Freeze-only versus fresh embryo transfer in a multicenter matched cohort study: contribution of progesterone and maternal age to success rates

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Objective: To compare implantation and ongoing pregnancy rates in freeze-only versus fresh transfer cycles.
Design: Retrospective matched cohort study.
Setting: Not applicable.
Patient(s): Women selected using a matching algorithm for similar distributions of clinical characteristics for a total of 2,910 cycles (1,455 fresh cohort and 1,455 freeze-only cohort).
Intervention(s): None.
Main Outcome Measure(s): Implantation and ongoing pregnancy rates.
Result(s): Implantation and ongoing pregnancy rates were statistically significantly higher in the freeze-only transfer cohort than in the matched fresh transfer cohort: ongoing pregnancy rate for freeze-only was 52.0% (95% confidence interval [CI], 49.4–54.6) and for fresh was 45.3% (95% CI, 42.7–47.9), odds ratio (OR) 1.31 (95% CI, 1.13–1.51). In a stratified analysis, the odds of ongoing pregnancy after freeze-only transfer were statistically significantly higher for women both above and below age 35 with progesterone concentration >1.0 ng/mL (age ≤35: OR 1.38 [1.11–1.71]; age >35: OR 1.73 [1.34–2.24]). For women with progesterone concentration ≤1.0 ng/mL, no statistically significant difference in freeze-only odds of ongoing pregnancy was observed in either age group. The sensitivity analysis revealed that increasing maternal age alone (regardless of progesterone) trended toward a more beneficial effect of freeze-only cycles. A lower progesterone concentration was associated with statistically significantly higher ongoing pregnancy odds for fresh but not freeze-only cycles.
Conclusion(s): Freeze-only transfer protocols are associated with statistically significantly higher ongoing implantation and pregnancy rates compared with fresh transfer cycles. This effect is most pronounced for cycles with progesterone >1.0 ng/mL at trigger and may also be stronger for older patients. (Fertil Steril® 2017;108:254–61. ©2017 by American Society for Reproductive Medicine.)
Key Words: Controlled ovarian stimulation, cryopreservation, freeze-only, fresh transfer, frozen embryo transfer

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In vitro fertilization (IVF) with frozen embryo transfer (FET) has become increasingly common in the United States. According to the Society for Assisted Reproductive Technology (SART), the number of FETs increased by over 80% from 2006 to 2012, which far outpaced the rate of increase for fresh cycles over the same period (1). Advances in cryopreservation of embryos have contributed to this trend, as newer vitrification techniques have improved embryo survival rates compared with slow freezing (2). In addition, there is increasing evidence that FET may lead to more favorable perinatal and live-birth outcomes, including a lower risk of preterm birth, low birth weight, placenta previa, and placental abruption (1, 3–7). As FET has become more common, freeze-only protocols have emerged in which all embryos are electively frozen and transferred in a later natural or medicated cycle.

Despite this growing trend, studies on the impact of freeze-only transfer versus fresh transfer protocols are limited. Two small, randomized, controlled trials (RCTs) by Shapiro et al. (8, 9) in normal and high responders reported increased pregnancy rates in freeze-only versus fresh transfers. Another RCT in patients with polycystic ovary syndrome found that freeze-only transfer was associated with higher live-birth rates compared with fresh transfer (10). A prospective cohort study of cleavage-stage embryos and a retrospective cohort study among women with prior implantation failure also found increased success rates in freeze-only transfer compared with fresh transfer protocols (11, 12).

Given the limited evidence base in the literature and the small cohort sizes of prior studies, we used a large multicenter data set to investigate the effects of freeze-only versus fresh transfer in a retrospective matched cohort. In addition, our study aimed to determine whether maternal age and progesterone (P) affected the relationship between freeze-only versus fresh transfer protocols and pregnancy outcomes. To our knowledge, this study is the largest to investigate outcomes of freeze-only versus fresh transfer and the first to stratify outcomes by maternal age and P concentration on the day of trigger.

MATERIALS AND METHODS

We performed a retrospective matched cohort study on patients from 12 fertility treatment centers in the United States who underwent IVF cycles between 2009 and 2015. We included cycles in which fresh embryo(s) were transferred (fresh) and cycles in which all embryos were frozen, followed by a later transfer (freeze-only). We excluded frozen transfers of supernumerary embryos, cancelled cycles, cycles in which preimplantation genetic screening (PGS) or preimplantation genetic diagnosis was used, and cycles that were missing any data used for matching between the fresh and freeze-only cohorts (patient characteristics, measures of ovarian reserve, and cycle details; see the section "Propensity Score Cohort Matching"). Our analysis included only blastocyst-stage transfers.

