Journal of Assisted Reproduction and Genetics (2021) 38:2925–2931
https://doi.org/10.1007/s10815-021-02307-w

ASSISTED REPRODUCTION TECHNOLOGIES

Progesterone and estrogen levels are associated with live birth rates following artificial cycle frozen embryo transfers

Ronit Beck-Fruchter[1,2] · Simon Nothman[1,2] · Shira Baram[1] · Yoel Geslevich[1] · Amir Weiss[1,2]

Received: 25 January 2021 / Accepted: 22 August 2021 / Published online: 18 September 2021

Abstract

Purpose Does an association exist between serum progesterone and estradiol levels and live birth rates in artificial cycle frozen embryo transfer (AC-FET)?

Methods Retrospective cohort study was based on prospectively collected data at a university-affiliated fertility center. Included were all cycles using an artificial endometrial preparation with estradiol hemihydrate (Estrofem, 2 mg/8 h) and vaginal progesterone (Endometrin 100 mg/8 h), autologous oocytes, and cleavage stage embryo transfers. Serum progesterone and estradiol levels were measured 14 days after FET. A total of 921 cycles in 568 patients from to December 2010 to June 2019 were investigated. Live birth was the primary outcome measure.

Results Significant association was found between live birth and progesterone as well as estradiol levels (progesterone 14.65 vs 11.62 ng/ml, $p=0.001$; estradiol 355.12 vs 287.67 pg/ml, $p=0.001$). A significant difference in live birth rate was found below and above the median progesterone level (10.9 ng/ml, $p=0.007$). Lower estradiol level was significantly associated with lower live birth rate (< 188.2 pg/ml 8.3%, > 263.1 pg/ml 16%, $p=0.02$).

Conclusions Serum progesterone and estradiol levels impact live birth rate in AC-FET.

Keywords Artificial cycle frozen embryo transfer (AC-FET) · Frozen embryo transfer (FET) · Live birth rate · Progesterone · Estrogen/estradiol

Introduction

The use of frozen embryo transfer cycles (FET) has progressively increased, and its proportion in the USA has doubled from 16 in 2007 to 32.7% in 2016 [1]. Some even advocate freeze all as the preferred treatment protocol [2].

Embryo quality is a key factor affecting assisted reproductive technology (ART) outcome, but successful implantation requires not just good quality embryos but also a receptive endometrium and a synchronized dialogue between the two [3]. Endometrial receptivity is achieved after sequential actions of estrogen and progesterone, either naturally secreted by the ovary or exogenously supplied. Several methods of endometrium preparation for FET have been developed, and regarding live birth rate, none has proven superior to others, although stimulated cycles may improve clinical pregnancy rate [4].

A common and effective method for endometrial preparation prior to frozen embryo transfer is a sequential regimen with estrogen and progesterone, aiming to mimic the endocrine exposure of the endometrium in a natural cycle. It is usual to administer estrogen until sufficient endometrial thickness is reached and then to add progesterone for the number of days as determined by the stage of development of the embryo being transferred [5]. It is assumed that this regimen results in receptive endometrium; however, this may not always be true. Even when euploid embryos are transferred, many of the cycles do not result in successful pregnancy (71% pregnancy rates, 57% live birth rate) [6], which may result from inappropriate endometrial receptivity.

Different routes and doses of estrogen administration have been used in order to provide adequate endometrial preparation [4]. Estrogen biology is complex, with multiple factors impacting estradiol kinetics (e.g., genetic variation, exposure to estrogen-like chemicals), which might cause

✉ Ronit Beck-Fruchter
Beck_r@technion.ac.il

[1] Fertility Unit, Department of Obstetrics and Gynecology, Emek Medical Center, Afula, Israel

[2] Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

personal variations in the biological targets affected by the hormone [7].

Progesterone and progestins can be administered by intramuscular, vaginal, oral, and rectal routes and recently also subcutaneously [1, 8]. Different progesterone levels and endometrial effect might result from the diverse administration routes and preparations, drug interaction, hormonal milieu, compliance, and metabolism. Vaginal progesterone leads to lower serum progesterone levels [9]. Whether those levels are related to the treatment outcome is of clinical importance.

Recent findings suggested that there is a minimum threshold of serum P levels that needs to be reached in order to obtain the best pregnancy results, and not all patients achieve this optimal level [10].

The aim of the present study was to investigate the impact of serum progesterone and estradiol levels on reproductive outcomes in AC-FET using oral estrogen and vaginal progesterone.

