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Original Article

Infertility

Season at the time of oocyte collection and frozen embryo transfer outcomes

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ABSTRACT

STUDY QUESTION: Does the meteorological season at the time of oocyte retrieval affect live birth rates in subsequent frozen embryo transfers?

SUMMARY ANSWER: Frozen embryo transfers resulting from oocytes retrieved in summer have 30% increased odds of live birth compared to frozen embryo transfers resulting from oocytes retrieved in autumn, regardless of the season at the time of embryo transfer.

WHAT IS KNOWN ALREADY: Season at the time of frozen embryo transfer does not appear to be associated with live birth rate. One study in the northern hemisphere found increased odds of live birth with frozen embryo transfer resulting from oocytes collected in summer when compared to those collected in winter.

STUDY DESIGN, SIZE, DURATION: Retrospective cohort study including all frozen embryo transfers performed by a single clinic over eight years, from January 2013 to December 2021. There were 3659 frozen embryo transfers with embryos generated from 2155 IVF cycles in 1835 patients. Outcome data were missing for two embryo transfers, which were excluded from analysis. Outcomes were analysed by the season, temperatures, and measured duration of sunshine at the time of oocyte collection and at the time of frozen embryo transfer.

PARTICIPANTS/MATERIALS, SETTING, METHODS: There were no significant differences between patients with oocyte collection or embryo transfers in different seasons. Meteorological conditions on the day of oocyte collection and the day of frozen embryo transfer, and in the preceding 14- and 28-day periods, were collected including mean, minimum, and maximum temperatures, and recorded duration of sunshine hours. Clinical and embryological outcomes were analysed for their association with seasons, temperatures, and duration of sunshine with correction for repeated cycles per participant, age at the time of oocyte retrieval, and quadratic age.

MAIN RESULTS AND THE ROLE OF CHANCE: Compared to frozen embryo transfers with oocyte retrieval dates in autumn, transfers with oocyte retrieval dates in summer had 30% increased odds of live birth (odds ratio (OR): 1.30, 95% CI: 1.04–1.62) which remained consistent after adjustment for season at the time of embryo transfer. A high duration of sunshine hours (in the top tertile) on the day of oocyte retrieval was associated with a 28% increase in odds of live birth compared to duration of sunshine hours in the lowest tertile (OR 1.28, 95% CI: 1.06–1.53). Temperature on the day of oocyte retrieval did not independently affect the odds of live birth. The odds of live birth were decreased by 18% when the minimum temperature on the day of embryo transfer was high, compared with low (OR: 0.82, 95% CI: 0.69–0.99), which was consistent after correction for the conditions at the time of oocyte retrieval.

LIMITATIONS, REASONS FOR CAUTION: This was a retrospective cohort study, however, all patients during the study period were included and data was missing for only two patients. Given the retrospective nature, causation is not proven and there are other factors that may affect live birth rates and for which we did not have data and were unable to adjust, including pollutants and behavioural factors. We were also not able to stratify results based on specific patient populations (such as poor- or hyper-responders) nor report the cumulative live birth rate per commenced cycle.

WIDER IMPLICATIONS OF THE FINDINGS: These findings may be particularly relevant for patients planning oocyte or embryo cryopreservation. Given the increasing utilization of cryopreservation, identification of factors that influence outcomes in subsequent frozen embryo transfers has implications for future therapeutic and management options. Further studies to clarify the physiology underlying the influence of sunshine hours or season on subsequent frozen embryo transfer outcomes are required, including identification of specific populations that may benefit from these factors.

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Introduction

Seasonal variations in human fecundability and birth rates have been well described around the world, however, the underlying cause remains unclear (Wesselink *et al.*, 2020). Proposed contributory factors include environmental impacts on gamete quality, miscarriage rates, and coital frequency, as well as sociological and behavioural effects. Evidence suggests that the effect is not purely behavioural or sociological, with seasonal variations being reported in women undergoing artificial insemination (Paraskevaides *et al.*, 1988).

Several groups have assessed how seasonal variations influence the success of ART, encompassing IVF, and ICSI. Some studies report a seasonal variation in the biochemical, clinical, and live birth rates (Stolwijk *et al.*, 1994; Chamoun *et al.*, 1995; Rojansky *et al.*, 2000; Weigert *et al.*, 2001; Wood *et al.*, 2006; Vandekerckhove *et al.*, 2016; Zhao *et al.*, 2019; Farland *et al.*, 2020; Mehrafza *et al.*, 2020; Chu *et al.*, 2022) whilst others have failed to demonstrate an association (Fleming *et al.*, 1994; Dunphy *et al.*, 1995; Revelli *et al.*, 2005; Wunder *et al.*, 2005; Kirshenbaum *et al.*, 2018; Xiao *et al.*, 2018; Liu *et al.*, 2019; Singh *et al.*, 2021; Carlsson Humla *et al.*, 2022).

Seasonal associations with the success of fresh embryo transfers, whereby an embryo generated in an IVF cycle is implanted in the same cycle as it is generated, have been more widely studied than frozen embryo transfers, whereby the embryo generated is cryopreserved and transferred into the uterus in a later cycle.

In Australia, there is a continuing trend towards the use of vitrification and frozen/thawed embryo transfers. In 2020, 32.6% of all initiated cycles were 'freeze-all' cycles, whilst the proportion of transfer cycles in which a frozen/thawed embryo was transferred was 60.5%. The live birth rate per transfer was 31.3% for frozen/thawed transfers and 25.3% for fresh embryo transfers (Newman *et al.*, 2022).