Patients underwent controlled ovarian stimulation (COS) according to established practice patterns at each clinic using one of several protocols (antagonist, long agonist, flare) and human chorionic gonadotropin (hCG) or leuprolide acetate (LA; Lupron; AbbVie) trigger. Oocytes were retrieved transvaginally 35 to 36 hours after hCG or LA administration (the trigger was administered when the largest follicle measured 18–24 mm) and were fertilized using either conventional IVF or intracytoplasmic sperm injection (ICSI). The embryos were then cultured to the blastocyst stage.

For fresh cycles, luteal support was initiated after retrieval, and the embryos were transferred into the uterus at the blastocyst stage. For freeze-only cycles, embryos were cryopreserved at the blastocyst stage according to the established practice protocols at each clinic. In a subsequent cycle, patients underwent FET in either a natural or medicated cycle (using estrogen and P supplementation). Indications for freeze-only cycles included, but were not limited to, premature P elevation, patient preference, and ovarian hyperstimulation syndrome (OHSS) risk.

Implantation rate was defined as the number of heartbeats divided by the number of embryos transferred. Ongoing pregnancy was defined as continued pregnancy at the time that the patient transferred care from a reproductive endocrinologist to an obstetrician (usually 8–12 weeks, depending on the clinic).

Propensity Score Cohort Matching

We used a propensity score method to match characteristics between the freeze-only and fresh transfer groups based on factors that may affect success of IVF cycles, using a logistic regression in which the treatment group is the outcome and the factors of interest are the predictors. The propensity score is the estimated probability that a given patient would have been assigned to the treatment group, given a particular set of variables.

Before matching, 13,791 cycles (1,455 freeze-only and 12,336 fresh) were available for analysis. The cycles were matched on characteristics that may influence the success of IVF cycles, including patient characteristics (age, body mass index, diagnosis, clinic, parity, gravidity), measures of ovarian reserve (antral follicle count, day-3 follicle-stimulating hormone [FSH], estradiol [E_2], luteinizing hormone [LH]), and cycle characteristics (gonadotropin dose, P concentration at trigger, oocytes retrieved, number of usable embryos, and number of embryos transferred) (Table 1).

After achieving a balanced cohort through propensity score matching, we performed regression analysis of the treatment groups on the outcome, while controlling for the propensity score. Mixed effects logistic regression was used to compute the odds ratios (ORs) between freeze-only and fresh cycles in the matched data. Clinics and patients were treated as random effects in the mixed model to account for repeated observations on patients and the within-clinic correlation of ongoing pregnancy rates. The distribution of key characteristics before and after matching is shown in Supplemental Table 1 and Supplemental Figure 1 (available online). Most characteristics differed substantially before matching. After matching, all characteristics were similar between the freeze-only and fresh groups.

TABLE 1

Baseline characteristics of fresh and freeze-only cohorts after propensity score matching.

Metric	Fresh	Freeze-only	*P* value
N	1,455	1,455	–
Age (y)	34.1 (4.0)	34.1 (4.3)	.98
BMI	24.8 (4.8)	25.0 (5.1)	.21
Parity	0.2 (0.6)	0.2 (0.6)	.58
Gravidity	0.7 (1.2)	0.7 (1.2)	.78
Basal AFC	17.8 (9.4)	17.9 (9.5)	.78
Day 3			
FSH	6.7 (2.1)	6.9 (2.1)	.11
E_2	51.5 (22.8)	51.4 (22.5)	.91
LH	7.3 (4.1)	7.4 (4.5)	.57
Gonadotropin dose	2,536.5 (1,698.7)	2,563.8 (1,589.9)	.66
P at trigger	1.5 (1.1)	1.5 (1.0)	.19
Oocytes retrieved	20.8 (10.3)	21.2 (11.4)	.37
No. of usable embryos	5.6 (3.9)	5.7 (3.9)	.57
Endometrial thickness (mm)	10.2 (2.2)	10.0 (2.3)	.46
No. of embryos transferred	1.5 (0.5)	1.5 (0.5)	.76
% ICSI	93.7%	91.6%	.94
Clinic distribution[a]	–	–	.82
Diagnosis			
DOR	8.5%	7.8%	.59
Endometriosis	6.0%	5.7%	.81
Idiopathic	4.3%	3.6%	.45
Male factor	20.8%	18.7%	.16
None provided	3.2%	3.3%	1
Other	18.8%	19.7%	.57
Ovulatory dysfunction	13.3%	15.0%	.2
PCOS	6.3%	6.3%	1
Tubal	17.9%	19.0%	.5
Uterine	1.0%	0.9%	.85

Note: Mean (± standard deviation) is shown for continuous variables, and percentages are shown for dichotomous variables. AFC = antral follicle count; BMI = body mass index; DOR = diminished ovarian reserve; FSH = follicle-stimulating hormone; E_2 = estradiol; LH = luteinizing hormone; P = progesterone; PCOS = polycystic ovary syndrome.

[a] *P* value is the result of a chi-square test for the difference in distribution of clinics between protocols.