Materials and methods

Retrospective cohort study based on prospectively collected data on AC-FET cycles was performed from December 2010 to June 2019. The study was approved by the hospital's institutional review board.

All cycles of patients undergoing FET using autologous oocytes on the protocol detailed below were screened. Patients without hormonal tests results 14 days after the embryo transfer were excluded from the study.

The primary endpoint was live birth. The secondary endpoints were pregnancy and implantation rate (the number of gestational sacs observed divided by the number of embryos transferred).

Treatment protocol Endometrial preparation started on days 1–3 of menstruation with oral administration of estradiol hemihydrate (Estrofem®, NOVO NORDISK A/S, DENMARK), 6 mg/day. After 8–10 days on estrogens, a vaginal 2D ultrasound was performed. Patients were considered ready for embryo transfer when endometrial thickness was ≥ 7 mm with triple layer pattern and ultrasound detected quiescent ovaries. If endometrium thickness was < 7 mm, estrogen therapy was prolonged and/or dose increased. At the time of ultrasound, if the endometrium is of a proliferative tri-phasic pattern and the ovaries are devoid of follicular or luteal cysts, progesterone is commenced, and embryo transfer is scheduled. P was administered 48–72 h before ET, corresponding with the age of the embryo (2–3 days). Progestogen supplementation was performed with vaginal tablets (ENDOMETRIN® Vaginal Insert, Ferring Pharmaceuticals Ltd, Israel) at a dose of 100 mg/8 h. Embryo transfer was performed under ultrasound guidance by one of 6 specialist physicians. No pretreatment (e.g., GnRH agonist, combined oral contraceptive) was used. Embryo scoring was performed on days 2 and 3 after OPU according to the scoring criteria of Alpha and ESHRE [11]; grade 1 embryos were considered of good quality. The freezing technique before 2017 was slow freeze. After 2019 the technique was vitrification. During the transition period between 2017 and 2019, some embryos underwent slow freeze, while others were frozen by vitrification.

Serum progesterone and estradiol levels were measured 14 days after FET using commercially available kits (ADVIA Centaur, Siemens). The ADVIA Centaur Progesterone assay measures progesterone concentrations up to 60 ng/mL with a minimum detectable concentration (analytical sensitivity) of 0.21 ng/mL. The ADVIA Centaur Enhanced Estradiol assay measures estradiol concentrations up to 3000 pg/mL. The functional sensitivity of the ADVIA Centaur Enhanced Estradiol assay is 19 pg/mL.

In conceptus cycles, the hormonal treatment was maintained until the 12th gestational week.

Statistical methods Continuous data were presented as mean and standard deviation (median and range) and categorical data as number and percent. Patient parameters were assessed via *t*-test or Mann–Whitney *U* test in the case of non-normally distributed data for continuous data or χ^2 or Fisher's exact tests where appropriate for categorical data. Generalized estimating equations were performed to account for multiple cycles from the same patient.

Generalized estimating equation model for a repeated measures logistic regression using GEE was performed in order to test whether progesterone or estradiol levels predicted live births or other secondary outcome measures.

Results

A total of 1344 FET cycles, in 747 patients, using the medical regimen investigated were performed during the study period. Of these, 921 cycles in 568 patients had progesterone/estradiol data and were included in the analysis.

The mean age of patients at freezing was 30.8 ± 5.6 [29.9; 20.0–45.0], and the mean age at study entry was 31.8 ± 5.7 [31.1; 20.5–49.6] years. The mean BMI was 25.53 ± 5.45 [24.38; 15.62–51.27] kg/m^2 (Table 1). The mean number of embryos transferred was 1.9 ± 0.7 [2; 1–4]. Two-thirds of the transfers were of day 2 embryos, and the rest were day 3.

Two hundred and seventy-three cycles (29.6%; 95% CI: 26.7–32.7%) resulted in a pregnancy (in 235 patients) and 124 (13.5%; 11.3–15.8%) in a live birth. Biochemical

Table 1 Patient demographics and cycle characteristics ($N=568$)

Age at study entry (years)	31.8 ± 5.7
Age at freezing (years)	30.8 ± 5.6
Infertility duration (years)	2.9 ± 2.4
BMI	25.5 ± 5.4
Gravidity	1.1 ± 1.2
Parity	0.5 ± 0.7
Endometrial thickness (mm)	8.9 ± 1.7
At least one good quality embryo transferred	330 (64.3)
Number of embryos transferred	1.9 ± 0.7 [2; 1–4]

Continuous data is mean ± sd; binary data is *N* (%)

pregnancy occurred in 74 treatment cycles (8.0%), 66 (7.2%) of pregnancies resulted in a miscarriage, 3 pregnancies were terminated, and in 6 (0.7%), an ectopic pregnancy was diagnosed. The implantation rate was 10.9% (95% CI: 9.2–12.6%). There was no statistically significant difference in the rate of live births over the study period ($\chi^2=5.44$, p = 0.66).

