SciMedCentral
Medical Journal of Obstetrics and Gynecology

Research Article

Annual Variations in ICSI Cycle Outcome in Temperate Zone: Is It Weather Temperature or Calendar Season related?

Mohamad Elsaid Ghanem[2], Adel Saad Helal[1,2]*, Mohamed Ahmed Emam[1,2], Laila Abd-Alhamid Al-Boghdady[1,2], Abd-Elgawad El-Metwally[2], and Ibrahim Abdelkhalek Albahloul[1,2]

[1]*Department of Obstetrics & Gynecology, Mansoura Faculty of Medicine, Egypt*
[2]*Mansoura Integrated Fertility Center, Egypt*

***Corresponding author**
Adel Saad Helal El-Sayed, Department of Obstetrics & Gynecology, 60 Elgomhoria street, Mansoura University, Mansoura, Egypt, Tel: 00201001655174; 00201000061501

Submitted: 05 February 2023
Accepted: 27 February 2023
Published: 28 February 2023
ISSN: 2333-6439

Keywords
- Annual Variations
- ICSI Cycle
- Temperate Zone
- Weather Temperature
- Calendar Season

Abstract

Our aim to study the annual variation of ICSI cycle outcome is it related to calendar seasons or weather temperature in our geolocation as retrospective study.

We analyzed 3586 fresh first completed ICSI cycles from (2011- 2016) in our center. Cycles were assigned to one of 4 calendar-seasons then to one of 2 temperature centiles: (≥ or < 50th centile: 20°C) depending on date of ovum pick-up. Regression models (MR and BNLR) were set to compare predictive values of 6 variables: female-age, number of grade A embryo transfer (NET), blastocyst ratio (RBL), calendar-season, temperature-centile, day-light-duration-centile for implantation and clinical pregnancy rates as primary outcomes.

No significant differences were found between cycle features or outcome of calendar seasons. While cycle features between temperature centiles were comparable, cycle outcome in cold and hot weathers respectively were implantation rate (IR) 12.7%, 14.5% {OR & 95% CI 0.85 (0.76-0.96), (P=0.0098)}, cumulative pregnancy rate (CPR) 39.8%, 43.8% OR & 95% CI 0.84 (0.73-0.96) (P= 0.009)}. Using multiple regression (MR) for IR & binomial logistic regression (BNLR) for CPR employing 6 variables significantly predicted both IR and CPR (p < .000) but, day- light duration, calendar-season did not significantly add to prediction (p>0.05). in this study Absolute RR = 4 and Relative RR = 9. %, Number-Needed -to -Treat = 25.

We concluded that Weather temperature not Calendar season independently predicts better implantation rate (IR) and cumulative pregnancy rate (CPR) in ICSI as shown by regression analysis.

INTRODUCTION

Although there is general agreement on the existence of annual variation in natural human conception and birth in different geographical regions [1,2]. There is some debate about the occurrence of annual variation in the outcome of assisted reproductive techniques. Many studies reported significant seasonal variation in the outcome of IVF/ICSI cycles [1-4]. However few studies denied such seasonality [5,6]. Furthermore, it has been documented that peak month for natural births change with latitude [7]. The mechanisms underlying periodicity of human natural and in vitro conception are still unclear. Whereas ecological factors have been implicated in annual variation of natural births [8]; the mechanisms underlying variation in ART are not clear. Many authors attributed annual variation in IVF/ICSI outcome to daylight length i.e., photoperiodicity [1,2,8]. Others linked it to weather temperature changes [9,10], and still some found no influence of temperature [11]. The current theory explaining the natural reproductive cyclicity is the amount of sunlight and the temperature and it has been shown that peak fertility times vary from one latitude and climate to another and that geographical discrepancies reflect when people in those places are exposed to a mix of day-light and temperature that most closely approximates ideal conditions for human conception [11].

The primary outcome of this study is to investigate if there is association between ICSI outcome and calendar year seasons (winter, spring summer, autumn), weather temperature and day-light changes in our geolocation.

MATERIAL AND METHODS

After obtaining IRB approval (MIFC -IRB approval Number 3-2016) we retrieved and retrospectively analyzed 3586 fresh ICSI cycles performed in our center (MIFC) by the same team of clinicians and embryologists over 6 years (from January 2011 to December 2016). After excluding repeat cycles and cycles where embryo transfer was cancelled for any reason, we had 3465 completed fresh first ICSI cycles. The cycles were assigned to one of the 4 meteorological 3- month seasons in our country which include winter (December—January February), spring (March-April-May), summer (June-July-August), and autumn (September-October-November). The cycle assignment to a particular season depended on the date of ovum pick-up. We included all female ages and all ICSI indications. The geolocation of our center is N 31.044183, E 31.378584300000057 as determined by the GPS coordinates finder (https://gps–coordinates org). The elevation related to see level is 10 meters height. Monthly weather data (temperature, humidity, and day-light hours) were extracted from the climate–data org web site and are shown in Table 1. We compared recorded relevant patient and cycle features, weather data and outcome in the 4 meteorological seasons. The average daily temperature in our geolocation ranged between 12.9° C and 26.8° C over the whole year and the 50th centile or median value was 20°C. It was noted that the average temperature of March and April (spring season) and November (autumn season) was < 20°C. On the other hand, average temperature of May (spring season) and September and October (autumn season) was ≥20°C. We considered the median values for annual ranges for temperature (12.9 -26.8°C & median 20°C), day-light hours (10.1-14.15 hours & median 11.9 hrs.), and relative humidity (68-73 %, median 71%) to classify the year into 2 seasons: cold season (below median values of temp. <20° C, daylight hours <11.9 and relative humidity <71%) and hot season ≥ the median values for temperature, daylight hours and relative humidity, respectively. Accordingly, the "cold season" in our locality included 6 calendar months (November -April) and the "hot season" included 6 calendar months (May-October). Therefore, according to the temperature range the year can be divided into two temperatures - seasons only (Cold and Hot).