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Statistical Analysis

Logistic regression was used to compute the ORs and estimates of both implantation and ongoing pregnancy rates in freeze-only versus fresh transfer cycles. Additionally, these outcomes were stratified by age and P concentration (ng/mL) on the day of hCG/LA trigger due to statistical evidence of a modification of the effect of a freeze-only protocol by these factors. The effect of age and P concentration was first studied using a stratified model with cutoff values. Optimal cutoff values for age and P (35 years old and 1.0 ng/mL, respectively) were chosen by receiver operating characteristic analysis, which balanced specificity and sensitivity for classifying cycles as an ongoing pregnancy (Supplemental Fig. 2A and B, available online). We also conducted a sensitivity analysis investigating the joint effect of age and P on freeze-only cycle outcomes in a continuous model. Analyses were conducted in R (version 3.2.4) (13). All tests were two-sided with statistical significance at the alpha = 0.05 level.

An institutional review board has determined that this research study does not constitute human subjects research. Accordingly, the study authors have received a letter of exemption from Western Institutional Review Board for this research.

RESULTS

After propensity score matching, the freeze-only and fresh transfer cohorts had similar baseline characteristics in terms of age, body mass index, infertility diagnosis (diminished ovarian reserve, endometriosis, idiopathic, male factor, other/none, ovulatory dysfunction, polycystic ovary syndrome, tubal factor, and uterine factor), clinic, parity, gravidity, antral follicle count, day-3 FSH/E_2/LH, gonadotropin dose, P concentration at trigger, oocytes retrieved, number of usable embryos, and number of embryos transferred. The average age for both the freeze-only and fresh cohorts was 34.1 years old. This matching process resulted in two groups of patients with comparable prognoses, as evidenced by similar measures of ovarian reserve and cycle characteristics as listed previously. Though endometrial thickness and percentage of ICSI were not characteristics included in the propensity score matching, these characteristics were also similar between the fresh and freeze-only cohorts (see Table 1).

In our matched cohort, the overall implantation and ongoing pregnancy rates were statistically significantly higher in the freeze-only compared with fresh transfer groups, with OR 1.21 (95% CI, 1.05–1.41; $P<.01$) for implantation and OR 1.31 (95% CI, 1.13–1.51; $P<.001$) for ongoing pregnancy (Table 2). The ongoing pregnancy rate was 6.7% higher (95% CI, 3.0%–10.4%) in the freeze-only (52.0%; 95% CI, 49.4%–54.6%) compared with the fresh transfer group (45.3%; 95% CI, 42.7%–47.9%).

In a stratified analysis by maternal age and P concentration at trigger, the difference in ongoing pregnancy rate between fresh and freeze-only cycles was dependent on P

TABLE 2

Pregnancy outcomes in matched data.

Outcome	Fresh	Freeze-only	Odds ratio (Freeze-only vs. Fresh)	*P* value
Ongoing pregnancy rate	45.3% (42.7%, 47.9%)	52.0% (49.4%, 54.6%)	1.31 (1.13, 1.51)	<.001
Implantation rate	42.0% (39.5%, 44.5%)	46.8% (44.2%, 49.4%)	1.21 (1.05, 1.41)	<.01

Note: Values in parentheses are 95% confidence interval.

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TABLE 3

Ongoing pregnancy rates comparison between freeze-only and fresh cycles by progesterone and age strata in matched data.

P at trigger	Age (y)	Fresh n	Fresh OPR (%)	Freeze-only n	Freeze-only OPR (%)	Odds ratio (Freeze-only vs. Fresh)	*P* value
≤1	≤35	302	56.4 (51.6, 61.2)	284	54.6 (49.7, 59.5)	0.93 (0.70, 1.23)	.61
	>35	203	45.1 (39.8, 50.5)	198	48.9 (43.5, 54.3)	1.17 (0.86, 1.58)	.33
>1	≤35	576	46.1 (42.3, 49.9)	578	54.1 (50.3, 57.9)	1.38 (1.11, 1.71)	<.01
	>35	374	35.2 (31.0, 39.5)	395	48.4 (44.0, 52.8)	1.73 (1.34, 2.24)	<.0001

Note: Values in parentheses are 95% confidence interval. OPR = ongoing pregnancy rates; P = progesterone.

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concentration at trigger (Table 3). For cycles with $P \leq 1.0$ ng/mL, the odds of ongoing pregnancy were not statistically significantly different between freeze-only and fresh cycles irrespective of age group (≤35 years: OR 0.93; 95% CI, 0.70–1.23, $P=.61$; >35 years: OR 1.17; 95% CI, 0.86–1.58, $P=.33$). However, for cycles with $P>1.0$ ng/mL, we observed statistically significantly higher odds of ongoing pregnancy in freeze-only compared with fresh transfer cycles in both age groups. In women age ≤35 years with $P>1.0$ ng/mL, the OR comparing freeze-only versus fresh ongoing pregnancy rate was 1.38 (95% CI, 1.11–1.71; $P<.01$). For women age >35 years with $P>1.0$ ng/mL, the difference in ongoing pregnancy between freeze-only and fresh cycles was even greater (OR 1.73; 95% CI, 1.34–2.24; $P<.0001$). Although age was not associated with a statistically significant difference in freeze-only versus fresh transfer outcomes, our sensitivity analysis demonstrated that at higher concentrations of P, there was a trend toward an increasing benefit of freeze-only cycles seen with advancing maternal age; for the same progesterone level, the OR for achieving an ongoing pregnancy in a freeze-only versus fresh cycle increased with maternal age (Fig. 1).