For fresh transfers, only one study has reported a seasonal variation in live birth rates, with more sunshine hours and fewer rainy days associated with increased live birth rates in Belgium (Vandekerckhove *et al.*, 2016). Improvements in embryological outcomes such as fertilization rates and embryo quality have been reported with increased daylight hours in Israel (Rojansky *et al.*, 2000) and autumn in Iran (Mehrafza *et al.*, 2020), whilst increased clinical pregnancy but not live birth rates have been reported with warmer weather and summer at the time of IVF in mainland China (Chu *et al.*, 2022), Hong Kong (Zhao *et al.*, 2019), the UK (Wood *et al.*, 2006), the USA (Chamoun *et al.*, 1995; Farland *et al.*, 2020), and the Netherlands (Stolwijk *et al.*, 1994). Conversely, increased pregnancy rates were seen in winter with fresh embryo transfer cycles in Austria and Hungary, though there was a significant impact of cycle programming for clinician and patient convenience in this study (Weigert *et al.*, 2001). Contrary to these findings, a number of studies have found no difference in embryological outcomes (Fleming *et al.*, 1994; Wunder *et al.*, 2005; Xiao *et al.*, 2018), implantation rates (Fleming *et al.*, 1994; Revelli *et al.*, 2005; Wunder *et al.*, 2005; Xiao *et al.*, 2018), pregnancy rates (Wunder *et al.*, 2005; Xiao *et al.*, 2018), or live birth rates (Xiao *et al.*, 2018; Singh *et al.*, 2021; Carlsson Humla *et al.*, 2022) across the UK, Switzerland, Sweden, Italy, China, and India.

As early as 1995, Dunphy *et al.* (1995) reported on 321 consecutive cryopreserved embryo transfers in Canada, finding no impact of season at the time of transfer on pregnancy and live birth rates. Kirshenbaum *et al.* (2018) reported on the outcomes of 1400 frozen embryo transfers in Israel, finding no impact of the season or calendar month at the time of embryo transfer on clinical pregnancy rates, whilst a total of 17 485 frozen embryo transfer (FET) cycles in studies in China found no association between season at transfer and live birth (Xiao *et al.*, 2018; Liu *et al.*, 2019). All of these studies analysed outcomes by season and conditions at the time of embryo transfer, rather than at the time of oocyte collection.

Most of the published studies have analysed outcomes based on the date of embryo transfer, whether fresh or frozen, rather than the date of oocyte collection (in the case of frozen embryo transfers). For fresh transfers, it is impossible to separate the effect of season on *in vivo* oocyte development, *in vitro* oocyte and embryo development, and implantation and embryonic development *in vivo*. However, for frozen embryo transfers, such studies assess the effect of environment on endometrial receptivity and embryonic development *in vivo*, rather than an environmental influence on oocyte growth, maturation, and competence. Furthermore, many studies assess proxy indicators of live birth as their primary outcomes, such as clinical or biochemical pregnancy, implantation rates, or embryological outcomes.

Recently, an association has been reported between meteorological season and temperature at the time of oocyte retrieval and subsequent live birth rate following frozen embryo transfers (Correia *et al.*, 2022). Oocytes collected during summer or on days with warmer temperatures were more likely to be associated with live birth and clinical pregnancy, whilst the temperature and season on the day of embryo transfer did not have an effect. This study was performed in Boston, MA, a city in the northern hemisphere with a humid subtropical climate (Köppen classification Cfa).

Our aim was to assess whether season, temperature, and number of hours of bright sunshine at oocyte retrieval and at embryo transfer were associated with improved outcomes in a southern hemisphere setting with substantially different weather patterns to the previous study. Our study is based in Perth, Western Australia, which has a Mediterranean climate (Köppen classification Csa).

Materials and methods

We performed a retrospective cohort study of all frozen embryo transfers performed at two locations of a single clinic between 1 January 2013 and 31 December 2021 for which the oocyte retrieval date was also within these dates. All patients were managed at Fertility Specialists of Western Australia, a private clinic based at two metropolitan sites in Perth, Western Australia. Patients provided written consent for use of their anonymized data in research and academic publications prior to commencing treatment with the clinic.

Patient and treatment characteristics

All IVF cycles and transfer cycles are recorded in the clinic database. Data were collected on patient and partner demographics and IVF cycle characteristics including peak oestradiol

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concentration, number of oocytes collected, method of fertilization (IVF or ICSI), number of oocytes fertilized, and number of usable blastocysts. For each frozen transfer cycle, data were collected on the type of cycle (natural, minimally stimulated, or hormone therapy cycle (HRT)), all medications used, and the duration of embryo cryopreservation.

Clinic and laboratory activity did not differ across seasons. During the study period, all clinical management decisions and procedures were undertaken by fully qualified specialists. Laboratory and operating theatre conditions were strictly controlled, with no seasonal variation in temperature or humidity. All embryology procedures were performed on a temperature-controlled workbench and microscope stage, with culture in humidified benchtop incubators.

All frozen embryo transfers in the study period with an oocyte collection date for the transferred embryo within the same period were included.

Weather and seasonality parameters

Weather data for Perth, Western Australia, were obtained from the Australian Bureau of Meteorology. For each day during the study period we collected data on average, maximum and minimum temperature, and the number of daylight hours (recorded as actual number of hours of bright sunshine, as opposed to calculated from sunrise and sunset times).

Each day in the study period was categorized by meteorological season: summer (December–February), autumn (March–May), winter (June–August), or spring (September–November).

Tertiles were created for the average temperature, maximum and minimum temperature, and the daily hours of bright sunshine at oocyte retrieval. These same tertile cut-offs were also applied to the weather data at the time of frozen embryo transfer. The tertile cut-offs were:

- Average temperature (°C): low: 7.9–15.5, medium: 15.6–20.9, high: 21.0–33.9
- Maximum temperature (°C): low: 13.2–21.2, medium: 21.3–27.4, high: 27.5–43.3
- Minimum temperature (°C): low: 0.1–9.8, medium: 9.9–14.4, high: 14.5–27.8
- Sunshine hours: low: 0–7.6, medium: 7.7–10.6, high: 10.7–13.3

We also performed a secondary analysis of outcomes by average temperature and average sunshine hours in 14 and 28 days prior to oocyte collection or embryo transfer, with conditions again divided into tertiles.

