Comparison of two endometrial preparation methods for frozen-thawed embryo transfer in anovulatory PCOS patients: impact on miscarriage rate.

Virginie Simon, Geoffroy Robin, Christine Decanter, Didier Dewailly, Sophie Jonard-Catteau, Anne-Laure Barbotin, Pauline Plouvier

Comparison of two endometrial preparation methods for frozen-thawed embryo transfer in anovulatory PCOS patients: impact on miscarriage rate.

Virginie Simon[a], Geoffroy Robin[a,b], Christine Decanter[a] , Didier Dewailly[b], Sophie Catteau-Jonard[a,b], Anne-Laure Barbotin[a,b], Pauline Plouvier[a]

Where the work was done: *Endocrine Gynaecology and Reproductive Medicine Center of the Lille University Hospital (France)*

Commercial product mentioned in title: none

Financial support: none

Department affiliations:

[a:] Lille University Hospital, Department of Reproductive Medicine, Hospital Jeanne de Flandre, F- 59000 Lille, France.

[b:] University of Lille, F-59000, Lille, France

Corresponding author: Virginie Simon, Service de Gynécologie Endocrinienne et Médecine de la Reproduction, Hôpital Jeanne de Flandre, Avenue Eugène Avinée, 59037 Lille, France. (phone: +33 668029686 ; e-mail: virginie1.simon@chu-lille.fr)

Comparison of two endometrial preparation methods for frozen-thawed embryo transfer in anovulatory PCOS patients: impact on miscarriage rate.

ABSTRACT

OBJECTIVE - Some studies have suggested that patients with polycystic ovary syndrome (PCOS) are at high risk of miscarriage. However, this still remains controversial. Several potential factors might explain this association: obesity, hyperinsulinemia and hyperandrogenism. Artificial and stimulated cycles appear to be comparable for endometrial preparation in frozen-thawed embryo transfer (FET) in PCOS patients. Only a few studies have assessed miscarriage rates specifically in PCOS. We have evaluated the impact of endometrial preparation on FET outcomes in anovulatory PCOS patients.

METHODS – A retrospective cohort study was conducted at the Lille University Hospital, including 255 FET cycles in 134 PCOS patients between January 2011 and December 2017. PCOS was defined by the presence of at least two of the three Rotterdam's criteria. Patients were under 35 years old. Two endometrial preparation protocol were studied: stimulated cycle (gonadotropins on the second day of the cycle and luteal phase support including natural progesterone 600 mg/day) and artificial cycle (6 mg oral estradiol valerate and 800 mg micronized vaginal progesterone daily).

RESULTS - 137 FET were performed under stimulated cycle and 118 FET under artificial cycle. Early pregnancy rates (30% versus 37.3%, p = NS), miscarriage rates (22% versus 25%, p = NS) and live birth rates (23.4% versus 26.3%, p = NS) were similar.

CONCLUSIONS - In anovulatory PCOS women, the type of endometrial preparation does not influence FET outcomes, specifically regarding the miscarriage rate.

KEYWORDS Polycystic ovary syndrome, frozen-thawed embryo transfer, endometrial preparation, miscarriage rate.

INTRODUCTION

Since the first frozen-thawed embryo transfer (FET) in 1983 [1], embryo cryopreservation has become a common practice in assisted reproductive technology (ART). According to the ESHRE report published in 2020, FET is the second most widely used technique in ART after in vitro fertilization with intracytoplasmic sperm microinjection (IVF-ICSI) [2].

Many studies have compared the pregnancy outcomes after fresh embryo transfer versus frozen-thawed embryo transfer (FET) and found similar pregnancy rates in both groups [3–6].

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5 to 20% of the female population [7].

Patients with PCOS are at high risk of ovarian hyperstimulation syndrome (OHSS) and a freeze-all strategy with the use of FET reduces that risk.

In order to maximize the chances of pregnancy during a frozen-thawed embryo transfer (FET), the embryonic development and endometrial growth must be synchronized. Several methods are available for endometrial preparation. The artificial cycle is the most commonly used. The other two endometrial preparation protocols used are stimulated cycle and natural spontaneous cycle. However, the natural cycle can only be used in patients with regular menstruations. Although, the advantage of one treatment over the other remains controversial. Many studies have not found any significant difference between artificial and stimulated cycle

[8–13]. Two recent reviews concluded that there is no consistent evidence to support the benefit of using one treatment over the other [14,15].

Most of studies have been focused on patients with regular normo-ovulatory cycles and few of them have specifically evaluated these methods in anovulatory PCOS patients. However, in patients with cycle disorders, only stimulated and artificial cycles can be performed. A meta-analysis recently published has specifically focused its attention on the endometrial preparation protocols for FET in PCOS patients and found no difference between artificial and stimulated cycles [16].

Some studies have suggested that PCOS is associated with a higher rate of early spontaneous miscarriage [17–21]. This association could be explained by insulin resistance and sometimes high BMI [22–24] and hyperandrogenism. However, this association still remains controversial [25]. A meta-analysis including 9 studies found no difference in terms of early spontaneous miscarriage rates between PCOS and non-PCOS patients [26].

The objective of this study was to evaluate the miscarriage rate following different type of endometrial preparation protocol in anovulatory PCOS patients.