Within all fresh cycles, we observed that $P>1.0$ ng/mL was associated with a decrease in the odds of ongoing pregnancy compared with $P \leq 1.0$ ng/mL (OR 0.66; 95% CI, 0.53–0.82; $P<.001$), irrespective of age group (Supplemental Table 2, available online). The adverse effect of elevated P was abrogated in freeze-only cycles; we found no evidence of an association between P concentration and ongoing pregnancy rates in freeze-only cycles (for $P>1.0$ ng/mL: OR 0.98; 95% CI, 0.79–1.22; $P=.85$).

DISCUSSION

In summary, in this multicenter, matched, retrospective cohort study of 1,455 fresh cycles and 1,455 freeze-only cycles, we found that freeze-only cycles were associated with statistically significantly higher implantation and ongoing pregnancy rates compared with fresh cycles. In particular, this association was observed for cycles with $P>1.0$ ng/mL. For women with $P>1.0$ ng/mL and age >35 years, the odds of pregnancy were over 1.7 times greater for freeze-only than fresh transfer cycles. The odds of pregnancy were not statistically significantly different in freeze-only compared with fresh cycles for women with $P \leq 1.0$ ng/mL both above and below the cutoff of 35 years old. However, in a sensitivity analysis, we observed that at higher concentrations of P, freeze-only cycles trended as more beneficial with increasing maternal age, and lower P at trigger was associated with higher pregnancy rates in fresh but not freeze-only cycles.

