 150 µg every 3 days transdermal patches (Evopad®, Janssen-Cilag, Spain) for an average of 2 weeks, followed by 200 mg/8 h (at 08:00, 16:00 and 00:00 h) of vaginal micronized progesterone (Utrogestan®, Seid, Spain) until plasma β-human chorionic gonadotrophin (β-HCG) determination. When indicated, a depot GnRH agonist (Decapeptyl®, 3.75 mg, Ipsen Pharma, Spain) injection for pituitary suppression was administered in the mid-luteal phase of the preceding cycle. In case of biochemical pregnancy, all exogenous hormonal treatment was continued until the 10th week of pregnancy.

Serum analysis and ultrasound assessment

On the day prior to embryo transfer, and after 4 days of vaginal progesterone administration, a blood sample was obtained from 08:00 to 19:00 h and immediately analysed. Hormone determinations of oestradiol and progesterone were performed in a single laboratory with an electrochemiluminescence immunoassay (Cobas® e-411 analyser, Roche Diagnostics, Germany). For oestradiol, the lower limit of detection was 5 pg/ml with intra- and inter-assay variation of 2.4–8.5% and 2.5–11.9%, respectively. For progesterone, the lower limit of detection was 0.05 ng/ml, with intra- and inter-assay variation of 1.2–11.8% and 3.6–23.1%, respectively.

Transvaginal ultrasound was performed to assess endometrial thickness and pattern. Only cycles with extremely thin endometrium (<5 mm) were considered for cycle cancellation.

Single blastocyst transfer was performed under ultrasound guidance as previously reported (*Coroleu et al., 2002*; *Kava-Braverman et al., 2017*).

Statistical analysis

Mean and SD were used for continuous variables and frequencies and percentage for categorical variables. All the results expressed were per single blastocyst transfer. A multivariate logistic regression was used to evaluate the effect of the following variables on LBR: age, weight, serum progesterone and oestradiol concentrations the day before FET, type of oestrogen administrated (oral or transdermal), days of oestradiol exposure before FET and the use of previous agonist administration. These effects were submitted with odds ratios (OR) and 95% confidence intervals (CI), respectively. The same factors plus the time of blood sample collection and history of previous artificially prepared FET cycle with progesterone concentrations <10 ng/ml (*Jones, 1991*) were analysed to find any correlations with progesterone concentrations using the Student's *t*-test or Pearson correlation. According to univariate analysis, a multivariate linear regression was carried out to estimate factors associated with progesterone concentrations. A *P*-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS Statistics for Windows, Version 22 (IBM Corp., Armonk, NY, USA).

TABLE 1 PATIENT DEMOGRAPHICS AND CYCLE CHARACTERISTICS

Age (years)	36.99 ± 4.06
Weight (kg)	61.86 ± 11.02
Progesterone day before transfer (ng/ml)	11.15 ± 4.57
Oestradiol (day before transfer) (pg/ml)	203.08 ± 94.88
Oestrogen (days)[a]	18.20 ± 1.43
Oestrogen type (%, *n*/*N*)	
Transdermal	9.3 (64/685)
Oral	90.7 (621/685)
Previous agonist (%, *n*/*N*)	
No	60.0 (411/685)
Yes	40.0 (274/685)

All data are presented as mean ±SD, unless otherwise stated.

[a] Days of exogenous oestrogen administration until the day of cryopreserved embryo transfer.

Ethical approval

The study was approved by the Institutional Review Board on 16 January 2019 (reference number: 012019011604).

RESULTS

Patient demographics and cycle parameters for the 685 FET cycles meeting inclusion criteria are shown in TABLE 1. Mean ± SD serum progesterone and oestradiol concentrations the day prior to blastocyst transfer were 11.15 ± 4.57 ng/ml and 203.08 ± 94.88 pg/ml, respectively. Mean endometrial thickness was 10.44 ± 1.9 mm (5–20 mm).

Factors associated with LBR in FET cycles

The overall LBR per single cryopreserved blastocyst transfer was 44.8%. The following clinical parameters were significantly associated with LBR: age (OR 0.93; 95% CI 0.89–0.96), duration of oestradiol treatment before FET (OR 0.96; 95% CI 0.92–0.99), serum progesterone concentrations the day before FET (OR 1.04; 95% CI 1.01–1.08) and patients who underwent PGT-A (OR 2.17; 95% CI 1.55–3.03).