Live birth was associated with maternal age (30.8 vs 31.8 years, $p=0.052$), age at freezing (29.5 vs 30.7 years, $p=0.02$), and the transfer of at least one good quality embryo ($p=0.001$). No association was found between live birth and endometrial thickness (8.91 vs 8.88 mm). Significant association was found between live birth and progesterone as well as estradiol levels (progesterone 14.65 vs 11.62 ng/ml, $p=0.001$; estradiol 355.12 vs 287.67 pg/ml, $p=0.001$) (Table 2). For every 1 ng/ml increase in progesterone level, the odds of a live birth increased by 4%, and for every 10 pg/ml increase in estradiol level, the odds of a live birth increased by 1.7%.

When a multivariate analysis was performed, after adjusting for infertility etiology, age at freezing, and transfer of at least one good embryo, progesterone remained associated with live birth ($\chi^2=15.14$, $p<0.001$; OR: 1.05, 95% CI: 1.03–1.08). With the addition of estradiol to the model, both progesterone and estradiol were associated with live birth ($\chi^2=9.88$, $p<0.002$; OR: 1.05, 95% CI: 1.02–1.08; $\chi^2=8.30$, $p<0.004$; for 10-point increase OR: 1.02, 95% CI: 1.005–1.027, respectively).

Spearman correlation revealed that progesterone level was statistically significantly negatively correlated with BMI ($p<0.001$). This was not the case for estradiol.

To further explore correlation between progesterone and estradiol levels and live births, serum levels of progesterone and estradiol were classified into four quartiles according to the 25th, 50th, and 75th percentiles. The serum *P* intervals for each quartile were Q1: < 8.35 ng/ml, Q2: 8.35–10.9 ng/ml, Q3: 10.91–14 ng/ml, and Q4: > 14 ng/ml. Implantation and pregnancy rates were statistically significantly lower for the Q1 as compared to the other quartiles ($p=0.004$, $p=0.02$, respectively). Regarding live birth, a significant difference was found

Table 2 Patient and treatment parameters by live birth

	Live birth	No live birth	χ^2	P	*OR (95% CI)*
Patient no	120	448			
Treatment cycle no	$N=124$	$N=797$			
Maternal age (years)	30.8 ± 5.3	31.8 ± 5.4	3.79	0.052	0.96 (0.93–1.00)
Age at freezing (years)	29.5 ± 5.1	30.7 ± 5.5	5.73	0.02	0.96 (0.92–0.99)
Infertility duration (years)	2.9 ± 2.1	2.9 ± 2.1	0.08	0.77	0.99 (0.91–1.07)
BMI	25.6 ± 5.7	25.5 ± 5.2	0.04	0.85	1.00 (0.97–1.04)
Gravidity	1.1 ± 1.2	1.3 ± 1.4	1.72	0.19	0.91 (0.80–1.05)
Parity	0.6 ± 0.7	0.6 ± 0.7	1.05	0.31	1.12 (0.90–1.41)
Endometrial thickness (mm)	8.9 ± 1.5	8.8 ± 1.6	0.04	0.84	1.01 (0.91–1.12)
Embryo stage at freezing					
48 h	88 (72.4)	521 (65.4)	2.21	0.14	1.00 (reference)
72 h	34 (27.6)	276 (34.6)			1.39 (0.90–2.13)
At least one good quality embryo	98 (79.7)	510 (64.5)	10.67	0.001	2.16 (1.36–3.43)
Number of embryos transferred	2.1 ± 0.7	2.0 ± 0.7	1.86	0.17	1.22 (0.92–1.62)
FSH (IU/L)	6.7 ± 2.6 (6.50; 2.60–21.81)	6.4 ± 2.4 (6.15; 0.03–34.79)	1.16	0.28	1.04 (0.97–1.13)
Progesterone level (ng/ml)	14.6 ± 9.4	11.6 ± 6.7	14.35	0.001	1.04 (1.02–1.07)
Estradiol level (pg/ml)	355.1 ± 229.5	287.6 ± 229.5	13.06	0.001	1.002 (1.001–1.003)

[1]Some patients appear in the live and non-live columns; otherwise, each patient appears once regardless of number of cycles

Continuous data is mean ± sd; binary data is *N* (%)

below and above the median (≤ 10.9 ng/ml, 10.9%; > 10.9 16.7%; $p = 0.007$) (Table 3).