Clinical outcomes

The primary clinical outcome was live birth per embryo transfer, defined as the delivery of at least one live neonate. Secondary clinical outcomes were gestational age at delivery, plurality, clinical pregnancy (defined as at least one gestational sac on ultrasound), biochemical pregnancy (defined as a quantitative serum β-hCG level >25 IU/l at least 11 days after embryo transfer), miscarriage (defined as loss of a clinical pregnancy prior to 20 weeks' gestation), and stillbirth (defined as a stillbirth at or after 20 weeks' gestation).

Embryological outcomes

Other secondary outcomes included the number of oocytes obtained per cycle, the fertilization rate (defined as the number of zygotes with two pronuclei detected the day following insemination divided by the number of oocytes collected) and the usable blastocyst development rate (defined as the number of blastocysts considered suitable for cryopreservation or transfer divided by the number of two pronuclear zygotes).

Statistical analysis

Clinical outcomes were measured per frozen embryo transfer and embryological outcomes were measured per IVF cycle. Continuous data were summarized using medians, interquartile ranges (IQR) and ranges (R), and categorical data using frequency distributions. Clinical and embryological outcomes were analysed for their association with seasons, average temperatures, and hours of sunshine using the generalized estimating equations method with an exchangeable working correlation to account for the correlation between repeated cycles on each participant.

Binary outcomes including live birth, biochemical pregnancy, clinical pregnancy, and miscarriage were analysed using a binomial distribution and logit link function; embryological outcomes including number of oocytes, fertilization, and usable blastocyst rates were analysed using a negative binomial distribution with a logarithmic link function, as there was overdispersion in the data.

Gestational age at birth was analysed as 'time to delivery' using Cox proportional hazards modelling with a robust variance estimator to account for the correlation between repeated cycles. Clinical pregnancies that reached viability, i.e. 20 weeks or more gestation, were included in the time to event analysis.

All clinical models were adjusted for repeated IVF cycles by the same patient, age at time of oocyte retrieval, and a quadratic age term. Embryological models were adjusted for peak oestradiol, age at oocyte retrieval and a quadratic age term. Odds ratios (OR), incidence rate ratios (IRR), and hazard ratios (HR) are presented along with 95% CIs.

Sensitivity analyses were conducted on ICSI and IVF subgroups to evaluate the robustness of live birth results across these groups, and on the endometrial preparation used for the frozen embryo transfer cycle to evaluate whether results were consistent among groups who may be expected to have poorer birth outcomes.

Stata version 16 statistical software (College Station, TX: StataCorp LLC) was used for data analysis.

Ethical approval

Exemption from formal ethical review was granted by the Human Research Ethics Office of the University of Western Australia (reference 2022/ET000980).

Results

Over the 8-year study period, there were 3659 frozen embryo transfers performed, with embryos generated from 2155 IVF cycles in 1835 patients. The median age at oocyte retrieval was 34.5 years (IQR: 31.6–37.3), and at FET was 36.1 years (IQR: 33.3–38.9). Embryos were cryopreserved for a median of 0.4 years (IQR: 0.2–1.6) and each participant had a median of two FET procedures performed (IQR: 1–3).

Blastocysts were cryopreserved and transferred after 5 days of embryonic development in 69.4% of instances, and after 6 days of embryonic growth in 30.5% of instances. Embryos were transferred after 3 days of embryonic growth in 0.1% of cases (n = 2). Embryos were transferred in either natural cycles (43.5%), minimally stimulated cycles (42.3%) or hormonally controlled cycles using exogenous oestradiol and progesterone (14.2%); 97.7% were single embryo transfers, and 2.3% double embryo transfers. All

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patients received supplemental progesterone following FET, with 94.9% receiving progesterone pessaries; less commonly prescribed progestogens included intravaginal progesterone gel, oral progesterone, and intramuscular progesterone. Combination progesterone treatment was used in 5.3% of patients. These findings were similar across seasons.

Patient demographic and clinical characteristics were similar across seasons at the time of oocyte retrieval and at the time of embryo transfer and are shown in Table 1. The median duration of cryopreservation was slightly longer for FET cycles in which oocyte retrieval occurred in summer ($P = 0.046$); there were no other statistically significant differences in the populations. All seasonal models were checked with the inclusion of log-transformed cryopreservation length, which was not statistically significant in any models and did not substantively change results.

Outcome data were missing for two FETs; these were excluded from analysis leaving 3657 FETs for analysis.

Primary clinical outcome

Autumn was designated as the reference season for all analyses, with the lowest live birth rate per frozen embryo transfer occurring when oocyte collection occurred in autumn. Compared to FET with oocyte retrieval dates in autumn, FET with oocyte retrieval dates in summer had 30% increased odds of live birth (OR: 1.30, 95% CI: 1.04–1.62, $P = 0.02$); this remained consistent when the model was adjusted for season at the time of FET (Fig. 1).

There was no change in the odds of live birth based on mean day temperature at the time of oocyte retrieval, however, we observed a 28% increase in odds of live birth when the number of sunshine hours was high (10.7–13.3 h) compared with low (0–7.6 h) on the day of oocyte retrieval (OR: 1.28, 95% CI: 1.06–1.53, $P = 0.008$); this remained consistent when adjusted for sunshine hours on the day of FET (Fig. 2).