Influence of progesterone concentrations the day before FET on LBR

A logistic regression model was performed to show LBR according to serum progesterone concentrations the day prior to blastocyst transfer in patients who did and did not undergo PGT-A, adjusted for age and duration of oestradiol treatment (FIGURE 1). LBR showed a linear increase in both types of cycles (with/without PGT-A) as serum progesterone concentrations rise.

Factors that affect serum progesterone concentrations on the day before FET

Among the factors analysed with univariate analysis: age (R = 0.092; P = 0.017), weight (R = –0.114; P = 0.007), history of a previous FET with serum progesterone concentrations <10 ng/ml (P < 0.001) and time of blood extraction (R = –0.090; P = 0.018) showed a significant correlation to serum progesterone concentrations on the previous day of FET (TABLE 2). A total of 72 cycles presented a history of low serum progesterone values (<10 ng/ml) in a previous FET attempt under the same treatment. In these cycles, the mean (± SD) progesterone values the day before FET was 7.99 ± 2.95 ng/ml, compared with 11.52 ± 4.59 ng/ml in those cycles without such previous history. The further apart the time of blood collection from the latest dose of vaginal progesterone administered, the lower the serum progesterone value (R = –0.090; P = 0.018) (FIGURE 2).

When a multivariate linear regression was performed to correct for potential confounders, increasing age presented a positive correlation to serum progesterone concentrations (β = 0.11; 95% CI 0.01–0.20). On the contrary, significant negative correlations to progesterone concentrations were shown with a previous history of FET with serum progesterone value <10 ng/ml (β = –3.13; 95% CI –4.45 to –1.81), higher weight (β = –0.05; 95% CI –0.08 to –0.01), and delaying the moment of blood sampling during the day (β = –0.13; 95% CI –0.25 to –0.01) (TABLE 3).

DISCUSSION

This is thought to be the first study to analyse clinical factors related to serum progesterone values before FET. The findings demonstrate that weight, age, time of blood sampling and a prior history of low progesterone concentrations (<10 ng/ml) are independent factors associated with serum progesterone concentrations the day before blastocyst FET.

The effect of progesterone concentrations on the LBR following FET has been thoroughly investigated over the last 2 years. This study is in line with current literature (*Brady et al., 2014*; *Cédrin-Durnerin et al., 2019*; *Gaggiotti-Marre et al., 2019*; *Labarta et al., 2017*) showing that progesterone concentrations before FET are an independent factor associated with LBR. Similarly to previous published data on IVF and FET outcomes, other factors associated with LBR in cycles of FET were found such as age (*Devesa et al., 2018*; *Moragianni and Penzias, 2010*; *Younis, 2012*), duration of oestradiol before transfer (*Bourdon et al., 2018*) and decision to pursue PGT-A (*Murphy et al., 2019*; *Neal et al., 2018*).

According to the results of this study, there are specific factors that affect progesterone concentrations on the day prior to embryo transfer. It is extremely important to highlight that some of these factors are associated with altered pharmacokinetics (age, weight and prior history of low progesterone concentrations in a previous FET cycle), while others do not depend on changes in drug absorption or metabolism (timing of blood sampling).

The effect of age on vaginal absorption of progesterone tablets has been previously analysed. In a prospective study by *Levy et al. (2000)*, women >40 years old demonstrated an enhanced rate of absorption of progesterone using vaginal tablets compared with younger patients. In agreement with these previous data, results of this study show that age is positively associated with serum concentrations of progesterone in an independent manner. The thinner and more atrophic vaginal mucosa of older women may lead to increased absorption of vaginal progesterone, explaining these findings.