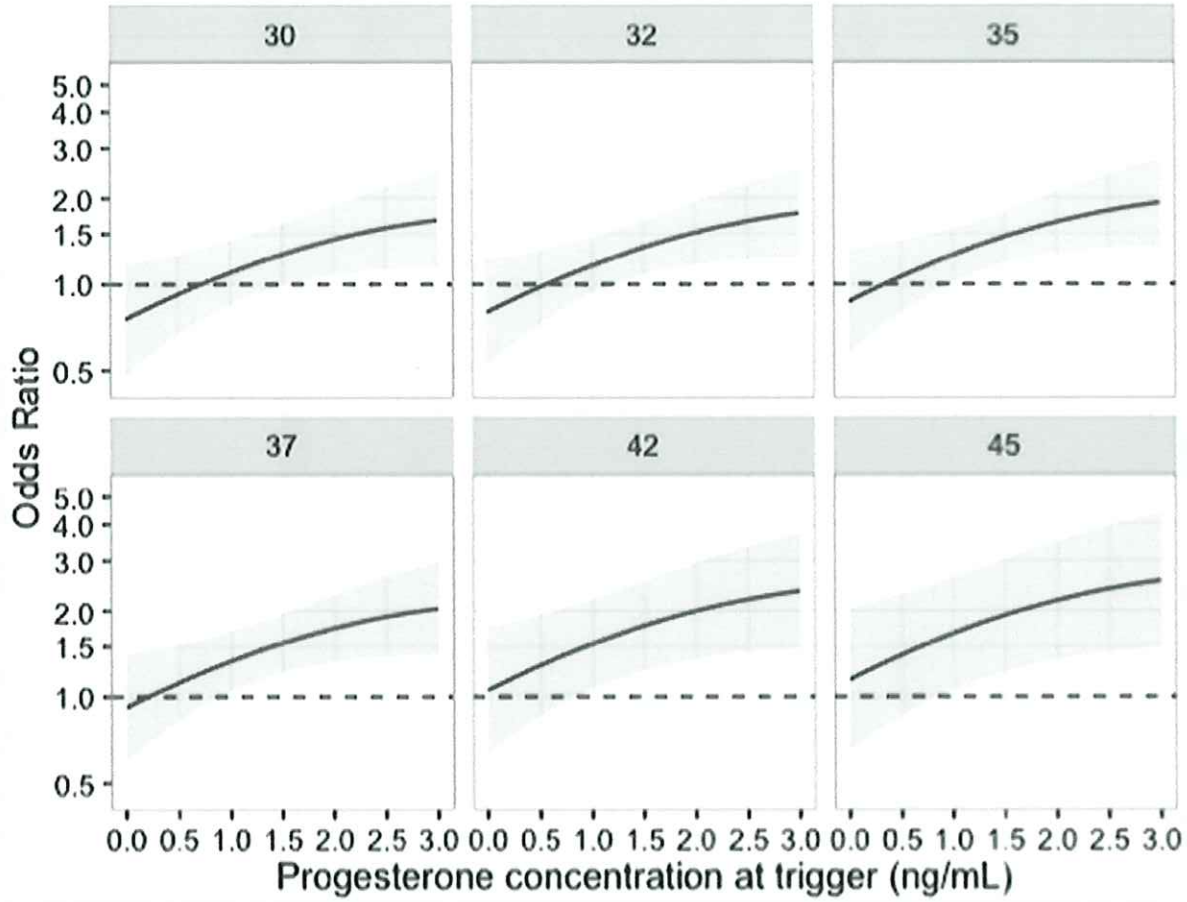
Plausible Biological Mechanisms

Freeze-only transfer protocols are thought to have several potential advantages over fresh transfer protocols. Multiple mechanisms have been suggested for the negative impact of COS on implantation and ongoing pregnancy. Hormone regulation plays a large role in endometrial receptivity, and high estrogen and P concentrations from COS may affect hundreds of genes involved in implantation (14–17). Further, high estrogen levels during ovarian stimulation have been hypothesized to interfere with endometrial angiogenesis (18–20). Additionally, studies have reported that an endometrium in an unstimulated cycle is more receptive to early placentation and embryogenesis than an endometrium during COS (19, 21, 22).

Asynchrony between the embryo and endometrium may result from COS because COS is associated with advanced histology and down-regulation of P receptors. Controlled ovarian stimulation may lead to a premature rise in P (23–26), which can result in premature maturation of the endometrium, disrupting the implantation window (27–29). In support of this hypothesis, several studies have demonstrated that in pregnancies resulting from fresh transfer, adverse pregnancy outcomes correspond with supraphysiologic estradiol levels (20, 21, 30–32). Furthermore, a study among oocyte donors and recipients in a shared program (where donors donated half their oocytes and kept the remaining oocytes for autologous transfer) reported higher implantation rates in recipients compared with donors during stimulated cycles; however, this difference was not statistically significant in frozen cycles, suggesting that COS has a negative effect on the endometrium (33).

It has also been hypothesized that the freeze-thaw process in freeze-only cycles serves as a filter for embryos of poorer quality, which may not survive the thaw (34). Freeze-only protocols can also reduce the risk for severe OHSS (35–38). Patients undergoing preimplantation genetic diagnosis or PGS may also need to freeze all embryos generated in a

FIGURE 1



Effect of patient age and progesterone (P) concentration at trigger on determining the odds ratio (OR) for ongoing pregnancy for freeze-only versus fresh cycles. Patient age and P concentration at trigger modify the OR for achieving ongoing pregnancy between a freeze-only versus a fresh cycle. The 95% pointwise confidence intervals are shown in *gray*. The *y*-axes are on the natural logarithmic scale. Each panel represents the effect of P on the OR at the indicated age (labeled at the top of the panel).

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given cycle if testing results cannot be available in time for a fresh embryo transfer.

Comparison with Existing Literature

This study adds to the body of literature suggesting that freeze-only cycles in comparison with fresh cycles may lead to superior outcomes for ongoing pregnancy in many patient cohorts. Although multiple studies have indicated similar or better transfer outcomes in FET cycles compared with fresh transfers (5, 39, 40), the literature on freeze-only versus fresh transfer is limited. This distinction is important because FETs may include transfer of supernumerary embryos (after the best embryos from the cohort were already transferred in a prior fresh or FET cycle); therefore, it is advantageous to specifically investigate freeze-only cycles.

To date, four RCTs have evaluated freeze-only versus fresh transfer, one of which has since been retracted. Shapiro et al. (8) reported that in a cohort of 103 "normal responders" (defined as age <41 years, FSH <10 mIU/mL, 8–15 antral follicles, first IVF attempt) undergoing an antagonist protocol, the clinical pregnancy rate per transfer was 84.0% in the cryopreservation group, which was statistically significantly higher than 54.7% in the fresh group. Shapiro et al. (9) also studied 122 high responders (>15 antral follicles) and found an higher pregnancy rate (although not statistically significant) in the freeze-only group, which was confounded by the fresh group having superior embryo quality. A meta-analysis on the subject included only these two aforementioned studies, as well as the retracted study, and concluded that freeze-only cycles had statistically significantly improved transfer outcomes compared with fresh cycles (41). Another RCT in 1,508 women with polycystic ovary syndrome found that freeze-only transfer of cleavage-stage embryos, compared with fresh transfer, was associated with a higher rate of live birth and a lower rate of OHSS (10).