The serum estradiol intervals were Q1: < 188.2 pg/ml, Q2:188.2–263.0 ng/ml, Q3: 263.1–364.5 ng/ml, and Q4 > 364.6 ng/ml). The implantation rate was statistically significantly lower for the Q1 of estradiol as compared to the fourth quartile ($p = 0.05$). The pregnancy rate was statistically significantly lower for the Q1 as compared to the other quartiles ($p = 0.02$). The live birth rate was statistically significantly lower for the Q1 as compared to the third and fourth quartiles (< 188.2 ng/ml, 8.3%; > 263.1 ng/ml, 16%, $p = 0.02$) (Table 4).

Seventy-seven patients had both progesterone and estradiol levels in the lower quartile; only one of them had a live birth (1.3%). Among the 65 patients with both values in the upper quartile, 16 (24.6%) gave birth ($p < 0.001$).

To define the predictive capability of serum progesterone and estradiol on the live birth, a receiving operating characteristic (ROC) curve was defined. Progesterone level above 10.71 correctly identified 62.5% of the live births (78 of the 124 live births); overall it classifies correctly 52.1% of the

Table 3 Quartiles of progesterone

Quartile	Q1	Q2	Q3	Q4	*p*
Progesterone ng/ml	< 8.35	8.35–10.90	10.91–14.00	> 14.00	
n	230	231	230	230	
Embryo stage at freezing					
48 h	143 (62.2)	142 (61.4)	166 (72.2)	159 (69.4)	0.05[#]
72 h	87 (37.8)	89 (38.6)	64 (27.8)	70 (30.6)	
At least one good quality embryo	150 (65.8)	151 (65.4)	159 (70.0)	148 (64.9)	0.63
Number of embryos transferred	2.0 ± 0.7 (1.9–2.1)	2.0 ± 0.7 (2.0–2.1)	2.0 ± 0.6 (1.9–2.1)	2.0 ± 0.6 (1.9–2.1)	0.61
Implantation rate	6.6 (4.0–9.2)	9.9 (6.6–13.2)	12.0 (8.5–15.6)	15.1 (10.9–19.3)	0.004*
Pregnancy	48 (20.9%)	72 (31.2%)	74 (32.2%)	79 (34.3%)	0.02[*]
Biochemical pregnancy	13 (5.7%)	25 (10.8%)	17 (7.4%)	19 (8.3%)	0.23
Clinical miscarriage	17 (7.4%)	13 (5.6%)	22 (9.6%)	14 (6.1%)	0.36
Live birth	18 (7.8%)	29 (12.6%)	32 (13.9%)	45 (19.6%)	0.007

Continuous data is mean ± sd [median, range]; binary data is *N* (%)

[#]Q1 as compared to Q3

*Q1 as compared to the other quartiles

Table 4 Quartiles of estradiol

Quartile	Q1	Q2	Q3	Q4	*p*
Estradiol (pg/ml)	< 188.2	188.2–263.0	263.1–364.5	> 364.6	
n	230	230	230	230	
Embryo stage at freezing					
48 h	162 (70.4)	154 (67.3)	142 (61.7)	151 (65.7)	0.26
72 h	68 (29.6)	75 (32.7)	88 (38.3)	79 (34.3)	
At least one good quality embryo	149 (65.6)	150 (65.5)	162 (71.1)	146 (63.8)	0.39
Number of embryos transferred	2.0 ± 0.7 (1.9–2.0)	2.0 ± 0.7 (1.9–2.1)	2.0 ± 0.7 (1.9–2.1)	2.0 ± 0.6 (2.0–2.1)	0.50
Implantation rate	7.5 (4.3–10.6)	10.1 (6.9–13.3)	11.6 (8.0–15.3)	14.5 (10.7–18.3)	0.05*
Pregnancy	50 (21.7%)	68 (29.6%)	76 (33.0%)	79 (34.3%)	0.02[#]
Biochemical pregnancy	21 (9.1%)	14 (6.1%)	18 (7.8%)	21 (9.1%)	0.60
Clinical miscarriage	7 (3.0%)	19 (8.3%)	25 (10.9%)	15 (6.5%)	0.01^
Live birth	19 (8.3%)	30 (13.0%)	33 (14.3%)	42 (18.3%)	0.02[&]