MATERIEL AND METHODS

Subjects

This is a retrospective study from prospectively collected datas of the French national ART registery named “JFIV” performed in the Reproductive Medicine Department of Lille University Hospital on 134 PCOS patients. As this study was retrospective and without intervention, the opinion of the Ethics Committee on the study was not required. All patients had given prior consent for the use of their clinical, hormonal and ultrasound datas. On December 16, 2019, the Institutional Review Board of the Lille University Hospital gave

unrestricted approval for the anonymous use of all patients' clinical, hormonal and ultrasound records (reference DEC20150715-0002). 255 FET cycles were performed in patients between January 2011 and December 2017. Patients were between 18 and 35 years old. PCOS was diagnosed on the revised Rotterdam criteria, which require the presence of at least two of the following three conditions [27] :

- Oligo and/or anovulation.
- Clinical and/or biochemical hyperandrogenism.
- Polycystic ovarian morphology (PCOM).

Oligomenorrhea was defined by the presence of an average cycle length of more than 35 days and included women with amenorrhea. Clinical hyperandrogenism was defined by the presence of hirsutism (modified Ferriman-Gallwey score over 6) and/or acne located in more than two areas. Biochemical hyperandrogenism was defined as a serum total testosterone level > 0.39 ng/ml and/or a serum androstenedione level > 1,75 ng/ml in our center. Polycystic ovarian morphology (PCOM) was defined by the presence of at least 19 follicles measuring 2 to 9 mm and/or ovarian volume ≥10 mL and/or ovarian surface area ≥ 5.5 cm^2, on at least one of the two ovaries. In addition, the AMH assay was used to replace the follicular count to define the PCOM with a threshold of 35 pmol/L or about 5 ng/mL [28,29]. Women with congenital adrenal hyperplasia, Cushing syndrome, androgen secreting tumor or hyperprolactinemia were excluded. The threshold of 12 follicles, initially described in the Rotterdam Consensus, was readjusted to 19 follicles of less than 9 mm in 2011 due to an improvement in the ultrasound performance (use of a General Electric Voluson E8 with high frequency endovaginal probe of 5 to 9 MHz) [29]. Patients with PCOS phenotype C (Hyperandrogenism + PCOM) were excluded from our study. In this study, we also excluded: patients with endometriosis at all stages, presence of one or two persistent hydrosalpinges, ICSI with use of surgical sperm or cryopreserved sperm and use of gamete donation.

Assay

The biological assessment included: estradiol, LH and FSH assays (ABBOTT's Automate Architect), AMH (Beckman Coulter Immunotech's second-generation AMH-EIA immunoenzyme kit (Villepinte, France)) [29], total testosterone, Δ4-androstenedione (by radioimmunoassay (RIA) and then from June 2013, by using liquid chromatography coupled to mass spectrometry (LC-MS / MS), 17-hydroxyprogesterone, SDHEA, SHBG, prolactinemia, fasting glucose and insulinemia.

Ultrasonographic (U/S) examination

The U/S examination was performed on the same day as the blood sampling, between day 2 and 5 at baseline, using a Voluson E8 Expert (General Electric Systems, VELIZY, France) and a 5-9 MHz transvaginal transducter as previously described. For each ovary, the total number of follicles smaller than 9 mm in diameter was counted by slow and continuous scanning of the entire ovary. Experienced sonographers performed all the ultrasounds. Patients were excluded from the analysis when transvaginal ultrasound was not possible.

Endometrial preparation

The choice of treatment was made in consultation with the referring doctor of the couple.

Stimulated cycle Patients were given injections of gonadotropins (FSH or HMG) on the second day of their cycle triggered by the sequential treatment of dydrogesterone. The starting dose, usually 50 to 75 IU / day, was determined according to the age of the patient, her Body Mass Index (BMI) and ovarian reserve. Ultrasound and hormone assays (estradiol, LH +/- progesterone) were performed between day 8 and day 10, and then repeated until the follicle reached 16 to 20 mm with a concordant hormone biology and a minimum endometrial thickness of 7 mm. Subcutaneous injection of recombinant hCG was then carried out in order to trigger final oocyte maturation (Ovitrelle®, Choriogonadotropin alpha, 250 µg, Merck

Serono, Lyon, France) and FET was performed 5 days later. Patients received luteal phase support beginning on the day of transfer. This treatment was based on the administration of vaginal progesterone (Progestan Gé®, progesterone, 200mg, Besins International: 200 mg in the morning and 400 mg in the evening) until the pregnancy test was performed. If positive, vaginal progesterone was continued until the first ultrasound was performed around 4-5 weeks of pregnancy.

Artificial cycle Oral or percutaneous natural estradiol treatment was started the first day of the cycle (Provames®, estradiol, Sanofi-Aventis, France, 2mg, three times a day or Vivelledot ®, estradiol, 75 µg / 24h: 2 patches, beginning the 1st day of the cycle and changed every 3 days, Novartis Pharma SA). Percutaneous administration of estrogens is preferred in cases of thromboembolic risk factors. Ultrasonography was performed at least 12 days after the start of testrogen treatment, and renewed if the endometrial thickness had not reached 7 mm during the first control. When the endometrium thickness reached at least 7 mm, treatment with vaginal progesterone was started (Progestan Gé®, Progesterone, 200 mg, 2 in the morning and 2 in the evening, Sanofi-Aventis, France) and frozen-thawed embryo transfer was planned on the fourth day of progesterone administration for cleaved-stage embryos. The treatment was continued until the pregnancy test was performed. If found positive, the treatment was continued until 12 weeks of pregnancy. None of the patients had received a previous gonadotropic desensitization with GnRH agonist.