In addition to these RCTs, a prospective observational cohort study of 530 patients undergoing antagonist protocol

and transfer of cleavage-stage embryos with $P \leq 1.5$ ng/mL found that freeze-only was associated with statistically significantly better pregnancy outcomes than fresh transfer (ongoing transfer 39.7% vs. 31.1%) (11). Also, a retrospective cohort study found that among 269 women with prior implantation failure, women who elected to undergo a freeze-only cycle had statistically significantly higher live-birth rates than women who elected to undergo another fresh cycle (OR 1.9; 95% CI, 1.1–3.3; $P=.03$) (12).

Similar to prior studies, our analysis reports statistically significantly higher ongoing pregnancy rates in freeze-only compared with fresh transfers, validating these previous findings in a much larger cohort (2,910 total cycles). Moreover, we include a wider patient population, sourced from multiple centers across the United States, thereby increasing the generalizability of our findings. We also did not exclude patients based on antral follicle counts, FSH levels, or P concentration at trigger. Importantly, we also report the results stratified by age and P, which was not done in prior studies.

Although our study was not a prospective RCT, we had access to extensive information on patient and cycle characteristics and were able to control for many potential confounders, including markers of ovarian function and oocyte quality. Though our findings in conjunction with prior studies are promising, the literature on freeze-only versus fresh transfers is limited overall, and additional large prospective studies are warranted. In particular, investigation of this question among PGS-tested embryos would be valuable in more directly controlling for embryo quality.

A premature rise in P has been found to negatively affect fresh transfer success rates in some studies (42, 43), making freeze-only protocols theoretically more beneficial in this population. Our study found that $P>1.0$ ng/mL was associated with statistically significantly lower ongoing pregnancy rates in fresh but not freeze-only protocols. The rise in P is thought to affect implantation through negatively affecting endometrial receptivity at the gene expression level and creating embryo–endometrium asynchrony (15, 29, 44–46). However, despite its effect on the endometrium, P concentration is not believed to correlate with embryo quality (47). Consistent with this idea, we found that freeze-only protocols resulted in statistically significantly higher ongoing pregnancy rates compared with fresh transfer protocols for women whose P concentration was >1.0 ng/mL. In our sensitivity analysis, we also observed a trend toward older women having better outcomes from freeze-only transfer cycles. This effect was most pronounced in the evaluation of the effect of freeze-only versus fresh transfer cycle in patients ≥35 years with $P>1.0$ ng/mL. Here, the OR for achieving ongoing pregnancy was the largest in any of the groups.

Although our study adds to the growing body of evidence suggesting increased success rates for freeze-only versus fresh embryo transfer, it is important to note that for clinical practice, multiple factors should be considered. Frozen cycles are generally associated with better perinatal and birth outcomes than fresh cycles, with a lower incidence of ectopic pregnancy, preterm birth, small for gestational age births, low birth weight, perinatal mortality, placental abruption, and placenta previa (1, 3–7). Frozen transfer cycles are also less likely to result in severe OHSS, which is a consideration for patients who are at higher risk for OHSS, including younger patients, patients with polycystic ovary syndrome, and patients with higher antral follicle counts (35, 38). The costs may be higher for freeze-only protocols because of cryopreservation/laboratory fees and possible additional medication costs, although one analysis found that FETs were more cost-effective than fresh transfers (48). Although vitrification has improved cryopreservation outcomes compared with slow freezing (2), a possibility remains for loss or damage to the embryo during the cryopreservation process, which has been estimated at 5% or less (2, 49, 50). Frozen transfer cycles also take additional time, which may add to the physical, emotional, and financial burdens of treatment.

Strengths and Limitations

The strengths of our study include the size of our large multicenter data set, the availability of data on P concentration at trigger, and the detailed information on potential confounders that allowed us to create a matched cohort incorporating markers of patient characteristics, ovarian reserve, cycle details, and embryo quality. Additionally, our study is the first to examine this question stratified by maternal age and P concentration on day of trigger. Limitations of the study include its retrospective format and inclusion of patients who underwent different protocols for ovarian stimulation, embryos generated in different laboratories with potentially different FET preparation protocols, lack of information on duration of infertility, and P concentrations assayed at different laboratories. Although we do not have information on the precision and intercoefficients and intracoefficients of P variation in multiple laboratories, we believe the differences in laboratories performing the progesterone assays are somewhat mitigated by the fact that we used a receiver operating characteristic analysis to determine optimal cutoffs in our data set for our analysis on the P effect on freeze-only cycles.

Another limitation is that we were unable to investigate live-birth and perinatal outcomes because the data were not available for the entire cohort. We also did not control for year of IVF. Additionally, it is also important to note that our fresh and freeze-only cohorts are blastocyst-transfer patients with a better-than-average prognosis (average age <35 years, basal antral follicle count >17, and number of usable embryos >5). Women whose embryos did not make it to the blastocyst stage were not included in the analysis. Therefore, the findings of our analysis may not be generalizable to poor responders, and this area warrants further study. Although we did not investigate the issue of cancellation rate in our analysis, it is likely that the cancellation rate in our study population would be low given the better-than-average prognosis of the patients.