We compared initially the cycle outcome in the 4 calendar seasons and then the outcome in the 2 weather seasons. In the 4 calendar and the 2 temperature seasons, we compared female ages, infertility duration, total stimulation doses and stimulation dose per egg retrieved. Our ICSI cycle protocol and embryo grading method has been previously published [12].

We compared the number of eggs retrieved, fertilization rate, number of grade A embryos transferred (NET), ratios of cleavage and blastocysts transferred (RBL), cycle outcome (Implantation and Clinical pregnancy rates) in calendar and temperature seasons. Stability of laboratory environment over the extended period of study was guaranteed by stability of embryology staff over time and the application of standard laboratory quality control protocols. A stable temperature for handling and culturing gametes and embryos was achieved by daily monitoring using an external thermometer for all equipment. Early in the morning we monitored temperature for incubators, heating stages, heating blocks, refrigerator/freezer and ambient temperature then recorded in the quality control log. This time was chosen because most equipment has been stabilized overnight & incubator doors and refrigerator/freezer doors have not been opened. Daily monitoring of PH and CO_2 was routine for all incubators as it was important for maintenance of media PH. We have no HVAC system as our IVF laboratory was designed to achieve the purpose of HVAC with many separate integrated units that perform the same function. In Table 5 we presented temporal cycle outcome in different seasons over the study period (2011-2016). We compared seasonal CPR in both calendar seasons and in cold and hot seasons to see if there are inter-annual differences in cycle outcome in the same seasons.

Statistical analysis

We used the parametric tests students T-test for comparisons between means of two parametric variables and one –way ANOVA test for comparing between means of more than 2 parametric variables. Post-hoc testing was further done to identify between group differences and the Bonferroni correction was used to correct for multiple comparisons. Therefore, p *value* was considered significant only when it was *< 0.0125* in multiple comparisons. For proportions we used Fisher exact test to compare 2 variables and the Chi square test (χ^2) for comparisons between more than 2 variables. We used Pearson and Spearman correlations to correlate between parametric and nonparametric variables, respectively. Binomial logistic and multiple regression analysis validated by ROC curve were used to compare the predictive values of different independent variables (age, number of grade A embryo transferred, ratios of blastocyst and weather data) for the dependent variables (clinical pregnancy and implantation rates) respectively. In all statistical tests the result was significant when p value was <0.05 in double and was < 0.0125 in multiple comparisons (Bonferroni correction). We used the statistical program SPSS 20 in analysis.

RESULTS

As shown in Table 3 no statistically significant differences were found in cycle features nor cycle outcome between the 4 calendar seasons. Contrarily, comparison between cold and hot weather (Table 4), showed that mean age was significantly lower

Table 1: Mansoura Climate Table // Historical Weather Data*

Month	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Average temp(°C)	12.9	13.5	15.8	19	23.6	25.7	26.6	26.8	25.3	22.8	19.4	14.9
Temp Range	6.8-19	7.3-19.8	9.2-22.4	11.8-26.3	15.2-32.1	18.4-33	20.6-32.6	20.4-33.3	18.7-32	17-28.6	13.9-25	9.1-20.7
Average humidity (%(	73	71	70	68	70	71	72	72	70	70	71	73
Average Daylight hours	10.4	11.1	11.9	12.9	13.7	14.15	13.9	13.3	12.4	11.4	10.6	10.1

*Source: climate-data org >Africa>Egypt>Dakahlia Governorate> Mansoura (https://en.climate-data.org/location/725/ accessed on 5-10-2017)

Table 2: Alexandria – Temporal Weather temperature over years (1991-2020)

Month	Min (°C)	Max (°C)	Mean (°C)
January	9	18	13.8
February	10	19	14.3
March	12	21	16.4
April	14	24	19.1
May	18	27	22.3
June	21	29	25.3
July	24	31	27.1
August	24	31	27.7
September	22	30	26.3
October	19	28	23.5
November	15	24	19.5
December	11	20	15.6
Year means	16.6	25.3	20.9

https://www.climatestotravel.com/climate/egypt/alexandr ia(accessed on 18-7-2021) (The mean temperature in the months May to October was above 20 **°C** ; in the other 6 months it was below 20 **°C**)