Techniques for freezing and thawing embryos

All the embryos were of good quality and contained between 4 and 5 blastomeres (at D2) and between 6 and 9 blastomeres (at D3) with less than 20% of cytoplasmic fragmentation. Embryos were cryopreserved according to a slow freezing protocol, using the Embryo Freezing Pack Kit (Origio, Målov, Denmark) according to the manufacturer's recommendations. Embryos were then packaged in straws (CryoBioSystem®) individually

and frozen according to a temperature descent program on an automatic freezing device (Planer Kryo560-16, United Kingdom). Embryos were thawed one day before transfer, except for the D3 embryos who were thawed the same day of embryo transfer. The embryonic survival (presence of at least 50% of intact blastomeres) was evaluated immediately after thawing. Any embryo whose survival was <50% was not transferred. Subsequently, the embryo(s) that were chosen to be transferred were placed in an oven at 37 °C in a culture medium (Global®, JCD, La Mulatiere, France) until the time of transfer. The embryonic quality was evaluated on the day of the transfer. The recovery rate, defined as the number of embryos transferred to the number of thawed embryos, was measured for each cycle.

The number of embryos transferred was determined with the couple, taking into account the woman's age, her history of medically assisted reproduction and any medical contraindications to twin pregnancies.

Transfer technique of embryos

A transfer test was performed in advance to measure the uterine height and ensure the feasibility of transfer. Embryo transfer was performed with the Elliocath® Angled Catheter (Ellios BioTek Laboratory, Paris, France). The catheter containing the embryo(s) was introduced into the uterine cavity and the embryo(s) were deposited at 1.5 - 2 cm from the uterine fundus with ultrasound guidance. Subsequently, the catheter was immediately examined under the microscope to ensure that the embryo did not accidentally remain in the catheter.

FET Outcomes

A pregnancy test was performed by assaying plasma quantitative hCG fourteen days post transfer. A pregnancy was defined by hCG level higher than 100 mIU / mL. Ongoing pregnancy was defined as the pregnancy that progressed beyond 12 weeks of amenorrhea. Live birth rate was defined as the birth of a child born alive beyond 24 weeks of amenorrhea

(SA). A spontaneous miscarriage was defined by the non-evolution of a pregnancy before 24 SA; early if the miscarriage occured before 12 SA and late if it occured between 12 and 24 SA. Biochemical pregnancies with hCG levels that never exceeded 100 mIU / mL were excluded from pregnancies and miscarriages.

Statistical Analysis

Quantitative values are expressed as a median with the 5th and 95th percentiles. The nonparametric Mann-Whitney test was used to compare the quantitative variables. The Chi-squared test was performed to compare the qualitative variables. These are represented as percentages with numbers. The differences were considered statistically significant when the p value was less than 0.05.

RESULTS

54% of transfers (n = 137) were performed using a stimulated cycle and 46% (n = 118) under artificial cycle.

The clinical characteristics of PCOS patients in each subgroup are shown in Table 1.

Patients were comparable in terms of age at time of thawing, BMI, presence or absence of smoking, presence of tubal infertility and rank of IVF attempt. Male infertility rate (51% versus 67%, p <0.05) was significantly lower in the artificial cycle group than in the stimulated cycle group.

The characteristics of FET cycles such as endometrial thickness, embryo recovery rate and number of embryos transferred were comparable between the two groups.

Miscarriage rate (22% versus 25%, p = NS) was no significantly different between the stimulated group and the artificial one.

Other FET outcomes (implantation rate, early pregnancy rate, ongoing pregnancy rate and

live birth rate shown in Table 2) are not significantly different between the two groups. An ectopic pregnancy and therapeutic interruption of pregnancy occurred in the artificial cycle group.

DISCUSSION

In this study, we did not find any statistically significant difference concerning miscarriage rates between the two protocols studied, i.e., stimulated cycle and artificial cycle after FET in anovulatory PCOS patients. This result is similar to a randomized study that had compared stimulated cycle and artificial cycle in patients with PCOS. Indeed, in this study [30], no difference was found between these two protocols in 576 PCOS patients who underwent a first cycle of FET. Clinical pregnancy rate, miscarriage rate and live birth rate were similar. However, patients with PCOS of phenotype C (normo-ovulatory PCOS) were also included and the threshold of 12 follicles (present on at least one of the two ovaries) was used to define PCOM, despite the use of a 6.5 MHz endovaginal probe.

A meta-analysis published in 2021 compared stimulated cycle and artificial cycle in PCOS patients and didn't find any difference in term of pregnancy outcomes between mild stimulation and AC groups [16].

