Seasonal Influence on Assisted Reproductive Technology Outcomes: A Retrospective Analysis of 1409 Cycles

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Background: It is well known that seasonal variations influence natural conception and birth rates variably in different populations. It has been hypothesised that similar seasonal influences may affect treatment outcomes following assisted reproductive technology (ART). However, most studies report conflicting results.

Aim: The aim of the study is to elucidate whether seasonality had any impact on the treatment success of the ART program.

Study Setting and Design: We conducted a retrospective cohort study at a university-level tertiary care hospital in South India.

Materials and Methods: All couples who underwent ART between January 2012 and December 2016 were included in the study. We divided the study population into three groups based on the seasonal differences experienced in our region. The primary outcome was live birth rate (LBR).

Statistical Analysis: Univariate and multiple logistic regression models were used to compare outcomes and results reported as odds ratio (OR) and 95% confidence interval (CI).

Results: Univariate analysis revealed no significant difference in LBR in monsoon season (174/651, 26.7% vs. 83/319, 26.0%; OR 1.04, 95% CI 0.77, 1.41; P = 0.81) as compared to summer. However, LBR was significantly higher in winter season (114/341, 33.4% vs. 83/319, 26.0%; OR 1.43; 95% CI: 1.02, 2.00; P = 0.04). Further, multivariate analysis following adjustment for various confounding factors revealed no significant statistical difference in LBR in monsoon (adjusted odds ratio [aOR], 0.92; 95% CI: 0.66, 1.26; P = 0.59) or winter (aOR 1.32; 95% CI: 0.92, 1.88; P = 0.13) as compared to summer season.

Conclusion: The current study found no significant effect of seasonal variation on LBR following ART.

Keywords: Assisted reproductive technology outcome, assisted reproductive technology, live birth rate, seasonal variation

INTRODUCTION

One in six couples in the reproductive age group fail to achieve a pregnancy after one year of unprotected sexual intercourse and at the end of their reproductive life, 2%–7% couples remain childless.[1] Assisted reproductive technology (ART) is considered the most effective treatment option for infertility. Over the years, the proportion of ART cycles has been steadily increasing with over 8.5 million cycles being performed annually in Europe alone.[2] However, in spite of various advances in the clinical and laboratory techniques and the introduction of several adjuncts aimed at improving ART outcomes, the live birth rate (LBR) remains at 22%–25%.[2-4] The success of the ART program is
considered multifactorial and is determined by clinical characteristics, controlled ovarian hyperstimulation protocols, in vitro gamete handling, culture conditions, and environmental factors.\(^5\)\(^6\)

Earlier studies have suggested that seasonal variations influence natural conception and birth rates variably in different populations.\(^7\)\(^8\) These changes are attendant to the effects of climate, nutritional, and sociocultural differences on the male and female reproductive physiology.\(^9\) It has been suggested that decreased melatonin secretion due to longer exposure to daylight may reduce gonadotrophin secretion, prolong the menstrual cycle, and alter endometrial function in women.\(^10\)\(^11\) Higher average temperature has been found to be associated with reduced semen quality and decreased sperm concentration.\(^12\) Sociocultural differences and seasonal holidays are also found to influence the frequency of intercourse and alter conception rates.\(^13\) In addition to this, the differences in dietary practices related to ethnic and geographical differences of couples may also affect the frequency of ovulation and menstrual cycle.\(^14\)

It has been hypothesised that similar seasonal influences may affect treatment outcomes following ART. In a quest for evaluating the effects of seasonal variation on ART, investigators have performed number of studies, however, most of the studies report conflicting results.\(^15\)\(^-\)\(^18\) While few studies reported poor fertilization and implantation rates in autumn and higher pregnancy rates following ART in spring,\(^15\)\(^16\) others reported significantly higher number of ART conceptions in the winter months.\(^17\)\(^18\) In addition, some investigators further reported no significant difference in fertilization, clinical pregnancy, or LBRs following ART in different seasons.\(^19\)\(^-\)\(^21\) The lack of consistency in the results may be attributed to the heterogeneity in study population, ART protocols, ethnicity, and regional variations in types and duration of seasons.