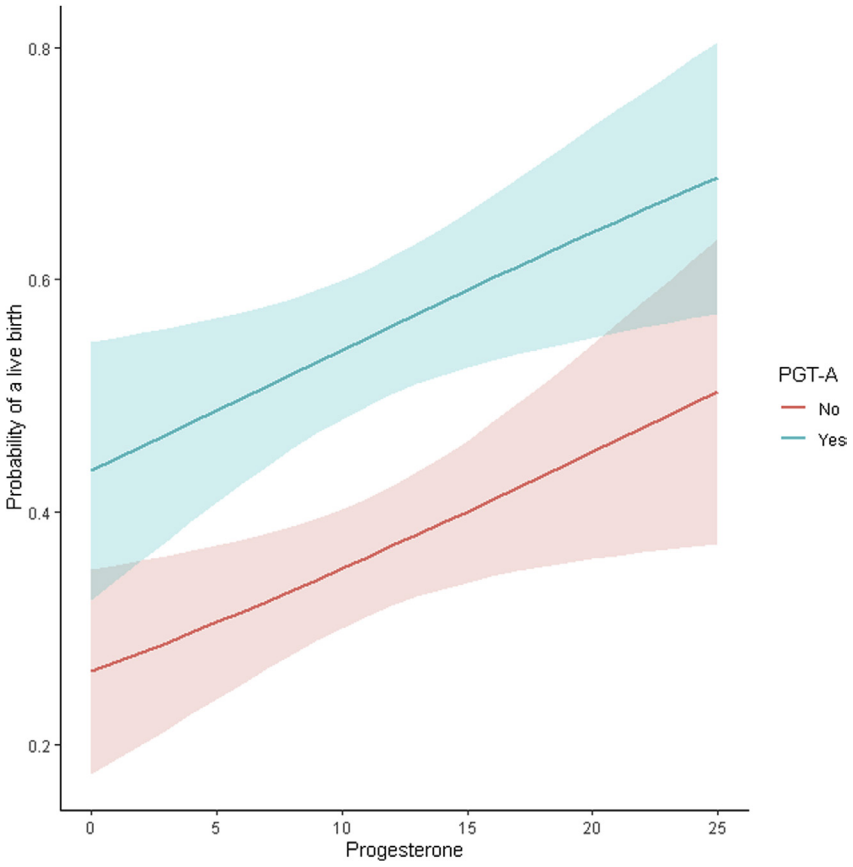

FIGURE 1 Logistic regression model, adjusted for age and duration of oestradiol treatment, showing probability of a live birth according to serum progesterone concentrations (ng/ml) the day before cryopreserved blastocyst transfer. The shading on the figure shows the 95% confidence interval.

TABLE 2 FACTORS ASSOCIATED WITH SERUM PROGESTERONE VALUES THE DAY BEFORE CRYOPRESERVED BLASTOCYST TRANSFER USING UNIVARIATE ANALYSIS

		Correlation coefficient	*P*-value
Oestrogen type			0.103
Oral	11.06 ± 4.62[a]		
Transdermal	12.04 ± 4.07[a]		
Previous agonist			0.146
Yes	10.86 ± 3.91[a]		
No	11.35 ± 4.96[a]		
Oestrogen (days)[b]	18.17 ± 3.7	−0.041	0.286
Progesterone in previous FET cycle			<0.001
<10 ng/ml (*n* = 72)	7.99 ± 2.95[a]		
≥10 ng/ml (*n* = 613)	11.52 ± 4.59[a]		
Oestradiol (pg/ml)	203.08 ± 94.88	−0.054	0.162
Age (years)	36.99 ± 4.06	0.092	0.017
Time extraction (time)	NA	−0.090	0.018
Weight (kg)	61.86 ± 11.02	−0.114	0.007

All data are presented as mean ± SD.

[a] Mean progesterone values (ng/ml) ± SD.

[b] Days of exogenous oestrogen administration until the day of FET.