Table 3: Cycle Features and Outcome in The 4 Calendar Seasons

Feature	Winter n=890	Spring n=843	Summer n=845	Autumn n=887	P	Notes
Age (yr.) $	**29.15±5.6**	**29.9±5.5**	**29.8±5.7**	**29.5±5.6**	**0.018***	Post-hoc: winter vs. spring summer=0.038.
Infertility Duration (yrs.) $	5.5±4.2	6.0±4.5	5.4±3.9	5.5±4.0	0.015*	Post-hoc: spring vs. summer =0.035
Total Dose (Amp)$	28.12± 8.98	28.26±8.72	28.32± 9.43	27.80±9.13	0.63*	
Dose/Egg Retrieved (Amps)$	3.9±4.9	3.8±4.57	4.2±5.66	3.7±4.10	0.16*	
Eggs Retrieved $	11.1±6.1	11.36 ±6.66	10.8±6.2	10.97±6.3	0.31*	
Fertility Rate $	66.15 ±23.2	66.7±22.9	65.8±22.9	63.9±22.7	0.06*	
Total Embryos Tr$	2.7±0.81	2.7±0.86	2.75±0.89	2.67±0.81	0.52*	
Embryo Tr A$	1.75±1.1	1.84±1.3	1.75± 1.2	1.80±1.3	0.35*	
Blastocyst n (%)	340 /890(38.2)	290/843 (34.4)	321/845(37.9))	333/887(37.5) (37.5)	0.32#	
Pregnancy (n)	373	346	361	363		
CPR (%)	41.9	41.1	42.7	40.9	0.86#	
Sum of ET	2429	2331	2305	2367		
Sum of sacs	336	305	326	313		
IR (%)	13.8	13.1	14.1	13.2	0.68#	
Temp ^{O} C $	13.8±0.86	19.45±2.3	26.40±0.46	22.49±2.4	0.000*	
Relative Humidity $	72.42±0.9	69.33±0.9	71.70±0.45	70.33±0.45	0.000*	
Light hours $	10.5±0.41	12.8±0.7	13.8±0.35	11.75±0.7	000*	

$ =(mean ± SD), * =Anova-test, # = χ2-test

in cold vs. hot weather (29.38±5.6, 29.83± 5.6 respectively, p = 0.016), and significantly lower IR and CPR in cold vs. hot weather IR (12.7 vs. 14.5), OR & 95% CI 0.85(0.76-0.96) p=0.009& CPR (39.5 vs.43.8) OR & 95% CI 0.84 (0.73-0.96) P= 0.009) respectively while other cycle parameters were not significantly different. Nonparametric correlation test between cycle outcome (IR, CPR) and calendar seasons was found to be insignificant, (r=0.003, p=0. 857, r=.005, p=0.773 respectively). Also cycle outcome (IR, CPR) was insignificantly correlated with relative humidity (r=-0.003, p=0.873 and r=-0.003, p=0.873 respectively) & day-light hours r=0.005, p=0.773, r=0.004, p=0.733) respectively. On the other hand, correlation revealed significant weak positive correlation between hot weather and cycle outcome (r= 0.036, p =0.035, r=0.043, p=0.011) respectively. As shown in Table 5 there were no inter-annual differences in CPR in the 4- calendar or the 2-weather seasons confirming stability and consistency of our outcome data. On the other hand while comparing annual and overall 4-calendar seasons showed insignificant differences in CPR, comparison of annual cold-hot seasons showed consistently numerically higher hot season CPR tending to be significantly so in some years. The smallness of number of cycles in annual comparisons rendered the difference insignificant (type 2 statistical errors). This is proved by the significant higher CPR hot season in the overall comparison (Tables 4 and 5).

Table 4: Cycle Features and Outcome in Cold Vs. Hot Weather

Feature	Cold (< 20° C) n=1747	Hot (≥ 20 ° C) n=1718	P
Age (yr.) [$]	29.38±5.6	29.83± 5.6	0.02*
BMI [$]	32.7 ±6.24	32.4 ±6.05	0.15*
Infertility Duration (yr.) [$]	5.69 ±4.266	5.51± 3.975	0.19**
Stimulation Dose (Amps)$	28.25 ±8.6	27.71±9.3	0.08*
Eggs Retrieved [$]	11.16 ±6.3	11.02±6.4	0.52*
Amps/Egg Retrieved $	3.92±4.09	3.94±4.81	0.89*
MII Eggs [$]	9.37±6.40	9.14±5.49	0.25*
Fertilization Rate[$]	76.61±22.02	76.63.52±22.0	0.97*
Total E Transferred$	2.73± 0.83	2.72 ±0.85	0.9*
E Transfer A [$]	1.87 ±1.14	1.9 ±1.12	0.34*
E Transfer B [$]	1.43±3.40	1.34 ± 1.18	0.29*
Blastocyst Stage(n)	655	662	
Cleavage Stage (n)	1092	1056	
Proportion Of Blastocyst (n) (%)	655/1747 (37.4)	662/1718 (38.5)	P=0.55[#]
Sum of ET (n)	4749	4683	
Sum of Sacs(n)	601	679	
Implantation Rate (%)	12.7	14.5	OR & 95% CI 0.83 (0.76-0.96) P=0.009[#]
Pregnancies (n)	690	753	
Cycle Pregnancy Rate (%)	39.5	43.8	OR & 95% CI 0 .84 (0.75-0.95) P= 0.009[#]
Multiple Pregnancy Rate (%)	131/440=29.7	151/486=31.1	OR & 95% CI 0.94 (0.72-1.12) p=0.7[#]
Daylight hours[$]	11.28 ±1.087	13.24±1.033	0.0093*
Mean temp° C[$]	15.9± 2.4	25.1±1.5	0.000*
Relative Humidity [$]	71.8±1.7	70.83±0.909	0.000*