It is important to note that most of the studies are reported in European\(^17\)\(^-\)\(^19\) and Middle eastern\(^15\)\(^,\)\(^20\) population as well as a study from China.\(^21\) There are no published studies on effects of seasonal variations on ART outcomes from South Asia. Considering the wide variation in seasons experienced in different countries, there is a need for studies from Asia and Australia. India broadly experiences three seasons of summer, monsoon (rainy season), and winter, and the duration of each season varies in different parts of the country. Due to the paucity of region-specific data, we planned a retrospective analysis of our ART program to elucidate whether the seasonality had any impact on treatment success.

### Materials and Methods

We conducted a retrospective cohort study at a university-level tertiary care hospital in South India. All couples who underwent autologous ART cycles with a fresh embryo transfer between January 2012 and December 2016 were included in the study. We excluded couples with female age >40 years and those who underwent a frozen embryo transfer. The study was approved by the institutional review board (IRB No. 10574). Written informed consent was taken from the couples undergoing ART. All procedures followed were in accordance with ethical standards laid down in the Helsinki Declaration.

We divided the study population into three groups based on the seasonal differences experienced in our region. Accordingly, the months from March to June constituted summer, (Group 1) (reference group); from July to November constituted monsoon (rainy season, Group 2), and from December to February constituted winter (Group 3).\(^22\) Each couple was assigned to the respective group according to the month in which the oocyte retrieval was conducted.

### Assisted Reproductive Technology Protocol

ART was carried out according to the institution protocol. We used the standard agonist (long, ultralong, short) or antagonist protocol using 100–300 IU recombinant follicle-stimulating hormone (Gonal-f, follitropin alfa, Merck Serono, Inc. Rockland, USA or Recagon, follitropin beta, Schering-Plough, USA). Ovulation was triggered using recombinant hCG (250 µg) (Ovitrelle, Merck Serono, Inc. Rockland, USA) or GnRh agonist (Leuprolide acetate 2 mg, Lupride, Sun Pharmaceuticals Industries, Ltd, India) subcutaneously when at least three follicles achieved a diameter of 17 mm. Transvaginal oocyte retrieval was performed under conscious sedation 35–36 h following trigger.

In regard to the ART procedure, sperms were obtained from fresh ejaculate or percutaneous epididymal sperm aspiration or testicular sperm aspiration samples collected on the day of oocyte retrieval. The samples were processed using the density gradient centrifugation method (80% and 40% Sydney in vitro fertilization [IVF] sperm gradient, Cook, Bloomington, USA) to obtain motile sperms, and the processed sample was incubated in the CO2 incubator for 1–2 h.

Following retrieval, oocytes were identified, graded, and incubated in culture media with oil overlay, and the culture dishes were incubated at 37°C with 6% CO2, 5% O2, and 89% N2 for 3–4 h for nuclear and cytoplasmic maturation of oocytes. Following this, mature oocytes
were identified and subjected to IVF or intracytoplasmic sperm injection (ICSI) technique for fertilization. The oocytes were checked for fertilization at 16–18 h following ICSI and 18–20 h following IVF. Fertilized oocytes were cultured in single media (One step, Vitromed, Jena, Germany) for 3–5 days. Embryos were graded according to the Istanbul consensus, 2010.[23] Between one and three embryos were transferred either at cleavage or blastocyst stage.

Luteal support was administered using micronised progesterone vaginally, 400 mg (Naturoigest, Zydis Healthcare, Ltd, India) twice daily and intramuscular progesterone 100 mg (Gestone, Ferring Pharmaceuticals, India) twice weekly. Pregnancy was diagnosed by a positive serum beta hCG test (≥5 mIU/L) and confirmed by transvaginal ultrasound conducted 2 weeks later. All women with an ongoing pregnancy were referred at 10 weeks to obstetric units for follow-up till delivery.

Information regarding clinical and laboratory variables such as age, indication, oocyte numbers, embryo quality, number of embryos transferred day of transfer (cleavage vs. blastocyst), and outcomes was obtained from the departmental ART database. Data regarding live birth were obtained through electronic media or phone interviews.