Regarding conditions on the day of FET, odds of live birth were decreased by 18% when the minimum temperature on the day of transfer was high (14.5–27.8°C) compared with low (0.1–9.8°C) (OR: 0.82, 95% CI: 0.69–0.99, $P = 0.040$); this remained after adjustment for the temperature on the day of oocyte collection. The odds of live birth were lower when FET occurred in spring when compared to autumn (OR: 0.80, 95% CI: 0.64–0.98, $P = 0.035$); however, these results were not consistent once the model was mutually adjusted for season at the time of oocyte collection (Fig. 1).

When live birth rate was analysed according to the average temperatures and sunshine hours in 14 and 28 days preceding oocyte retrieval or embryo transfer, there was no statistical difference in the odds of live birth.

Secondary clinical outcomes by conditions at oocyte collection

There were no differences observed in the odds of biochemical pregnancy, clinical pregnancy, miscarriage, or gestation at birth based on season or the number of hours of sunshine on the day of oocyte collection.

Miscarriage rates were lowest when the average temperature or the maximum temperature on the day of oocyte collection was in the middle tertile when compared to the lowest tertile (average temperature: OR: 0.70, 95% CI: 0.49–0.99, $P = 0.041$; maximum temperature: OR: 0.66, 95% CI: 0.47–0.93, $P = 0.019$), however, no difference was seen in biochemical or clinical pregnancy rates, or gestation at birth for these groups (Fig. 3).

Table 1. Demographic and clinical characteristics per frozen embryo transfer cycle (n = 3663) by season at the time of oocyte retrieval.

	Autumn (n = 945)	Winter (n = 1031)	Spring (n = 938)	Summer (n = 745)
Patient characteristics				
Age at OPU (years)	34.2 (31.4–37.1)	34.3 (30.9–37.2)	34.1 (31.2–36.6)	34.3 (31.3–36.9)
Age at FET (years)	36.3 (33.6–39.0)	36.1 (33.1–39.2)	35.9 (33.3–38.5)	36.4 (33.4–38.9)
Parity	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Gravidity	0 (0–1)	0 (0–1)	0 (0–1)	1 (0–1)
Oocyte collection cycle characteristics				
Peak oestradiol (pmol/l)	5852 (4149–8422)	6683 (4649–8881)	6222 (4195–9008)	6281 (4394–8830)
Fertilization method				
IVF	439 (46.5)	494 (47.9)	496 (52.9)	394 (52.9)
ICSI	506 (53.5)	537 (52.1)	442 (47.1)	351 (47.1)
Number of oocytes retrieved	11 (8–15)	12 (9–16)	12 (8–16)	12 (9–15)
Number of 2PN	7 (5–9)	8 (5–10)	7 (5–11)	7 (5–10)
Usable blastocysts	4 (3–6)	4 (3–6)	4 (3–6)	4 (3–6)
Frozen embryo transfer cycle characteristics				
Cryopreservation length (years)	0.4 (0.2–1.6)	0.4 (0.2–1.2)	0.4 (0.2–1.2)	0.5 (0.2–1.8)
Transfer cycle type				
Natural	415 (43.9)	422 (40.9)	456 (48.6)	298 (40.1)
Low dose stimulation	392 (41.5)	478 (46.4)	354 (37.7)	324 (43.5)
HRT	138 (14.6)	131 (12.7)	128 (13.6)	122 (16.4)
Progesterone supplementation				
Vaginal gel	40 (4.2)	54 (5.2)	30 (3.2)	22 (3.0)
Oral	43 (4.5)	36 (3.5)	31 (3.3)	30 (4.0)
Intramuscular	13 (1.4)	26 (2.5)	19 (2.0)	23 (3.1)
Pessary	893 (94.5)	972 (94.3)	898 (95.7)	709 (95.2)
Combination	48 (5.1)	59 (5.7)	45 (4.8)	42 (5.6)
Embryo age				
Day 3	2 (0.2)	0 (–)	0 (–)	0 (–)
Day 5	636 (67.3)	715 (69.4)	651 (69.4)	538 (72.2)
Day 6	307 (32.4)	316 (30.6)	287 (30.6)	207 (27.8)

Data are presented as median (interquartile range) for quantitative variables and n (%) for categorial variables.
OPU, oocyte pick up; FET, frozen embryo transfer.