In a retrospective study including 1926 FET cycles, Hatoum *et al*. compared the live birth and miscarriage rates between stimulated cycle and artificial cycle (normal or dysovulatory patients) [31]. They found a significantly higher rate of live birth and a significantly lower rate of early miscarriages (which included biochemical pregnancies and clinical pregnancies) in the stimulated group. However, luteal phase support in the artificial cycle consisted of administration of only 200 mg vaginal micronized progesterone 2 to 3 times daily; which is possibly insufficient, as published recently [32,33]. This increase of miscarriage rate after artificial cycle has been reported in several studies [10,13,31,34–38] without necessarily

being associated with a decrease of live birth rate [13,36]. NC seems to be associated with better pregnancy outcomes [37–40] and/or be associated with less pregnancy complications than AC [41,42] as confirmed in recent meta-analysis and reviews [40,43] but obviously it's not possible to use it in anovulatory PCOS patients. Recently, a multicenter French cohort study showed higher early pregnancy rates with AC compared with natural cycle or stimulated cycle [44]. However in this study, ovulation disorders were significantly higher in AC group.

Indeed, most of these studies did not discriminate anovulatory from normo-ovulatory patients [10,31,36,44], which may induce a bias towards PCOS patients for whom the artificial cycle is currently used. Finally, most of them compared the artificial cycle with the spontaneous cycle and not with the stimulated one.

In the study by Wright *et al.,* 199 FET cycles were randomized and no significant difference was found between stimulated and artificial cycle in normo-ovulatory and anovulatory patients [45]. More recently, a Cochrane published in 2020 suggested that stimulated cycle may improve pregnancy outcomes compared to artificial cycle but there is low-quality evidence [46]. Likewise, higher LBR were reported with stimulated cycle compared to artificial cycle in a recent meta-analysis but the authors concluded that there is very-low quality of evidence [40,47]. Again, these studies were not specifically dedicated to PCOS patients.

It is important to define which type of endometrial preparation for FET is better in PCOS patients. Indeed, several recent studies have shown that PCOS patients might have significantly higher live birth rates in FET than in fresh embryo transfer. Two recent meta-analysis studies published in 2019, including 5265 and 5379 patients respectively [4,48], have shown a significant increase in live birth rate in FET in "higher-responder" patients in general. In a study by Wei *et al.* involving 3665 patients from two multicenter randomized trials, when

supra-physiologic levels of estradiol (> 3000 pg / ml) were noted on the day of triggering and/or the oocyte count on oocyte retrieval exceeded 16, live birth rates were found to be significantly higher and miscarriage rates to be significantly lower (11.6% versus 26.3%, OR: 0.37 (0.23-0.57)) in FET than in fresh embryo transfer but only in PCOS patients [49]. This suggests that factors present in PCOS patients may induce an increased susceptibility to high levels of estradiol and high numbers of oocytes in these patients. In fact, several studies suggest a possible alteration of the endometrial receptivity in PCOS patients [50,51]. The endometrial overexpression of the estrogen receptor (ERα) in PCOS patients, which is normally inhibited by increase of progesterone levels, is mentioned in some studies [52]. Estradiol would inhibit the expression of several endometrial factors involved in implantation [51]. Endometrial resistance to progesterone [53,54] and endometrial overexpression of androgen receptors are also mentioned [55]. However, a loss of inhibition of the expression of estrogen and androgen receptors during the implantation window, associated with a concomitant decrease in the expression of important endometrial proteins such as glycodelin and integrin αvβ3, could lead to an increase of miscarriage rate in PCOS patients [56].

In a multicentric study in PCOS patients, 1508 patients were randomly subjected to fresh embryo transfer or FET in a first IVF cycle [57]. The live birth rate was significantly higher related to a significantly lower miscarriage rate (22% versus 32.7%, p <0.001) in the FET group. In the latest recommendations from the international guidelines for the management of PCOS [58,59], it is recommended to consider the use of a freeze-all strategy for PCOS patients who underwent IVF or ICSI.

The potential impact of PCOS on early spontaneous miscarriage is not a recent finding. Balen *et al.* [60] found a higher early miscarriage rate close to 40% in patients with PCOS who had a pregnancy after in vitro fertilization in a retrospective study evaluating 1,060 pregnancies.

The miscarriage rate during the spontaneous pregnancy in general population is about 10 to 16% [61,62]. This seems to be higher in the ART population with a miscarriage rate close to 29% in a study by Farr *et al.* [63] which was mostly correlated with the age of the patients during the ART trial.

A meta-analysis published in 2006 did not find any difference between PCOS and non-PCOS patients in term of miscarriage rate [26]. However, this association is still widely studied. Several studies have found a higher level of miscarriage rate in PCOS population [18,20,60], independent of the BMI [21] and the risk of embryonic aneuploidy [19]. But these studies are mostly retrospective and potentially present a bias. In a study analyzing 8058 embryo transfers (fresh or frozen-thawed), the miscarriage rate was significantly higher in the PCOS group but the endometrial preparation protocols were different according to the group of patients [18]. Non-PCOS patients had a spontaneous cycle while PCOS patients had received artificial cycle.

However, the independent effect of PCOS on the miscarriage rate is still debated [62,64] because of confounding factors known to increase miscarriages such as high BMI (obesity), insulin resistance [24] and hyperandrogenism [65], which are frequently found in PCOS patients and could explain the risk of miscarriages [24,66,67]. In case of hyperandrogenism, testosterone has a negative influence on the uterine expression of HOXA-10, which is essential for endometrial receptivity [68,69]. Low levels of HOXA-10 have been found in patients with repeated implantation failure and recurrent miscarriages [68].

In 2012, the Amsterdam consensus [70] did not recognize an increase of miscarriages in PCOS patients in a context of spontaneous pregnancy. Recently, the ESHRE [71] recognized a possible association between PCOS and the risk of miscarriage. Nevertheless, PCOS is not considered as a significant risk factor for recurrent miscarriages.