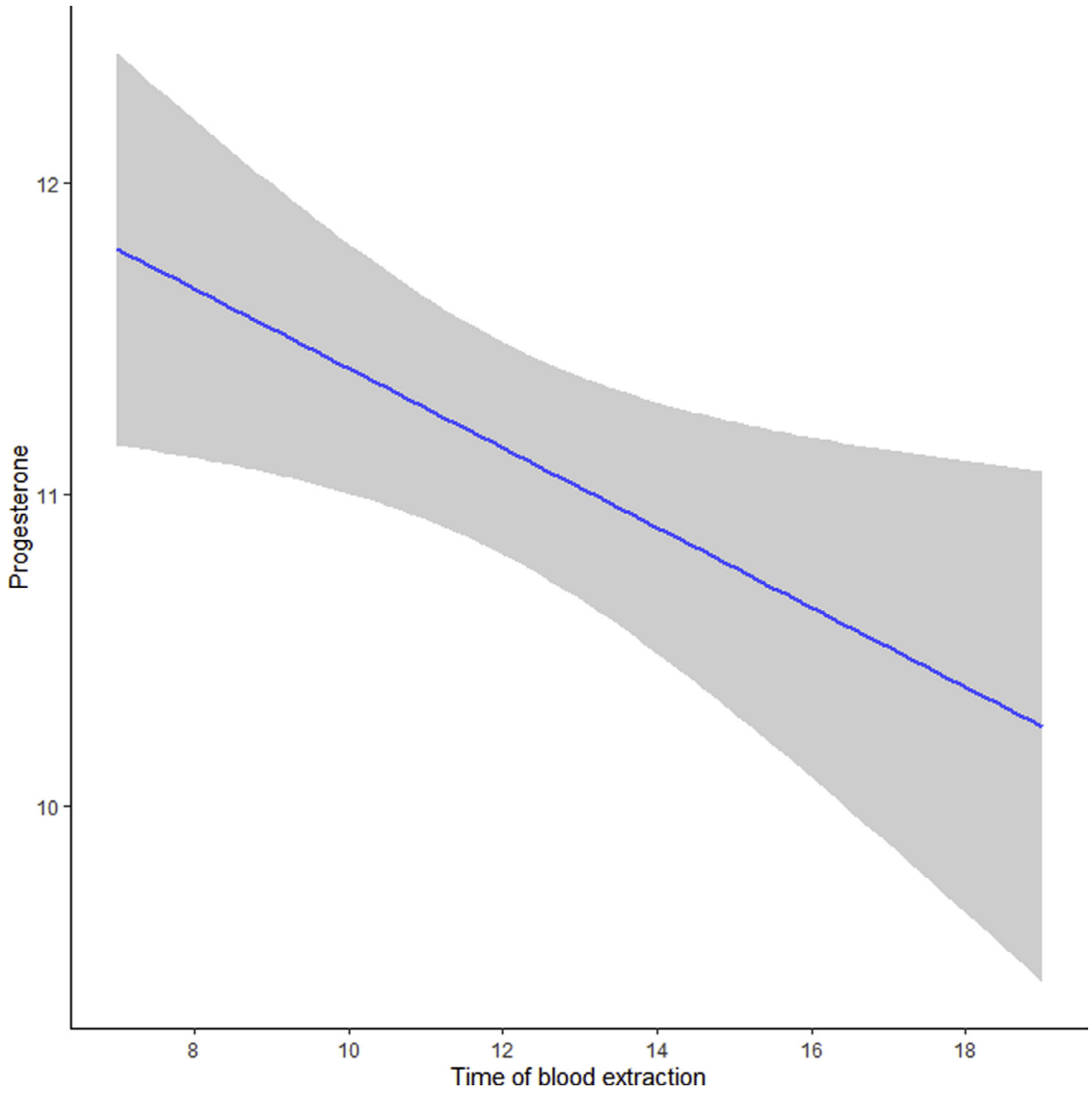

FIGURE 2 Regression model analysis showing the relationship between serum progesterone values (ng/ml) and time of blood extraction on the day prior to embryo transfer (from 08:00 to 19:00 h). $R = -0.090$; $P = 0.018$. The shading on the figure shows the 95% confidence interval.

On the contrary, the effect of body weight on serum progesterone concentrations of artificially prepared cycles is not yet clear. Previous research on 50 post-menopausal women who received 50 or 100 mg/day of vaginal micronized progesterone showed no significant difference in pharmacokinetic behaviour of serum progesterone in relation to weight (*Levy et al., 1999*). However, a more recent study on 229 oocyte recipient cycles using intramuscular progesterone found that serum progesterone concentrations on the day of embryo transfer were lower in overweight and obese women compared with those of normal weight (*Brady et al., 2014*). The findings here are in line with the latter study, showing that body weight is an independent factor that affects serum progesterone concentrations after 4 days of vaginal progesterone administration. These results are biologically plausible as body weight is a determinant factor that influences drug absorption, distribution, metabolism and elimination (*Edelman et al., 2010*).

TABLE 3 MULTIVARIATE LINEAR REGRESSION FOR FACTORS ASSOCIATED WITH SERUM PROGESTERONE CONCENTRATIONS ON THE DAY BEFORE CRYOPRESERVED BLASTOCYST TRANSFER

	β	95% CI
Age	0.11	0.01 to 0.20
Weight	−0.05	−0.08 to −0.01
Time of blood sampling	−0.13	−0.25 to −0.01
Low progesterone (< 10 ng/ml) in previous cycle	−3.13	−4.45 to −1.81

Interestingly, the current analysis showed that previous agonist down-regulation, oestradiol concentrations, type and duration of oestrogen did not affect serum progesterone values.