n= number, [$]= Mean ±SD, * =T-test, [#]= Fisher exact-test

Table 5: Temporal depiction of CPR

Year/ Season	Winter n (%)	Spring n (%)	Summer n (%)	Autumn n (%)	Cold n (%)	Hot n (%)
2011	101/272(37.6)[1]	115/270(42.6)[2]	108/260 (41.5)[3]	88/222(39.6)[4]	195/510(38.2)[a]	210/494 (42.5)[b]
2012	48/107(44.8)[1]	36/90(40.0)[2]	58/130(44.6)[3]	55/135 (40.7)[4]	110/246(44.7)[a]	101/221 (45.7)[b]
2013	41/84(48.8)[1]	40/92(43.4)[2]	25/58(43.1)[3]	49/117(41.8)[4]	78/196 (39.7)[a]	75/168(44.6)[b]
2014	57/125(45.6)[1]	45/123(36.5)[2]	44/110 (40.0.)[3]	53/122(43.4)[4]	100/255(39.2)[a]	103/236(43.6)[b]
2015	68/155 (43.8)[1]	60/140(42.8)[2]	58/140(41.4)[3]	46/114(40.4)[4]	98/255(38.4)[a]	130/284(45.6)[b]
2016	58/147 (39.5)[1]	50/128(39.1) [2]	68/147 (46.2)[3]	72/`177 (40.6)[4]	109/285 (38.2)[a]	134/315(42.5)[b]
P	[1] p=0.31*	[2] p=0.85*	[3] p=0.91*	[4] p= 0.53*	[a] p=0.62*	[b] p=0.92*
overall season CPR (n,%)	373/890(41.9) *	346/843(41.1) *	361/845(42.7) *	363/887(40.9) *	690/1747 (39.5) [#]	753/1718(43.8) [#]
p	Overall calendar seasons χ 2 = 0.74, df =3 p= 0.862*				Overall cold vs. hot χ 2=6.5, df=1, P=0.009 OR & 95 % CI: 0.83 (0.73 to 0.95) [#]	

* χ 2-test insignificant differences between CPR in individual years calendar seasons &cold and hot seasons
#= Fisher exact test for annual cold vs. hot (a vs. b) p: 2011 = 0.17; 2012=0.83; 2013 =0.39; 2014= 0.35 ; 2015=0.08; 2016=0.31 (see text for more explanation)

A multiple regression model was run to predict implantation rate from wife age, number of embryos grade A transferred, embryo stage at transfer (cleavage vs. blastocyst,) calendar seasons, temperature centile, day- light duration centile. These variables statistically significantly predicted implantation rate, $F(6, 3395) = 22.063$ $p< .000$, $R2 = .038$. Not all 6 variables added statistically significantly to the prediction (wife age, number of grade A embryos transferred, embryo stage at transfer, temperature centile made significant contribution to prediction (p=0.000,0.000,0.000, 0.005 respectively) while (day- light duration centile ,and calendar season did not add to prediction in this model (p= 0.323 and, 0 .212 respectively).

A logistic regression was performed to ascertain the effects of the same 6 independent variables on the likelihood of pregnancy as dependent variable. The logistic regression model was statistically significant {Chi square (6 df) =204.7 P=0.000), The model explained 7.8% (Nagelkerke R2) of the variance of cycle outcome and correctly classified 60.6% of cases. The Wald criterion demonstrated that female age, number of grade A embryos transferred, embryo stage at transfer, temperature centile made significant contribution to prediction (p=0.000,0.000,0.000,0.016 respectively) (Table 6). The corresponding Exp(B) & 95% CI are 0.975 (0.962-0.987) for wife age ;1.421 (1.332-1.516) for number of grade A embryos transferred; 1.622 (1.405-1.872) for embryo stage at transfer & 1.279 (1.046-1.565) for weather temperature centile. On the other hand, calendar season and day-light duration centile did not contribute significantly to the model (p=0.147, 0.472 respectively). The corresponding Exp(B) & 95% CI are 0 .943 (0.871-1.021) for calendar season & 0.941 (0.798-1.110) for day light duration. From the logistic regression model, we know that older wife age negatively affects CPR, and that blastocyst stage embryo transfer and larger number of grade A cleavage embryo transfer and higher weather temperature centile (respectively) independently positively affect CPR in this order of weight (Table 7).