Finally, there is insufficient data on early miscarriages rates in studies that evaluated endometrial preparation methods. Many of these studies do not mention this association [9,11,72]. In addition, the definition of early spontaneous miscarriage is very different from one study to another, making their interpretations difficult. Some studies define miscarriage only when the diagnosis of clinical pregnancy has been previously established by the presence of a least one gestational sac and at least one embryo with cardiac activity [13,30], whereas the others define a miscarriage by just the presence of a positive hCG level [32]. Prospective randomized studies are needed to ensure the equivalence in terms of efficacy of both treatments in anovulatory PCOS patients.

To conclude, in anovulatory PCOS patients, the method of endometrial preparation for FET does not appear to have any impact on miscarriage rate and live birth rate. The association between PCOS and early spontaneous miscarriage remains controversial. In the literature, it still remains unclear whether PCOS is considered to be a risk factor for miscarriages, regardless of the confounding factors that are often associated with PCOS such as android obesity and insulin resistance.

ACKNOWLEDGEMENT

We thank Mrs Lydie Lombardo and Sylvie Vanoverschelde for their help in collecting the blood samples and caring for patients. We thank Pallavi Singh for her help to correct english translation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 1983;305:707–9.

[2] De Geyter C, Calhaz-Jorge C, Kupka MS, Wyns C, Mocanu E, Motrenko T, et al. ART in Europe, 2015: results generated from European registries by ESHRE. Hum Reprod Open 2020;2020:hoz038. https://doi.org/10.1093/hropen/hoz038.

[3] Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011;96:344–8. https://doi.org/10.1016/j.fertnstert.2011.05.050.

[4] Bosdou JK, Venetis CA, Tarlatzis BC, Grimbizis GF, Kolibianakis EM. Higher probability of live-birth in high, but not normal, responders after first frozen-embryo transfer in a freeze-only cycle strategy compared to fresh-embryo transfer: a meta-analysis. Hum Reprod 2019;34:491–505. https://doi.org/10.1093/humrep/dey388.

[5] Wong KM, van Wely M, Mol F, Repping S, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev 2017;3:CD011184. https://doi.org/10.1002/14651858.CD011184.pub2.

[6] Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, et al. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. N Engl J Med 2018;378:126–36. https://doi.org/10.1056/NEJMoa1705334.

[7] Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril 2016;106:6–15. https://doi.org/10.1016/j.fertnstert.2016.05.003.

[8] Groenewoud ER, Cohlen BJ, Macklon NS. Programming the endometrium for deferred transfer of cryopreserved embryos: hormone replacement versus modified natural cycles. Fertil Steril 2018;109:768–74. https://doi.org/10.1016/j.fertnstert.2018.02.135.

[9] Groenewoud ER, Cantineau AEP, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. Hum Reprod Update 2013;19:458–70. https://doi.org/10.1093/humupd/dmt030.

[10] Guillén V, Ayllón Y, Domingo J, Jáuregui J, Santana A, Pellicer A. Thawed embryo transfer: natural or replaced endometrial cycle? A 5000 patients observational study. Fertil Steril 2011;96:S270. https://doi.org/10.1016/j.fertnstert.2011.07.1031.

[11] 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.

[12] Groenewoud ER, Cohlen BJ, Al-Oraiby A, Brinkhuis EA, Broekmans FJM, de Bruin JP, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. Hum Reprod 2016;31:1483–92. https://doi.org/10.1093/humrep/dew120.

[13] Cerrillo M, Herrero L, Guillén A, Mayoral M, García-Velasco JA. Impact of Endometrial Preparation Protocols for Frozen Embryo Transfer on Live Birth Rates. Rambam Maimonides Med J 2017;8. https://doi.org/10.5041/RMMJ.10297.

[14] Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev 2017. https://doi.org/10.1002/14651858.CD003414.pub3.