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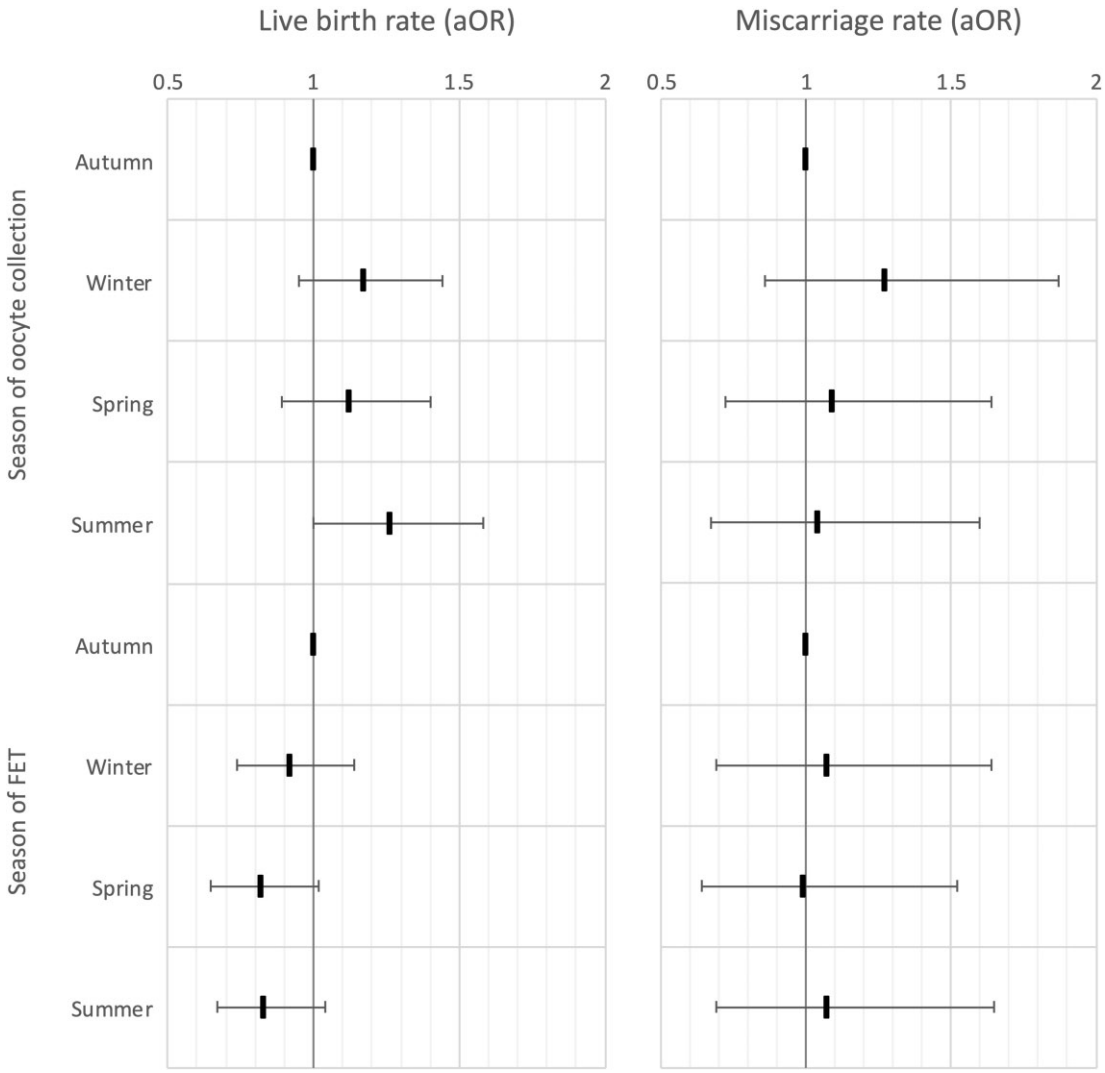

Figure 1. Adjusted odds ratios (aOR) for live birth rate and miscarriage rate by season of oocyte collection and season of frozen embryo transfer (FET). Autumn is the reference season. OR adjusted for age, quadratic age, and multiple IVF cycles in the same patient. OR mutually adjusted for the season of oocyte collection/FET. Error bars represent 95% confidence intervals.

Secondary clinical outcomes by conditions at frozen embryo transfer

Biochemical pregnancy rates were increased when the number of sunshine hours was in the middle tertile at the time of FET compared to the lowest tertile (OR: 1.21, 95% CI 1.02–1.43, $P = 0.028$). They were decreased when FET occurred in spring as compared to autumn, however, as for live birth rates, this difference did not persist after adjustment for season at the time of oocyte collection.

No difference was observed in clinical pregnancy rate, miscarriage rate, or gestation at birth based on season, sunshine hours, or average temperature on the day of FET. However, the odds of miscarriage were increased by 42% when FET occurred on a day with a maximum temperature in the top tertile (27.5–43.3°C) compared to the lowest tertile (13.2–21.2°C) (OR: 1.42, 95% CI 1.02–1.98, $P = 0.039$).

Gestational age at delivery for pregnancies that progressed to 20 weeks' gestation or greater was not associated with season, weather, or sunshine hours in any models.

Stillbirths were rare (0.2%, n = 8), as were multiple births (twins occurred in 0.7%, n = 25). Due to their low prevalence, they were not further analysed.

Embryological outcomes by conditions at oocyte collection

Peak oestradiol levels were higher in winter ($P = 0.010$). Peak oestrogen was statistically significant in all models and influenced seasonal effects, consequently, all embryological models were adjusted for log-transformed peak oestradiol, as well as age and a quadratic term for age.

Increased fertilization rates were associated with average 14-day sunlight hours in the highest tertile (10.7–13.3 h) (IRR: 1.04, 95% CI: 1.00–1.07, $P = 0.048$), compared with lowest (0–7.6 h). There were no effects of seasonality, sunlight hours or temperatures including 24-h, 14-, and 28-day average, minimum, or maximum temperatures on the number of oocytes or usable blastocyst rates per IVF cycle.

Sensitivity analyses

Predetermined sensitivity analyses were performed on the primary outcome only. We assessed the method of fertilization (ICSI or IVF) and FET cycle type (HRT or natural/minimal stimulation).

Within the ICSI group, there were increased odds of live birth when oocyte collection occurred in summer or in spring; this effect remained when adjusted for the season of FET. Consistent