Testing the predictive value of the same independent variables on cycle outcome (pregnant state) by the AUC (Table 4 & Figure 1) revealed nearly similar results. The independent variables had the following AUC and 95% CI, P respectively {female age: 0.456 (0 .436-0.475) p=0.000; number of embryo transfer grade A: 0.605 (0.586-0.623) p=0.000; embryo stage at transfer :0.565(0.545-0.584) p=0. 000; temperature centile :0.517(0.498-0.537) p=0.08; calendar season: 0.499(0.479-0.519) p=0.934; day-light duration :0.500 (0.480-0.519) p=0.985.

Figure 2 shows monthly total births in Egypt plotted against our monthly ICSI -CPR. It can be noted that while the ICSI outcome peaks in May –June (hot months) then plateaus to October when it drops, the monthly total births in Egypt peaks in January (cold months) and once again a lesser peak in August (hot months). The peak births in January (cold month) reflect peak conceptions in summer months which lends credibility to our study. The Egyptian national birth data (derived from UN statistical department data over the specified years) reflect births allover Egypt whose location extends between latitudes 22 and 31 north. Therefore, we cannot extrapolate exactly seasonality in our geolocation situated in Lower Egypt (Mansoura) to the whole country.

Furthermore, we reviewed our IUI data in the same 9 years' time of the current study (years 2011-2019) (unpublished data). We could retrieve 1510 completed IUI cycles with documented cycle outcome (clinical pregnancy). Overall CPR was 11.4% (173/1510). Comparing CPR in hot (months May-Oct) 13.2% (82/621), and cold weathers (months Nov-April) 10.2% (91/889), the OR & 95 CI of pregnant outcome was 1.33 (0.97-1.83) p=0. 0.07, which tended to be significant. This gives support to our concept explaining the annual variation of ICSI outcome by weather temperature not by calendar season or day –light duration. With larger IUI sample the difference between hot and cold seasons in IUI cycle outcome would have been clearly significant (type II statistical error).

DISCUSSION

The published studies on seasonal variation of ICSI/IVF outcome are conflicting. There are many reasons for this conflict. Firstly: variation in geographical latitude zone where the study was undertaken whether tropical zone [9], or temperate zone [1,13]. No available publications from cold zone (latitude between 60-90 North and South of the equator line). Secondly: the definition of seasons {4 calendar 3-months seasons [1,6], or 2 photoperiods 6-month seasons [3]}. Thirdly: the type of ART technique: IVF cases only [1,14], or ICSI only cases [13]. Fourthly: the arbitrary point of season assignment of included cases: whether stimulation starting day [1], ovulation trigger day [15], or ovum-pickup day [4,16]. Fifthly: the studies vary in power with the number of cases ranging from few hundreds [5,9], to many thousands [4,8]. In our study we retrospectively analyzed 3465 completed first fresh ICSI cycles as mentioned in the material and methods section. We assigned the cases to a particular season according to the day of egg retrieval because

Table 6: Logistic Regression outcome

Independent Variables	B	Wald	df	significance	Exp(B)	95% C.I. for EXP(B)
Wife age	- 0.025	15.117	1	0.000	0.975	0.962 - 0.987
Embryo Transfer A number	0.351	113.206	1	0.000	1.421	1.332-1.516
Blastocyst stage	0.484	43.541	1	0.000	1.622	1.405-1.872
Temp. Centile	0.246	5.758	1	0.016	1.279	1.046- 1.5651
Calendar Seasons	- 0.059	2.103	1	0.147	0.943	0.871-1.021
Day Light Centile	-0.061	.518	1	0.472	0.941	0.798-1.110
Constant	-0.404	3.136	1	0.077	0.668	

Table 7: AUC for the 6 independent variables

Test Result Variable(s)	Area	Std. Error	Asymptotic Significance	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Wife Age	0.456	0.010	0.000	0.436	0.475
Embryo Transfer A	0.605	0.010	0.000	0.586	0.623
Blastocyst Stage	0.565	0.010	0.000	0.545	0.584
Temp. Centile	0.517	0.010	0.084	0.498	0.537
Calendar Seasons	0.499	0.010	0.934	0.479	0.519
Day Light Centile	0.500	0.010	0.985	0.480	0.519

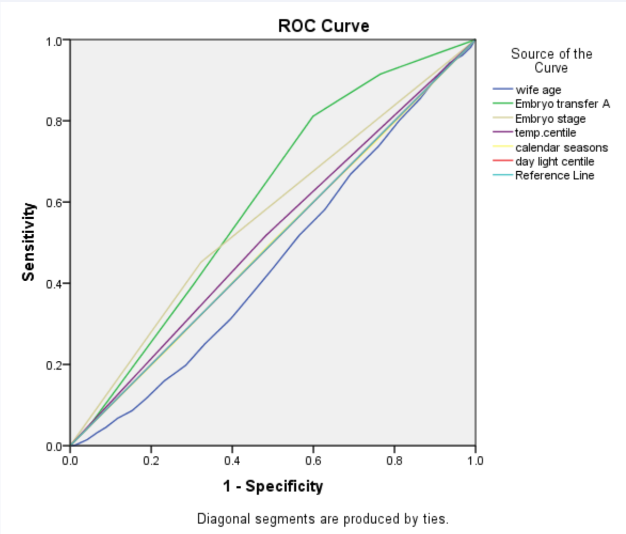

Figure 1 Diagonal segments are produced by ties.