[15] Yarali H, Polat M, Mumusoglu S, Yarali I, Bozdag G. Preparation of endometrium for frozen embryo replacement cycles: a systematic review and meta-analysis. J Assist Reprod Genet 2016;33:1287–304. https://doi.org/10.1007/s10815-016-0787-0.
[16] Zeng MF, Zhou X, Duan JL. Stimulated cycle versus artificial cycle for frozen embryo transfer in patients with polycystic ovary syndrome: a Meta-analysis. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2021:1–6. https://doi.org/10.1080/09513590.2020.1867976.
[17] Bu Z, Hu L, Su Y, Guo Y, Zhai J, Sun Y-P. Factors related to early spontaneous miscarriage during IVF/ICSI treatment: an analysis of 21,485 clinical pregnancies. Reprod Biomed Online 2020;40:201–6. https://doi.org/10.1016/j.rbmo.2019.11.001.
[18] Liu L, Tong X, Jiang L, Li TC, Zhou F, Zhang S. A comparison of the miscarriage rate between women with and without polycystic ovarian syndrome undergoing IVF treatment. Eur J Obstet Gynecol Reprod Biol 2014;176:178–82. https://doi.org/10.1016/j.ejogrb.2014.02.041.
[19] Luo L, Gu F, Jie H, Ding C, Zhao Q, Wang Q, et al. Early miscarriage rate in lean polycystic ovary syndrome women after euploid embryo transfer - a matched-pair study. Reprod Biomed Online 2017;35:576–82. https://doi.org/10.1016/j.rbmo.2017.07.010.
[20] Palomba S, Falbo A, Russo T, Tolino A, Orio F, Zullo F. Pregnancy in women with polycystic ovary syndrome: the effect of different phenotypes and features on obstetric and neonatal outcomes. Fertil Steril 2010;94:1805–11. https://doi.org/10.1016/j.fertnstert.2009.10.043.
[21] Rees DA, Jenkins-Jones S, Morgan CL. Contemporary Reproductive Outcomes for Patients With Polycystic Ovary Syndrome: A Retrospective Observational Study. J Clin Endocrinol Metab 2016;101:1664–72. https://doi.org/10.1210/jc.2015-2682.
[22] Joham AE, Boyle JA, Ranasinha S, Zoungas S, Teede HJ. Contraception use and pregnancy outcomes in women with polycystic ovary syndrome: data from the Australian Longitudinal Study on Women's Health. Hum Reprod 2014;29:802–8. https://doi.org/10.1093/humrep/deu020.
[23] Sun Y-F, Zhang J, Xu Y-M, Cao Z-Y, Wang Y-Z, Hao G-M, et al. High BMI and Insulin Resistance Are Risk Factors for Spontaneous Abortion in Patients With Polycystic Ovary Syndrome Undergoing Assisted Reproductive Treatment: A Systematic Review and Meta-Analysis. Front Endocrinol 2020;11:592495. https://doi.org/10.3389/fendo.2020.592495.
[24] Tian L, Shen H, Lu Q, Norman RJ, Wang J. Insulin resistance increases the risk of spontaneous abortion after assisted reproduction technology treatment. J Clin Endocrinol Metab 2007;92:1430–3. https://doi.org/10.1210/jc.2006-1123.
[25] Palomba S, Falbo A, Daolio J, Battaglia FA, La Sala GB. Pregnancy complications in infertile patients with polycystic ovary syndrome: updated evidence. Minerva Ginecol 2018;70:754–60. https://doi.org/10.23736/S0026-4784.18.04230-2.
[26] Heijnen EMEW, Eijkemans MJC, Hughes EG, Laven JSE, Macklon NS, Fauser BCJM. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. Hum Reprod Update 2006;12:13–21. https://doi.org/10.1093/humupd/dmi036.
[27] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 Consensus on Diagnostic Criteria and Long-Term Health Risks Related to Polycystic Ovary Syndrome. Fertil Steril 2004;81:19–25.
[28] Fraissinet A, Robin G, Pigny P, Lefebvre T, Catteau-Jonard S, Dewailly D. Use of the serum anti-Müllerian hormone assay as a surrogate for polycystic ovarian morphology: impact on diagnosis and phenotypic classification of polycystic ovary syndrome. Hum Reprod 2017;32:1716–22. https://doi.org/10.1093/humrep/dex239.

[29] Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod 2011;26:3123–9. https://doi.org/10.1093/humrep/der297.
[30] Yu J, Ma Y, Wu Z, Li Y, Tang L, Li Y, et al. Endometrial preparation protocol of the frozen-thawed embryo transfer in patients with polycystic ovary syndrome. Arch Gynecol Obstet 2015;291:201–11. https://doi.org/10.1007/s00404-014-3396-0.
[31] Hatoum I, Bellon L, Swierkowski N, Ouazana M, Bouba S, Fathallah K, et al. Disparities in reproductive outcomes according to the endometrial preparation protocol in frozen embryo transfer : The risk of early pregnancy loss in frozen embryo transfer cycles. J Assist Reprod Genet 2018;35:425–9. https://doi.org/10.1007/s10815-017-1078-0.
[32] Devine K, Richter KS, Widra EA, McKeeby JL. Vitrified blastocyst transfer cycles with the use of only vaginal progesterone replacement with Endometrin have inferior ongoing pregnancy rates: results from the planned interim analysis of a three-arm randomized controlled noninferiority trial. Fertil Steril 2018;109:266–75. https://doi.org/10.1016/j.fertnstert.2017.11.004.
[33] Alsbjerg B, Polyzos NP, Elbaek HO, Povlsen BB, Andersen CY, Humaidan P. Increasing vaginal progesterone gel supplementation after frozen-thawed embryo transfer significantly increases the delivery rate. Reprod Biomed Online 2013;26:133–7. https://doi.org/10.1016/j.rbmo.2012.10.012.
[34] Guan Y, Fan H, Styer AK, Xiao Z, Li Z, Zhang J, et al. A modified natural cycle results in higher live birth rate in vitrified-thawed embryo transfer for women with regular menstruation. Syst Biol Reprod Med 2016;62:335–42. https://doi.org/10.1080/19396368.2016.1199064.
[35] Peigné M, Devouche E, Ferraretto X, Gricourt S, Luton D, Patrat C, et al. Higher live birth rate with stimulated rather than artificial cycle for frozen-thawed embryo transfer. Eur J Obstet Gynecol Reprod Biol 2019;243:144–9. https://doi.org/10.1016/j.ejogrb.2019.10.040.
[36] Tomás C, Alsbjerg B, Martikainen H, Humaidan P. Pregnancy loss after frozen-embryo transfer—a comparison of three protocols. Fertil Steril 2012;98:1165–9. https://doi.org/10.1016/j.fertnstert.2012.07.1058.
[37] Vinsonneau L, Labrosse J, Porcu-Buisson G, Chevalier N, Galey J, Ahdad N, et al. Impact of endometrial preparation on early pregnancy loss and live birth rate after frozen embryo transfer: a large multicenter cohort study (14 421 frozen cycles). Hum Reprod Open 2022;2022:hoac007. https://doi.org/10.1093/hropen/hoac007.
[38] Li J, Sun Q, Zhang M, Fu X, Zhang Y, Gao S, et al. Natural cycles achieve better pregnancy outcomes than artificial cycles in non-PCOS women undergoing vitrified single-blastocyst transfer: a retrospective cohort study of 6840 cycles. J Assist Reprod Genet 2022. https://doi.org/10.1007/s10815-022-02424-0.
[39] Mensing L, Dahlberg ES, Bay B, Gabrielsen A, Knudsen UB. Endometrial preparation methods prior to frozen embryo transfer: A retrospective cohort study comparing true natural cycle, modified natural cycle and artificial cycle. Arch Gynecol Obstet 2022. https://doi.org/10.1007/s00404-021-06371-6.
[40] Mumusoglu S, Polat M, Ozbek IY, Bozdag G, Papanikolaou EG, Esteves SC, et al. Preparation of the Endometrium for Frozen Embryo Transfer: A Systematic Review. Front Endocrinol 2021;12:688237. https://doi.org/10.3389/fendo.2021.688237.
[41] Fu Y, Chen D, Cai B, Xu Y, Zhu S, Ding C, et al. Comparison of two mainstream endometrial preparation regimens in vitrified-warmed embryo transfers after PGT. Reprod Biomed Online 2022;44:239–46. https://doi.org/10.1016/j.rbmo.2021.09.009.