**Outcomes**

The primary outcome was LBR defined as delivery of a live fetus after 22 completed weeks of gestation.[24] The secondary outcomes were clinical pregnancy rate, miscarriage rate, fertilization rate, embryo cleavage rate, and implantation rate. Clinical pregnancy rate was defined as one or more intrauterine or extraterine gestational sacs diagnosed using transvaginal ultrasound.[24] Miscarriage rate was the proportion of clinical pregnancies that underwent a spontaneous loss at <22 weeks of gestation.[24] Fertilization rate was the proportion of injected or inseminated mature oocytes with two pronuclei on the day after IVF or ICSI.[24] Embryo cleavage rate was defined as the number of embryos that underwent cleavage divided by the number of fertilized oocytes.[25] Blastocyst development rate was defined as the proportion of cleavage stage embryos that develop into a blastocyst. Implantation rate was calculated as the total number of gestational sacs divided by the total number of embryos transferred in each group.[25]

**Statistical methods**

In view of retrospective nature of the study and no available data in the Indian setting, sample size calculation was not done before initiation of study. The power of the study was calculated as post hoc analysis based on results obtained in the current study. Data were reported as mean, standard deviation for normally distributed continuous variables and as median, interquartile range for nonnormally distributed continuous variables. Frequency (percentages) was reported for categorical variables. Fertilization rate, embryo cleavage rate, and blastulation rate were compared between study groups using proportion test. Clinical pregnancy and live birth being dichotomous outcomes were analyzed by simple logistic regression. Multiple logistic regression models were constructed to control for potential confounders such as female age, body mass index (BMI), oocyte morphology, embryo quality, number and grade of embryos transferred, mode of fertilization (IVF or ICSI), and day of transfer (cleavage or blastocyst). The effect was reported as odds ratio (OR) with 95% confidence interval (CI). Data were analyzed using SPSS, version 21.0 (Armonk, NY, USA: IBM Corp).

**RESULTS**

A total of 1705 ART cycles were carried out during the study period. Out of these, 257 cases underwent elective cryopreservation of embryos and 39 cases resulted in nonretrieval or retrieval of immature oocytes and hence excluded. Finally, 1409 cases were included for the study out of which 1359 cases underwent fresh embryo transfer. Embryo transfer was not done in 50 cases due to various reasons such as fertilization failure, cleavage arrest, and nonavailability of adequate quality embryos for...
transfer [Figure 1]. The cases were assigned into each of the three groups based on the month of oocyte retrieval. Accordingly, 337 cases were included in the summer group, 715 cases in the monsoon group, and 357 cases in the winter group. The baseline characteristics have been reported in Table 1. Significant difference was noted among the cohorts for variables, namely female BMI (P 0.002), mode of ART (IVF or ICSI) (P 0.006), and the number of embryos transferred (P 0.001) [Table 1]. The

| Table 1: Baseline comparison between the study groups |
|------------------------------------------------------|
| Group 1 summer (reference) (n=337) | Group 2 monsoon (n=715) | Group 3 winter (n=357) | P |
|-------------------------------------|-------------------------|------------------------|---|
| Female agea | 32.08±4.3 | 32.24±4.5 | 32.38±4.3 | 0.68 |
| BMIa | 24.85±4.2 | 25.75±4.3 | 25.89±4.2 | 0.002* |
| Infertilityb | | | | |
| Primary | 252 (74.8) | 500 (69.9) | 246 (68.9) | 0.18 |
| Secondary | 85 (25.2) | 215 (30.1) | 111 (31.1) | |
| ART stimulation protocol | | | | |
| Antagonist | 214 (63.5) | 456 (63.8) | 243/357 (68.1) | 0.78 |
| Long | 47 (13.9) | 98 (13.7) | 41 (11.5) | |
| Long depot | 43 (12.8) | 81 (11.3) | 38 (10.6) | |
| Short | 33 (9.8) | 80 (11.2) | 35 (9.8) | |
| Number of oocytec | 7 (4.11) | 7 (4.11) | 7 (4.11) | 0.33 |
| Oocyte morphologyb | | | | |
| Normal | 107 (31.8) | 195 (27.3) | 84 (23.5) | 0.07 |
| Heterogenous | 156 (46.3) | 382 (53.4) | 202 (56.6) | |
| Complete dysmorphism | 74 (22.0) | 138 (19.3) | 71 (19.9) | |
| ART-ICSI cycles | 271/337 (80.4) | 631/715 (88.3) | 314/357 (88.0) | 0.006* |
| Day of embryo transfery | | | | |
| Cleavage stage | 266 (83.1) | 598 (87.3) | 294 (83.8) | 0.20 |
| Blastocyst stage | 57 (16.9) | 87 (12.7) | 57 (16.2) | |
| Embryo transfer numberc | 2 (2.2) | 2 (2.3) | 2 (2.3) | 0.001* |
| Top quality embryosd | 146/323 (45.2) | 344/685 (50.2) | 185/351 (52.7) | 0.14 |