Continuous data is mean ± sd [median, range]; binary data is *N* (%)

*Q1 as compared the Q4

[#]Q1 as compared to the other quartiles

^Q1 as compared to the second and third quartiles

[&]Q1 as compared to the third and fourth quartiles

cases. Likewise, estradiol level above 259.4 correctly identified 61.3% of the live births (76/124); overall it classifies 51.6% of the cases.

Discussion

The current study found a significant correlation between serum progesterone and estradiol levels 2 weeks after embryo transfer and live birth rate in AC-FET. A similar correlation was noted for pregnancy and implantation rate. Live birth rate was significantly lower in cycles in which the progesterone level was below 10.9 ng/ml or the estradiol level was below 188.2 pg/ml. When progesterone and estradiol levels were both in the lower quartiles, the live birth rate was negligible.

During the natural cycle, measuring the serum progesterone level is of limited value, due to the pulsatile release of progesterone from the corpus luteum, echoing the pulsatile release of LH from the pituitary. Serum progesterone levels can fluctuate eightfold in a 90-min period during the mid-luteal phase and range from 2.3 to 40.1 pg/mL [12]. In fresh ART cycles, involving controlled ovarian hyperstimulation and oocyte aspiration, the luteal phase is dysfunctional, but the presence of corpora lutea with pulsatile, although defective, progesterone release mitigates the usefulness of serum progesterone measurements. In AC-FET, no CL is formed; thus, there is no endogenous source of progesterone, so measuring its serum levels represent the absorption, distribution, and metabolism of the drug and may be beneficial.

Few studies investigated the correlation between progesterone levels in AC-FET and clinical outcome. The treatment protocols, the medical preparation, and the administration route vary between studies and so do their conclusions. When using vaginal micronized progesterone, lower pregnancy rates in correlation with lower progesterone levels were observed by some [13], but not by others [14–16]. Additionally, lower progesterone levels were correlated with lower implantation rates [15], higher miscarriage rates [14, 16, 17], lower ongoing pregnancy rates [13, 15, 18, 19], and lower live birth rates [20–22]. Under vaginal micronized progesterone treatment, levels between 8.8 and 11 ng/l were offered as cut-offs [13–15, 21]. Optimal progesterone levels between 22 and 31 ng/l were reported when using progesterone pessary [23]. When intramuscular progesterone was used for luteal support, high levels of progesterone (> 20 ng/ml) were correlated with higher clinical pregnancy and live birth rate in some studies [24–26]but with lower ongoing pregnancy and live birth rate in another [27].

The effect of BMI on serum progesterone concentrations was studied previously. Levy et al. found no significant difference in pharmacokinetics in relation to weight, height, or BMI in post-menopausal women [28]. However, others found a negative correlation between progesterone levels and BMI when administered either via intramuscular or vaginal route [18–20, 24]. We describe similar results, showing that BMI is an independent factor that affects serum progesterone concentrations. This might be one of the mechanisms explaining the known negative correlation between BMI and live birth rate [6].

The timing of the progesterone measurements differs between the studies. In our study, the assay was performed 2 weeks after embryo transfer. When administered vaginally, progesterone is preferentially absorbed by uterine endometrial tissue, whereas a small percentage is distributed into the systemic circulation. On multiple dosing, steady-state concentrations are attained within approximately 1 day after initiation of treatment [29, 30]. For this reason, the diversity in progesterone values between the different studies cannot be explained by the variability in the assay timing. The wide range of progesterone serum values between different patients may be explained by the inter-individual variability in vaginal progesterone uptake, distribution, and metabolism that have been described previously [31, 32].

Data about estradiol levels and clinical outcome in AC-FET cycles are conflicting. Contrary to our findings, other studies found no correlation between estradiol levels in AC-FET cycles and clinical outcomes [13, 18, 33–35]. Moreover, one study suggested that elevated E2 levels in artificial autologous FET cycles are associated with lower ongoing pregnancy and live birth rates [36]. The inconsistent results may be due to the different regimes used (e.g., GnRH agonist down regulation, monitoring estradiol levels, and adjusting the dose or canceling the cycle with low levels). It is also important to note that estradiol and progesterone levels are not independent variables, rather adequate estrogen levels increase vaginal progesterone absorption, further confounding the picture [37].