An abundance of evidence supports that the concentrations of progesterone

are not steady during the day. Intra-day variability of serum progesterone concentrations has been reported not only in the spontaneous cycle of normal women (*Bungum et al., 2013*; *Filicori et al., 1984*; *Fujimoto et al., 1991*; *Kerkhof et al., 2015*), but also in the late follicular and mid-luteal phases of gonadotrophin-stimulated cycles for IVF (*González-Foruria et al., 2019*; *Thomsen et al., 2018*). In artificially prepared cycles, insights from endocrinological studies in progesterone pharmacokinetics show a rapid absorption when using vaginal tablets, reaching mean peak plasma concentrations after 3–6 h, and also a fast mean elimination half-life of 13 h from administration (*Archer et al., 1995*; *Corleta et al., 2004*; *Levy et al., 1999*). The results of this study demonstrate once more that progesterone concentrations vary during the day, even when exogenous hormone replacement is given. These findings perfectly reflect the previous data on vaginal progesterone tablet pharmacokinetics, showing that mean progesterone values are lower, the further from the last administration of vaginal progesterone the blood sample was obtained.

A history of late follicular phase progesterone elevation on the day of ovulation triggering in ovarian stimulation for IVF is the most important factor to predict the same outcome in a subsequent cycle (*Venetis et al., 2016*). In a similar manner, and according to our results using hormone replacement therapy for FET, the history of low progesterone concentrations (<10 ng/ml) under the same treatment is the strongest predictor of progesterone concentrations in a subsequent cycle. Although the origin of serum progesterone concentrations in artificial endometrial preparation cycles for FET is completely different from those of ovarian stimulation cycles, the previous history remains the most determining factor in predicting both clinical outcomes. Thus, the current results suggest that patient intrinsic characteristics, regarding the vaginal absorption of progesterone and the distribution and metabolism of this sex steroid, are of utmost importance in determining serum progesterone concentrations.

The main strength of this study is the novelty of the topic, as it is the first study to analyse clinical factors associated with progesterone concentrations before FET, apart from confirming previous data on the relevance of serum progesterone concentrations in FET cycles. Interestingly, all FET cycles included in the analysis were performed in a single centre, under the same clinical setting and laboratory conditions. All patients underwent the same protocol of vaginal progesterone administration, regarding the doses (200 mg/8 h) and posology (08:00, 16:00 and 00:00 h). In addition, only cycles performed with a patient's own eggs and with single-embryo transfer were included to avoid potential confounders when analysing LBR. Despite a robust and strict design, this work has some shortcomings that need comment. The main limitation is its retrospective design, leading to higher risk of patient selection bias. Using this approach it is not possible to explain what the reasons were for receiving oral or transdermal oestrogens, to undergo more or less days of oestrogens and to undergo GnRH agonists in the preceding cycle. Although patients were advised to administer vaginal tablets of 200 mg of micronized progesterone at three set times of the day (08:00, 16:00 and 00:00 h), there was no way to verify that the treatment was really performed in such a manner and under those precise instructions. Also, even though patients were advised not to have sexual intercourse after starting vaginal progesterone tablets, it was not possible to confirm sexual abstinence during this period. In relation to this, an interesting randomized controlled trial demonstrated that vaginal absorption of progesterone was dramatically reduced in cases of immediate intercourse after vaginal progesterone administration (*Merriam et al., 2015*).

The results of this study have important implications in clinical practice that should be highlighted. First of all, it provides more evidence demonstrating the important effect of serum progesterone concentrations before FET on LBR. As FET is nowadays becoming more widely used in clinical practice (*De Geyter et al., 2018*; *Devesa et al., 2018*; *Moragianni and Penzias, 2010*; *Younis, 2012*), we believe that more efforts should be directed towards improving the chances of success under this approach. Furthermore, the study has shown that certain clinical characteristics of the patient are associated with progesterone concentrations. These findings may help clinicians to personalize the luteal phase support in artificially prepared FET cycles, depending on patient characteristics. In this regard, future research should be directed towards the validation of these results and, more importantly, to individualize endometrial artificial preparation for FET, because so far no cycle regimen has been shown to be superior (*Ghobara et al., 2017*). An interventional study assessing LBR after the addition of more exogenous progesterone when serum concentrations are low before FET would be of remarkable value.