*Statistically significant, aPresented as mean±SD, bPresented as frequency (%), cPresented as median (IQR). BMI=Body mass index, ART=Assisted reproductive technology, ICSI=Intracytoplasmic sperm injection, IQR=Interquartile range

| Table 2: Outcome characteristics |
|----------------------------------|
| Group 1 summer (reference) | Group 2 monsoon | Group 3 winter | P |
| Fertilization ratea | 75.27±23.9 | 75.05±23.9 | 76.73±20.7 | 0.52 |
| Embryo cleavage ratea | 94.77±15.4 | 95.53±13.7 | 96.7±11.8 | 0.17 |
| Blastulation rateb | 37.5 (0-57.1) | 30.3 (0-59.6) | 36.3 (0-61.8) | 0.49 |
| Implantation rate (%) | 128/651 (19.7) | 347/1502 (23.1) | 196/757 (25.9) | 0.02* |
| Clinical pregnancy ratec | 108/323 (33.4) | 260/685 (37.9) | 148/351 (42.1) | 0.06 |
| Live birth ratec | 83/319 (26.0) | 174/651 (26.7) | 114/341 (33.4) | 0.05 |
| Miscarriage ratec,d | 17/108 (15.7) | 42/260 (16.2) | 20/148 (13.5) | 0.76 |

*Statistically significant, aPresented as mean±SD, bPresented as median (IQR), cPresented as frequency (%), dCalculated per clinical pregnancy. IQR=Interquartile range, SD=Standard deviation

| Table 3: Comparison of outcomes between Group 1 (summer) and Group 2 (monsoon) |
|--------------------------------------|
| Group 1 summer (reference) | Group 2 monsoon | OR (95% CI) | P |
| Fertilization ratea | 75.27±23.9 | 75.05±23.9 | 1.01 (0.77-1.30) | 0.98 |
| Embryo cleavage ratea | 94.77±15.4 | 95.53±13.7 | 0.97 (0.67-1.39) | 0.87 |
| Blastulation rateb | 37.5 (0-57.1) | 30.3 (0-59.6) | 0.81 (0.56-1.13) | 0.21 |
| Implantation rate (%) | 128/651 (19.7) | 347/1502 (23.1) | 1.23 (0.98-1.54) | 0.08 |
| Clinical pregnancy ratec | 108/323 (33.4) | 260/685 (37.9) | 1.22 (0.92-1.61) | 0.16 |
| Live birth ratec | 83/319 (26.0) | 174/651 (26.7) | 1.04 (0.77-1.41) | 0.81 |
| Miscarriage ratec,d | 17/108 (15.7) | 42/260 (16.2) | 1.03 (0.56-1.91) | 0.92 |

*Presented as mean±SD, bPresented as median (IQR), cPresented as frequency (%), dCalculated per clinical pregnancy. OR=Odds ratio, CI=Confidence interval, IQR=Interquartile range, SD=Standard deviation
ART outcomes among the study groups have been enumerated in Table 2. Live birth rate (P 0.05) and clinical pregnancy rate (P 0.06) were found to be comparable among the study groups [Table 2].

Outcomes

Univariate analysis was done with summer season as the reference group. There was no significant difference in LBRs in monsoon (174/651, 26.7% vs. 83/319, 26.0%; OR: 1.04, 95% CI: 0.77, 1.41; P = 0.81) versus summer season [Table 3]. However, significantly higher LBR was seen in the winter season (114/341, 33.4% vs. 83/319, 26.0%; OR: 1.43; 95% CI: 1.02, 2.00; P = 0.04) as compared to the summer season [Table 4]. The clinical pregnancy was also significantly higher in winter as compared to the summer season (148/351, 42.1% vs. 108/323, 33.4%; OR: 1.45, 95% CI: 1.06,1.99; P = 0.02) [Table 4]. Although there was a trend toward higher clinical pregnancy in the monsoon season as compared to the summer season, it did not achieve statistical significance (260/685, 37.9% vs. 108/323, 33.4%; OR: 1.22; 95% CI: 0.92, 1.61; P = 0.16) [Table 3].