Since endogenous progesterone and estradiol levels in conceptus cycles do not start to rise before 16 days after embryo transfer [38], the levels measured in this study represent the sole effect of the medication and the personal uptake and metabolism of the patient.

The current study adds to the knowledge gathered recently regarding the importance of luteal phase monitoring in AC-FET. Strengths of this study are the inclusion of a large number of ART cycles and the setting of live birth outcomes as the primary outcome. It presents the outcome of a single uniform FET protocol, with cleavage stage embryos. Cleavage stage embryos are still used worldwide[39] as clear benefit of blastocyst transfer, especially in frozen cycles, has not been demonstrated [4]. The main limitations of the study are its retrospective design and covering a long period.

In summary, low serum progesterone and estradiol levels in AC FET cycles may negatively impact the clinical outcome. Optimization of ART success rates relies not only on

the creation of high-quality embryos but also on the establishment of a receptive endometrium. Although significant research has been directed toward optimization of AC-FET cycles, the ideal protocol has not been identified yet. The reason might be that no single regimen fits all, requiring a personalized approach. Measuring progesterone and estradiol levels and adjusting the treatment accordingly might improve the clinical outcome of AC-FET. Larger prospective studies are needed to confirm our results and further elucidate the optimal way to proceed in the presence of low serum hormonal levels.

Contributions Ronit Beck Fruchter and Amir Weiss contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ronit Beck Fruchter, Simon Nothman, Shira Baram, Yoel Geslevich and Amir Weiss. The first draft of the manuscript was written by Ronit Beck Fruchter and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Code availability Not applicable.