In conclusion, this study confirms previous data showing that serum progesterone concentrations before FET are associated with LBR, and demonstrates that such concentrations depend on certain clinical characteristics of the patient. These findings highlight the importance of future research on the individualization of luteal phase support in artificially prepared FET cycles.

ACKNOWLEDGEMENTS

This research was performed under the auspices of the Càtedra d'Investigació en Obsterícia i Ginecologia of the Department of Obstetrics, Gynecology and Reproductive Medicine, Dexeus University Hospital, Universitat Autònoma de Barcelona.

REFERENCES

Archer, D.F., Fahy, G.E., Viniegra-Sibal, A., Anderson, F.D., Snipes, W., Foldesy, R.G. **Initial and steady-state pharmacokinetics of a vaginally administered formulation of progesterone.** Am. J. Obstet. Gynecol. 1995; 173: 471–478. doi:10.1016/0002–9378(95)90268–6

Asoglu, M.R., Celik, C., Karakis, L.S., Findikli, N., Gultomruk, M., Bahceci, M. **Comparison of daily vaginal progesterone gel plus weekly intramuscular progesterone with daily intramuscular progesterone for luteal phase support in single, autologous euploid frozen-thawed embryo transfers.** J. Assist. Reprod. Genet. 2019; 36: 1481–1487. doi:10.1007/s10815–019–01482–1

Bourdon, M., Santulli, P., Kefelian, F., Vienet-Legue, L., Maignien, C., Pocate-Cheriet, K., de Mouzon, J., Marcellin, L., Chapron, C. **Prolonged estrogen (E2) treatment prior to frozen-blastocyst transfer decreases the live birth rate.** Hum. Reprod. 2018; 33: 905–913. doi:10.1093/humrep/dey041

Brady, P.C., Kaser, D.J., Ginsburg, E.S., Ashby, R.K., Missmer, S.A., Correia, K.F., Racowsky, C. **Serum progesterone concentration on day of embryo transfer in donor oocyte cycles.** J. Assist. Reprod. Genet. 2014; 31: 569–575. doi:10.1007/s10815–014–0199-y

Bungum, L., Franssohn, F., Bungum, M., Humaidan, P., Giwercman, A. **The Circadian Variation in Anti-Müllerian Hormone in Patients with Polycystic Ovary Syndrome Differs Significantly from Normally Ovulating Women.** PLOS ONE 2013; 8: e68223. doi:10.1371/journal.pone.0068223

Cédrin-Durnerin, I., Isnard, T., Mahdjoub, S., Sonigo, C., Seroka, A., Comtet, M., Herbemont, C., Sifer, C., Grynberg, M. **Serum progesterone concentration and live birth rate in frozen–thawed embryo transfers with hormonally prepared endometrium.** Reprod. Biomed. Online 2019; 38: 472–480. doi:10.1016/j.rbmo.2018.11.026

Corleta, H., von, E., Capp, E., Ferreira, M.B.C. **Pharmacokinetics of Natural Progesterone Vaginal Suppository.** Gynecol. Obstet. Invest. 2004; 58: 105–108. doi:10.1159/000078842

Coroleu, B., Barri, P.N., Carreras, O., Martínez, F., Veiga, A., Balasch, J. **The usefulness of ultrasound guidance in frozen-thawed embryo transfer: a prospective randomized clinical trial.** Hum. Reprod. Oxf. Engl. 2002; 17: 2885–2890

De Geyter, Ch., Calhaz-Jorge, C., Kupka, M.S., Wyns, C., Mocanu, E., Motrenko, T., Scaravelli, G., Smeenk, J., Vidakovic, S., Goossens, V., Gliozheni, O., Strohmer, H., Obruca, Kreuz-Kinderwunschzentrum, S.P.G., Petrovskaya, E., Tishkevich, O., Wyns, Christine, Bogaerts, K., Balic, D., Sibincic, S., Antonova, I., Vrcic, H., Ljiljak, D., Pelekanos, M., Rezabek, K., Markova, J., Lemmen, J., Sõritsa, D., Gissler, M., Tiitinen, A., Royere, D., Tandler—Schneider, A., Kimmel, M., Antsaklis, A.J., Loutradis, D., Urbancsek, J., Kosztolanyi, G., Bjorgvinsson, H., Mocanu, Edgar, Scaravelli, Giulia, de Luca, R., Lokshin, V., Ravil, V., Magomedova, V., Gudleviciene, Z., Belo Iopes, G., Petanovski, Z., Calleja-Agius, J., Xuereb, J., Moshin, V., Simic, T.M., Vukicevic, D., Romundstad, L.B., Janicka, A., Calhaz-Jorge, Carlos, Laranjeira, A.R., Rugescu, I., Doroftei, B., Korsak, V., Radunovic, N., Tabs, N., Virant-Klun, I., Saiz, I.C., Mondéjar, F.P., Bergh, C., Weder, M., De Geyter, Christian, Smeenk, J.M.J., Gryshchenko, M., Baranowski, R. **ART in Europe, 2014: results generated from European registries by ESHRE The European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE).** Hum. Reprod. 2018; 33: 1586–1601. doi:10.1093/humrep/dey242