A significantly higher implantation rate was reported in winter season (25.9% vs. 19.7%, OR: 1.43, 95% CI: 1.11, 1.84; P = 0.006) as compared to summer season [Table 4]. However, there was no statistical difference in the implantation rate between the monsoon and summer season (23.1% vs. 19.7%, OR: 1.23, 95% CI: 0.98,1.54; P = 0.08) [Table 3]. The miscarriage rate was comparable among the seasonal groups (P = 0.76) [Table 2]. The mean fertilization rate (P = 0.52), embryo cleavage rate (P = 0.17), and the blastulation rate (P = 0.49) were also not significantly different among the groups [Table 2].

We adjusted for significant confounding factors (female age, BMI, number of oocytes, oocyte morphology, mode of fertilization, day of embryo transfer, number of embryos transferred, top quality embryo. AOR=Adjusted odds ratio, CI=Confidence interval, BMI=Body mass index.

Outcomes

Univariate analysis was done with summer season as the reference group. There was no significant difference in LBRs in monsoon (174/651, 26.7% vs. 83/319, 26.0%; OR: 1.04, 95% CI: 0.77, 1.41; P = 0.81) versus summer season [Table 3]. However, significantly higher LBR was seen in the winter season (114/341, 33.4% vs. 83/319, 26.0%; OR: 1.43; 95% CI: 1.02, 2.00; P = 0.04) as compared to the summer season [Table 4]. The clinical pregnancy was also significantly higher in winter as compared to the summer season (148/351, 42.1% vs. 108/323, 33.4%; OR: 1.45, 95% CI: 1.06,1.99; P = 0.02) [Table 4]. Although there was a trend toward higher clinical pregnancy in the monsoon season as compared to the summer season, it did not achieve statistical significance (260/685, 37.9% vs. 108/323, 33.4%; OR: 1.22; 95% CI: 0.92, 1.61; P = 0.16) [Table 3].

A significantly higher implantation rate was reported in winter season (25.9% vs. 19.7%, OR: 1.43, 95% CI: 1.11, 1.84; P = 0.006) as compared to summer season [Table 4]. However, there was no statistical difference in the implantation rate between the monsoon and summer season (23.1% vs. 19.7%, OR: 1.23, 95% CI: 0.98,1.54; P = 0.08) [Table 3]. The miscarriage rate was comparable among the seasonal groups (P = 0.76) [Table 2]. The mean fertilization rate (P = 0.52), embryo cleavage rate (P = 0.17), and the blastulation rate (P = 0.49) were also not significantly different among the groups [Table 2].

We adjusted for significant confounding factors (female age, BMI, number of oocytes, oocyte morphology, mode of fertilization (IVF or ICSI), top quality embryo, stage of embryo (cleavage or blastocyst), and number of embryos transferred). Multivariate analysis revealed no significant statistical difference in LBR in monsoon (adjusted odds ratio [aOR]: 0.92; 95% CI: 0.66, 1.26; P = 0.59) or winter (aOR: 1.32; 95% CI: 0.92, 1.88; P = 0.13) as compared to summer season [Table 5]. Similarly, there was no statistical difference in the clinical pregnancy rate in monsoon season (aOR: 1.07; 95% CI: 0.79, 1.43; P = 0.67) and winter season (aOR: 1.28; 95% CI: 0.92, 1.78; P = 0.14) as compared to summer season [Table 6].

We further analysed the data to assess the effects of the various potential confounding factors on LBR. It was found that a significantly higher proportion of cases in the winter group had multiple embryo transferred...
as compared to the summer group (85.3% vs. 78.4%; \( P = 0.02 \)). This may have contributed to the significantly higher clinical pregnancy and LBR noted in winter on univariate analysis which was alleviated on multivariate analysis to reveal no statistically significant difference.