Declarations

Ethics approval This research study was conducted retrospectively from data obtained for clinical purposes. Approval was obtained from the ethics committee of HaEmek medical center.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Turkgeldi E, Hanege BY, Yildiz S, Keles I, Ata B. Subcutaneous versus vaginal progesterone for vitrified–warmed blastocyst transfer in artificial cycles. Reprod Biomed Online. 2020;41:248–53. https://doi.org/10.1016/j.rbmo.2020.04.007.
2. Vuong LN, Dang VQ, Ho TM, Huynh BG, Ha DT, Pham TD, et al. IVF transfer of fresh or frozen embryos in women without polycystic ovaries. N Engl J Med. 2018;378:137–47. https://doi.org/10.1056/nejmoa1703768.
3. Simón C, Martín JC, Pellicer A. Paracrine regulators of implantation. Bailliere's Best Pract Res Clin Obstet Gynaecol. 2000. https://doi.org/10.1053/beog.2000.0121.
4. Glujovsky D, Pesce R, Sueldo C, QuinteiroRetamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2020;10:CD006359. https://doi.org/10.1002/14651858.CD006359.pub3.
5. Mackens S, Santos-Ribeiro S, van de Vijver A, Racca A, Van Landuyt L, Tournaye H, et al. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. Hum Reprod. 2017;32:2234–42. https://doi.org/10.1093/humrep/dex285.
6. Boynukalin FK, Gultomruk M, Cavkaytar S, Turgut E, Findikli N, Serdarogullari M, et al. Parameters impacting the live birth rate per transfer after frozen single euploid blastocyst transfer. PLoS ONE. 2020;15:1–15. https://doi.org/10.1371/journal.pone.0227619.
7. Sier JH, Thumser AE, Plant NJ. Linking physiologically-based pharmacokinetic and genome-scale metabolic networks to understand estradiol biology. BMC Syst Biol. 2017;11:1–16. https://doi.org/10.1186/s12918-017-0520-3.
8. Labarta E, Rodríguez C. Progesterone use in assisted reproductive technology. Best Pract Res Clin Obstet Gynaecol. 2020. https://doi.org/10.1016/j.bpobgyn.2020.05.005.
9. Miles RA, Paulson RJ, Lobo RA, Press MF, Dahmoush L, Sauer MV. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. Fertil Steril. 1994;62:485–90. https://doi.org/10.1016/s0015-0282(16)56935-0.
10. Alsbjerg B, Labarta E, Humaidan P. Serum progesterone levels on day of embryo transfer in frozen embryo transfer cycles—the truth lies in the detail. J Assist Reprod Genet. 2020;37:2045–6. https://doi.org/10.1007/s10815-020-01851-1.
11. Balaban B, Brison D, Calderón G, Catt J, Conaghan J, Cowan L, et al. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26:1270–83. https://doi.org/10.1093/humrep/der037.
12. Mesen TB, Young SL. Progesterone and the luteal phase: a requisite to reproduction. Obstet Gynecol Clin North Am. 2015. https://doi.org/10.1016/j.ogc.2014.10.003.
13. Cédrin-Durnerin I, Isnard T, Mahdjoub S, Sonigo C, Seroka A, Comtet M, et al. Serum progesterone concentration and live birth rate in frozen–thawed embryo transfers with hormonally prepared endometrium. Reprod Biomed Online. 2019;38:472–80. https://doi.org/10.1016/j.rbmo.2018.11.026.
14. Gaggiotti-Marre S, Martinez F, Coll L, Garcia S, Álvarez M, Parriego M, et al. 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;35:439–42. https://doi.org/10.1080/09513590.2018.1534952.
15. Alsbjerg B, Thomsen L, Elbaek HO, Laursen R, Povlsen BB, Haahr T, et al. Progesterone levels on pregnancy test day after hormone replacement therapy-cryopreserved embryo transfer cycles and related reproductive outcomes. Reprod Biomed Online. 2018;37:641–7. https://doi.org/10.1016/j.rbmo.2018.08.022.
16. Commissaire M, Epelboin S, Vigan M, Tubiana S, Llabador MA, Gauché-Cazalis C, et al. Serum progesterone level and ongoing pregnancy rate following frozen-thawed embryo transfer after artificial endometrial preparation: a monocentric retrospective study. J Gynecol Obstet Hum Reprod. 2020. https://doi.org/10.1016/j.jogoh.2020.101828.
17. Basnayake SK, Volovsky M, Rombauts L, Osianlis T, Vollenhoven B, Healey M. Progesterone concentrations and dosage with frozen embryo transfers – what's best? Aust New Zeal J Obstet Gynaecol. 2018;58:533–8. https://doi.org/10.1111/ajo.12757.
18. 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;32:2437–42. https://doi.org/10.1093/humrep/dex316.
19. Labarta E, Mariani G, Paolelli S, Rodriguez-Varela C, Vidal C, Giles J, et al. Impact of low serum progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study in artificial cycles with vaginal progesterone. Hum Reprod. 2021;36:683–92. https://doi.org/10.1093/humrep/deaa322.
20. González-Foruria I, Gaggiotti-Marre S, Álvarez M, Martínez F, García S, Rodríguez I, et al. Factors associated with serum