Our study is also limited by the inherent differences that may be present in the patient populations when comparing freeze-only with fresh transfer; for example, freeze-only transfers may be more likely among women with higher antral follicle count, who therefore have a higher OHSS risk. However, although we cannot exclude that underlying

differences may be present in the patient populations, we believe that these limitations are diminished because we were able to match on an extensive amount of potential confounders, including patient characteristics, markers of ovarian function and embryo quality, indication for IVF, cycle details, endometrial thickness, and clinic-specific factors. This extensive cohort matching increases the validity of our findings and provides confidence that the two patient populations were similar on important drivers of IVF success.

CONCLUSION

Our study found that freeze-only transfer protocols have higher ongoing implantation and pregnancy rates than fresh transfer protocols, a finding that is consistent with prior studies suggesting that COS may negatively impact the endometrium's ability to support implantation. Freeze-only protocols appear to be particularly beneficial for older women who have a premature rise in P. Elevated P at trigger is a prognostic factor for poorer outcomes in fresh transfer cycles but not for freeze-only cycles. However, multiple elements should be taken into account when deciding on the best protocol for a particular patient, including patient preference, cost, and timing. Prospective RCTs should further expand on these findings as well as investigate the effect of freeze-only protocols on success rates of PGS-screened embryos and on live-birth outcomes.

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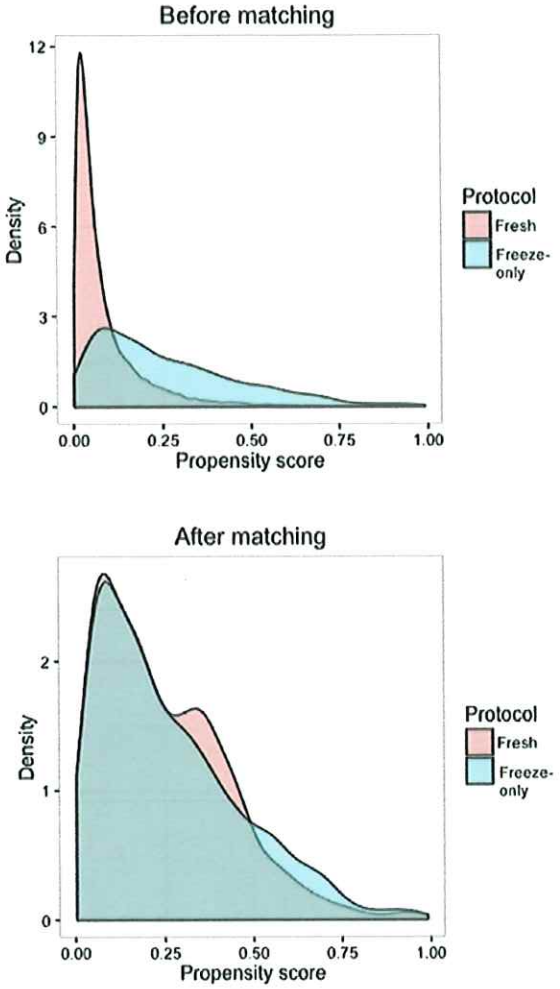
SUPPLEMENTAL FIGURE 1



Propensity score matching for fresh and freeze-only cohorts. From the distribution of the propensity score and inspection of the distribution of metrics, we achieved good balance between the treatment and control groups.

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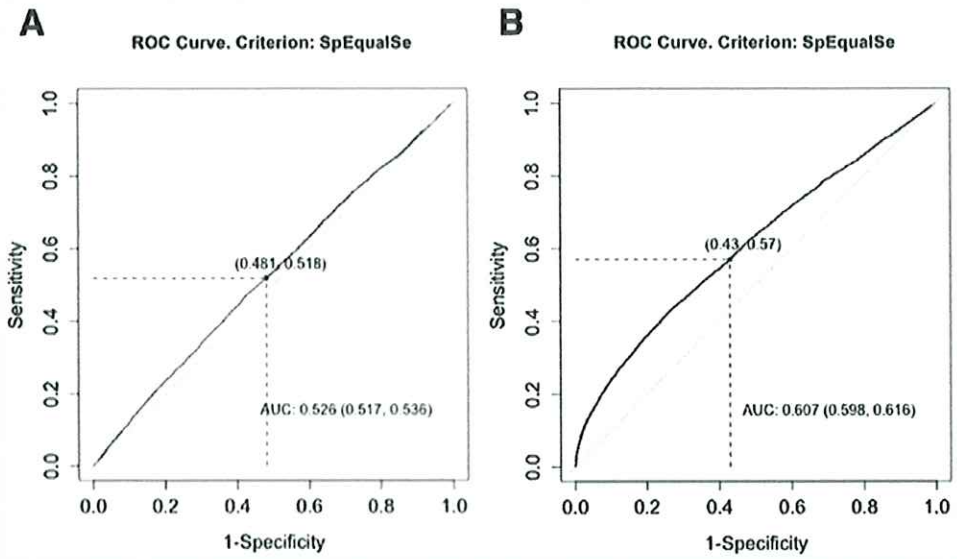
SUPPLEMENTAL FIGURE 2