**Discussion**

The current study findings suggest that after adjustment of potential confounders, there is no statistical difference in LBRs and clinical pregnancy rates among the seasonal groups. The embryo cleavage rate and the blastulation rate were also found to be comparable among the groups.

In an earlier retrospective analysis of 8184 ART cycles conducted in Europe, Weigert et al.\(^{[18]}\) reported significantly higher pregnancy rates in the colder months September–April (32.2%) versus May–August (28.5%). Investigators concluded that the difference may be attributed to the difference in semen parameters in different seasons. Other outcomes such as fertilization rate and embryo cleavage rate were not reported in the study. These findings are broadly in agreement with the current study.

A large retrospective cohort study conducted in China included 38,476 women who underwent either fresh or frozen embryo transfer.\(^{[21]}\) The study groups were divided into 3 monthly groups into spring, summer, autumn, and winter. The unadjusted model showed a significant difference in clinical pregnancy rates (\( P = 0.027 \)) and embryo cleavage rate (\( P = 0.033 \)) with significantly higher rates in summer. However, there was no significant difference in LBRs (\( P = 0.11 \)) among the study groups. The possible reasons for the contradictory findings in this study could be the inclusion of both fresh and frozen transfer cycles, difference in ethnicity, and regional variation in the average temperature and humidity in the different seasons.

A recent study by Kirshenbaum et al.\(^{[20]}\) from Israel analyzed a cohort of 4420 ART cycles out of which 3020 comprised fresh embryo transfer and 1400 comprised vitrified-warmed embryo transfers. The investigators found no difference in clinical pregnancy rates in consecutive calendar months for a period of 3 years. The inclusion of both fresh and vitrified warmed embryo transfer cycles and the categorization of participants based on calendar months may have influenced the outcomes contributing to the dissimilar findings reported in the current study.

In another retrospective study from Israel (\( n = 305 \)), authors reported higher fertilization rates (74.7% vs. 65.6%) and higher embryo quality (54.2% vs. 33.6%) in spring as compared to autumn.\(^{[15]}\) The clinical pregnancy rates were found to be comparable (22% vs. 30%). The contradictory findings obtained may have been due to the inclusion of IVF cycles only since the study was conducted before the introduction of ICSI in ART. This may have considerable influence on outcomes, especially fertilization rate contributing to the difference in results.

This study is one of the only studies which evaluates the effects of seasonality on ART outcomes in South India. We have evaluated clinically relevant outcomes such as LBR and clinical pregnancy along with important laboratory parameters. In addition, we have adjusted for multiple possible covariates including stage (cleavage or blastocyst) and number of embryos transferred which may otherwise influence ART outcomes. Importantly, this study is one of the few to have assessed the influence of a tropical climate on ART outcomes.

The effects of seasonality are found to be pronounced on natural conceptions than ART-IVF cycles due to the possible effect of temperature on sperm quality and the decrease in gonadotrophin secretion due to alteration in dark-light duration. The utilisation of gonadotrophin stimulation and ICSI accompanied by tight control on temperature and humidity within the ART laboratory may possibly blunt the effects of the external environment. Maintenance of a strict “cold chain” for transport and delivery of drugs and laboratory consumables to the center are also essential for optimum performance of an ART unit, and the impact of such external factors could not be assessed in the current study. The study may be limited by its retrospective design and small sample size causing type II error. The power of the study using current sample size was found to be 59%. In addition, we have adjusted for important confounders to alleviate the effect of any selection bias. Data from various studies are still conflicting and it could be considered too early to come to a definite conclusion regarding the effects of seasons on ART outcomes. It would therefore be prudent to say that larger prospective trials from different climatic zones are needed to contribute to the existing data.

**Conclusion**

The current study found no significant effect of seasonal variation on LBR following ART in the study population on multivariate analysis. The results need to be confirmed through larger region-specific prospective studies, possibly with registry-based data.

**Data availability statement**

The data will be made available at suitable request pending regulatory permission.
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Nil.

Conflicts of interest
Dr. Mohan S Kamath is the Deputy Editor for Journal of Human Reproductive Sciences. However, for the current manuscript, he was not involved in the editorial decisions or blind peer review process.

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