progesterone concentrations the day before cryopreserved embryo transfer in artificial cycles. Reprod Biomed Online. 2020;40:797–804. https://doi.org/10.1016/j.rbmo.2020.03.001.
21. Labarta E, Mariani G, Paolelli S, Rodriguez-Varela C, Vidal C, Giles J, Bellver J, Cruz F, Marzal A, Celada P, Olmo I, Alamá P, Remohi J, Bosch E. Impact of low serum progesterone levels on the day of embryo transfer on pregnancy outcome: a prospective cohort study in artificial cycles with vaginal progesterone. Hum Reprod. 2021;36(3):683–92. https://doi.org/10.1093/humrep/deaa322.
22. Volovsky M, Pakes C, Rozen G, Polyakov A. Do serum progesterone levels on day of embryo transfer influence pregnancy outcomes in artificial frozen-thaw cycles? J Assist Reprod Genet. 2020;37:1129–35. https://doi.org/10.1007/s10815-020-01713-w.
23. Yovich JL, Conceicao JL, Stanger JD, Hinchliffe PM, Keane KN. Mid-luteal serum progesterone concentrations govern implantation rates for cryopreserved embryo transfers conducted under hormone replacement. Reprod Biomed Online. 2015;31:180–91. https://doi.org/10.1016/j.rbmo.2015.05.005.
24. Brady PC, Kaser DJ, Ginsburg ES, Ashby RK, Missmer SA, Correia KF, et al. Serum progesterone concentration on day of embryo transfer in donor oocyte cycles. J Assist Reprod Genet. 2014;31:569–75. https://doi.org/10.1007/s10815-014-0199-y.
25. Boynukalin FK, Gultomruk M, Turgut E, Demir B, Findikli N, Serdarogullari M, et al. Measuring the serum progesterone level on the day of transfer can be an additional tool to maximize ongoing pregnancies in single euploid frozen blastocyst transfers. Reprod Biol Endocrinol. 2019;17:1–8. https://doi.org/10.1186/s12958-019-0549-9.
26. Liu YF, Wang F, Huang GY, Mao XG. The relationship between the hormone levels before transplantation and the outcomes of hormone replacement therapy frozen embryo transfer. Minerva Endocrinol. 2018. https://doi.org/10.23736/S0391-1977.17.02660-8.
27. Kofinas JD, Blakemore J, McCulloh DH, Grifo J. Serum progesterone levels greater than 20 ng/dl on day of embryo transfer are associated with lower live birth and higher pregnancy loss rates. J Assist Reprod Genet. 2015;32:1395–9. https://doi.org/10.1007/s10815-015-0546-7.
28. Levy T, Gurevitch S, Hava B, Ashkenazi J, Magazanik A, Homburg R, et al. Pharmacokinetics of natural progesterone administered in the form of a vaginal tablet. Hum Reprod. 1999;14:606–10. https://doi.org/10.1093/humrep/14.3.606.
29. Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. Maturitas. 1993;16:185–202. https://doi.org/10.1016/0378-5122(93)90064-O.
30. Paulson RJ, Collins MG, Yankov VI. Progesterone pharmacokinetics and pharmacodynamics with 3 dosages and 2 regimens of an effervescent micronized progesterone vaginal insert. J Clin Endocrinol Metab. 2014;99:4241–9. https://doi.org/10.1210/jc.2013-3937.
31. Archer DF, Fahy GE, Viniegra-Sibal A, Anderson FD, Snipes W, Foldesy RG. Initial and steady-state pharmacokinetics of a vaginally administered formulation of progesterone. Am J Obstet Gynecol. 1995;173:471–8. https://doi.org/10.1016/0002-9378(95)90268-6.
32. Wu G, Chen J, Hu X, Zhou H, Liu J, Lv D, et al. Pharmacokinetic properties of three forms of vaginal progesterone administered in either single or multiple dose regimen in healthy post-menopausal Chinese women. Front Pharmacol. 2017;8:1–9. https://doi.org/10.3389/fphar.2017.00212.
33. Mackens S, Santos-Ribeiro S, Orinx E, De Munck N, Racca A, Roelens C, et al. Impact of serum estradiol levels prior to progesterone administration in artificially prepared frozen embryo transfer cycles. Front Endocrinol (Lausanne). 2020;11:1–8. https://doi.org/10.3389/fendo.2020.00255.
34. Remohi J, Ardiles G, Garcia-Velasco JA, Gaitán P, Simón C, Pellicer A. Endometrial thickness and serum oestradiol concentrations as predictors of outcome in oocyte donation. Hum Reprod. 1997;12:2271–6. https://doi.org/10.1093/humrep/12.10.2271.
35. Bocca S, Real EB, Lynch S, Stadtmauer L, Beydoun H, Mayer J, et al. Impact of serum estradiol levels on the implantation rate of cleavage stage cryopreserved-thawed embryos transferred in programmed cycles with exogenous hormonal replacement. J Assist Reprod Genet. 2015;32:395–400. https://doi.org/10.1007/s10815-014-0402-1.
36. Fritz R, Jindal S, Feil H, Buyuk E. Elevated serum estradiol levels in artificial autologous frozen embryo transfer cycles negatively impact ongoing pregnancy and live birth rates. J Assist Reprod Genet. 2017;34:1633–8. https://doi.org/10.1007/s10815-017-1016-1.
37. 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. 2000;65:645–9. https://doi.org/10.1016/S0039-128X(00)00121-5.
38. Neumann K, Depenbusch M, Schultze-Mosgau A, Griesinger G. Characterization of early pregnancy placental progesterone production by use of dydrogesterone in programmed frozen–thawed embryo transfer cycles. Reprod Biomed Online. 2020;40:743–51. https://doi.org/10.1016/j.rbmo.2020.01.019.
39. Neuhausser WM, Neuhausser WM, Neuhausser WM, Neuhausser WM, Neuhausser WM, Vaughan DA, et al. Non-inferiority of cleavage-stage versus blastocyst-stage embryo transfer in poor prognosis IVF patients (PRECiSE trial): study protocol for a randomized controlled trial. Reprod Health. 2020;17:1–10. https://doi.org/10.1186/s12978-020-0870-y.