Devesa, M., Tur, R., Rodríguez, I., Coroleu, B., Martínez, F., Polyzos, N.P. **Cumulative live birth rates and number of oocytes retrieved in women of advanced age. A single centre analysis including 4500 women ≥38 years old.** Hum. Reprod. 2018; 33: 2010–2017. doi:10.1093/humrep/dey295

Devroey, P., Polyzos, N.P., Blockeel, C. **An OHSS-Free Clinic by segmentation of IVF treatment.** Hum. Reprod. 2011; 26: 2593–2597. doi:10.1093/humrep/der251

Edelman, A.B., Cherala, G., Stanczyk, F.Z. **Metabolism and pharmacokinetics of contraceptive steroids in obese women: a review.** Contraception 2010; 82: 314–323. doi:10.1016/j.contraception.2010.04.016

Evans, J., Hannan, N.J., Edgell, T.A., Vollenhoven, B.J., Lutjen, P.J., Osianlis, T., Salamonsen, L.A., Rombauts, L.J.F. **Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence.** Hum. Reprod. Update 2014; 20: 808–821. doi:10.1093/humupd/dmu027

Filicori, M., Butler, J.P., Crowley, W.F. **Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion.** J. Clin. Invest. 1984; 73: 1638. doi:10.1172/JCI111370

Fujimoto, V.Y., Clifton, D.K., Cohen, N.L., Soules, M.R. **Variability of serum prolactin and progesterone levels in normal women: The relevance of single hormone measurements in the clinical settings.** Int. J. Gynecol. Obstet. 1991; 34: 287. doi:10.1016/0020–7292(91)90375-F

Gaggiotti-Marre, S., Martinez, F., Coll, L., Garcia, S., Álvarez, M., Parriego, M., Barri, P.N., Polyzos, N., Coroleu, B. **Low serum progesterone the day prior to frozen embryo transfer of euploid embryos is associated with significant reduction in live birth rates.** Gynecol. Endocrinol. 2019; 35: 439–442. doi:10.1080/09513590.2018.1534952

Ghobara, T., Gelbaya, T.A., Ayeleke, R.O. **Cycle regimens for frozen-thawed embryo transfer.** Cochrane Database Syst. Rev. 2017. doi:10.1002/14651858.CD003414.pub3

González-Foruria, I., Rodríguez, I., Martínez, F., Rodríguez-Purata, J., Montoya, P., Rodríguez, D., Nicolau, J., Coroleu, B., Barri, P.N., Polyzos, N.P. **Clinically significant intra-day variability of serum progesterone levels during the final day of oocyte maturation: a prospective study with repeated measurements.** Hum. Reprod. 2019; 34: 1551–1558. doi:10.1093/humrep/dez091

Jones, GS **Luteal phase defect: a review of pathophysiology.** Current opinion in obstetrics and gynecology 1991; 3: 641–648

Kava-Braverman, A., Martínez, F., Rodríguez, I., Álvarez, M., Barri, P.N., Coroleu, B. **What is a difficult transfer? Analysis of 7,714 embryo transfers: the impact of maneuvers during embryo transfers on pregnancy rate and a proposal of objective assessment.** Fertil. Steril. 2017; 107: 657–663. doi:10.1016/j.fertnstert.2016.11.020

Kerkhof, L.W.M.van, Dycke, K.C.G.V., Jansen, E.H.J.M., Beekhof, P.K., Oostrom, C.T.M.van, Ruskovska, T., Velickova, N., Kamcev, N., Pennings, J.L.A., Steeg, H.van, Rodenburg, W. **Diurnal Variation of Hormonal and Lipid Biomarkers in a Molecular Epidemiology-Like Setting.** PLOS ONE 2015; 10e0135652. doi:10.1371/journal.pone.0135652