[42] Hu K-L, Zhang D, Li R. Endometrium preparation and perinatal outcomes in women undergoing single-blastocyst transfer in frozen cycles. Fertil Steril 2021;115:1487–94. https://doi.org/10.1016/j.fertnstert.2020.12.016.
[43] Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen-thawed embryo transfer cycles: a systematic review and network meta-analysis. J Assist Reprod Genet 2021;38:1913–26. https://doi.org/10.1007/s10815-021-02125-0.
[44] Vinsonneau L, Labrosse J, Porcu-Buisson G, Chevalier N, Galey J, Ahdad N, et al. Impact of endometrial preparation on early pregnancy loss and live birth rate after frozen embryo transfer: a large multicenter cohort study (14 421 frozen cycles). Hum Reprod Open 2022;2022:hoac007. https://doi.org/10.1093/hropen/hoac007.
[45] Wright KP, Guibert J, Weitzen S, Davy C, Fauque P, Olivennes F. Artificial versus stimulated cycles for endometrial preparation prior to frozen–thawed embryo transfer. Reprod Biomed Online 2006;13:321–5. https://doi.org/10.1016/S1472-6483(10)61434-4.
[46] Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev 2010:CD006359. https://doi.org/10.1002/14651858.CD006359.pub2.
[47] Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen–thawed embryo transfer cycles: a systematic review and network meta-analysis. J Assist Reprod Genet 2021;38:1913–26. https://doi.org/10.1007/s10815-021-02125-0.
[48] Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril 2013;99:156–62. https://doi.org/10.1016/j.fertnstert.2012.09.003.
[49] Wei D, Yu Y, Sun M, Shi Y, Sun Y, Deng X, et al. The Effect of Supraphysiological Estradiol on Pregnancy Outcomes Differs Between Women With PCOS and Ovulatory Women. J Clin Endocrinol Metab 2018;103:2735–42. https://doi.org/10.1210/jc.2018-00613.
[50] Palomba S, Piltonen TT, Giudice LC. Endometrial function in women with polycystic ovary syndrome: a comprehensive review. Hum Reprod Update 2020. https://doi.org/10.1093/humupd/dmaa051.
[51] Tomaszewski C, Dewailly D. The role of the endometrium in the infertility of women with polycystic ovary syndrome. Médecine Reprod 2012;14:121–34. https://doi.org/10.1684/mte.2012.0396.
[52] Piltonen TT. Polycystic ovary syndrome: Endometrial markers. Best Pract Res Clin Obstet Gynaecol 2016;37:66–79. https://doi.org/10.1016/j.bpobgyn.2016.03.008.
[53] Li X, Feng Y, Lin J-F, Billig H, Shao R. Endometrial progesterone resistance and PCOS. J Biomed Sci 2014;21:2. https://doi.org/10.1186/1423-0127-21-2.
[54] Savaris RF, Groll JM, Young SL, DeMayo FJ, Jeong J-W, Hamilton AE, et al. Progesterone Resistance in PCOS Endometrium: A Microarray Analysis in Clomiphene Citrate-Treated and Artificial Menstrual Cycles. J Clin Endocrinol Metab 2011;96:1737–46. https://doi.org/10.1210/jc.2010-2600.
[55] Apparao KBC, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod 2002;66:297–304.
[56] Giudice LC. Endometrium in PCOS: Implantation and predisposition to endocrine CA. Best Pract Res Clin Endocrinol Metab 2006;20:235–44. https://doi.org/10.1016/j.beem.2006.03.005.