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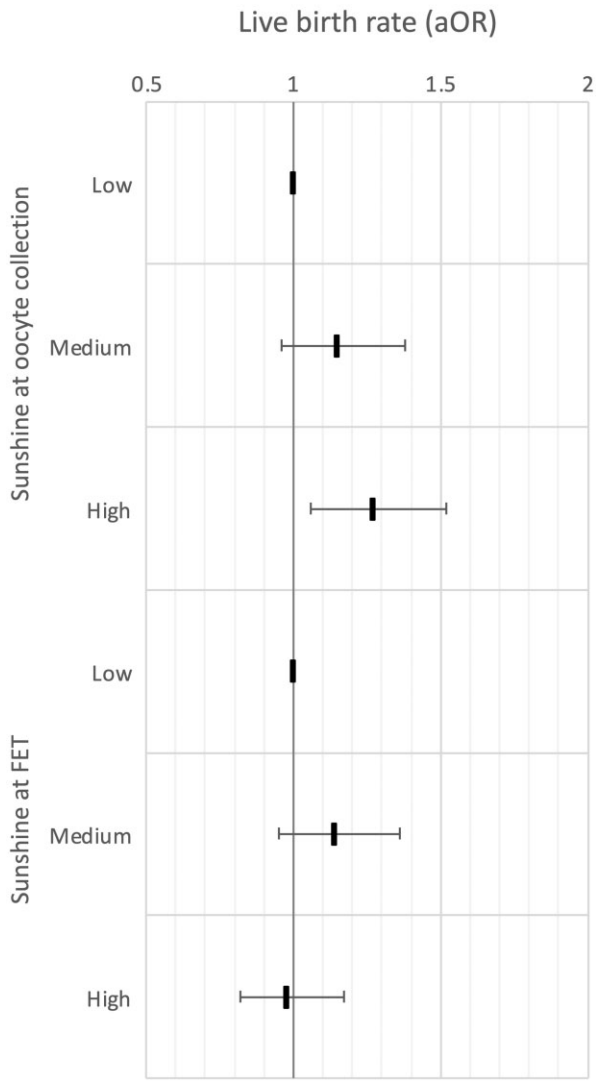

Figure 2. Adjusted odds ratios (aOR) for live birth rate by recorded number of sunshine hours (tertiles) on the day of oocyte collection and frozen embryo transfer (FET). The lowest tertile is the reference tertile. OR adjusted for age, quadratic age, and multiple IVF cycles in the same patient, and mutually adjusted for sunshine hours at the time of oocyte collection/FET. Error bars represent 95% confidence intervals.

with the whole group findings, the odds of live birth were increased with sunshine hours in the highest tertile on the day of oocyte collection but were not affected by average temperature on the day of oocyte collection.

Sensitivity analysis of the IVF group (conventional fertilization without ICSI) contrasted with the whole group findings, with season at the time of oocyte collection not affecting live birth rate. Hours of sunshine and average temperatures on the day of oocyte collection also did not affect live birth rates. Live birth rates in this group were reduced when FET occurred in spring or summer.

Overall, the whole group findings appear to be driven by the effect of seasonality and sunshine hours in the ICSI group. Live birth was reduced with FET in spring and summer uniquely in the IVF group.

Sensitivity analysis by FET cycle type was consistent with whole group findings. There were increased odds of live birth with oocyte retrieval in summer for patients undergoing HRT cycles (n = 518), which was consistent after adjustment for the season on the day of FET. Sunshine hours on the day of oocyte retrieval increased the odds of live birth in the HRT group but did not reach statistical significance ($P < 0.1$), and there was no effect of average temperature.

Discussion

Our results confirm that the season and weather conditions at the time of oocyte collection affect live birth rates in patients undergoing frozen embryo transfer, favouring summer, and more sunshine hours. The odds of live birth with oocyte collection in summer were 30% higher when oocyte retrieval occurred in summer as compared with autumn, and 28% higher when oocyte collection occurred on a day when the number of sunshine hours was high as compared with low. These findings were independent of the season and conditions on the day of embryo transfer.

These findings are consistent with previously published data on FETs, which found no association between season at the time of embryo transfer and clinical pregnancy or live birth rates (Dunphy *et al.*, 1995; Kirshenbaum *et al.*, 2018; Xiao *et al.*, 2018; Liu *et al.*, 2019). Along with the recently published data of Correia *et al.* (2022), our findings suggest that seasonal or environmental conditions affect oocyte development and/or maturation, and ultimately embryo competence and subsequent live birth rates, rather than impacting on uterine receptivity and early pregnancy development.

Compared to the study performed by Correia *et al.* in Boston our temperature tertiles were substantially higher, with daily average temperatures in degrees Celsius (°C) of 7.9–15.5 versus <6.7 in the first tertile, 15.6–20.9 versus 6.7–17.2 in the second tertile, and 21.0–33.9 versus >17.2 in the third tertile. Whilst day lengths determined by sunrise and sunset times were reported in the Boston study, we instead used actual recorded hours of bright sunshine, which has a more robust physiological basis for impact on oocyte development.

Correia *et al.* suggested that in their population ambient temperature on the day of oocyte retrieval, and not day light hours, was the underlying driver of the seasonal variation in FET success rates. This is at odds with our findings, which found the duration of bright sunshine on the day of oocyte retrieval to be driving the seasonal variations, whereas ambient temperature was not associated with clinical outcomes. This may be related to the fact that we used the actual measured duration of sunshine hours rather than calculated daylight hours. Although sunshine hours and temperature were correlated, over the duration of our study the degree of correlation between daily sunshine hours and daily mean, maximum, and minimum temperatures was, respectively, low ($r = 0.47$), moderate ($r = 0.58$), and very low ($r = 0.17$) using Pearson's correlation coefficient. This supports the validity of our finding of an independent relationship between sunshine hours and outcome, irrespective of temperature parameters.

Melatonin has been suggested as a positive mediator of oocyte maturation and competence through its antioxidant, autocrine, and paracrine actions (Reiter *et al.*, 2014). Though the data are of poor quality, melatonin supplementation appears to improve embryological outcomes but not live birth rate in IVF cycles (Hu *et al.*, 2020). Pineal melatonin secretion occurs at night, and the duration of secretion and peak levels are higher in winter than in summer, making increases in circulating melatonin levels an unlikely cause of the observed effects in this study (Wehr, 1997). Whether melatonin production in the follicular granulosa cells and the oocyte itself follow the same seasonal variation remains uncertain (Reiter *et al.*, 2014).