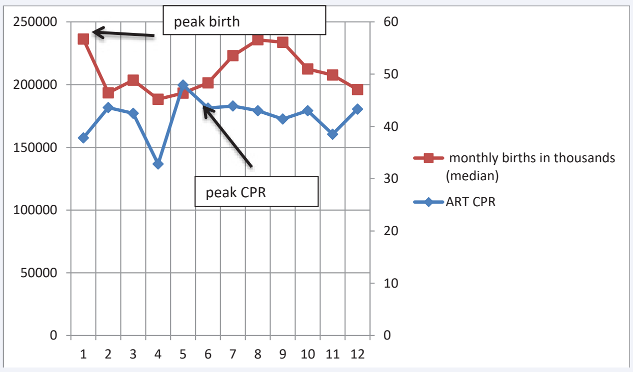

Figure 2 Figure 2: Monthly our ART CPR vs Monthly median Total births in Egypt Source: UNSD Demographic Statistics link (http://data.un.org/Data.aspx?d=POP&f=tableCode%3A55#POP) (accessed on 5-10-2017).

studies with largest series used this timing [4,16]. The rationale for this is that IVF/ICSI–ET provides unique controlled conditions for the study of seasonal influences on the human reproductive process. Although laboratory conditions are standardized to maintain stable optimal temperature, humidity, and gas tension, indirect environmental influences cannot be excluded in analogy with natural conception. Although these influences can act through gametes, it can also act through embryos. McElgunn [17], reported that the critical period prior to conception and during pregnancy are important periods for adverse influence on fertility and pregnancy outcome, and that environmental exposures and indoor air quality, are the most common concerns of women in their places of work. Some of these environmental exposures may affect IVF outcomes. It is therefore conceived that the in vitro phase of IVF/ICSI is the most vulnerable stage. As shown in table (III) the cycle parameters were comparable in the 4-calendar seasons except for mean age which was statistically lower in winter (p=0.038) and infertility duration which was statistically longer in spring (p=0.035). However according to Bonferroni correction for multiple comparisons these values are not statistically significant (as detailed previously in statistical analysis section). Our results differ from that of Rojansky et al, (2000)] in Israel although we have comparable latitude and seasonal weather. They found significantly higher fertilization rate and higher proportion of good quality embryos in spring season and correlated it with absolute daylight hours. They found no correlation between fertilization rate and embryo quality with temperature or humidity. This may be due to their small sample size (305 cases), inclusion of IVF-only cycles (no ICSI) and mechanical tubal factor infertility. Furthermore, if longer light hours were the true explanation of the differences in fertilization rate and embryo quality, summer season should have the peak values compared with other seasons as it has the longest light hours. Wunder et al. [6], reported that there were no statistically significant differences between the seasons concerning the fertilization rate, pregnancy or implantation rates that agrees with our results. Revelli et al, [5], agree with us in their study which found that ovarian responsiveness to gonadotropins, quality of gametes and embryos, and fertilization rate and implantation processes were not significantly affected by calendar seasonality and concluded that calendar season is not a relevant factor to be considered when planning an IVF treatment. Also, Wood et al. [3], in their study in Liverpool UK, found no significant differences in fertilization, implantation, or pregnancy rates between the 4 calendar seasons like our results although they noticed lower gonadotropin units per oocyte retrieved in summer vs. winter season and ascribed this to differences in daylight duration and its effect on melatonin secretion. However, when Wood et al. [3], re-analyzed their data comparing months with maximal daylight (April-September) against those occurring during the darker winter months (October-March), they found significantly higher implantation and clinical pregnancy rates in the maximal daylight months (April-September). Despite differences in geolocation our results are comparable. Only one study by Braga et al. [16], from São Paulo (coordinates 23.5505° S, 46.6333° W) in southern hemisphere linked seasonal variability in fertilization after ICSI with calendar seasons, where fertilization was higher during the spring than any other time. In Braga et al.[16], study patients undergoing ICSI were assigned to a season group according to the day of oocyte retrieval and concluded that fertilization rate was increased during the spring ($p < 0.01$). In fact, a nearly 50% increase in the fertilization rate during the spring was observed (odds ratio 1.45, confidence interval 1.20-1.75; $p < 0.01$). However, the dissimilarity between our geolocation and that of Braga et al. [16], study render comparison between their results and ours not valid. Braga et al. [16], demonstrated a seasonal variability in fertilization after ICSI, where fertilization is higher during the spring than at any other time and did not study the effect of different weather components on cycle outcome e.g. (temperature, day-light hours). Vandekerckhove et al. [14], evaluated if weather conditions determined by temperature, rain, and sunshine at the start of ovarian stimulation influenced the outcome of IVF in terms of number of mature and fertilized oocytes, pregnancy, and live birth rates. In a retrospective study they analyzed all fresh cycles (N = 9865) in Fertility Center, University Hospital, Gent, Belgium. There was a clear trend towards better results when the “early” weather conditions (one month before the treatment cycle) were good. There was a statistically significant negative correlation between the number of rainy days (Pearson Correlation -0.326; $p < 0.01$) and the rain flow (Pearson Correlation -0.262; $p < 0.05$) on the one hand and the live birth rate per cycle on the other. In other words, better weather conditions in terms of higher temperature and less rain were associated with better outcome.