[57] Chen Z-J, Shi Y, Sun Y, Zhang B, Liang X, Cao Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. N Engl J Med 2016;375:523–33. https://doi.org/10.1056/NEJMoa1513873.
[58] Costello MF, Misso ML, Balen A, Boyle J, Devoto L, Garad RM, et al. Evidence summaries and recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome: assessment and treatment of infertility. Hum Reprod Open 2019;2019. https://doi.org/10.1093/hropen/hoy021.
[59] Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018;33:1602–18. https://doi.org/10.1093/humrep/dey256.
[60] Balen AH, Tan SL, MacDougall J, Jacobs HS. Miscarriage rates following in-vitro fertilization are increased in women with polycystic ovaries and reduced by pituitary desensitization with buserelin. Hum Reprod 1993;8:959–64.
[61] Nybo Andersen AM, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708–12.
[62] Wang JX, Davies MJ, Norman RJ. Polycystic ovarian syndrome and the risk of spontaneous abortion following assisted reproductive technology treatment. Hum Reprod 2001;16:2606–9.
[63] Farr SL, Schieve LA, Jamieson DJ. Pregnancy loss among pregnancies conceived through assisted reproductive technology, United States, 1999-2002. Am J Epidemiol 2007;165:1380–8. https://doi.org/10.1093/aje/kwm035.
[64] Palomba S, de Wilde MA, Falbo A, Koster MPH, La Sala GB, Fauser BCJM. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update 2015;21:575–92. https://doi.org/10.1093/humupd/dmv029.
[65] Yang W, Yang R, Lin M, Yang Y, Song X, Zhang J, et al. Body mass index and basal androstenedione are independent risk factors for miscarriage in polycystic ovary syndrome. Reprod Biol Endocrinol RBE 2018;16:119. https://doi.org/10.1186/s12958-018-0438-7.
[66] Luo L, Wang Q, Chen M, Yuan G, Wang Z, Zhou C. IGF-1 and IGFBP-1 in peripheral blood and decidua of early miscarriages with euploid embryos: comparison between women with and without PCOS. Gynecol Endocrinol Off J Int Soc Gynecol Endocrinol 2016;32:538–42. https://doi.org/10.3109/09513590.2016.1138459.
[67] Jakubowicz DJ, Essah PA, Seppälä M, Jakubowicz S, Baillargeon J-P, Koistinen R, et al. Reduced serum glycodelin and insulin-like growth factor-binding protein-1 in women with polycystic ovary syndrome during first trimester of pregnancy. J Clin Endocrinol Metab 2004;89:833–9. https://doi.org/10.1210/jc.2003-030975.
[68] Yang Y, Chen X, Saravelos SH, Liu Y, Huang J, Zhang J, et al. HOXA-10 and E-cadherin expression in the endometrium of women with recurrent implantation failure and recurrent miscarriage. Fertil Steril 2017;107:136-143.e2. https://doi.org/10.1016/j.fertnstert.2016.09.016.
[69] Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88:238–43. https://doi.org/10.1210/jc.2002-021072.
[70] Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Hum Reprod 2012;27:14–24. https://doi.org/10.1093/humrep/der396.
[71] Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, Middeldorp S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open 2018;2018. https://doi.org/10.1093/hropen/hoy004.

[72] Peeraer K, Couck I, Debrock S, De Neubourg D, De Loecker P, Tomassetti C, et al. Frozen-thawed embryo transfer in a natural or mildly hormonally stimulated cycle in women with regular ovulatory cycles: a RCT. Hum Reprod 2015;30:2552–62. https://doi.org/10.1093/humrep/dev224.

TABLE 1: Clinical characteristics of patients and FET.

PCOS	STIMULATED CYCLE (n= 137)	ARTIFICIAL CYCLE (n= 118)	P
Age (years)	31 [26-35]	30 [25-35]	NS
BMI (kg/m^2)	23 [18-32]	23 [18-31]	NS
Smoking	12% (n=16)	15.3% (n=17)	NS
Etiology : Tubal Male	 9 % (n=13) 67 % (n=92)	 17.8 % (n=21) 51 % (n=60)	 NS **p=0.02**
Rank of IVF attempt	1 [1-3]	1 [1-3]	NS
Type : IVF ICSI	 34.3 % (n=47) 65.7 % (n=90)	 49.3% (n=58) 50.8 % (n=60)	 **p=0.03**
Endometrium thickness (mm)	9 [7-12]	9 [7-12]	NS

Embryo recovery rate (number of embryos transferred / number of frozen-thawed embryos)	79.3 %	79.7%	NS
Number of embryos transferred -1 -2	 57.6 % (n=79) 42.4 % (n=58)	 63.6 % (n=75) 36.4 % (n=43)	 NS

NS = non significant

TABLE 2: FET outcomes in PCOS anovulatory patients.

PCOS	STIMULATED CYCLE (n=137)	ARTIFICIAL CYCLE (n=118)	p
Implantation rate	23 %	26 %	NS
Early pregnancy rate	30 % (n= 41)	37.3 % (n=44)	NS
Ongoing pregnancy rate	23.4 % (n= 32)	27 % (n= 32)	NS
Miscarriage rate	22 % (n=9)	25 % (n=11)	NS
Live birth rate	23.4 % (n=32)	26.3 % (n=31)	NS