Labarta, E., Mariani, G., Holtmann, N., Celada, P., Remohí, J., Bosch, E. **Low serum progesterone on the day of embryo transfer is associated with a diminished ongoing pregnancy rate in oocyte donation cycles after artificial endometrial preparation: a prospective study.** Hum. Reprod. 2017; 32: 2437–2442. doi:10.1093/humrep/dex316

Levy, T., Gurevitch, S., Bar-Hava, I., Ashkenazi, J., Magazanik, A., Homburg, R., Orvieto, R., Ben-Rafael, Z. **Pharmacokinetics of natural progesterone administered in the form of a vaginal tablet.** Hum. Reprod. 1999; 14: 606–610. doi:10.1093/humrep/14.3.606

Levy, T., Yairi, Y., Bar–Hava, I., Shalev, J., Orvieto, R., Ben-Rafael, Z. **Pharmacokinetics of the progesterone-containing vaginal tablet and its use in assisted reproduction.** Steroids, Progestins, Progesterone Receptor Modulators, and Progesterone Antagonists 2000; 65: 645–649. doi:10.1016/S0039–128X(00)00121–5

Martinez, F., Rodriguez, I., Devesa, M., Buxaderas, R., Gómez, M.J., Coroleu, B. **Should progesterone on the human chorionic gonadotropin day still be measured?.** Fertil. Steril. 2016; 105: 86–92. doi:10.1016/j.fertnstert.2015.09.008

Merriam, K.S., Leake, K.A., Elliot, M., Matthews, M.L., Usadi, R.S., Hurst, B.S. **Sexual Absorption of Vaginal Progesterone: A Randomized Control Trial.** Int. J. Endocrinol. 2015. doi:10.1155/2015/685281

Moragianni, V., Penzias, A. **Cumulative live-birth rates after assisted reproductive technology. Curr. Opin. Obstet.** Gynecol 2010; 22: 189–192. doi:10.1097/GCO.0b013e328338493f

Murphy, L.A., Seidler, E.A., Vaughan, D.A., Resetkova, N., Penzias, A.S., Toth, T.L., Thornton, K.L., Sakkas, D. **To test or not to test? A framework for counselling patients on preimplantation genetic testing for aneuploidy (PGT-A).** Hum. Reprod. 2019; 34: 268–275. doi:10.1093/humrep/dey346

Neal, S.A., Morin, S.J., Franasiak, J.M., Goodman, L.R., Juneau, C.R., Forman, E.J., Werner, M.D., Scott, R.T. **Preimplantation genetic testing for aneuploidy is cost-effective, shortens treatment time, and reduces the risk of failed embryo transfer and clinical miscarriage.** Fertil. Steril. 2018; 110: 896–904. doi:10.1016/j.fertnstert.2018.06.021

Shapiro, D.B., Pappadakis, J.A., Ellsworth, N.M., Hait, H.I., Nagy, Z.P. **Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts.** Hum. Reprod. 2014; 29: 1706–1711. doi:10.1093/humrep/deu121

Solé, M., Santaló, J., Boada, M., Clua, E., Rodríguez, I., Martínez, F., Coroleu, B., Barri, P.N., Veiga, A. **How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes.** Hum. Reprod. 2013; 28: 2087–2092. doi:10.1093/humrep/det242

Thomsen, L.H., Kesmodel, U.S., Andersen, C.Y., Humaidan, P. **Daytime Variation in Serum Progesterone During the Mid-Luteal Phase in Women Undergoing In Vitro Fertilization Treatment.** Front. Endocrinol. 2018; 9. doi:10.3389/fendo.2018.00092

Venetis, C.A., Kolibianakis, E.M., Bosdou, J.K., Lainas, G.T., Sfontouris, I.A., Tarlatzis, B.C., Lainas, T.G. **Basal serum progesterone and history of elevated progesterone on the day of hCG administration are significant predictors of late follicular progesterone elevation in GnRH antagonist IVF cycles.** Hum. Reprod. 2016; 31: 1859–1865. doi:10.1093/humrep/dew141

Younis, J. **Ovarian aging and implications for fertility female health.** Minerva Endocrinol. 2012; 37: 41–57

Received 20 January 2020; received in revised form 26 February 2020; accepted 3 March 2020.