Correlation analysis in our study found significant, weak, positive correlation between temperature centiles. (r= 0.036, p =0.035) and both implantation and pregnancy rates. Our regression models including wife age, number of grade A embryos transferred, stage of embryo transferred, temperature centile, light duration (≥ 50th centile or <) and calendar seasons as independent predictors of cycle outcome showed that wife age, embryo grade A number and embryo stage and temperature centile independently added to the prediction while calendar season and light duration centile did not add. The findings of the regression models were confirmed by the ROC curve where light duration and calendar seasons AUC were insignificant while that for temperature centile tended to be significant. The lack of inter- calendar season differences in cycle outcome in our study can be explained by the overlap of temperature ranges between months and between seasons in our geolocality (Tables I and II). Comparing the outcome between cold (November –April) and hot (May-October) seasons proved significantly lower IR and CPR in colder compared with warmer weather (12.7 vs. 14.5) OR & 95% CI 0.85(0.76-0.96) P=0.009 & (39.8 vs.43.8) OR & 95% CI 0 .84 (0.73-0.96) P= 0.009 respectively. In this respect we agree with Vandekerckhove et al. [14]. Analyses of epidemiological studies have shown distinct geographical differences on the impact of seasonality on natural conceptions as reported by Rojansky et al [1]. In more temperate latitudes a distinct difference is noted, with most studies showing the major peak in birth rate occurring in spring, consistent with a peak of conception during the summer months, and a smaller secondary peak of births occurring in September [19-21]. Lam and Miron [22], reported that regressions of monthly births on a flexible specification of lagged monthly temperature show that temperature has quantitatively important effects on both seasonal and non-seasonal variation in births. Martinez-Bakker et al. [7], in an epidemiologic study found that the timing of annual birth pulses followed a latitudinal gradient, with northern states of USA exhibiting spring/summer peaks and southern states exhibiting autumn peaks, a pattern which is observed throughout the Northern Hemisphere. Additionally, the amplitude of United States birth seasonality was more than two-fold greater in southern states versus those in the north consistent with weather temperature patterns. Lam and Miron [22], postulated that regarding seasonality of natural human conception there is the possibility that temperature effect depends on the magnitude with hot and cold weather suppress fecundity while moderate temperature may have no effect. Demographers have implicated a host of social, environmental, and physiological factors that may interact to drive natural birth seasonality. While a consensus has not yet been reached, mechanisms vary geographically, and hypothesized drivers include income, culture, race, holidays, rainfall, cold winters, and seasonally variable sperm quality [22]. Although Maryam Asgharnia et al. [23], study more or less similar to our study as regard sample size and methodology they reported that seasonal variation occurs in both summer and winter may be a result of transfer of high-quality embryos, this does not agree with our results, and this may be due to that we are differ in latitudes. Again, Xitong Liu et al. [24], study does not agree with our results although it is a very big study and that is explained by the study includes all types of IVF/ICSI (fresh and frozen cycles) with all indications and also, difference in latitudes. From the previous discussion we can conclude that annual variation or seasonality of human conceptions either by assisted reproductive techniques (ART) or by natural conception vary with the geolocation of the place and is related to weather temperature changes and not to calendar seasons. From our data we can say that in our geolocation ART results in cold weather months are significantly lower than hot months which do not match exactly with calendar seasons.

Among the merits of this study is the big sample size (3465) of completed ICSI cycles in a single center that means consistency and stability of clinical and laboratory aspects. However, among the limitations is that the study spanned over long time (6 years) which render it vulnerable to annual changes in weather data. Although we demonstrated stability of mean weather temperature, we remain skeptic about consistency of these records. Another limitation of this study is the absence of standard HVAC system in the laboratory. Although we reported techniques and logs to guarantee stability of laboratory environment over time, a standard HVAC would have been an advantage.

CONCLUSION

In conclusion, annual variation in ICSI cycle outcome in our geolocation seems to be the same as the annual variation in natural conception in the same region with peak conception rate in summer and is explained mainly by weather temperature.

RECOMMENDATIONS

We recommend a larger multicenter national study to correlate annual variation in ICSI live births with annual variation in natural live births. This will unequivocally prove the hypotheses of the similarity of annual variation of live birth whether natural or by ICSI/IVF techniques.

ROLE OF AUTHORS

Mohamed Elsaid Ghanem role is writing, editing, collection of data, planning of the protocol and statistics

Adel Saad Helal role is writing, editing, collection of data, planning of the protocol and statistics and is the corresponding Author

Laila Al-boghdady, **Mohamed Ahmed Emam, Ibrahim Abdelkhalek Elbahloul and Abd-Elgawad El-Metwally** sharing in collection of data and planning of the protocol

ACKNOWLEDGMENT

We thank our IVF- laboratory staff headed by Mr. Emad Sedeek, MSC, an MD student for their laboratory endeavor. We also thank our Statistical Advisor Prof Dr. Magdy Ibrahiem MD for his valuable advices on statistical presentation of our results.