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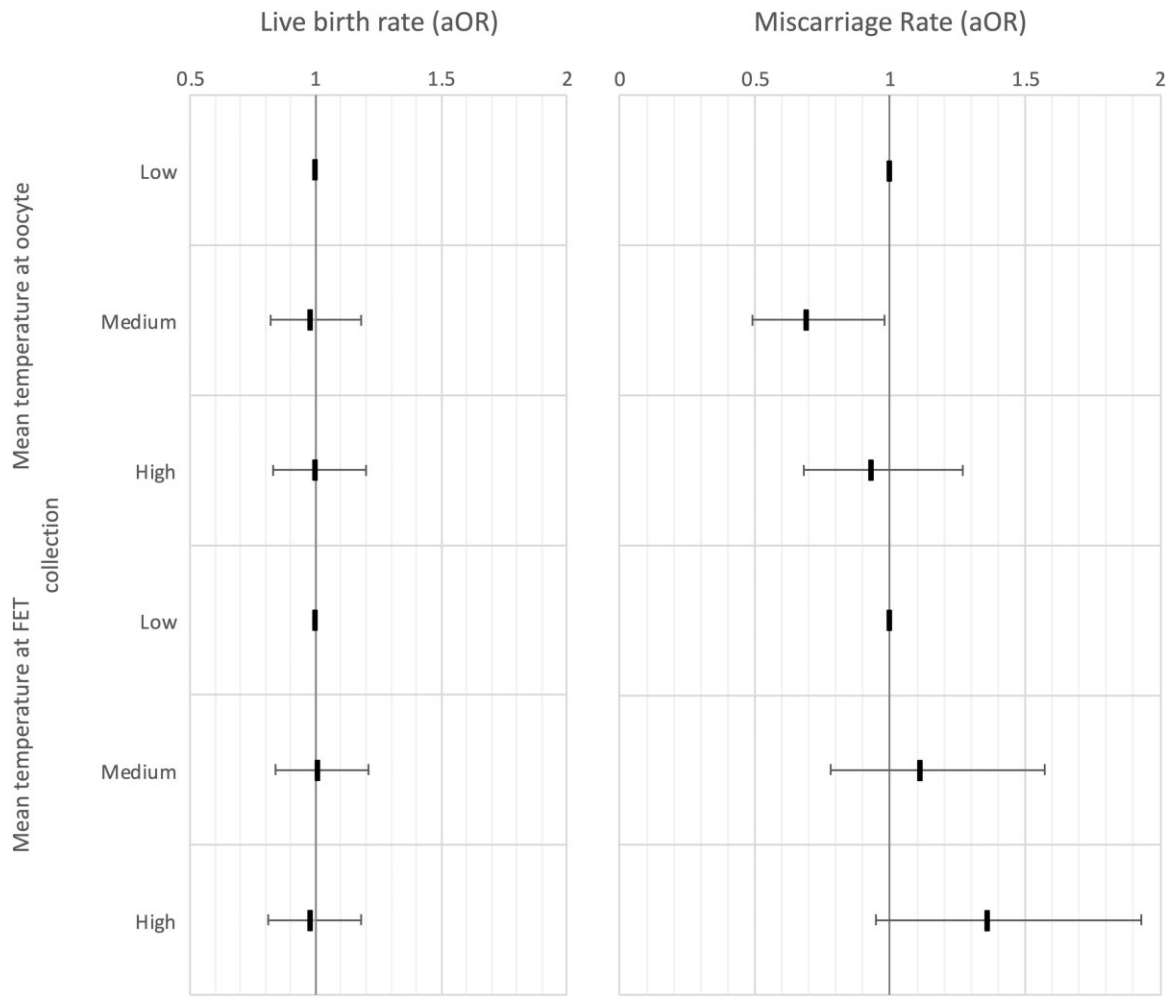

Figure 3. Adjusted odds ratios (aOR) for live birth rate and miscarriage rate by average temperature on the day of oocyte collection and frozen embryo transfer (FET). The lowest tertile is the reference tertile. OR adjusted for age, quadratic age, and multiple IVF cycles in the same patient, and mutually adjusted for temperature at the time of oocyte collection/FET. Error bars represent 95% confidence intervals.

Vitamin D levels have also been proposed to influence IVF outcomes (Paffoni *et al.*, 2014), though it has been speculated that this may be through an endometrial effect (Rudick *et al.*, 2012). In an Australian population, 25(OH)D levels are comparable in summer and autumn, before dropping precipitously in winter (Voo *et al.*, 2020). Given the lowest live birth rates were seen with oocytes collected in autumn, vitamin D levels at the time of oocyte collection are unlikely to play a substantial role. Furthermore, a recent systematic review and meta-analysis found no significant impact of Vitamin D levels on live birth rate (Cozzolino *et al.*, 2020).

Oocyte collection occurs in the morning in our centre, so most sunshine hours on a given day will occur after the collection. Our secondary analysis using an average number of sunshine hours in the 14 and 28 days prior to oocyte collection did not reveal a significant impact on live birth rates, suggesting that sunshine exposure during folliculogenesis may not be the main driving factor in our findings.

It is possible that there are differences in activity, diet, and lifestyle in different seasons which could underlie the observed differences in live birth rates, though such data were not collected in this study. It is also possible that other environmental factors including pollutants may impact clinical outcomes.

The lower live birth rate observed when FET was performed in spring may reflect the median duration of embryo cryopreservation of 0.4 years, meaning that these embryos were more likely to have been created from oocytes collected in autumn, the population in which the lowest success rates were seen. This would be consistent with the finding that the difference did not persist once the model was mutually adjusted for the season at oocyte collection.