Receiver operating characteristic (ROC) curves for determining the optimal age and progesterone (P) concentration at trigger cutoff points. Optimal cutoff points were chosen to balance specificity and sensitivity for classifying patients as achieving ongoing pregnancy. (**A**) ROC curve for P at trigger. A cutoff value of 1.0 ng/mL yielded a sensitivity and specificity each equal to 0.48. (**B**) ROC curve for age. A cutoff value of 35 years old yielded a sensitivity and specificity each equal to 0.43. AUC = area under the curve.

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SUPPLEMENTAL TABLE 1

Clinical metrics before and after matching.

	Before matching			After matching		
Metric	**Fresh**	**Freeze-only**	***P* value**	**Fresh**	**Freeze-only**	***P* value**
N	12,336	1,455	–	1,455	1,455	–
Age (y)	34.9 (4.4)	34.1 (4.3)	<.001	34.1 (4)	34.1 (4.3)	.98
BMI	25.7 (5.3)	25 (5.1)	<.001	24.8 (4.8)	25 (5.1)	.21
Parity	0.2 (0.5)	0.2 (0.6)	.02	0.2 (0.6)	0.2 (0.6)	.58
Gravidity	0.7 (1.1)	0.7 (1.2)	<.01	0.7 (1.2)	0.7 (1.2)	.78
Basal AFC	16 (8.9)	17.9 (9.5)	<.001	17.8 (9.4)	17.9 (9.5)	.78
Day 3						
FSH	7 (2.5)	6.9 (2.1)	.02	6.7 (2.1)	6.9 (2.1)	.11
E_2	52.1 (23.1)	51.4 (22.5)	.27	51.5 (22.8)	51.4 (22.5)	.91
LH	5.6 (3.2)	7.4 (4.5)	<.001	7.3 (4.1)	7.4 (4.5)	.57
Gonadotropin dose	2,839.6 (1,924.3)	2,562 (1,594.7)	<.001	2,536.5 (1,698.7)	2,563.8 (1,589.9)	.66
P at trigger	1.1 (0.6)	1.5 (1)	<.001	1.5 (1.1)	1.5 (1)	.19
Oocytes retrieved	15.7 (8.2)	21.2 (11.4)	<.001	20.8 (10.3)	21.2 (11.4)	.37
No. of usable embryos	4.1 (2.9)	5.7 (3.9)	<.001	5.6 (3.9)	5.7 (3.9)	.57
Endometrial thickness (mm)	10.4 (2.2)	10 (2.3)	.01	10.2 (2.2)	10 (2.3)	.46
Embryos transferred	1.7 (0.7)	1.5 (0.5)	<.001	1.5 (0.5)	1.5 (0.5)	.76
% ICSI	87.9%	93.7%	<.0001	93.7%	91.6%	.94
Clinic distribution[a]	–	–	<.01	–	–	.82
Diagnosis						
DOR	10.5	7.8	<.01	8.5	7.8	.59
Endometriosis	5.7	5.7	1.0	6.0	5.7	.81
Idiopathic	6.7	3.6	<.0001	4.3	3.6	.45
Male factor	22.4	18.7	<.01	20.8	18.7	.16
None provided	4.4	3.3	.05	3.2	3.3	1.0
Other	22.8	19.7	<.01	18.8	19.7	.57
Ovulatory dysfunction	9.5	15.0	<.0001	13.3	15.0	.2
PCOS	5.4	6.3	.16	6.3	6.3	1.0
Tubal	11.8	19.0	<.0001	17.9	19.0	.5
Uterine	0.8	0.9	.76	1	0.9	.85

Note: Mean (± standard deviation) is shown for continuous variables; percentages are shown for dichotomous variables. AFC = antral follicle count; BMI = body mass index; DOR = diminished ovarian reserve; E_2 = estradiol; FSH = follicle-stimulating hormone; ICSI = intracytoplasmic sperm injection; LH = luteinizing hormone; P = progesterone; PCOS = polycystic ovary syndrome.

[a] *P* value is the result of a chi-square test for the difference in distribution of clinics between protocols.

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SUPPLEMENTAL TABLE 2

Ongoing pregnancy odds ratios between progesterone concentration at trigger (> 1 versus < 1) stratified by protocol in matched data.

Group	Odds ratio (95% CI) ($P > 1.0$ compared with $P \leq 1.0$)	*P* Value
Fresh	0.66 (0.53, 0.82)	< .001
Freeze-only	0.98 (0.79, 1.22)	.85

Note: CI = confidence interval; P = progesterone.

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