REFERENCES

1. Rojansky N, Benshushan A, Meirsdorf S, Lewin A, Laufer N, Safran A. Seasonal variability in fertilization and embryo quality rates in women undergoing IVF. Fertil Steril. 2000; 74: 476-481.
2. Bronson FH. Are human seasonally photoperiodic? J Biol Rhythms. 2004; 19: 180-192.
3. Wood S, Quinn A, Troupe S, Kingsland C, Lewis-Jones I. Seasonal variation in assisted conception cycles and the influence of photoperiodism on outcome in in vitro fertilization cycles. Hum Fertil (Camb). 2006; 9: 223-229.
4. Winchester PD, Proctor C, Xie C, Gonzalez F. Successful IVF pregnancy is linked to seasonality that differs by maternal race, Fertility and Sterility. 2015; 104: e101 - e102.
5. Fleming C, Lynne Nice, Hughes AO, Hull MGR. Pregnancy: Apparent lack of seasonal variation in implantation rates after in-vitro fertilization. Human Reproduction. 1994; 9: 2164-2166.
6. Wunder DM1, Limoni C, Birkhäuser MH; Swiss FIVNAT-Group. Lack of seasonal variations in fertilization, pregnancy and implantation rates in women undergoing IVF. Human Reproduction. 2005; 20: 3122-3129.
7. Martinez-Bakker M, Bakker KM, King AA, Rohani P. Human birth Seasonality: latitudinal gradient and interplay with childhood disease dynamics. Proc R Soc.B. 2014; 281: 20132438.
8. Weigert M, Feichtinger W, Kulin S, Kaali SG, Dorau P, Bauer P. Seasonal influences on in vitro fertilization and embryo transfer. J Assist Reprod Genet. 2001; 18: 598-602.
9. Chang SY, Lan KC, Chen CW, Huang FJ, Tsai MY, Chang CY. The influences of weather on patients with different ovarian responses in the treatment of assisted reproductive technology. J Assist Reprod Genet. 2005; 22: 191-198.
10. Bronson FH. Climate change and seasonal reproduction in mammals. Philos Trans R SocLond B Biol Sci. 2009; 364: 3331-3340.
11. Cipriani L, Bianchi A, Bazzocchi A, Fabbri F, Damiano G, Ciotti P. Can global temperature affect in vitro fertilization cycles. Fertil Steril. 2016; 106: e286.
12. Ghanem ME, Sadek EE, Elboghdady LA, Helal AS, Gamal A, Eldiasty A, et al. The effect of luteal phase support protocol on cycle outcome and luteal phase hormone profile in long agonist protocol intracytoplasmic sperm injection cycles: a randomized clinical trial. Fertil Steril. 2009; 92: 486-493.
13. Stolwijk AM, Reuvers MJCM, Hamilton CJCM, Jongbloet PH, Hollanders JMG, Zielhuis GA. Infertility: Seasonality in the results of in-vitro fertilization, Human Reproduction. 1994; 9: 2300-2305.
14. Vandekerckhove F, Van der Veken H, Tilleman K, De Croo I, Van den Abbeel E, Gerris J, et al. Seasons in the sun: the impact on IVF results one month later. Facts Views Vis Obgyn. 2016; 27: 75-83.
15. Revelli Alberto, Giovanni Battista La Sala, Gianluca Gennarelli, Laura Scatigna, Cinzia Racca, Marco Massobrio. Seasonality and human in vitro fertilization outcome. Gynecol Endocrinol. 2005; 21: 1.
16. Braga DP, Setti A, FigueiraRde C, Iaconelli A, Jr, Borges E Jr. Seasonal variability in the fertilization rate of women undergoing assisted reproduction treatments. Gynecol Endocrinol. 2012; 28: 549-552.
17. Mc Elgunn B. Reproductive and developmental hazards in the workplace Clin Excell Nurse Pract. 1998; 2: 140-145.
18. Rojansky N, Brzezinski A, Schenker JG. Seasonality in human reproduction: an update Hum Reprod. 1992; 7: 735-745.
19. James WH. Seasonal variation in human births. J Biosoc Sci. 1990; 22: 113-119.
20. Lerchl A, Simoni M, Nieschlag E. Changes in seasonality of birth rates in Germany from 1951 to 1990. Natur wissenschaften. 1993; 80: 516-518.
21. Eriksson AW, Fellman J. Seasonal variation of livebirths, stillbirths, extramarital births, and twin maternities in Switzerland. Twin Res. 2000; 3: 189-201.
22. Lam DA, Miron JA. The effects of temperature on human Fertility. Demography. 1996; 33: 291-305.
23. Asgharnia M, Mehrafza M, Raoufi A, Hosseinzadeh E, Samadnia S, Atrkar Roushan Z. The effect of seasonality on reproductive outcome of patients undergoing intracytoplasmic sperm injection: A descriptive cross-sectional study. Int J Reprod BioMed. 2020; 18: 989-994.
24. Xitong Liu, Haiyan Bai, Ben W. Mol, Wenhao Shi, Ming Gao, Juanzi Shi. Seasonal variability does not impact in vitro fertilization success. Sci Rep. 2019; 9: 17185.