Though the season at the time of FET did not impact live birth rates after correction, a modest reduction in live birth rate was seen when the minimum temperature on the day of embryo transfer was high, as well as an increase in miscarriage rates when maximum temperature on the day of embryo transfer was high. The increased odds of miscarriage seen when maximum temperatures are in the highest tertile of 27.5–43.3°C is consistent with epidemiological studies, which have found that rates of miscarriage are higher in summer months (Hajdu and Hajdu, 2021; Wesselink *et al.*, 2022). Interestingly, our data suggest that this association persists even when corrected for the conditions at the time of oocyte collection, suggesting that it is high temperatures in early pregnancy rather than during oocyte development that underlie this finding.

Given the increasing utilization of cryopreservation and frozen embryo transfers, patients and clinicians may opt to collect oocytes and cryopreserve embryos in the summer months when daylight hours are higher with a view to increasing live birth rates per embryo transfer. However, further questions remain to be

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answered regarding the impact of seasonal and environmental influences on the success of fertility treatment.

Firstly, there is increasing utilization of oocyte cryopreservation for the purpose of fertility preservation in patients who are likely to have accelerated diminution of their ovarian reserve, whether iatrogenic or natural, or who wish to delay conception. Whether the environmental conditions at the time of oocyte collection impact reproductive success for such patients remains unknown and should be evaluated.

Secondly, the impact season and environmental influences have on sperm number and function is uncertain. It is known that seminal parameters vary across the seasons (Levitas *et al.*, 2013), and that environmental exposures can influence sperm quantity, quality, and DNA integrity (Pizzol *et al.*, 2021). Given our findings in the sensitivity analyses of contrasting outcomes in the conventional IVF and the IVF–ICSI population, evaluation of seminal parameters and sperm DNA damage, and environmental factors should be investigated further. Noting that the main findings of our study appeared to be driven by the ICSI group, and that these findings were abrogated in the conventional IVF group, it is likely that sperm quality is influencing our results. Existing evidence suggests that higher temperatures are associated with impaired seminal parameters, and we would therefore expect sperm factors to reduce the observed seasonal effects in this study, which could explain the reduced effect in IVF cycles without ICSI. We did not evaluate or correct for the use of frozen or fresh sperm in this study.

Thirdly, our study has considered conditions at the time of oocyte collection or embryo transfer, and in the weeks prior, however, oocyte development and spermatogenesis occur over a period of months prior to ovulation or ejaculation. Given the nadir in success rates was seen with oocytes collected in autumn, it is possible that the higher temperatures of the preceding summer during the period of oocyte activation and growth, and spermatogenesis, may have a detrimental impact on the quality of the resulting gametes. Differentiating the most relevant and environmentally sensitive time frames for gamete development would be challenging and would require a complex interventional experimental model that is unlikely to be feasible, however, the resulting insights into the physiology of gametogenesis would be enlightening. Such an observation may also explain, for example, the role of seasonally variable physiological factors such as endogenous melatonin levels, whose role during the period of ovarian stimulation may be less important than their role during the preceding pre-antral oocyte development.

Finally, factors other than temperature, meteorological season, and number of daylight hours may vary across the year and might have contributed to our findings. Air quality has been associated with impaired reproductive outcomes with regard to natural conception and ART outcomes. Particulate matter $<10\,\mu m$ (PM_{10}) or $< 2.5\,\mu m$ ($PM_{2.5}$) has been associated with reduced fecundity and increased likelihood of infertility, as well as reduced live birth rates and increased miscarriage rates in patients undergoing IVF (Checa Vizcaino *et al.*, 2016; Li *et al.*, 2021). *In vitro* exposure of mouse oocytes to PM_{10} also results in impaired maturation capacity, increased cell cycle arrest, as well as increased oxidative stress, DNA damage, mitochondrial dysfunction, and apoptosis (Jo *et al.*, 2020). Nitrogen dioxide (NO_2) levels have been linked to increasing miscarriage rates in the general population as well as a decrease in live birth rates for those undergoing IVF, whilst sulphur dioxide (SO_2) is associated with DNA damage *in vitro* and increased rates of miscarriage *in vivo* (Checa Vizcaino *et al.*, 2016), and has also been linked to an increased risk of poor ovarian response in patients undergoing IVF (Wu *et al.*, 2022). Carbon monoxide exposure is associated with increased miscarriage rates (Checa Vizcaino *et al.*, 2016).

Air quality in Perth is generally very high, though evaluation of local air quality data at the time of oocyte collection and the time of frozen embryo transfer may help to delineate the effects of these pollutants on oocyte development and on embryo implantation and early pregnancy.

Conclusions

This is the first study to analyse frozen embryo transfer outcomes using actual measured hours of sunshine, and the first to analyse outcomes by meteorological season in the southern hemisphere. Optimal conditions for live birth appear to be associated with summer and increased sunshine hours on the day of oocyte retrieval, rather than the average temperature on the day of oocyte retrieval. In contrast, high minimum temperatures on the day of FET are associated with reduced live births. Other environmental factors as well as the underlying physiology warrant further investigation.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

S.L. conceived and designed the study, contributed to data collection, and wrote the final manuscript. C.R. assisted with data collection. M.W. assisted with study design, provided the data, and assisted with data cleaning. E.N. performed the statistical analyses. R.H. provided assistance with the study design. All authors contributed to the interpretation of the data and writing of the final article, including the discussion and conclusions.

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Conflict of interest

S.L. has received educational travel assistance from Besins, Merck, and Organon outside the submitted work. R.H. is the National Medical Director of City Fertility and the Medical Director of Fertility Specialists of Western Australia, has received honoraria from MSD, Merck Serono, Origio, and Ferring and travel support from Merck Serono and MSD outside the submitted work, and has equity interests in CHA SMG. C.R., M.W., and E.N. declare that they have no conflicts of interest.

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